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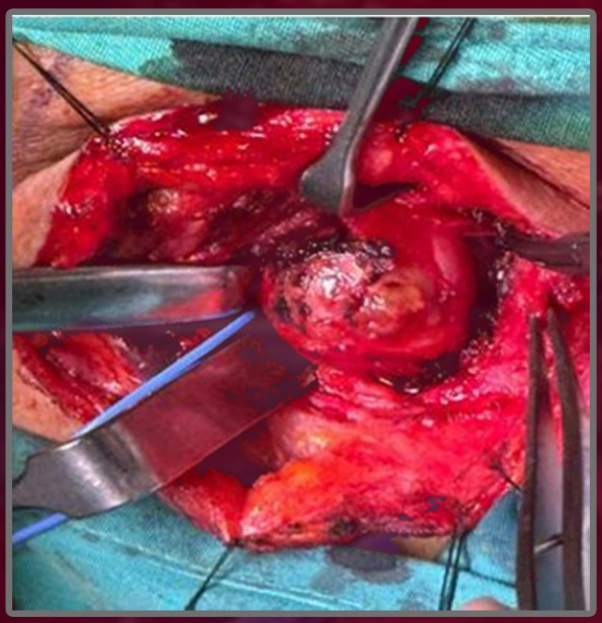

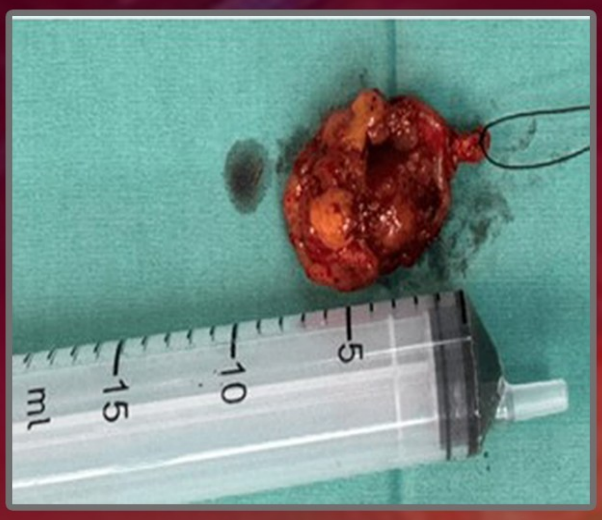

IMJM
IIUM MEDICAL JOURNAL MALAYSIA

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EDITORIAL

Volume 25 Number 1, January 2026

The Enduring Enigma of Spinal Tuberculosis in Modern Diagnostic Era and The Critical Role of Magnetic Resonance Imaging

Tuberculosis (TB), a disease of antiquity, is endemic in Malaysia and remains a public health problem despite advances in medical care. The ongoing burden is immense; in Malaysia it reached a four-decade high in 2022 with incidence rates of 113 cases per 100,000 population.[1] Of the patients with TB, about 30% have extrapulmonary involvement and among all extrapulmonary manifestations, about 10% are spinal TB.[2] Historically known as Pott's disease, spinal TB remains the most common and the most dangerous forms of skeletal TB.[2] Despite its antiquity and well-documented pathology, spinal TB persists as an enduring enigma in the modern diagnostic landscape, particularly due to persistent challenges in achieving a timely diagnosis and the devastating neurological consequences of delayed treatment.

The primary concern regarding spinal TB is the profound morbidity and mortality associated with delayed identification and management. Failure to recognize and treat the infection at an early stage often leads to severe complications and debilitating neurological deficits such as paraparesis or paraplegia. The clinical presentation of Pott's disease is often characterized by a profound lack of specificity and an indolent course, which is the cornerstone of its diagnostic enigma. Patients frequently present with non-specific symptoms such as back pain.[2] Constitutional symptoms like malaise, night sweats, and weight loss can also be present, but these are highly variable. The lack of classic, distinguishing features means that the median time from symptom onset to definitive diagnosis remains protracted, ranging between four and six months in many studies, a significant delay that directly correlates with a worse prognosis.[2]

The road to a definitive diagnosis for spinal TB is fraught with challenges, beginning with the ambiguity of the clinical picture and extending into the difficulty of pathogen confirmation. Abnormal laboratory results such as white cell count, erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP) is commonly seen. However, these are merely indicators of inflammation and are not specific to tuberculosis. Tuberculin skin test (Mantoux Test) is a test indicating prior exposure or infection and its value declines in endemic areas. In recent years, the GeneXpert MTB assay is used for diagnosis, however the cost of the test remains high and may also not be available in all hospitals in Malaysia.[1] Biopsy plays a valuable role in the diagnosis of spinal TB. However, culturing the organism, *Mycobacterium tuberculosis*, from biopsy specimen is slow and can be negative due to low organism load. The main challenge of biopsy is to obtain adequate tissue sample for complete testing (culture, histopathology and tuberculous polymerase chain reaction-PCR). In many suspected cases, no organism can be cultured despite multiple attempts. Indeed, a review from the National Tuberculosis Centre found that bacteriological or histopathological confirmation was achieved in only 29.0% of spinal TB cases, highlighting a major bottleneck in definitive diagnosis.[3] This means clinicians must often rely on presumptive diagnosis based on a combination of clinical presentation, laboratory and radiological findings.

In the current modern diagnostic era, Magnetic Resonance Imaging (MRI) has emerged as the imaging modality of choice for the diagnosis, assessment of extent, and follow-up of spinal TB.[4] MRI is highly sensitive for the early detection of the disease and is unmatched in its ability to visualize soft tissue involvement, as well as critical neural compression. MRI facilitates early detection and provides crucial morphological information that aids in differentiation from a spectrum of other spinal pathologies, which it often mimics. These include pyogenic spondylodiscitis, metastatic lesions, multiple myeloma, lymphoma and

various degenerative disorders. Key imaging features of tuberculous spondylitis (Pott's disease) include multilevel involvement and skip lesions, late preservation of the disc spaces, presence of paraspinal or psoas abscesses and anterior vertebral corner destruction causing progressive kyphotic deformity. The ability of MRI to provide such detailed anatomical and pathological information is essential, particularly in cases presenting with neurological deficits (paraplegia/paraparesis), where the need for urgent surgical intervention to decompress the spinal cord is critical.

While the clinical utility of MRI is undeniable, its role is intrinsically linked to the availability of resources. MRI is a high-cost imaging with limited resources. There is a substantial growth in the volume of MRI examinations performed across various specialty in recent years in Malaysia. One of the biggest burdens affecting MRI service in a resource-limited region is long waiting time for the patients. Based on an online survey, the current state of insufficient resources and increasing demand for MRI services in hospitals in Southeast Asia including Malaysia is recognised.[5] Almost all respondent hospitals stated the lack of MRI equipment and the need for more MRI systems and provision of advanced MRI protocols. Some of these hospitals also indicate the need for MRI system upgrade, to increase government funding and to remove red tape with procurement processes to avoid delays especially with repairing and purchasing parts of existing MRI scanner. This study emphasizes that while MRI is the preferred modality, access can be a significant barrier.[5] The establishment of advanced and affordable MRI diagnostic facilities is vital to reinforce tuberculosis control strategies.

Spinal TB remains a persistent and complex challenge, a poignant reminder of an old disease resisting full control of physicians in the modern era. The key to mitigating the morbidity lies in aggressive and timely diagnosis. The optimal diagnostic strategy for spinal TB is therefore a seamless synergy between high clinical suspicion, followed by the use of MRI to establish the extent and nature of the infection. And finally, molecular and microbiological methods (often guided by imaging) to confirm the pathogen and initiate the necessary anti-tubercular drug regimen needed. MRI is instrumental in this process and the benefits of this advanced technology must be made accessible. The call for advanced and affordable diagnostic facilities across all endemic regions must be heeded.[5] Only through a sustained, synergistic approach integrating robust clinical awareness with state-of-the-art imaging and pathogen-specific testing can the enduring enigma of spinal TB be finally resolved, thus ensuring favourable outcomes and improving the quality of life for the affected patient population.

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EXPERT OPINION

Breast Fillers on the Rise: A Silent Epidemic of Unregulated Aesthetic Trend

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ABSTRACT

The use of breast fillers for aesthetic enhancement is gaining popularity over the past decade, driven by social media influence, accessibility, and the perception of minimal invasiveness. However, unlike approved breast implants, many injectable breast fillers remain unregulated and often administered by unqualified personnel in non-medical settings. This growing trend is accompanied by a striking lack of public awareness regarding its potential complications. Reported complications range from localized inflammatory reactions, granuloma formation, chronic pain, and infection to devastating sequelae such as tissue necrosis, migration of filler material, breast deformity, and systemic embolic phenomena. Breast fillers also cause profound difficulties to routine breast cancer surveillance and diagnosis. These complications often results in substantial physical, psychological and socioeconomic burden to affected individuals. This article highlights the emerging public health concerns surrounding the use of unregulated breast fillers, emphasizing the gap between increasing popularity and insufficient patient education on safety and long-term consequences. Thus, greater regulatory enforcement, improved public awareness, and multidisciplinary collaboration between clinicians, radiologists, and policymakers are urgently needed to curb these unsafe practices, in order to safeguard patient welfare in the era of rapidly expanding aesthetic medicine.

Keywords:
breast filler, breast augmentation, siliconomas, breast cancer, complications

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INTRODUCTION

Breast augmentation is increasingly becoming popular in Malaysia, driven by societal influences that prioritizes certain physical attributes, including breast size and shape, as part of prevailing beauty standards among women. Driven by demands from patients for less invasive and cheaper alternatives to traditional surgical procedures, this aesthetic field has undergone a transformative evolution. This dynamic landscape has fuelled the development and introduction of numerous types of tissue fillers for breast contouring. These tissue filler substances, mostly pose significant, and often severe long-term safety risks.[1,2] This presently widespread, and often unregulated use of breast fillers as breast augmentation alternatives has simultaneously ushered in a silent public health epidemic, particularly in the context of oncological surveillance carried out by screening mammogram. This necessitates immediate academic and regulatory scrutiny to be put in place.

Historical perspective and current trends

Historically, injectable breast augmentation is fraught with examples of many materials initially hailed as outstanding substances, only to be later abandoned due to catastrophic complications. Materials like paraffin, that was used in the early 20th century, were ultimately abandoned because they led to migrating foreign body reactions, fibrosis, and the formation of *paraffinomas*. Similarly, the use of liquid silicone injection and polyacrylamide hydrogel (PAAG), prevalently used in the earlier decades, is now prohibited substance in many countries due to established safety concerns.[2,3,4]

Collagen fillers, which were initially popular due to its natural origin and relatively safe, now also shows significant number of cases of allergic reactions in patients. Another breast filler, the hyaluronic acid (HA) which is a naturally occurring substance in the human

body is currently reported as a good alternative with better biocompatibility and reversibility. HA is approved by the Food and Drug Administration (FDA) United States of America and the European Union Nations as fillers for facial areas, but not for breast augmentation. In this case, a considerably larger volume of filler is required for breast augmentation using HA. Pure HA is commercially available but, due to the high cost, HA products are often compounded with silicone. Due to this mixing, a significant risk of unforeseen complications and permanent tissue damage has been reported with the procedures using HA.[4,5] It is possible that patients may be inadequately informed about the complications prior to the procedure, as both the provider and patients may be unaware of the presence of silicone in the formulation (Figure 1).

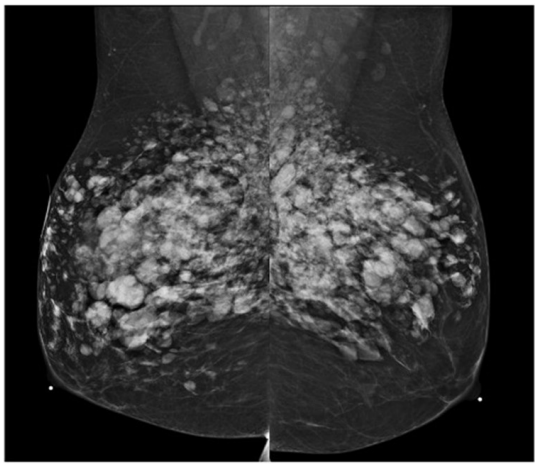

Figure 1: A 50-year-old woman presented for routine screening mammography and was surprised to learn that a supplementary breast MRI was required due to the obscuring effect of previously injected breast fillers. She had undergone the procedure more than a decade earlier and she had been informed at the time that the fillers were not permanent.

Clinical complications

The common clinical complications reported due to these breast fillers include infection, hematoma and seroma formation, induration and pain.[6] Extensive migration and displacement of the materials can cause serious medical complications such as pulmonary embolism and stroke.[7,8,9] Furthermore, this foreign material can trigger a chronic inflammatory response in the surrounding breast tissue. This intense inflammatory response and foreign body reaction may then result in granuloma formation, tissue necrosis, and breast asymmetry, and finally posing a significant source of morbidity for the affected patients.[5,7] Recent studies report that PAAG injections are associated with high rates of complication which include induration or nodules (60%), pain (27.4%), migration (13.3%), infection (5.2%), and calcification (3.7%). The above complications often manifest months or even years after the procedure has been done, with some cases being reported up to 20 years later.[1,3]

Perhaps the most critical and often overlooked danger of unregulated injectable augmentation is the profound challenge it poses to routine breast cancer surveillance and diagnosis.[8,10] The presence of these synthetic materials and the subsequent inflammatory and foreign body reaction fundamentally alter the radiological appearance of the breast, creating a diagnostic quagmire for radiologists. Worryingly, breast cancer in augmented breasts is notoriously difficult to detect, as the injected materials often obscure lesions on conventional screening modalities like mammography and sonography.[11,12] Sometimes, the use of unidentified injection substances, often by unverified operators, can present with radiological findings that mimic suspicious breast microcalcifications. These unidentified materials may often appear as asymmetric, rounded amorphous densities with scattered dystrophic calcifications, making it difficult for radiologists to differentiate post-augmentation changes from true malignancy. In many cases radiologists need to use additional and more advanced multimodality imaging such as Magnetic Resonance Imaging (MRI) and Contrast-Enhanced Spectral Mammography (CESM) which are more expensive and less widely available to resolve this issue. [10,12,13] (Figure 2).

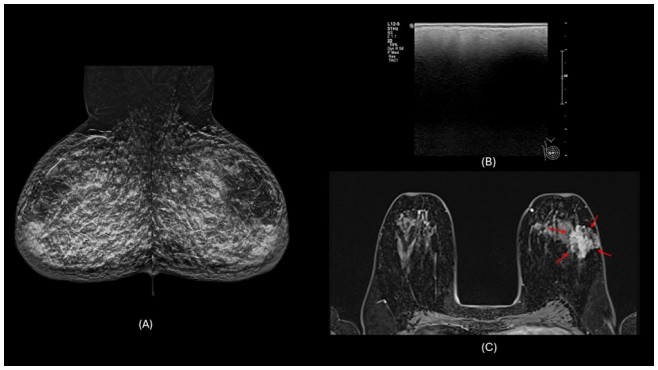

Figure 2: A 64-year-old woman, who had experienced persistent "lumpy breasts" following a prior breast augmentation procedure, presented late after noticing a significant increase in lump size. Mammography (A) and ultrasound (B) failed to reveal any suspicious lesion. Subsequent MRI (C) identified a suspicious mass in the left breast (arrows). The lesion was biopsied, and histopathology confirmed invasive carcinoma.

It is important to also note here that not all patients experience positive psychological outcomes following breast fillers procedures. This is so particularly in those patients who have experienced complications, as these patients have reported a lower level of satisfaction and self-esteem. Unfortunately, in the majority of the above cases, the risks were not adequately explained to patients prior to the procedure. Many women who experience such adverse events following unregulated breast augmentation often choose not to come forward or seek justice due to a complex interplay of emotional, social, cultural and systemic barriers. Many of them fear of being judged for their choices of going for breast augmentation. Among Muslim women, this cosmetic procedure is often regarded as religiously impermissible[14], leading many patients to seek to undergo such procedures in private centres and demand strict confidentiality. Consequently, the affected individuals hesitate to report complications due to fear of potential repercussions, while many others remain unaware of their rights or the legal avenues available to them.

Despite all the above concerns, contemporary unregulated injectable breast fillers continue to enter the market as the demand grows. The surge of such breast fillers represents a serious and growing public health crisis. The harmful effects of unregulated breast fillers are not merely cosmetic but they encompass various forms of complications, which includes the necessity for more complicated surgical intervention in certain cases. And most importantly, the profound challenge to oncological surveillance that delays or obscures the diagnosis of life-threatening breast cancer.

In our experience at our centres, we are seeing more and more cases each year, and especially in younger patients involved in these procedures. In previous years, women in 40s and above usually undergo this procedure. But now, patients as young as in their late twenties already opt for this type of breast augmentation. The psychological impact of the adverse event is also worrying with many women suffering in silence and these unlicensed practitioners seldom face any kind of accountability. While the global academic evidence unequivocally highlights the long-term dangers of these injectable materials, there is a notable lack of scientific data from Malaysia. This gap highlights the urgent need for more comprehensive research in Malaysia and a regulatory paradigm shift to address safety concerns and thus protect patients.

Regulatory landscape and Recommendations

Guidelines by the Ministry of Health Malaysia (MOH) pertaining to aesthetic procedures have been introduced but lack of specific regulation addressing the use of fillers is one of the main concerns.[15] Reports of unlicensed practitioners offering breast filler injections also raise the issue of lack in enforcement and monitoring system of the existing regulations. To address these challenges, there is a need for collaboration between MOH, healthcare professionals, academicians, researchers and industry stakeholders. Regulatory authorities must implement stricter controls on the use of these materials and the procedures. An informed comprehensive counselling should be mandated to patients before the procedure and must be done by adequately trained providers. A standardised, long-term follow-up guidelines including psychological assessment should be formulated for all patients with a history of injection augmentation mammoplasty. Public awareness campaigns must be launched to educate prospective female patients of the grave, long-term consequences of these procedures and to counteract the often, unrealistic portrayals of these procedures in the media and advertising. Only through such concerted efforts across regulatory, medical, and public health sectors can this silent epidemic of unregulated aesthetic trends of unregulated breast fillers be addressed in order to protect patients from what are often devastating and avoidable long-term health consequences.

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REVIEW ARTICLE

The Impact of Qur'anic Reading and Listening Duration and Frequency on Cognitive Performance and Hippocampal Function: A Systematic Review

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ABSTRACT

Engaging with the Qur'an, whether through reading or listening, necessitates complex cognitive processing of the written or spoken Arabic language. This activity demands concentration and attention, thereby engaging cognitive functions such as selective attention, Arabic language processing, and auditory perception. The purpose of this paper is to review the effects of reading and listening to the Qur'an on hippocampal cognitive function. A literature search was conducted using online databases: ScienceDirect, PubMed/ Medline, and Scopus. The keywords used in the literature search were "cognitive", "Qur'an", and "hippocampus". This approach included six full-length articles. A manual search of the cited references was also used to find additional considerations for the discursive analysis of each topic discussed in this review. As a sacred text, the Qur'an engages both reading and listening skills and involves cognitive processes that impact memory and comprehension. Regular engagement with the Qur'anic text and its recitation can significantly improve brain activity, memory, and cognitive development. Reading and listening are essential skills for language comprehension and communication. When a person reads the Qur'an, he or she engages in visual Arabic language processing, while when listening to its recitation, the person is involved in auditory language processing. These activities stimulate different parts of the brain, contributing to a more comprehensive understanding of the Qur'an text.

Keywords:
Qur'an, cognitive function, hippocampus, reading, listening, neuroplasticity.

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INTRODUCTION

The hippocampus is an important brain structure that supports not only memory functions but also broader cognitive domains, including executive function, working memory, processing speed, and information integration.[1–3] Optimal learning depends on the interplay of these functions, and hippocampal neurogenesis plays a significant role in both cognition and behaviour[4,5] Structurally, the hippocampus comprises several subfields that may be differentially sensitive to specific aspects of cognitive performance.[4]

Reading and listening represent two fundamental modalities of language comprehension. Although distinct, they share overlapping neural substrates that process semantic meaning. The temporal lobe contributes primarily to phonological awareness and comprehension, while the hippocampus underlies declarative memory and higher-order cognition.[1,3,5] Evidence suggests that listening and reading are linked to auditory processing and cognitive mechanisms. However, studies directly examining their relationship with hippocampal-dependent cognitive functions remain limited.[6] Moreover, cognitive and metacognitive strategies such as planning, monitoring, and attentional control are essential for enhancing comprehension in both modalities, particularly during language learning.[7]

Attention plays a particularly important role in listening comprehension, as auditory information unfolds rapidly and requires immediate processing. By contrast, reading comprehension benefits from extended exposure and the gradual construction of a situation model. These

differences indicate that reading and listening rely on overlapping but partly distinct cognitive processes, with attention being more critical for listening than for reading.[8,9]

Importantly, engagement with the Qur'an through reading, listening, and memorization has been associated with unique cognitive benefits. Studies show that older adults who regularly recite or memorize the Qur'an experience enhanced memory, attention, and overall cognitive performance, with potential protective effects against dementia.[10–12] Neurophysiological findings further support this, demonstrating increased theta-wave synchronization within temporal–hippocampal networks during Qur'an recitation and listening.[13,14] However, current research remains fragmented: different cognitive tools are applied across studies (e.g., MMSE vs. MoCA-Ina), outcomes are inconsistent, and critically, no study has directly assessed hippocampal morphology or function using neuroimaging techniques such as MRI volumetry.

Despite growing evidence that Qur'an engagement benefits cognition, existing studies rely mainly on indirect cognitive measures rather than direct assessments of hippocampal function. There is limited understanding of how reading and listening to the Qur'an, especially in terms of duration and frequency, differentially influence hippocampus-related cognitive functions. Furthermore, methodological inconsistencies across studies hinder the comparability of results, and the absence of neuroimaging data leaves underlying neural mechanisms largely unexplored.[2,10–15] To our knowledge, this is the first systematic review to synthesize and critically evaluate evidence on the effects of Qur'an reading and listening on hippocampal-dependent cognitive functions. By addressing both behavioural and neurophysiological findings, this review highlights Qur'an engagement as a culturally relevant cognitive intervention and identifies pathways for future studies to integrate standardized cognitive assessments and neuroimaging. This approach provides a novel foundation for understanding the role of Qur'an engagement in supporting cognitive resilience and healthy aging.

METHOD AND METHODS

Protocol and Reporting Framework

This review was conducted in accordance with the *Preferred Reporting Items for Systematic Reviews and Meta-Analyses Protocols (PRISMA-P)* guidelines. The review process took place between October 1, 2024, and January 2025. Findings are reported following the PRISMA 2009 statement, ensuring transparency and reproducibility.

Eligibility Criteria

Inclusion criteria

Eligible studies were randomised controlled trials (RCTs), quasi-experimental studies, cohort studies, and cross-sectional studies that objectively measured cognitive or neurobiological outcomes. Specifically, studies evaluating the effects of Qur'an reading (*tilawah*) or listening (*sama'*) on hippocampus-related cognitive functions were included. Only studies published in English or Indonesian within the past 10–15 years were considered to ensure relevance to recent advances in neuroscience and religious research.

Exclusion criteria

Studies were excluded if they involved animal models, case reports, correspondence, review articles, editorials, or expert opinions.

PICO Framework

- **Population:** Adolescents and adults aged 18–65 years, with or without cognitive impairment.
- **Intervention/Exposure:** Reading (*tilawah*) or listening (*sama'*) to the Qur'an.
- **Comparison:** No exposure to Qur'anic recitation, or exposure to alternative auditory stimuli (e.g., music, non-religious texts).
- **Outcome:** Changes in hippocampus-related cognitive functions (e.g., memory, executive function, processing speed, neurophysiological measures).

Search Strategy

A systematic search was performed in PubMed/ MEDLINE, Scopus, and Science Direct. Boolean operators (AND/OR) were applied to refine results. Reference lists of eligible studies were also screened to identify additional relevant publications. Search string for Pubmed/MEDLINE database: ("Quran"[MeSH Terms] OR "Qur'an"[Title/Abstract] OR "Koran "[Title/Abstract]) AND ("Cognition"[MeSH Terms] OR "Cognitive Function "[Title/Abstract] OR "Memory" [Title/Abstract] OR "Executive Function" [Title/Abstract]) AND ("Hippocampus "[MeSH Terms] OR "Hippocampal"[Title/Abstract]). Scopus database: (TITLE-ABS-KEY (qur'an OR quran OR koran) AND TITLE-ABS-KEY (cognit* OR memory OR "executive function") AND TITLE-ABS-KEY (hippocampus OR hippocampal)) AND PUBYEAR >2008 AND (LIMIT-TO (LANGUAGE , "English") OR LIMIT-TO (LANGUAGE , "Indonesian")). Science Direct database: ("Qur'an" OR "Quran") in Title, Abstract, or Keywords; AND ("cognit*" OR "memory") in Title, Abstract, or Keywords; AND ("hippocampus") in Title, Abstract, or Keywords. Filters applied: Publication years 2009-2024; Article type: Research articles.

Study Selection

Three reviewers (UR, STP, LAF) independently screened studies in three phases: title screening, abstract screening, and full text screening. Discrepancies were resolved through discussion, and unresolved disagreements were referred to an additional reviewer. A PRISMA flow diagram was used to document the selection process, including reasons for exclusion.

Data Extraction

A standardised data extraction form (Microsoft Excel) was developed prior to the review. Extracted information included: study characteristics (author, year, country, design), participant characteristics (age, sample size, health status), intervention details (reading or listening modality, duration, frequency), comparator details, cognitive or neurobiological outcome measures, and main findings. Data were independently extracted by the reviewers and cross-checked for accuracy.

Data Synthesis

Given the expected heterogeneity of study designs and outcomes, a narrative synthesis was performed. Findings were summarised according to type of exposure (reading vs. listening), duration and frequency of exposure, and cognitive outcomes related to hippocampal function.

RESULT

Study Selection

The search identified 85 unique studies. After excluding duplicates, 59 abstracts were screened. Only 33 articles were assessed for eligibility. Of the 12 studies, nine were experimental, two were observational, and one was a brain wave study. Full details on the included studies are in **Figure 1**.

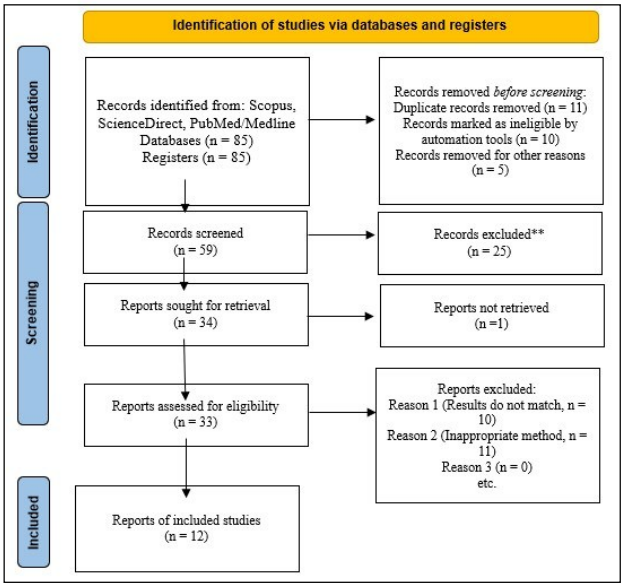

Figure 1. Figure 1: PRISMA flow chart of the inclusion process

Our analysis of the twelve included studies indicated that the majority demonstrated positive effects of Qur'anic reading or listening on various cognitive domains. Several cross-sectional studies and quasi-experimental designs demonstrated significant improvements in MMSE or MoCA-Ina scores, indicating enhanced memory and overall cognitive performance.[10,11,16]

EEG-based studies showed increased brain wave activity

Table 1. Reading and listening to the Qur'an on cognitive

No	Author (Year)	Study design	Participants	Assessment Tool	Key Findings	Ref
1	Irawati (2019)	Cross-sectional.	96	MMSE	The duration of reading the Koran affects cognitive	10
2	Nurfiani (2018)	Quasi Experiment	50	MMSE, MoCA-Ina	There is a relationship between reading the Qur'an and improving memory	11
3	Julianto (2011)	Brain waves	4	EEG	Significant increase in brain wave activity after reading the Qur'an, which has the potential to improve short-term memory abilities	12
4	Choirunnisa (2020)	Quantitative	39	Instrument	Reading the Qur'an improves cognitive	20
5	Indrijaningrum (2020)	Case-control	34	MoCA-Ina	The duration and frequency of reading the Qur'an have no relationship with cognitive	13
6	Samhani (2022)	Experiment	28	EEG	Surah Al-Fatihah (Qur'an) improves memory	15
7	Slamet (2019)	Experiment	10	Pre-post treatment	The cognitive intelligence score of children was 25.40 after the treatment	17
8	Sirin (2021)	Quantitative	33	Instrument	Significant difference in verbal learning, visual learning, attention speed, and phonemic and semantic fluency before and after memorisation training	19
9	Faqihuddin (2024)	Mixed-Methods	50	In-depth interviews	The tradition of Qur'an memorization remains strong in Muslim families and is increasingly applied to early childhood	21
10	Munawaroh (2023)	Quasi Experiment	22	Instrument MMSE	Increased scores for the word-list recall test. No significant improvement in CG. However, there was an improvement in the HQ group, represented by a significantly higher score after the treatment.	16
11	Muthohharoh (2023)	Qualitative Study	30	Children memorise the Qur'an and compare the contribution of each systemic cognitivist modelling (SCM) indicator to their average ability	The children's memorisation ability was quite good	18
12	Hussain (2021)	Experiment	50	listened to Qur'an recitation	A significant increase in working memory performance among participants in the experimental group	22

following Qur'an reading or listening, potentially strengthening short-term memory.[12,15] Research involving children reported good memorisation abilities and improved cognitive intelligence scores after memorisation training.[17,18] Interventions involving listening to the Qur'an demonstrated significant improvements in working memory among participants in the intervention group.[23] Similar findings were reported by Sirin, with improvements in verbal learning, visual learning, attention speed, and both phonemic and semantic fluency.[19]

However, one study found no significant association between the duration/frequency of Qur'anic reading and cognitive function (p= 0.089).[13] This variation in findings may be attributed to differences in study design, intervention duration, measurement tools, and participant characteristics. Overall, these findings support the hypothesis that reading and listening to the Qur'an can provide neurocognitive benefits, particularly in memory, attention, and executive functions involving the hippocampus.

Effects on Cognitive Functioning Based on Psychometric Assessments (MMSE/MoCA)

There were mixed findings from studies using cognitive screening tools. Two studies reported a significant positive association. A cross-sectional study by Irawati found that the duration of reciting the Qur'an significantly affected cognitive function in the elderly (p=0.001).[10] Similarly, a study by Nurfiani showed an association between reading the Qur'an and improved memory function as measured by MMSE and MoCA-Ina (p=0.000).[11] In contrast, a case-control study by Indrijaningrum found no significant relationship between the duration and frequency of reading the Qur'an and cognitive function measured using MoCA-Ina (p=0.089).[13]

Effects on Brain Activity and Memory (EEG)

Studies using neurophysiological measurements consistently show positive effects. Julianto reported a significant increase in brainwave activity after reciting the Qur'an, potentially improving short-term memory ability

($p<0.05$).[12] In parallel, an experimental study by Samhani found that listening to *Surah Al-Fatihah* also significantly improved memory, as evidenced by EEG data ($p<0.050$).[15] These findings provide objective evidence of changes in brain activity associated with memory improvement, both through reading and listening modalities

Other Effects

One experimental study by Slamet focusing on a paediatric population showed that Qur'anic memorisation interventions can improve cognitive intelligence scores.[15]

DISCUSSION

This systematic review synthesizes evidence from twelve studies investigating the relationship between Qur'anic engagement (reading and listening) and hippocampus-related cognitive functions. While the majority of included studies reported positive effects, a critical appraisal reveals a landscape of evidence that is promising yet preliminary. The significant heterogeneity in study designs, ranging from cross-sectional surveys in elderly populations to small EEG experiments in young adults, precludes definitive conclusions and highlights the need for cautious interpretation. Furthermore, the overreliance on psychometric screening tools like the MMSE, which may lack sensitivity to subtle hippocampal-dependent changes, and the scarcity of direct neuroimaging data, limit the depth of our current understanding. The central challenge, therefore, is to reconcile these varied findings by integrating them with established neuroscience to propose plausible mechanistic pathways.

Cognitive and Hippocampus

The hippocampus is a critical brain structure that undergoes several structural changes, both grossly and at the cellular level, with age. These changes have been correlated with cognitive decline in older adults. In non-demented older individuals, regional hippocampal morphology is associated with specific memory abilities and broader cognitive domains[2]. It is also involved in general information processing, including spatial information processing, temporal sequencing, and formulating relationships between objects in the environment.[3] The hippocampus is an important structure in cognitive aging, playing a role in episodic memory and broader cognitive domains.[1] The hippocampus undergoes several structural changes both grossly and at the cellular level with age, and these have been correlated with cognitive decline in older adults. In non-demented older individuals, regional hippocampal morphology is associated with specific memory abilities and broader cognitive domains.[2] The hippocampus is also involved in creating and storing representations of the physical environment, but it also plays a role in many other aspects of memory. The view that the human hippocampus acts primarily to create and store representations of the physical environment should be interpreted in the context of the abundant evidence showing that the human hippocampus plays a role in many other aspects of memory.[2,5,23,24]

The hippocampus plays an important role in supporting spatial navigation and memory. It performs these functions through Cognitive Mapping, where it is responsible for creating and maintaining mental representations of the physical environment. These maps allow us to navigate and remember spatial information, such as the location of objects or landmarks.[25,26] The hippocampus organises spatial information relationally, which helps us understand the relationship between objects and locations, thereby forming accurate and detailed spatial memory. This structure also enables cognitive map flexibility, allowing us to update and adapt our spatial representations based on new information or changes in the environment. This flexibility is important for efficient navigation and memory formation.[26] The hippocampus interacts with other memory systems, such as the dorsal striatum, to support spatial navigation. These interactions contribute to various navigation scenarios and allow for the integration of different types of memory, including spatial and episodic memory.[25] Spatial navigation training has been shown to protect the hippocampus from age-related changes. Studies have found that hippocampal volume decreases with age, but spatial navigation training can help mitigate these changes and preserve cognitive function.[27]

Cognitive, Listening, and Hippocampus Mechanism

The hippocampus supports cognitive processes by forming and comparing relational memory representations. This mechanism allows for the integration of different types of information, including visual and auditory inputs, from specialised cortical regions. It enables the hippocampus to combine disparate inputs and contribute to perception and online processing.[28] This type of memory is essential for cognitive listening as it allows individuals to retain and retrieve information they have heard. The interplay between the hippocampus and other brain regions, such as the prefrontal cortex, is crucial for memory consolidation.[25] Coordinated replay between the hippocampus and neocortical areas is believed to be a key mechanism in consolidating memories,[2] which helps in the long-term retention of information obtained through cognitive listening. While the traditional view of the hippocampus is that it creates a mental map of physical space, recent research suggests that it may also play a role in mapping social space.[5] This cognitive mapping function can contribute to understanding and navigating social interactions, which are essential aspects of cognitive listening in social contexts. The hippocampus is involved in general information processing, including spatial information processing, temporal sequencing, and formulating relationships between objects in the environment.[3] These cognitive processes are relevant to cognitive listening as they contribute to comprehending and interpreting auditory information.

Cognitive listening refers to actively and attentively engaging with auditory information, such as speech or music, to understand and interpret its meaning. It involves various cognitive processes, including attention, perception, memory, and comprehension. The hippocampus, a brain structure located in the medial temporal lobe, is related to mental listening through its involvement in several cognitive functions; the hippocampus plays a critical role in encoding and retrieving memories, including verbal and auditory information.[2,25] It helps form new memories of what has been heard during cognitive listening and retrieve those memories when needed. Declarative memory, which involves recalling facts and events, is supported by the hippocampus.[5] The hippocampus is involved in relational processing, which refers to the ability to understand and integrate relationships between different elements of information.[28]

This is relevant to cognitive listening as it enables the comprehension and interpretation of auditory information by connecting and organising different pieces of information. While traditionally associated with creating cognitive maps for physical space, recent research suggests that the hippocampus may also play a role in mapping social space.[5] This cognitive mapping function can contribute to understanding and navigating social interactions, which are essential aspects of cognitive listening in social contexts.

The hippocampus is involved in general information processing, including spatial information processing, temporal sequencing, and formulating relationships between objects in the environment.[3] These cognitive processes are relevant to cognitive listening as they contribute to comprehending and interpreting auditory information.

Listening comprehension plays a prominent role in reading comprehension.[5] Evidence has shown that oral language comprehension is important for reading comprehension across different languages.[5] Regarding cognitive skills, vocabulary, and word reading fluency are shared contributors to reading and listening comprehension.[29] A study compared the brain activation patterns associated with comprehending written and spoken sentences in Portuguese. The results showed modality effects and individual differences in language comprehension.[12] Regarding the reading versus listening debate, a neuroscientist explains that both reading and listening have brain benefits in common. In both situations, the brain is working to connect the puzzle pieces, making sense of the plot and attempting to predict what will happen next.

Research suggests that listening and reading activate almost identical brain activity and that the brain's representation of meaning does not depend on which

sense acquires the words that convey it. Both reading and listening comprehension share some cognitive components, such as language comprehension and decoding.[29,30] However, they also have some differences. For example, reading requires the decoding of text, while listening does not.[30] The cognitive process involves constructing meaning from text and requires skills such as attention, visual discrimination, sequential processing, immediate memory, and working memory. These skills must be automatic for successful reading; many struggling readers lack these skills. Some common cognitive biases that can impact listening include confirmation bias, anchoring bias, availability bias, hindsight bias, and overconfidence bias.[31] To overcome these biases, individuals can actively seek additional information, consider alternative perspectives, and seek feedback from others.[29]

Reading and listening to the Qur'an can have cognitive benefits and enhance cognitive abilities. Memorising the Qur'an, in particular, is believed to develop several cognitive skills, strengthening an individual's aptitude.[8] However, the specific cognitive benefits of reading the Qur'an have yet to be extensively studied. Reading the Qur'an involves cognitive skills such as attention, visual discrimination, sequential processing, immediate memory, and working memory.[32] These skills are essential for successful reading and comprehension. The cognitive benefits of reading Al-Qur'an may extend beyond the development of basic cognitive skills. For many individuals, reading and reciting the Qur'an has spiritual and psycho-spiritual significance. The Qur'an is considered sacred, and its recitation is often associated with religious devotion and spiritual growth.[32]

Reading and listening to Al-Qur'an can have potential cognitive benefits, particularly for seniors. Here are some points related to reading, listening to the Al-Qur'an, and cognitive function: A review of the literature suggests that listening to Al-Qur'an has potential as a psycho-spiritual therapy and may have positive effects on cognitive function.[8]

Evidence suggests that oral language comprehension is essential for reading comprehension, and the two are closely related.[29,31,33] This relationship may be particularly relevant for seniors who may experience declines in cognitive function. Online resources are available for learning to read and listen to Al-Qur'an, including one-to-one classes and websites that offer reading and listening practice. Reading and listening to Al-Qur'an requires cognitive skills such as attention, memory, and visual processing.[29] Some studies suggest that reading, listening, and memorizing Al-Qur'an may stimulate the brain's nerves and positively impact cognitive function.[34]

The Qur'an possesses a unique harmony that other sentences do not have. The verses in the Qur'an contain words full of goodness so that they have a positive effect and give peace.[35] When listening to the recitation of the Qur'an, a series of sound waves from the Qur'an that reaches the brain will positively affect the responsiveness of its cells. Following the nature of Allah SWT, the brain will respond in the right direction.

Towards a Mechanistic Understanding: Potential Pathways

Based on the synthesized findings and established neuroscience, we propose several non-mutually exclusive hypotheses for how Qur'anic reading and listening might influence hippocampal function:

1. Auditory Entrainment and Theta Synchronisation: As discussed above, the rhythmic and melodic nature of Qur'anic recitation may serve as an auditory driver for cortical and hippocampal theta rhythms. This entrainment could optimise the brain's internal state for learning and memory consolidation, a mechanism similar to that proposed for certain types of music therapy.
2. Modulation of Neurotrophic Factors: Regular cognitive engagement, particularly complex tasks like memorising a foreign language (Classical Arabic), is known to upregulate Brain-Derived Neurotrophic Factor (BDNF), a key protein supporting hippocampal neurogenesis and synaptic plasticity. The intense cognitive training involved in Qur'anic memorisation may exert its long-term benefits, as suggested in studies with children and the elderly,[17, 19] through this BDNF-mediated pathway.

3. Stress Reduction and Neuroendocrine Effects: Listening to the Qur'an has been associated with reduced anxiety and inducing a state of peace.[35] Psychological stress elevates cortisol levels, which have a known neurotoxic effect on the hippocampus. It is plausible that the stress-reducing (psycho-spiritual) effect of engaging with a sacred text protects the hippocampus from glucocorticoid-related damage, thereby preserving cognitive function and potentially slowing age-related decline.[10,11,16] This pathway may be particularly relevant for the positive findings in elderly populations.

LIMITATIONS

This systematic review has several limitations that should be considered when interpreting the findings. First, the included studies themselves were methodologically heterogeneous, with variations in design, sample size, and cognitive assessment tools, preventing a meta-analysis. Secondly, many of the studies, particularly the EEG investigations, had very small sample sizes, increasing the risk of type II errors and reducing the generalisability of the results. Third, the reliance on behavioural and neurophysiological measures means the direct evidence linking Qur'anic engagement to hippocampal morphology or function via neuroimaging (e.g., fMRI) is still absent. Finally, the potential for publication bias, wherein studies with null findings are less likely to be published, cannot be ruled out. Future research should prioritize larger, randomized controlled trials with standardised outcome measures and incorporate direct neuroimaging to elucidate the underlying neural mechanisms.

CONCLUSIONS

Reading and listening are essential skills for language comprehension and communication. When a person reads the Qur'an, he or she engages in visual language processing, whereas when the person listens to the recitation of the Qur'an, he or she are involved in auditory language processing. These activities stimulate different brain parts, contributing to a more comprehensive understanding of the Qur'an text.

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CONFLICT OF INTEREST

The author declares there is no conflict of interest.

INSTITUTIONAL REVIEW BOARD (ETHICS COMMITTEE)

None.

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AUTHOR CONTRIBUTIONS

Conceptualization was contributed by UR, STP, LAF, and FM; Design was carried out by UR and FM; Supervision was provided by UR; Data collection was conducted by UR, STP, and LAF; Data analysis was performed by UR and FM; Manuscript writing was carried out by UR and FM. All authors have read and agreed to the published version of the manuscript.

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REVIEW ARTICLE

Agarwood's Role in Inflammatory-related Conditions: A Systematic Review of Animal Models

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ABSTRACT

Agarwood (*Aquilaria* spp.) is a resinous wood traditionally used in various medicinal systems across Asia for treating inflammation-related ailments. Despite its longstanding ethnopharmacological use, scientific validation of its anti-inflammatory effects remains fragmented. This scoping review aims to systematically evaluate and synthesize current evidence from animal studies investigating the anti-inflammatory potential of agarwood. A comprehensive literature search was conducted using PubMed, Scopus, and Web of Science. Inclusion criteria focused on original animal studies assessing the anti-inflammatory effects of agarwood extracts, essential oils, or derivatives. Data on study design, animal models, agarwood species, treatment dosage, duration, biomarkers, and outcomes were extracted and summarized narratively due to methodological heterogeneity. Eight studies met inclusion criteria, involving models of inflammation-related conditions such as pain, neuroinflammation, gastrointestinal injury, cancer, and toxicity. Agarwood treatment consistently reduced pro-inflammatory cytokines (e.g., IL-1β, IL-6, TNF-α), modulated oxidative stress markers (e.g., NO, SOD, GSH), and regulated signalling pathways including NF-κB, p38 MAPK, and Nrf2–ARE. Notably, improvements were observed in behavioural and histological outcomes across models, with evidence of dose-dependent effects in several studies. In conclusion, preclinical evidence supports agarwood's broad-spectrum anti-inflammatory and antioxidant properties across multiple organ systems. These findings provide mechanistic insights and a scientific basis for its traditional use. However, variability in species, extraction methods, and study designs highlights the need for standardised protocols and clinical validation to advance agarwood as a potential therapeutic agent.

Keywords:
agarwood, Aquilaria, anti-inflammation, inflammatory, oxidative, animal model

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INTRODUCTION

Agarwood (*Aquilaria* spp., *Thymelaeaceae*) is a highly valued, fragrant, resinous wood with a history dating back to approximately 1400 BCE. Revered in religious texts, classical literature, cultural rituals, and historical trade, agarwood is most prized for the aromatic heartwood formed, which forms in response to injury and emits a distinctive scent when burned.[1] While widely known for its role in perfumery, agarwood is also used in wood chips, carvings, resins, and traditional medicine.[2]

Due to high demand and overharvesting, wild agarwood populations have declined significantly. The International Union for Conservation of Nature (IUCN) has classified several species as threatened, including *Aquilaria malaccensis* (Critically Endangered), *A. microcarpa* (Endangered), *A. sinensis* (Vulnerable), and *A. subintegra* (Data Deficient).[3] Consequently, international trade is regulated under the Convention on International Trade in Endangered Species (CITES), spurring interest in sustainable cultivation and alternative products such as seedlings, inducers, fertilizers, hydrosols, and leaf-based extracts.

Traditionally, agarwood has held therapeutic value in Chinese, Ayurvedic, Unani and Malay medicine for ailments including coughs, rheumatism, jaundice, and postpartum disorders, often serving as stimulant, tonic,

and carminative agent.[2,4] Historical medical texts such as Avicenna's *Canon of Medicine* and China's *Compendium of Materia Medica* document its medicinal applications.[5,6] In Malay traditional medicine, *A. malaccensis* (locally known as *kayu gaharu* or *kayu gaharu lempong*) is used in boiled preparations and liniments for treating pain, inflammation, and female reproductive conditions.[2] Historical Malay medical manuscripts such as *Kitab Tib MSS 2515*, *MSS 2999 Kitab Tib: Pandangan dan Tafsiran Perubatan Moden Terhadap Manuskrip Perubatan Melayu* (Modern Medical Perspectives and Interpretations of Malay Medical Manuscripts), and *Kitab Tib Muzium Terengganu* document its therapeutic significance and integrate spiritual practices such as Quranic recitations alongside herbal remedies, underscoring agarwood's deep cultural and medicinal significance in Malay ethnomedicine.[7]

Recent research has focused on the phytochemical constituents of agarwood and their pharmacological potential, particularly anti-inflammatory effects.[2,8,9] Inflammation is a vital physiological defence mechanism through which the immune system identifies and eliminates harmful or foreign stimuli to promote tissue repair and healing.[10,11] However, chronic or dysregulated inflammation contributes to numerous diseases, including cancer, atherosclerosis, rheumatoid arthritis, and sepsis.[12,13]

Despite an extensive body of research particularly those using *in vitro* approach, the anti-inflammatory effects of agarwood remain inconclusive due to variability in study design, extraction methods, and bioactive compound characterization. While *in vitro* models offer efficiency and ethical advantages, they lack the complexity of whole organisms.[14] Animal models, by contrast, provide essential insights into systemic effects and therapeutic relevance.[15] Nevertheless, animal-based studies on agarwood remain limited, hindering translational progress towards developing a modern therapeutic agent. Therefore, this scoping review aims to assess existing animal studies investigating agarwood's anti-inflammatory effects, elucidate underlying mechanisms, and provide a scientific foundation for the development of agarwood-derived therapeutic agents.

MATERIALS AND METHODS

This review was conducted in accordance with the framework established by a previous benchmark study and adhered to the PRISMA-ScR (Preferred Reporting Items for Systematic Reviews and Meta-Analyses extension for Scoping Reviews) guidelines.[16,17] The methodology consisted of the following steps: (1)identifying the research questions, (2)identifying relevant studies, (3) selecting studies, (4)charting the data, and (5)collating, summarising, and reporting the results.

Identification of research questions

The central research question guiding this review was: "What are the effects of agarwood on inflammation-related conditions in animal models?" This broad approach includes studies in which agarwood was evaluated as a potential therapeutic agent for inflammation-related conditions, ranging from mild inflammation to more severe diseases such as arthritis and cancer.[2,13]

Identification of relevant studies

A systematic literature search was conducted on three electronic databases: PubMed, Scopus, and Web of Science. No restriction was set on publication year, and all available studies up to the date of the search were considered. The included studies retrieved spanned from 2003 to 2023. Several keyword combinations were used to ensure comprehensive coverage of relevant studies. For PubMed, the search string was: "((agarwood OR agilawood OR aloeswood OR eaglewood) AND (cancer OR (Inflammation OR inflammatory OR antiinflammatory OR anti-inflammatory)))". The same search string was applied in Scopus. For Web of Science, the following string was used: "((ALL=(agarwood OR agilawood OR aloeswood OR eaglewood)) AND ALL=(cancer OR inflammation OR inflammatory OR antiinflammatory OR anti-inflammatory))".

Article selection process

The Endnote software (version 20.4.1, Clarivate, London, UK) was used to sort the references and eliminate

duplicates, followed by a manual verification step. Following initial screening by title and abstract, full-text articles were assessed for eligibility using predefined inclusion and exclusion criteria. The inclusion and exclusion criteria are listed in Table I.

Table I: Article identification and selection was based on these defined inclusion-exclusion criteria.

Criterion	Characteristics
Inclusion	Original/research articles published in peer-reviewed journals
	Studies that included animal models
	Studies using any type of agarwood as sample
	Studies which were compliant to animal study ethics
	English written articles
Exclusion	Reviews, perspectives, commentaries, letters to the editor, case study, proceedings, early access type articles, books and book chapters
	In vitro only articles
	Out of scope articles
	Non-English written articles

Only peer-reviewed, English-language original research articles involving animal models and agarwood samples were included. Reviews, commentaries, conference abstracts, *in vitro*-only studies, and non-English articles were excluded. A manual search of references from selected articles was also conducted to identify any additional relevant studies. Discussions were held between the authors to resolve any disagreements and to finalise the selection of articles.

Data charting and quality assessment

Data were extracted into a standardised Excel spreadsheet, capturing details such as authorship, publication year, animal model, disease type, agarwood sample, dosage, administration route, duration, and outcomes. Study quality was assessed using the ARRIVE guidelines (Animal Research: Reporting of *In Vivo* Experiments), with each criterion scored from 0 (inaccurate) to 2 (accurate). Risk of bias was evaluated using the SYRCLE tool (Systematic Review Centre for Laboratory Animal Experimentation), focusing on elements like randomisation, blinding, and selective reporting.

Collating, summarizing and reporting the results

Due to substantial heterogeneity in study designs, disease models, intervention protocols, and reported outcomes, a meta-analysis was not feasible. Instead, a scoping review approach was employed to map the existing evidence and summarise the progress in the field. Findings were synthesized narratively, with studies grouped by disease category and discussed in relation to agarwood dosage, treatment duration, affected cytokines, and relevant signalling pathways.

RESULTS

Article identification and selection

From a total of 231 articles identified through PubMed (47), Scopus (94), and Web of Science (90), 110 duplicates were removed. Title and abstract screening were conducted on the remaining 121 articles, of which 111 were excluded based on the inclusion and exclusion criteria. Ten articles proceeded to full-text screening, after which two non-English articles and one with questionable methodology were excluded. One additional article was included via manual reference checking, resulting in a final selection of eight studies. The selection process is illustrated in Figure 1.

A total of eight animal studies were included in this review. Considerable variation was noted in the reported *Aquilaria* species, extraction methods, sample applied on animal model, and inoculation status. In several studies, species identification or inoculation status was not provided, which may affect reproducibility, and the phytochemical consistency of the extracts used. These details are summarized in Table II.

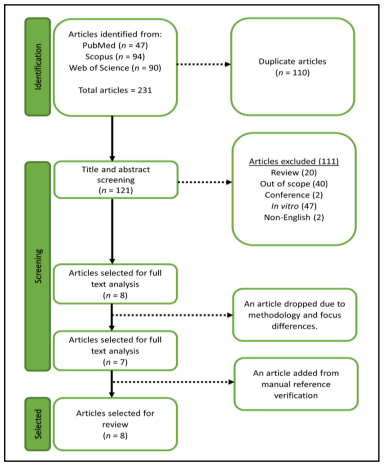


Figure 1: Summary of process of article identification, screening, and selection.

Quality and risk-of-bias assessment

All eight included studies scored above 80 % on the ARRIVE checklist, indicating high reporting quality as shown in Table III. Consistently high scores were seen in areas such as study design, ethical statement, and

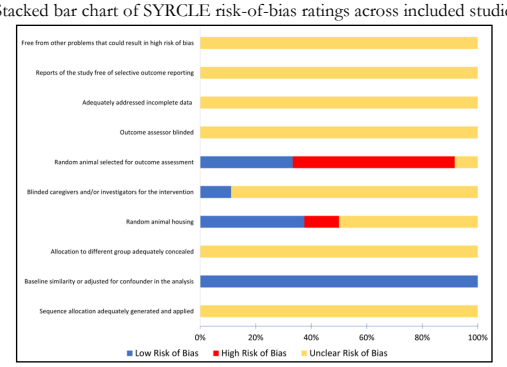
outcome reporting. However, the implementation of blinding was rarely reported, with only one study referencing it, and even then, the application was unclear. This resulted in a blinding score of just 6.25% across the included studies. Risk-of-bias assessment using the SYRCLE tool showed that while baseline group similarity (n=8;100%) and outcome measures were generally well-handled, most studies lacked detailed reporting on randomisation, allocation concealment, and selective outcome reporting, as illustrated in Figure 2.

Table II: Aquilaria species, extraction methods, and inoculation status reported in the included studies.

Included articles	Aquilaria species	Extraction method	Sample applied on animal model	Inoculation status
Article 1[18]	Sinensis	2-h reflux with ethanol (50% v/v).	Extract was dissolved in dimethylsulfoxide (DMSO)	NR
Article 2[18]	Crassna	Hydrodistillation (48h)	Essential oil and essential oil extract (suspended in 1% Tween-80)	NR
Article 3[19]	Not specified	Hydrodistillation (12h)	Essential oil suspended in 1% Tween-80	Artificial inoculation reported
Article 4[20]	Agallocha	Methanol extraction (3×1h)	Extract was dissolved in distilled water	NR
Article 5[21]	Not specified	Absolute ethanol extraction (2 days)	Ethanolic extract.	NR
Article 6[9]	Not specified	95% ethanol extraction (2h) + reflux (3×1h)	Alcoholic extract.	Artificial inoculation reported
Article 7[22]	Not specified	95% ethanol extraction (2h) + reflux (2 ×1h)	Ethanolic extract.	Artificial inoculation reported
Article 8[23]	Crassna	Ethanol extraction	Ethanolic extract.	NR

*NR-not reported

Figure 2: Stacked bar chart of SYRCLE risk-of-bias ratings across included studies.



Anti-inflammatory effects of agarwood in animal models

Eight studies evaluated the anti-inflammatory effects of agarwood using various animal models involving conditions such as analgesia, toxicity, cancer, psychiatric disorders, neurodegenerative diseases, and gastrointestinal injury. Agarwood was administered in different forms including ethanolic extracts, essential oils, and powdered heartwood from species such as A. crassna, A. sinensis, and A. agallocha. Details on experimental designs, dosages, durations, and outcomes are summarized in Table IV.

Despite variability in disease models, sample types, and experimental protocols, consistent reductions in inflammatory readouts were reported. The following subsections outline the pharmacological effects and molecular mechanisms identified across these studies.

Analgesic and anti-inflammatory effects

The earliest study reviewed demonstrated the analgesic and anti-inflammatory effects of A. sinensis leaf ethanolic extract in mice. Using inflammatory models such as writhing, hot plate, ear and paw oedema, and leukocyte migration, the extract (424 and 848 mg/kg) showed significant in vivo activity.[8] These findings were supported by in vitro LPS-stimulated assays (50-200 µg/mL), marking one of the first studies to validate agarwood's traditional use through both in vivo and in vitro approaches.

Toxicity and anti-cancer effects

Additionally, article 2 assessed the safety and anticancer potential of *A. crassna* essential oil (EO) in Swiss mice.[18] Acute (2000 mg/kg) and sub-chronic (100 and 500 mg/kg for 28 days) toxicity tests showed no treatment-related mortality. In tumour-xenografted mice (HCT 116 model), EO treatment significantly reduced tumour size and vascularization, with histological evidence of necrosis and decreased cell density.

Article 5 examined the protective effects of agarwood chip ethanolic extract (100 mg/kg daily for 35 days) against methanol-induced brain and liver toxicity.[21] Detoxification of alcohols, xenobiotics, and drugs elevates reactive oxygen species (ROS), contributing to oxidative stress, inflammation, apoptosis, and tissue damage.[24] In this case, methanol-exposed models increased levels of oxidative and inflammatory markers, including nitric oxide (NO), malondialdehyde (MDA), acetylcholinesterase (AChE), cyclooxygenase-2 (COX-2), lipoxygenase (LOX), caspase-3, tumour necrosis factor-alpha (TNF-α), monoamine oxidase (MAO), and DNA fragmentation. Agarwood treatment restored these biomarkers to near-control levels, suggesting its utility in mitigating ROS-driven tissue damage in hepatic and neurodegenerative contexts.[25]

Table III: Assessment of agarwood animal model inflammatory related **study using ARRIVE framework.**

ARRIVE Framework	Article 1[8]	Article 2[18]	Article 3[19]	Article 4[20]	Article 5[21]	Article 6[9]	Article 7[22]	Article 8[23]	Framework total percentage (%)
Abstract	2	2	2	2	2	2	2	2	100
Introduction or background	2	2	2	2	2	2	2	2	100
Objectives	2	2	2	2	2	2	2	2	100
Ethical statement	2	2	2	2	2	2	2	2	100
Housing and husbandry	2	2	2	2	2	2	2	2	100
Animal care and monitoring	2	2	2	2	2	2	2	2	100
Interpretation or scientific implications	2	2	2	2	2	2	2	2	100
Generalizability or translation	1	1	1	1	1	1	1	1	50
Protocol registration	1	1	1	1	1	1	1	1	50
Data access	2	1	1	1	1	1	2	1	62.5
Declaration of interests	2	2	2	2	2	2	2	2	100
Study designs	2	2	2	2	2	2	2	2	100
Sample size	2	2	2	2	2	2	2	2	100
Inclusion and exclusion criteria	1	1	1	1	1	1	1	1	50
Randomization	2	1	2	1	2	1	2	1	75
Blinding	0	0	1	0	0	0	0	0	6.25
Outcome measures	2	2	2	2	2	2	2	2	100
Statistical method	2	2	1	2	2	2	2	1	87.5
Experimental animals	2	2	2	2	2	2	2	2	100
Experimental procedures	2	2	2	2	2	2	2	2	100
Results	2	2	2	2	2	2	2	2	100
Article total percentage (%)	88.1	83.33	85.71	83.33	85.71	83.33	88.1	80.95	

Stress-related disorders

In addition to its effects on systemic toxicity and tumour growth, agarwood has also shown promise in modulating central nervous system inflammation. Article 3 demonstrated that Aquilaria EO exhibits anxiolytic and antidepressant effects in a restraint stress-induced mouse model. EO was administered daily at 10, 20, and 40 mg/kg for 10 days, 5 minutes prior to a 3-hour stress exposure.[19] Restraint stress elevated pro-inflammatory cytokines and NO, contributing to hyperactivation of the hypothalamic-pituitary-adrenal (HPA) axis, a key pathway in stress-related disorders.[26] EO treatment dose-dependently suppressed serum levels of interleukin-1α (IL-1α), IL-1β, and IL-6, while also reducing nNOS expression in both the cerebral cortex and hippocampus. It also significantly downregulated corticotropin-releasing factor (CRF), CRF receptors (CRFR), adrenocorticotropic hormone (ACTH), and corticosterone (CORT), indicating suppression of HPA axis activity.[27]

Neurodegenerative disorders

Article 8 investigated the effects of agarwood on Alzheimer's disease and dementia by measuring beta-amyloid (Aβ) and tau protein (τ) levels in brain tissue.[23] The accumulation of Aβ and τ is known to trigger neuroinflammation and oxidative stress, as activated microglia release pro-inflammatory cytokines and reactive oxygen species, ultimately contributing to neuronal dysfunction and cell death.[28] Neurofibrillary tangles formed by τ-protein disrupt synaptic transmission, impairing cognition.[29] In a high-fat diet-induced dementia mouse model, agarwood supplementation (1 mL/day for 16 weeks) significantly reduced Aβ and τ expression. Markers of microglial activation and oxidative stress were also lower in treated groups compared with controls.

Gastrointestinal disorders

Several studies have explored agarwood's protective effects on gastrointestinal tissues under inflammatory and chemotherapeutic stress. Article 6 successfully demonstrated that different types of agarwood extracts wild (WAAE), burning-chisel-drilling (FBAAE), and whole-tree inducing technique (WTAAE) effectively ameliorated intestinal injury induced by 5-fluorouracil (5-FU) in a murine model.[9] Over 14 days, treated animals showed improved body weight, intestinal propulsion, and tissue architecture. Biochemically, agarwood reduced NO, IL-17, and IL-33, while increasing antioxidant enzymes (GSH, SOD) and IL-10. Upregulation of Nrf2, Keap1, HO-1, and NADPH-related genes was observed. Concurrently, pro-inflammatory mediators were reduced in agarwood-treated groups relative to controls, including TNF-R, TRAF6, MyD88, IKKβ, IκB-β, and NF-κB.

Table IV: Summary of agarwood inflammatory-related studies using animal model administered either orally or injected intraperitoneally (IP).

Author	Sample	Animal	Model and sample dose	Administration route and duration	Sample size [group(n)]	Outcome measured	Findings
Article 1[8]	Aquilaria sinensis leaf ethanolic extract (50 % v/v twice reflux)	Male and female 18-22 -gram ICR mice	Acetic-acid-induced writhing response in mice. Sample dose: 424 mg/kg 848 mg/kg	1 hour before acetic acid injection. Route: Oral	n =10	Frequency of writhing occurring was recorded 15 min after the injection of acetic acid. Indomethacin was administered to mice to the positive control group.	Agarwood samples group (424 and 848 mg/kg)) showed restrained writhing with inhibition rate of 62.2 % and 66.9 %, respectively. Positive control (20mg/ kg) also markedly reduced writhing time.
			Hot plate latency assay in mice Sample dose: 424 mg/kg 848 mg/kg	1 hour and 2 hours before hot plate latencies recording. Route: Oral	n =10	The time that elapsed until the occurrence of either a hind paw licking or a jump off the plate surface was recorded as the hot-plate latency (5< t <30 seconds). Measurement was conducted at 1 and 2 hours after oral administration of samples.	Agarwood sample (848 mg/kg) increased pain threshold by 57.1 % measured at 2 hours after oral administration. Indomethacin (20 mg/ kg) also markedly increased mice latency.
			Xylene-induced ear swelling in mice Sample dose: 424 mg/kg 848 mg/kg	1 hour before xylene injection on anterior and posterior right ear lobe. Route: Oral	n = 10	Ear swelling degree caused by xylene induced injection was measured Indomethacin was administered to the positive control group. Left lobe as control.	At a dose of 848 mg/kg, the sample reduced xylene-induced ear swelling in a dose-dependent manner, with a 51.0% inhibition rate. Positive control (20 mg/ kg) also showed potent swelling reducing effect.
			Carrageenan-induced paw oedema in mice Sample dose: 424 mg/kg 848 mg/kg	1 hour before subcutaneous injection of carrageenan. Route: Oral	n = 10	Paw oedema caused by 2 % (v/v) carrageenan solution was measured using plethysmometer. Indomethacin was administered to the positive control group.	Sample at 848 mg/kg notably inhibited paw oedema in mice administered orally at 1, 3, and 5 hours after carrageenan injection showing similar effect as positive control (20 mg/kg).
			Carboxymethyl-cellulose-sodium (CMC-Na) induced leukocyte emigration in mice Sample dose: 424 mg/kg 848 mg/kg	1 hour before the injection of the CMC-Na solution. Route: Oral	n =10	An hour after oral administration of drugs, leukocyte cell count (stained with 0.01% crystal violet in 3% acetic acid) was conducted to observe whether the treatment enhances or reduced the leukocyte emigration in the mice peritoneal cavity. Dexamethasone was introduced as positive control in this test.	CMC-Na (375 mg/kg) significantly enhanced leukocyte emigration in the mice peritoneal cavity. Sample dose-dependently inhibited leukocyte emigration with inhibition percentage of 90.6 % (848 mg/kg) comparable to dexamethasone, 96.84 % (20 mg/kg).
			lipopolysaccharide (LPS)-stimulated NO release from macrophages in vitro Sample dose for assay: 50 µg/mL 100 µg/mL 200 µg/mL	48 hours	N/A	All assays were performed on thioglycolate-elicited mouse peritoneal macrophages. Measuring the LPS-induced massive amount of NO in macrophages. Also, the test was meant to observe the toxicity effect after 48 hours of exposure to samples. 0.1 µM hydrocortisone as positive control.	Agarwood sample dose-dependently reduced the NO-induced release at concentration of 50, 100, and 200 µg/ mL resulting in IC_{50} of 80.4 µ/mL. Additionally, samples showed no obvious toxicity effect on the viability of macrophages during 48 hours period of incubation.
Article 2[18]	Essential oil hydrodistillated from Aquilaria crassna stem bark (after 1 week maceration in distilled water at room temperature)	Male and female 8 to 12 weeks Swiss mice	Acute toxicity. Sample dose: 2000 mg/kg	Single dose and observed for 16 days. Route: Oral	n = 9	Mortality and toxicity signs such as apathy, hyperactivity, dizziness, vomiting, diarrhoea, excessive salivation, loss of fur, anxiety, convulsions, lethargy, and morbidity	Agarwood samples showed no treatment-related mortality at the limit test dose (2000 mg/kg). No significant changes in mice behaviour during the 14 days observation period. Also, no abnormal changes attributable to treatment had been noticed in body weights and treatment related changes like respiration rate and heart rate.
			Sub-chronic toxicity Sample dose: 100 mg/kg 500 mg/kg	Daily dose over 28 days. Route: Oral	3(n = 10)	Observations were made on general behaviour, hematological, and biochemical parameters. Signs such as piloerection, diarrhoea, sedation, loss of fur, and alterations in locomotor activity or mortality were recorded.	Swiss mice showed no signs of toxicity recorded during the 28 consecutive days of treatment at the doses of 100 and 500 mg/kg. Also, no changes had been recorded in the hematological and biochemical indices.
		6 to 8 weeks Athymic-NCR nu/nu mice	In vivo anti-tumour (HCT-116 cell) Sample dose: 50 mg/kg 100 mg/kg 200 mg/kg	Daily dose over 6-8 weeks period. Route: Oral	4(n = 6)	Cross-section of xenografted tumour to measure size and observe any histological changes when treated	Treatment caused significant reduction in the tumour size compared to those in the untreated group. Apparent differences in the extent of necrotic regions. Histological feature showed loss of cell compactness and severe necrosis with areas of low density of blood vessels, as well as many pools of tumour cells compared to control group.
Article 3[19]	Hydrodistillated agarwood essential oil from whole tree agarwood-inducing technique (species not mentioned)	Adult male ICR mice	Elevated Plus Maze (EPM) Test Sample dose: 10 mg/kg 20 mg/kg 40 mg/kg	Daily dose for 10 days before stress induction. Route: IP	7(n = 12)	Time spent, distance moved, and number of entries in the open arms from the central elevated platform was recorded.	Agarwood treated group and positive control group showed markedly increased the time spent, distance moved and entries in open arms. 20 and 40 mg/kg agarwood showed comparable effects as 2.5 mg/kg diazepam suggesting anxiolytic effect.
			Light Dark Exploration (LDE) Test Sample dose: 10 mg/kg 20 mg/kg	Daily dose for 10 days before stress induction. Route: IP		Time spent, distance moved, and number of transitions between dark and light compartment were recorded.	Agarwood treatment increased the time spent and distance in light compartment including increased the transition to light compartment. 40 mg/kg agarwood displayed comparable effects to 2.5 mg/ kg diazepam indicating the anxiolytic effect.
			Open Field (OF) Test Sample dose: 10 mg/kg 20 mg/kg 40 mg/kg	Daily dose for 10 days before stress induction. Route: IP		Mice placed in an open field cage for 2 mins for acclimatization. Then, the next 10 mins, the movement were recorded with a threshold selected at 6.5 cm/s. Time spent and distance moved were recorded.	Agarwood treatment (20 and 40 mg/kg) increased time in the center/open arms compared with control; values were comparable to diazepam.

cont'd

			Tail Suspension (TS) Test Sample dose: 10 mg/kg 20 mg/kg 40 mg/kg	Daily dose for 10 days before stress induction. Route: IP		A system consists of eight suspension units divided by walls where mouse was suspended by the tail using an adhesive tape for 6 min, and the immobility time during the final 4 min was recorded automatically by software.	Depressed mice showed increased immobility, while paroxetine significantly decreased immobility. Agarwood at 20 and 40 mg/kg also reduced immobility compared with control, with effects comparable to paroxetine (10 mg/kg).
			Forced Swimming (FS) Test Sample dose: 10 mg/kg 20 mg/kg 40 mg/kg	Daily dose for 10 days before stress induction. Route: IP		Mice were individually placed into a plastic cylinder (20 cm height, 18 cm diameter) filled with 12 cm high water (24 ± 1 ℃). All animal were forced to swim for 6 mins. The immobility time during the final 4 mins was recorded.	Immobility time was described as time spent by mouse floating in the water without struggling and making only small movement to keep its head above water. Agarwood at 40 mg/kg and paroxetine at 10 mg/kg showed similar immobility inhibitory effect suggesting the potential anti-depressant effect.
			RT-PCR Sample dose: 10 mg/kg 20 mg/kg 40 mg/kg	N/A	N/A	This method evaluates the mRNA levels of neuronal nitric oxide synthase (nNOS), corticotropin releasing factor (CRF), and corticotropin releasing factor receptor (CRFR).	Repeated restraint stress increased the nNOS gene and protein expression in the cerebral cortex and hippocampus. Agarwood treatment (40 mg/kg) markedly inhibited the mRNA levels of nNOS in cerebral cortex and hippocampus. The treatment also significantly inhibited the nNOS protein level in the hippocampus but unobvious in the cerebral cortex.
			Western blot Sample dose: 10 mg/kg 20 mg/kg 40 mg/kg	N/A	N/A	This method measures the protein levels of nNOS and CRFR.	Agarwood treatment significantly reduced the expression of CRF and protein CRFR in the cerebral cortex and hippocampus. Also, the concentrations of ACTH and CORT downstream of the HPA axis were reduced.
Article 4[20]	Methanolic extract of Aquilaria agallocha heartwood	250 to 280 grams of Wistar rats	Blood analysis Sample dose: 3 g/kg	Pre and post treatment (1 hour after) Route: Oral	n = 6	Blood samples of rats were collected pre-dose and 1-hour post-dose after oral administration of methanolic agarwood extract (3g/kg). Samples were analyzed by UHPLC-Q-TOF/MS.	10 compounds detected in rat blood plasma out of the 22 compounds identified from the methanolic extract of Aquilaria agallocha heartwood.
		22 to 25 grams of Kunming mice	5-fluorouracil (5-Fu) intestinal mucositis induction Sample dose: 200 mg/kg 400 mg/kg 800 mg/kg	Sample daily dose 30 minutes prior to 5-Fu. Total duration of 7 days Route: Oral	6(n = 10)	Body weight, food intake, and stool scoring were recorded to assess the severity of intestinal mucositis. The following tests includes histopathological evaluation, immunohistochemical analysis, superoxide dismutase (SOD) and malondialdehyde (MDA) analysis including cytokines evaluation via RT-PCR and western blot. IM induce using 5-Fu dose at 60 mg/kg/day for 5 days Loperamide 4 mg/kg.	Agarwood reduced the severity of 5-Fu induced IM displayed by the improvement in body weight, food intake, and diarrhoea status. Agarwood treated mice showed relatively intact mucosa structure with notably better villus height and crypts depth. SOD levels were increased while MDA levels were significantly reduced by the treatment. The treatment also reduced the COX-2 and TNF-α expression. These agarwood effects recorded were similar to the effects shown by the positive control, loperamide.
Article 5[21]	Ethanolic extract of agarwood chip (species not mentioned)	Male 100-110 grams of Sprague -Dawley rats	Agarwood protective effects against methanol toxicity Sample dose: 100 mg/kg	Sample daily dose for 35 days Route: Oral	4(n = 10)	Animal separated into 4 groups i.e., control (untreated), methanol (model), agarwood, and agarwood-methanol groups. Methanol (3 g/kg) was injected once per week for three weeks. Agarwood was orally administered daily at 100 mg/kg. After 35 days, feeding was stopped for 12 hours prior to anesthetized and sacrificed for brain and liver tissue collection.	Methanol caused elevation of NO, MDA, ACHE, COX-2, LOX, TNF-α, Caspase-3, MAO and DNAF in brain and liver compared to control. Treatment with agarwood pre, during, and post-methanol administration managed to improve the liver and brain biological parameters compared to the control.
Article 6[9]	Alcohol extract of wild agarwood extract, whole tree inducing agarwood extract, and burning-chisel-drilling agarwood extract. Species not mentioned	Male 18 to 20 grams of ICR mice	Agarwood protective effect against intestinal injury by fluorouracil (5-Fu) Sample dose: WAAE 2.84 g/kg FBAAE 2.84 g/kg WTAAE: 0.71 g/kg 1.42 g/kg 2.84 g/kg	Sample daily dose. Model 5-Fu once/2 day. 14 days duration. Route: Oral	7(n = 10)	Animals divided into 7 groups i.e., Control Model 5-Fu (25 mg/kg) Wild agarwood (WAAE) treated (2.84 g/kg) + 5-Fu (25 mg/kg) Burning-chisel-drilling agarwood (FBAAE) treated (2.84 g/kg) + 5-Fu (25mg/kg) Whole tree inducing agarwood (WTAAE) treated group (0.71, 1.42, and 2.84 g/kg) + 5-Fu (25 mg/kg). Analysis conducted includes body weight observation, intestinal propulsion rate analysis, colon tissue histopathological evaluation, lipid peroxidation analysis (NO, GSH, and SOD), cytokines analysis (IL-17, IL-33, and IL-10), and RT-PCR for anti-oxidant and inflammation.	Agarwood treated group showed increased body weight in the 13th day (especially WTAAE treated). Model group showed loss of body weight. Intestinal propulsion rate of agarwood treated groups were significantly increased (WTAAE and WAAE showed better rates than FBAAE). Model group showed low intestinal propulsion rate. Agarwood significantly relieves the colon histopathological injury caused by 5-Fu. Treatment protected colon from damage characterized by villi shortening and crypt disruption, inflammatory cell infiltration, goblet cell reduction, mucosa and muscle layer thinning as shown by model group. NO level was reduced while GSH and SOD levels were significantly increased by agarwood treatment. Agarwood markedly reduced the levels of IL-17 and IL-33 while significantly increased the level of IL-10. Increased expression of Nrf2-ARE–related genes and proteins was observed following agarwood treatment. NF-κB activation and downstream targets were reduced in treated groups compared with controls.

cont'd

Article 7[22]	Alcohol extract of wild agarwood extract, whole tree inducing agarwood extract, and burning-chisel-drilling agarwood extract. Species not mentioned	Male 4 to 6 weeks of ICR mice	Agarwood protective effect against gastric ulcer Sample dose: WAAE 2.84 g/kg FBAAE 2.84 g/kg WTAAE: 0.71 g/kg 1.42 g/kg 2.84 g/kg	Daily pre-treatment with sample for 7 days Route: Oral	7(n = 10)	Animals were divided into 7 groups.[9] Pre-treatment with agarwood was done daily for 7 days while control and model groups were pre-treated with distilled water (20 mL/kg). After pre-treatment and 24 hours food deprivation with water ad libitum, mice were infected by oral gavage absolute ethanol at 0.015 mL/g except for normal group. An hour after that, blood was collected for analysis and sacrificed. Stomach was immediately removed and fixed with formaldehyde solution for gastric lesion index and histopathological analysis.	Pre-treatment with agarwood reduced ethanol-induced mucosal damage and ulcer index. The model group exhibited severe gastric ulcers with linear haemorrhages and ulceration craters. Agarwood protected against gastric lesions, preventing submucosal oedema, haemorrhagic injury, mucosal degradation, epithelial loss, inflammatory infiltration, and necrosis. Dose-dependent treatment decreased NO levels while increasing GSH and SOD, highlighting antioxidant effects. Agarwood reduced proinflammatory cytokines (IL-1β, IL-6) and elevated anti-inflammatory IL-10 levels. Immunohistochemical analysis showed downregulation of NF-κB and p-38 MAPK phosphorylation, confirming its potent anti-inflammatory activity.
Article 8[23]	Aquilaria crassna ethanolic extract (sample type not mentioned).	Female 6 weeks of ICR mice	Agarwood effect against Alzheimer's and other dementia related diseases. Working under the hypothesis of critical association between obesity, diabetes, and Alzheimer's. Sample dose: 1 mL/day added to high-fat energy diet.	Ad lib food supply with sample for 16 weeks. Route: Oral	2(n = 10)	Mice were fed with high-fat energy diet plus agarwood ethanolic extract (1 mL/day) for the treated group. Control was fed with high-fat energy diet only. Body weight changes were measured weekly. Blood analysis was conducted at the termination point of experiment. Aβ and τ-protein expression analysis (western blot)	Agarwood treatment increased body weight over time. However, the difference was not significant at the end of 16 weeks. Weight changes after week 9 were almost asymptotically plateau. Blood analysis showed slightly higher levels of HDL, LDL, and cholesterol values which were unexpected. Aβ and τ-protein expression were reduced (>50%) versus control.

Similarly, in an ethanol-induced gastric ulcer model, agarwood pre-treatment reduced mucosal damage, oedema, and hemorrhage.[22] Levels of GSH and SOD were elevated, while IL-1β, IL-6, and NO were suppressed. IL-10 was upregulated, alongside inhibition of phosphorylated NF-κB and p38 MAPK.

Another study using powdered A. agallocha extract (200-800 mg/kg) confirmed protection against 5-FU-induced intestinal mucositis, with improved clinical symptoms, structural preservation, and decreased COX-2 and TNF-α levels.[20]

Selection of agarwood sample and animal model

Agarwood sample selection often reflected the researchers' geographic location; studies from Indochina primarily used A. crassna, while those from China used A. sinensis. However, several studies did not specify species or inoculation status, the latter being critical as artificial inoculation alters phytochemical profiles.[30, 31]

Animal model selection aligned with study objectives. For example, mice were used for stress models due to their heightened sensitivity, while rats were chosen for procedures requiring larger blood volumes. Figure 3 summarises animal and sample types. Rats are preferred for surgical and imaging studies due to their size, while mice are commonly used in pharmacological research for cost-efficiency and lower dosing requirements.[32]

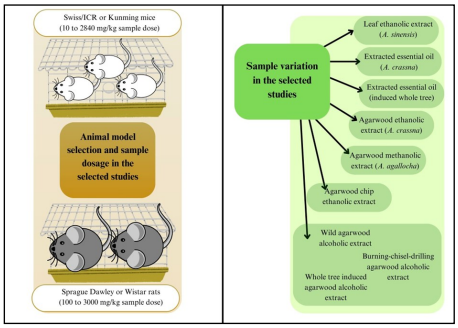


Figure 3: (a) On the left: Animal models used in the selected studies, predominantly Swiss/ICR or Kunming mice and Sprague Dawley or Wistar rats. Agarwood sample dosages ranged from 10 to 2840 mg/kg in mice and 100 to 3000 mg/kg in rats. (b) On the right: Variations in agarwood sample types, including extracts from different plant parts and essential oils. Sample selection was influenced by research objectives and the geographical affiliations of the researchers.

DISCUSSION

Inflammation is a physiological response to harmful stimuli such as infection, injury, or certain pathological conditions, often presenting with symptoms like fever, sore throat, and nasal congestion. Under normal conditions, these responses are self-limiting, and tissue homeostasis is restored. However, in chronic inflammatory diseases, this process becomes dysregulated, preventing resolution and leading to persistent tissue damage.[33] Chronic inflammation is now widely recognized as a key contributor to a range of non-communicable diseases commonly associated with modern lifestyles and aging, including cancer, obesity, cardiovascular disorders, and neurodegenerative conditions.[34]

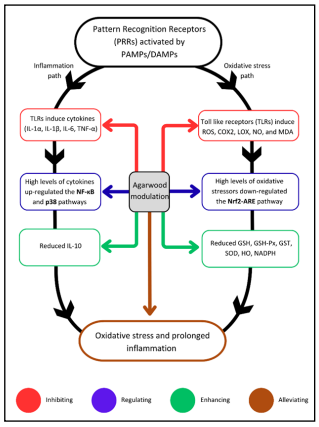


Figure 4: Proposed mechanisms underlying the anti-inflammatory effects of agarwood. Agarwood may modulate inflammation directly or indirectly through oxidative stress-related pathways. These effects may involve inhibition, regulation, or activation of molecular signalling cascades associated with immune and oxidative responses. Color codes: red=inhibition, purple=regulation, green=enhancement, brown=alleviation.

The current review highlights consistent findings from animal models indicating that agarwood exerts anti-inflammatory effects through multiple biological pathways, including the Nrf2–ARE and NF-ϰB signalling cascades (Figure 4), which are central to oxidative stress and inflammation control.

Bioactive phytochemicals in agarwood are postulated to modulate inflammation by influencing key molecular signals. One major pathway involves the activation of pattern recognition receptors (PRRs) by pathogen or damage associated molecular patterns (PAMPs/DAMPs),[35] triggering downstream cascades that recruit immune cells and stimulate cytokine production.[36] This leads to the release of pro-inflammatory mediators such as TNF-α, IL-1, IL-6, and type I interferons. These cytokines also influence neuroendocrine and neurotransmitter function, contributing to illness behaviours and psychiatric conditions, including anxiety and anhedonia.[37] These substances can induce fever, decrease food intake, sexual activity, and social exploration, as well as provoke anxiogenic-like effects, interfering with the brain's ability to respond to pleasurable stimuli.[38]

In stress-induced models, agarwood treatment suppressed HPA axis hyperactivity that was activated by cytokines production by downregulating CRF expression in the cerebral cortex and hippocampus, and reducing ACTH secretion from the pituitary and adrenocortical hormone excretion from the adrenal cortex, indicating effective modulation of cytokine-driven stress responses.[39] These behavioural changes are consistent with an anxiolytic-like profile, as agarwood treatment increased time spent in the centre or open arms compared with controls, with values comparable to diazepam. Findings also align with an antidepressant-like effect, as agarwood reduced immobility times in a manner comparable to paroxetine. In addition, stress-related cytokine levels (IL-1α, IL-1β, IL-6) were reduced with treatment, further supporting anti-inflammatory contributions to these behavioural outcomes. Overall, these patterns are consistent with anxiolytic and antidepressant like effects, though their clinical relevance remains to be established. In neurodegeneration models, agarwood reduced microglial activation and oxidative stress. These findings motivate more targeted studies on neuroinflammatory pathways to clarify the mechanisms underlying its neuroprotective potential.

In gastrointestinal models, agarwood mitigated ethanol- and drug-induced mucosal injury by restoring antioxidant levels (GSH, SOD, IL-10) and reducing pro-oxidant and pro-inflammatory markers (NO, IL-1β, IL-6).[19] These molecular changes included upregulation of Nrf2, Keap1, HO-1, and NADPH-related genes, along with suppression of NF-ϰB and p38 MAPK signalling.[40, 41] The concurrent antioxidant and anti-inflammatory responses support a dual-action hypothesis for agarwood's effects in gastrointestinal inflammation, although confirmation across different models is still needed. Similar protective effects were observed in chemotherapy-induced intestinal damage, where agarwood enhanced the expression of Nrf2, Keap1, HO-1, GST, and NADPH, thereby reducing oxidative stress, promoting mucosal protection, and modulating immune responses.[42] This is particularly relevant given the bidirectional relationship between inflammation and oxidative stress, in which elevated cytokines such as TNF-α and IL-1β promote ROS production, while oxidative stress further amplifies inflammatory signalling.[43] The molecular evidence also supports this dual action: agarwood enhanced the expression of Nrf2–ARE–related genes, consistent with antioxidant activation, while suppressing NF-ϰB signalling, a central regulator of inflammatory responses. Together, these shifts provide a mechanistic basis for its observed antioxidant and anti-inflammatory effects. By

modulating both cytokine levels and antioxidant enzymes such as SOD and GSH, agarwood appears to act on this interlinked pathological axis. NF-ϰB inhibition, in particular, contributed to reduced inflammation and tissue damage across both gastrointestinal and neuroinflammatory models. Given that NF-ϰB regulates cytokines involved in stress and mood-related pathways, its downregulation may underlie agarwood's observed effects on neuroendocrine modulation, particularly in anxiety and depression-like behaviours.[44] Likewise, the suppression of p38 MAPK phosphorylation an important mediator of cytokine production and epithelial injury further supports agarwood's therapeutic potential in addressing gastric ulceration and systemic inflammatory responses.[41]

Despite variability in disease models, agarwood type, and experimental protocols, several consistent outcomes were observed across the reviewed studies:

- Reduction in pro-inflammatory cytokines (e.g., IL-1β, IL-6, TNF-α) indicating suppression of key mediators involved in acute and chronic inflammatory responses.
- Modulation of oxidative stress markers (e.g., GSH, SOD, NO) highlighting agarwood's antioxidant capacity in mitigating reactive oxygen species (ROS)-induced damage.
- Regulation of inflammatory signalling pathways (NF-ϰB, p38-MAPK, Nrf2-ARE) suggesting a mechanistic basis for agarwood's systemic anti-inflammatory and cytoprotective effects.

In addition to these anti-inflammatory and antioxidant outcomes, one study demonstrated reduced tumour growth in HCT116 xenograft models following agarwood essential oil treatment. These results point to possible anti -tumour activity that warrants further investigation in preclinical cancer studies.

Taken together, these outcomes across different models suggest that agarwood's effects are mediated, at least in part, through shared inflammatory pathways such as NF-ϰB and Nrf2, which play central roles in inflammation and oxidative stress. Overall, the evidence indicates that agarwood exhibits broad-spectrum anti-inflammatory and antioxidant activities across multiple organ systems and disease contexts. This highlights its promise as a candidate for the development of novel therapeutics targeting inflammation-driven disorders.

Beyond efficacy, safety is an equally important consideration for the clinical translation of agarwood. Only a few of the included studies reported toxicity outcomes. For example, *A. crassna* essential oil showed no acute toxicity at 2,000 mg/kg or sub-chronic toxicity at 100–500 mg/kg for 28 days in mice, with no treatment-related mortality or notable behavioural or biochemical changes.[18] Another study found that agarwood ethanolic extract actually improved liver and brain biomarkers in a methanol-induced toxicity model, suggesting possible protective rather than harmful effects.[21] While these results are encouraging, most studies did not evaluate standard safety parameters such as liver enzymes, kidney function, or histopathology. This gap is particularly relevant for solvent-based extracts like ethanol, which may have toxicological implications in humans.

It is also worth noting that many of the included studies had methodological shortcomings. Randomization, blinding, and allocation concealment were rarely reported, which points to a moderate–high risk of bias. These weaknesses limit how confidently the results can be interpreted and underline the need for better reporting and study design in future preclinical work. Future studies should prioritize the use of random allocation, allocation concealment, and blinded outcome assessment to reduce bias and strengthen the robustness and reproducibility of findings. In addition, species identification and inoculation status were not consistently reported across studies, which hinders reproducibility and complicates cross-study comparisons. Addressing this gap will require future studies to provide complete details on species, extraction protocols, and inoculation status to ensure reproducibility and phytochemical consistency.

Although the preclinical findings are promising, moving agarwood-based therapies into human use presents several challenges. At present, little is known about their pharmacokinetics, making it difficult to predict how these

extracts are absorbed, distributed, metabolized, or excreted in humans. Without such data, establishing safe and effective dosing remains speculative.[45] Extrapolating dosing from animals to humans is not straightforward due to physiological differences; robust methods such as allometric scaling or physiologically based pharmacokinetic modeling are preferred over simple weight-based formulas.[46] Finally, regulatory approval of herbal therapies demands standardized preparations, reproducible phytochemical profiles, and comprehensive safety testing, including genotoxicity and long-term toxicity assessments. In several countries, including Malaysia, herbal products must also comply with GMP and registration procedures to be legally marketed.[47] These considerations underscore the need for well-designed pharmacological and clinical studies before agarwood can be confidently advanced as a therapeutic option.

LIMITATIONS

While this review supports the anti-inflammatory potential of agarwood, several limitations should be noted. There is considerable variability in species, extraction methods, and phytochemical content, and many studies lacked details such as plant origin, inoculation status, and standardization. As highlighted in Table II, incomplete reporting of species and inoculation status represents a significant limitation that hinders reproducibility and cross-study comparisons. Future studies should ensure detailed reporting of Aquilaria species, extraction protocols, and inoculation status to improve reproducibility and maintain phytochemical consistency across studies. Most data were derived from preclinical models, limiting clinical relevance, and only a few studies conducted dose response or long-term evaluations.

Although the included studies generally achieved high scores on the ARRIVE checklist, the limited use of blinding and randomization raises concerns about internal validity. The absence of these measures increases the potential for performance and detection bias, which may lead to an overestimation of treatment effects.[48, 49] Consequently, the findings should be interpreted with caution, as the robustness of the reported outcomes may be reduced. Future studies should integrate rigorous methodological safeguards, including random allocation, allocation concealment, and blinded outcome assessment. These practices are essential to reinforce study rigor, minimize bias, and improve the reproducibility and translational value of preclinical research.[50]

Information on safety and toxicity was also limited. While a small number of studies reported no obvious adverse effects or even protective outcomes, most did not include standard assessments such as liver enzymes, renal function, or histopathology. This gap makes it difficult to draw firm conclusions about the safety profile of agarwood extracts, particularly those prepared using solvents like ethanol, which may pose toxicological risks if translated to humans. Comprehensive toxicity evaluation should therefore be incorporated into future preclinical work alongside efficacy testing.

To ensure broad coverage, three major databases (PubMed, Scopus, and Web of Science) were searched; however, relevant studies from other platforms may have been missed. In addition, journal indexing can change over time, potentially affecting study inclusion. Finally, as is typical of scoping reviews, no formal quality appraisal was conducted, since the objective was to map available evidence rather than assess study rigor.[17]

CONCLUSIONS

Although limited in number, existing preclinical studies provide promising evidence for the anti-inflammatory effects of agarwood across diverse animal models, including those simulating analgesic, anticancer, neurodegenerative, psychiatric, and gastrointestinal conditions. These findings support its traditional medicinal use and highlight its potential for future therapeutic development targeting inflammation-related disorders.

Further research is essential to validate these findings, identify optimal dosing strategies, and assess the long-term safety and efficacy of agarwood-based interventions. Mechanistic studies and standardized formulations will be

crucial in advancing its clinical applicability.

To date, no registered clinical trials have evaluated agarwood or its derivatives in humans. While some botanical trials exist, the composition and relevance to agarwood remain unclear. Given its pharmacological potential, well-designed clinical studies are urgently needed to confirm efficacy, establish safety profiles, and explore its role in modern therapeutic settings.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article are available from the corresponding author upon reasonable request and will be provided in Excel format.

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CONFLICT OF INTEREST

The authors declare that the research was conducted without any commercial or financial relationships that could be perceived as a potential conflict of interest.

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REVIEW ARTICLE

Exploring Neonatal NaV1.5 Voltage-Gated Sodium Channel as a Therapeutic Target in Cancer

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ABSTRACT

Voltage-gated sodium channels (VGSCs) play pivotal roles in cancer progression and have emerged as promising therapeutic targets and biomarkers. VGSCs comprise multiple subtypes with distinct tissue distributions, influencing tumour characteristics in different ways. Among these, the tetrodotoxin-sensitive α-subunits and the β1 subunit, commonly found in breast cancer, have been implicated in metastasis and tumour aggressiveness. The NaV1.5 channel and its neonatal variant (nNaV1.5) are overexpressed in aggressive cancers such as breast, prostate, colorectal, and lung cancers, thereby enhancing their invasive capacity. nNaV1.5 is particularly significant due to its tumour-specific expression and strong association with poor prognosis, especially in breast cancer, where it regulates cell proliferation, invasion, and tumour microenvironment remodelling. This review highlights nNaV1.5 as a critical ion channel that drives metastasis through ion regulation, extracellular acidification, and cytoskeletal remodelling. We further evaluate current therapeutic strategies, including siRNA, monoclonal antibodies, and small-molecule inhibitors, while addressing translational challenges such as tumour heterogeneity, drug delivery limitations, and off-target cardiotoxicity due to its similarity with the adult isoform. In addition, we explore the potential of nNaV1.5 as a biomarker subject to epigenetic regulations by factors including RE1-silencing transcription factor (REST) and histone deacetylase 2 (HDAC2), which may facilitate patient stratification and treatment optimization. By integrating mechanistic insights, therapeutic opportunities, and translational challenges, this review goes beyond descriptive summaries to provide a framework for advancing nNaV1.5 research from preclinical studies toward clinical application in cancer therapy.

Keywords:
Voltage-gated sodium channel, neonatal NaV1.5, metastasis, targeted therapy, biomarker.

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INTRODUCTION TO VOLTAGE-GATED SODIUM CHANNELS (VGSCS) AND CANCER

Voltage-gated sodium channels (VGSCs) are essential for the generation and conduction of electrical signals in excitable cells, including neurons and muscle cells. These channels consist of pore-forming α-subunits and auxiliary β-subunits, which regulate channel activity. VGSCs belong to a wider superfamily of ion channels that also includes voltage-gated potassium and calcium channels. The α-subunits of VGSCs are classified according to their tetrodotoxin (TTX) sensitivity and tissue-specific characteristics.[1] The β1 subunit, highly expressed in breast cancer tissues, can be a significant modulator of tumour cell behaviour and interactions within the tumour microenvironment.[2] VGSCs exist in three conformational states: open, closed, and inactivated, which control Na^+ ion conductance and enable fast inactivation within milliseconds.[3]

Structural research has significantly improved our knowledge of VGSCs, demonstrating how voltage sensors regulate gating charge movements, while the selectivity filter mediates Na^+ conductance through a water-lined channel.[4] Furthermore, it was unravelled that slow

inactivation mechanisms are controlled by conformational changes on the intracellular side of VGSC, and are crucial in cellular excitability and physiological activities.[5] VGSCs possess multiple toxin- and drug-binding sites, which differently influence the channel function.[6] This structural and functional diversity highlights the therapeutic value of VGSCs. To date, nine functional α-subunits and four β-subunits have been characterized in mammals.[7] The subtypes of α-subunits are shown in Table 1.

Table 1: The list of Subtypes of VGSCs6

α-Subunits	Gene Symbol	Chromosomal Location	TTX-S/R	Predominant Location	Expression in DRG	Effect of Mutation
$Na_{V1.1}$	SCN1A	M:2 H:2q24	TTX-S	PNS	+++	Epilepsy
$Na_{V1.2}$	SCN2A	M:2 H:2q23-24	TTX-S	CNS	+	Epilepsy
$Na_{V1.3}$	SCN3A	M:2, H:2q24	TTX-S	CNS (embryonic)	Upregulated after axotomy	None reported
$Na_{V1.4}$	SCN4A	M:11, H:17q23-25	TTX-S	Skeletal muscle	-	Myotonia, periodic paralysis
$Na_{V1.5}$	SCN5A	M:9, H:3p21	TTX-R	Heart muscle	-	Long-QT, Brugada 'syndrome, Progressive familial heart block
$Na_{V1.6}$	SCN8A	M:15, H:12q13	TTX-S	CNS, PNS, glia nodes of Ranvier	+++	Cerebellar atrophy
$Na_{V1.7}$	SCN9A	M:2, H:2q24	TTX-S	PNS Schwann cell	+++	Increased and decreased pain sensitivity
$Na_{V1.8}$	SCN10A	M:9, H:3p22-24	TTX-R	PNS (sensory neurons)	+++	None reported
$Na_{V1.9}$	SCN11A	M:9, H:3p21-24	TTX-R	PNS	+++	None reported

PNS: Peripheral Nervous System, CNS: Central Nervous System, Tetrodotoxin-Sensitive: TTX-S, Tetrodotoxin-Resistant: TTX-R, DRG: Dorsal Root Ganglion

In the last two decades, VGSCs have been a focus of attention as therapeutic targets because of their involvement in cancer metastasis. According to a study, VGSC expression was found to be greater in tumours than in normal tissues.[8] The tumour microenvironment also showed higher levels of K^+ and Na^+ and a reduced pH.[9] Hence, VGSCs have been reported to be upregulated in multiple studies concerning numerous carcinomas, including prostate, breast, lung, colon, cervical, brain, and ovarian cancers .[10-16] VGSC expression has also been identified in gliomas, with specific subtypes that are prevalent in tumours, such as $Na_V1.1$, 1.2, and 1.3.[17] In general, high-grade gliomas exhibit fewer Na^+ channel subtypes. $Na_V1.6$, which is strongly expressed in pilocytic astrocytomas, is almost absent in glioblastomas, highlighting the potential of VGSCs as diagnostic markers.

Additionally, VGSCs have been linked to the metastatic potential of various cancers. Studies have demonstrated that VGSC activity promotes cancer cell migration and invadopodia formation, which are critical metastatic processes.[18,19] Several studies have associated VGSCs with poor prognosis in breast cancer.[1] The overexpression of $Na_V1.5$ in breast cancer has also been linked to poor prognosis and increased invasiveness.[20] Moreover, VGSCs enhance the invasiveness of lung cancer cells, and highly metastatic cell lines express functional sodium channels, where sodium influx disrupts sodium homeostasis and its signalling pathways.[12] In another analysis, VGSC activity was found to facilitate prostate cancer metastasis *in vivo*[14] via Na^+ channel proteins expressed in malignant prostate cells. Hence, elevated Na^+ channel expression is thought to correlate with increased invasiveness.[21]

Different VGSC subtypes are overexpressed in different cancer cells, with $Na_V1.5$ and 1.7 being the dominant isoforms. The upregulation of these subtypes influences the migration and invasion of cells, hence increasing metastatic potential.[22] VGSCs are also functionally expressed in cervical cancer with differential expression of Na_V 1.2 and 1.7,[11] suggesting their utility as prognostic markers for cervical cancer.

VGSC Mechanism of Invasiveness in Cancer

VGSC activity promotes cancer cell migration and invasion by increasing the influx of Na^+, which leads to the formation of invadopodia, which are cellular protrusions that secrete proteolytic enzymes, such as metalloproteases and cathepsins, to break down the extracellular matrix (ECM) and enable metastasis (refer to Figure 1).[16,19,23] In breast cancer, the association between neonatal $Na_V1.5$ ($nNa_V1.5$) and sodium/hydrogen exchanger-1 (NHE-1) escalate this invasive mechanism. Additionally, VGSC expression enhances endocytic membrane activity, as observed in small-cell lung cancer (SCLC), where uptake of horseradish peroxidase (HRP) is more than four times higher than in normal cells and correlates strongly with metastatic potential.[24] VGSCs are

controlled by hormones and growth factors, such as the epidermal growth factor (EGF), which increases the channel expression and metastatic behaviour at both transcriptional and post-translational levels.[25] Moreover, VGSCs affect signalling pathways via abnormal ion transport mechanisms and cause depolarization of cancer cells.[26] This, in turn, activates Rac1 and initiates cytoskeletal reorganization.[27] Rac1 is intracellularly anchored to the plasma membrane and functions as a protein that controls cell shape and movement.[26] As a result, VGSCs promote cancer by regulating ion flux, membrane dynamics, and intracellular signalling, which ultimately leads to metastasis.

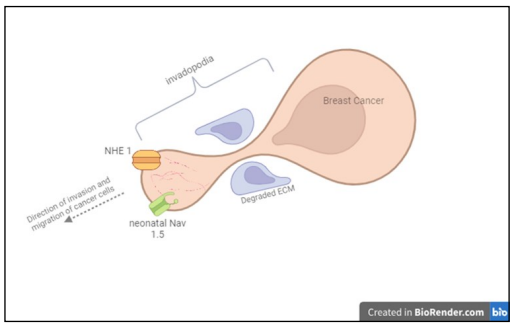

Figure 1: Schematic of invadopodia formation in breast cancer cells. Neonatal NaV1.5 (nNaV1.5) regulates Na+ influx, which activates sodium/hydrogen exchanger-1 (NHE-1), leading to extracellular acidification and protease activity that degrades the extracellular matrix.[20]

VGSC Inhibitors and Blockers in Cancer Therapy

Ion channel inhibitors, especially VGSC blockers, are gaining traction in oncology for their potential to suppress tumour progression. *In vitro* studies have demonstrated that inhibiting VGSC expression reduces cancer cell invasiveness. For example, phenytoin and riluzole significantly suppressed proliferation in prostate cancer cell lines.[27] Some VGSC inhibitors not only suppress tumour growth but also enhance immune responses.[9] Specific subtypes like $Na_V1.5$ and its neonatal variant ($nNa_V1.5$) have been targeted in breast cancer, where NP siRNA and monoclonal antibody mAb-$nNa_V1.5$ effectively reduced invasion and metastasis.[28,29]

However, achieving tumour specificity remains a challenge. Combination therapies such as tamoxifen with VGSC inhibitors or si-$Na_V1.6$ to block TNF-α, have shown promise in targeting both hormonal and ion channel pathways.[30-32] Nonetheless, not all combinations are effective; for instance, both propranolol and ranolazine failed to demonstrate synergistic effects.[33]

Other VGSC blockers include tramadol, which reversibly inhibits $Na_V1.7$ and $Na_V1.5$;[34] lidocaine, which induces apoptosis in ovarian cancer cells; and DHA,[35] which reduced migration in MDA-MB-231 breast cancer cells by 26%.[10] Agents like phenytoin and ranolazine have shown anti-metastatic properties, with the latter also providing cardio-neuroprotection.[26,37] Additionally, naringenin reduces $Na_V1.7$ expression and suppresses cancer cell motility.[38]

In conclusion, VGSC inhibitors hold significant therapeutic promise, but their roles in modulating chemoresistance and driving tumour progression warrant further studies.[8]

Exploring the Uniqueness of Neonatal $Na_V1.5$

$nNa_V1.5$ Structure and Expression

The $nNa_V1.5$ is a splice isoform identified in humans, rats, and mice, which features a sequence distinct from the adult $Na_V1.5$ primarily found in cardiac tissues.[39] Unlike the adult form, $nNa_V1.5$ is selectively expressed in various cancers but not in normal tissues, making it a promising candidate for early cancer diagnosis and targeted anti-metastatic therapies.[23] This cancer-specific expression pattern sets $nNa_V1.5$ apart from other sodium channels, whose splice variants differ only slightly, limiting their therapeutic selectivity. Although there may be apparent homogeneity between the adult and neonatal forms, they are pharmacologically different, allowing for the design of drugs that specifically target $nNa_V1.5$ without affecting the adult form.[28]

The transition from the adult to the neonatal form results from alternative splicing of exon ^{6}, leading to the substitution of seven amino acids within the S3–S4 segment of the voltage sensor domain I (VSDI).[40] Notably, this includes the replacement of a negatively charged aspartate at position 211 with a positively charged lysine in $nNa_V1.5$. This lysine is located in a helix -turn-helix motif in the voltage-sensing region, which is exposed on the cell surface. This particular site is a

potential target for peptide toxins from animal venoms, which can selectively bind with and modify gating behaviour in a subtype-specific manner,[41] thus adding to the therapeutic benefit of nNa$_V$1.5.

nNa$_V$1.5 Roles

The nNa$_V$1.5 is highly expressed in neonates and is re-expressed in various cancers, especially breast cancer, where it is associated with metastasis. In contrast to the adult variant, nNa$_V$1.5 is capable of promoting cancer cell invasion, particularly in acidic tumour microenvironments, because of its higher acid resistance.[23,42] nNa$_V$1.5 activity is regulated by Protein Kinase A (PKA); inhibition with KT5720 has been shown to reduce nNa$_V$1.5-mediated expression and metastasis.[2] In breast cancer, nNa$_V$1.5 expression is associated with oestrogen receptor (ER) status and increased levels of glutamate, which further promote metastasis.[28,43]

Epigenetic control by RE1-silencing transcription factor (REST) and histone deacetylase 2 (HDAC2) also has been established, where they repress nNa$_V$1.5 expression at low levels. In MCF-7 cells, and histone deacetylase (HDAC) inhibitors such as Trichostatin A (TSA) decrease the level of REST and HDAC2, thereby increasing the expression of nNa$_V$1.5, making it more aggressive.[44] In colorectal cancer, the levels of nNa$_V$1.5 correlate with the progression-free survival and the Tumour-Node-Metastasis (TNM) breast cancer stage.[45]

Outside cancer, nNa$_V$1.5 is also present in dorsal root ganglia (DRG) neurons where it is responsible for causing neuropathic pain.[46] Compared to the adult isoform, nNa$_V$1.5 has a 50% greater Na^+ influx, activates at more positive voltages, and exhibits slower activation and recovery kinetics.[40] These properties reinforce its value as a cancer-specific therapeutic target.[28,47] Figure 2 shows a comparison of the protein sequences, illustrating the functional architecture of the general α-subunit of the voltage-gated sodium channel and the VSDI S3–S4 region of adult and neonatal Na$_V$1.5.

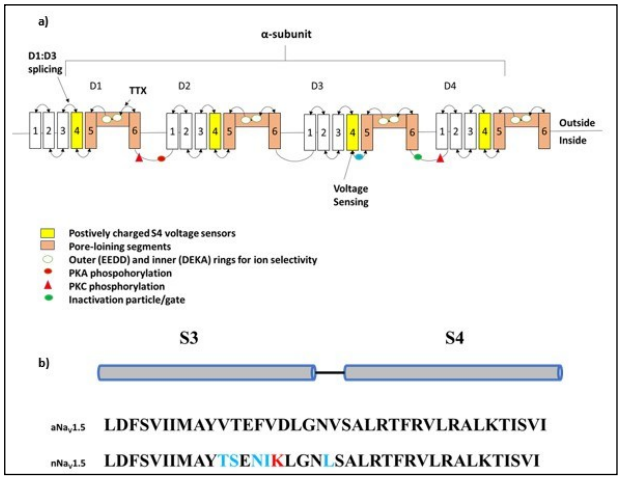

Figure 2: Protein sequence comparison of (a) functional architecture of the general α-subunit voltage-gated sodium channel; (b) Adult (aNa$_V$1.5) and Neonatal (nNa$_V$1.5) isoforms in the voltage-sensing domain (VSDI): S3-S4 region with important residues highlighted in blue and red[23,40]

Expression Patterns of nNa$_V$1.5 in Cancers

The nNa$_V$1.5 has emerged as a critical contributor to cancer progression, particularly in breast, colorectal, and brain cancers. In triple-negative breast cancer (TNBC), nNa$_V$1.5 is significantly upregulated and strongly associated with metastasis.[28,29,42] Elevated anti-nNa$_V$1.5 antibody levels in advanced breast cancer patients suggest its potential as a biomarker.[20] These antibodies may influence immune responses, possibly contributing to tumour progression. Functionally, inhibition of nNa$_V$1.5 reduces lymph node metastasis, reinforcing its role in invasiveness.[39] To date, most supporting evidence comes from *in vitro* studies using models such as MDA-MB-231 cells and limited serum antibody analyses. While these findings indicate strong clinical relevance, validation in larger patient cohorts is still lacking. In particular, the prognostic value of circulating anti-nNa$_V$1.5 antibodies needs to be tested against established clinical markers such as ER, progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER2). Future research should focus on integrating nNa$_V$1.5 expression data with clinical outcomes in well-annotated cohorts to determine its reliability as a prognostic and predictive biomarker.

In colorectal cancer, nNa$_V$1.5 expression is significantly elevated in advanced conditions and correlates with poor prognosis, highlighting its potential as a biomarker and

therapeutic target in resistant cases.[45] However, much of the data relies on immunohistochemistry with relatively small sample sizes, and mechanistic insights into how nNa$_V$1.5 drives invasion under hypoxia or chemotherapy resistance remain incomplete. Expanding studies to multi-center cohorts and integrating transcriptomic analyses is crucial for validating nNa$_V$1.5 as a clinically useful biomarker in colorectal cancer. Meanwhile, in brain cancer, nNa$_V$1.5 was initially identified via exon 6A,[48] with later studies confirming its high expression in astrocytomas and correlation with tumour grade.[15,49-51] Several Na$_V$1.5 isoforms have been identified in the human brain.[46,52] Additionally, Na$_V$1.6 expression in gliomas offers broader therapeutic avenues.[32] Using ion channel gene (iCG) signatures, including Na$_V$1.5 and Na$_V$1.6, studies have shown that higher expression levels predicted poorer glioma survival and aided risk stratification.[53]

In addition, Na$_V$1.5 increases NHE-1 pH sensitivity, which increases Li^+ uptake in acidic tumour microenvironments.[54] nNa$_V$1.5-driven extracellular acidification may modulate immune evasion by increasing regulatory T cell (Treg) activity and suppressing cytotoxic responses.[55] Additionally, nNa$_V$1.5 was associated with enhanced glutamate secretion in TNBC cells, which facilitates a pro-metastatic state. This effect was later abolished by TTX treatment, supporting the functional role of nNa$_V$1.5 in metastasis.[28] Evidence for nNa$_V$1.5 in gliomas highlights its association with higher tumour grade and poorer survival, but these studies are largely observational and often lack functional validation in vivo. A major barrier to translation is drug delivery across the blood-brain barrier, which remains poorly addressed in current preclinical work. Innovative delivery systems such as nanobody- or nanoparticle-based approaches should be tested in animal models to establish the feasibility of targeting nNa$_V$1.5 in brain cancers. Table 2 presents a summary of the expression patterns and clinical associations of nNa$_V$1.5 across various cancer types.

Table 2: Expression patterns and clinical associations of nNaV1.5 across cancer types

Cancer Type	Expression Pattern of nNa$_V$1.5	References
Breast Cancer (esp. Triple-Negative)	Strong upregulation in invasive and metastatic cells; detectable circulating antibodies in advanced disease	Brisson et al., 2013; Azahar et al., 2022; Rajaratinam et al., 2022; Sharudin et al., 2022
Colorectal Cancer	Elevated in advanced disease stages; expression correlates with TNM stage progression	Lastraioli et al., 2021; Guzel et al., 2019
Brain Tumours (Gliomas, Astrocytomas, Glioblastoma)	Overexpression in high-grade gliomas and astrocytomas; exon 6A isoform/nNa$_V$1.5 confirmed in brain tissue.	Xing et al., 2014; Wang et al., 2015; Schrey et al., 2002
Prostate Cancer	Na$_V$1.5 and nNa$_V$1.5 functionally expressed in malignant prostate cells.	Yildirim et al., 2012; Smith et al., 1998
Ovarian Cancer	Functional contribution of Na$_V$1.5/ nNa$_V$1.5 in tumour cells.	Liu et al., 2021
Cervical Cancer	Functional VGSC expression, including Na$_V$1.5 and nNa$_V$1.5	Díaz et al., 2007
Lung Cancer (non-small cell, small cell)	Functional Na$_V$1.5 expression in highly metastatic cell lines	Roger et al., 2007; Onganer & Djamgoz, 2005

Therapeutic Targeting of nNa$_V$1.5

Targeting nNa$_V$1.5 to Prevent Breast Cancer Invasion

In breast cancer, particularly in aggressive subtypes such TNBC, nNa$_V$1.5 contributes to a distinct set of pro-metastatic processes. Its activity enhances glycolysis, drives extracellular acidification that activates proteases, and alters ion dynamics to promote cell migration. Inhibition of nNa$_V$1.5 disrupts these pathways, thereby reducing the invasive behaviour of breast cancer cells and offering a rationale for therapeutic intervention.[1] Moreover, antibodies specifically targeting nNa$_V$1.5 have shown promise as therapeutic agents and have been demonstrated to effectively inhibit its function, thereby limiting metastatic behaviour.[56] Their efficacy has also been tested using 3D spheroid invasion assays, providing more physiologically relevant models of breast cancer metastasis.

Preclinical studies support the anti-metastatic potential of targeting nNa$_V$1.5. For instance, siRNA-mediated knockdown of the SCN5A gene, which encodes nNa$_V$1.5, significantly reduced breast cancer cell migration and invasion.[47] Pharmacological agents like ranolazine, originally developed to treat angina, have demonstrated strong inhibitory effects on VGSC activity, particularly against Na$_V$1.5 and its neonatal variant. In addition,

ranolazine significantly reduced invasiveness in both 2D and 3D models of breast cancer, especially within acidic tumour microenvironments, where nNa$_V$1.5 expression is elevated.[42] Combination therapies have also shown potential; for example propranolol, a β-blocker, inhibited lateral motility and invasion in MDA-MB-231 cells and exhibited enhanced anti-metastatic effects when combined with ranolazine.[57]

Epigenetic mechanisms also regulate nNa$_V$1.5 expression. HDAC inhibitors such as TSA have been found to modulate Na$_V$1.5 expression, reducing its oncogenic activity in metastatic breast cancer.[44] Since treatment with TSA led to a reduction in nNa$_V$1.5 levels and metastatic potential in cancer cells, this suggests that epigenetic therapies may represent a viable strategy for controlling metastasis.

Other inhibitors with distinct pharmacological characteristics, such as phenytoin and DAPT (N-[N-(3,5-difluorophenacetyl)-L-alanyl]-S-phenylglycine-t-butyl ester), have been found to inhibit the proliferation, migration, and invasion of MDA-MB-231 breast cancer cells.[33,58] These results strengthen the therapeutic potential of nNa$_V$1.5. However, more research is needed to fully understand the genetic and epigenetic regulation of nNa$_V$1.5 and to translate these findings into targeted clinical approaches for metastatic breast cancer.

Targeting nNa$_V$1.5 to Inhibit Brain Tumour Invasion

Different research works have emphasised the significance of nNa$_V$1.5 in cancer cell motility, highlighting its role in regulating ion exchange that drives migration and invasion within the tumour microenvironment.[31] In brain tumours such as glioblastoma, nNa$_V$1.5 overexpression is strongly associated with extracellular matrix degradation and is correlated with higher tumour grade and aggressiveness. This close link to malignancy underscores its value as a therapeutic target.[15] Thus, blocking this channel can potentially reduce cancer cell invasion and metastasis.

Inhibition of nNa$_V$1.5 and other ion channels as a therapeutic strategy in glioblastoma has been explored.[31] A study has demonstrated that inhibition of nNa$_V$1.5 reduced the motility of glioblastoma cells, a key characteristic of this very invasive cancer. Drug delivery systems that employ nanobodies have also shown increased specificity and efficacy. Nanobodies are small single-domain antibody fragments derived from camelid antibodies, noted for their ability to penetrate tissues and cross the blood-brain barrier (BBB), making them promising for targeting CNS tumours.[59] Nanobodies present a new therapeutic approach by delivering drugs directly to tumour cells that express nNa$_V$1.5, thereby attenuating off-target effects and enhancing therapeutic precision. This strategy can be particularly valuable in the management of aggressive cancers like glioblastoma, where the BBB is a limiting factor to effective drug delivery.

Potential Role of nNa$_V$1.5 as a Diagnostic Marker

Beyond its therapeutic potential, nNa$_V$1.5 can also serve as an early diagnostic marker of aggressive cancers. Upregulation of nNa$_V$1.5 has been associated with poor prognosis and high metastatic potential in breast and colorectal cancers.[23] Screening of nNa$_V$1.5 expression may allow early detection of metastatic disease and the timely initiation of treatments targeting VGSC activity. In addition, the search for anti-metastatic agents that selectively block nNa$_V$1.5 function is a potentially rewarding approach for developing novel therapeutics.

The possibility of using anti-nNa$_V$1.5 antibodies as biomarkers of breast cancer progression has been investigated.[55] In the study, the detection of neonatal Na$_V$1.5 antigens in the blood samples enabled the identification of antibodies that correlated with breast cancer metastasis. Pro-inflammatory cytokines (IL-6) and anti-nNa$_V$1.5 antibodies could also be used as biomarkers for monitoring tumour development and immune system functioning. Furthermore, it was suggested that the immunogenicity of nNa$_V$1.5 may be used as a marker of immune surveillance, associating nNa$_V$1.5 with the triad interplay between breast cancer, metastasis, and the immune system.[20] These findings highlight the potential of nNa$_V$1.5 as a cancer immunotherapy target and a promising direction for further studies.

LIMITATIONS AND FUTURE PERSPECTIVES

Despite the promising therapeutic potential of targeting nNa$_V$1.5 in cancer metastasis, several significant challenges remain. First, most studies on nNa$_V$1.5-targeted therapies have been conducted in preclinical models, and only a few clinical trials have evaluated their effectiveness in cancer patients. Currently, nNa$_V$1.5-targeted strategies remain at the preclinical stage. Monoclonal antibodies (mAb-nNa$_V$1.5) and siRNA approaches have shown promise *in vitro* and animal models, but no registered clinical trials have yet advanced beyond exploratory preclinical work. The translation of these preclinical findings into clinical application is challenging due to the heterogeneity of tumour biology and patient-specific responses. Moreover, considering that anti-nNa$_V$1.5 antibodies and VGSC inhibitors (including TTX and ranolazine) primarily demonstrate *in vitro* activity, their off-target effects and potential toxicity in humans are of concern because of the essential roles of VGSCs in cardiac and neural function.

Another major limitation is the heterogeneity of VGSC expression in various types of cancers, as well as the variability of nNa$_V$1.5 expression within tumours. Such diversity complicates the development of therapeutic approaches that are broadly applicable. Furthermore, the mechanisms underlying epigenetic regulation of nNa$_V$1.5 expression is poorly understood, and thus, more studies are required to clarify how HDACs and other epigenetic therapies can be leveraged in cancer treatment.

Future research should address these limitations by conducting well-designed clinical trials to demonstrate the safety and efficacy of Na$_V$1.5–targeted therapies. A key obstacle that needs to be overcome is drug delivery, especially in brain cancers such as glioblastoma, where the BBB restricts access of large molecules like antibodies. In addition, the high homology between the neonatal and adult isoforms poses a risk of off-target cardiac toxicity, underscoring the urgent need for highly selective therapeutic agents. Tumour heterogeneity represents another challenge, as intra-tumoural differences in nNa$_V$1.5 expression and inter-patient variability may reduce therapeutic consistency and complicate biomarker validation.

The development of more specific VGSC inhibitors and improved delivery systems, such as nanobody- or nanoparticle-based platforms, could enhance therapeutic precision and minimize systemic side effects. Furthermore, exploring combination strategies that integrate nNa$_V$1.5-targeted therapies with immunotherapy or chemotherapy may provide synergistic benefits in metastatic cancers. Finally, advancing this field will require defining the role of nNa$_V$1.5 across cancer types and establishing its application as a clinically reliable biomarker for early diagnosis, prognosis, and treatment stratification.

CONCLUSION

The neonatal isoform of Na$_V$1.5 plays a critical role in cancer metastasis, particularly in breast, colorectal, and brain tumours. With its ability to promote cancer cell migration, invasion, and survival, it represents a unique and highly promising therapeutic target. Preclinical studies have demonstrated that inhibition of nNa$_V$1.5 through specific antibodies, VGSC inhibitors, or epigenetic regulators can reduce invasiveness and metastatic potential, underscoring its value as both a therapeutic target and a biomarker of aggressive cancers. Nonetheless, major challenges remain in advancing this approach to the clinical stage. Future research should focus on developing highly selective inhibitors, validating their efficacy in clinical trials, and investigating strategic combination therapies. Advancing the understanding of nNa$_V$1.5's role in cancer biology may ultimately enable new and effective treatment strategies for metastatic tumours and improve survival outcomes for cancer patients.

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invasion in metastatic breast cancer. *Breast Cancer Res Treat* 2012; 143(2):603-15. Doi: 10.1007/s10549-012-2102-9

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REVIEW ARTICLE

Arabic Language and Medical Terminology in Education: A Systematic Review

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ABSTRACT

The use of Arabic in medical education and healthcare communication remains limited despite growing linguistic needs in Arabic-speaking populations. Issues related to translation accuracy, cultural sensitivity, and curriculum alignment hinder its integration. The objective of this study is to systematically review how Arabic medical terminology has been translated, validated, and applied in educational and clinical contexts. This systematic review followed the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) 2020 guidelines. Searches were conducted in May 2025 across three databases: Scopus, Web of Science (WoS), and the Education Resources Information Center (ERIC). A total of 769 records were retrieved, and 19 peer-reviewed articles were selected based on inclusion criteria. A six-point quality appraisal framework was applied to ensure methodological rigour prior to thematic synthesis. Three major themes emerged: (1) translation and cultural adaptation, highlighting the importance of expert validation and semantic accuracy; (2) psychometric evaluation, where tools such as the Postgraduate Hospital Educational Environment Measure (PHEEM) demonstrated strong internal consistency and contextual clarity; and (3) educational application, showing varied success in enhancing communication, comprehension, and learner confidence. Gaps in terminology standardisation and the limitations of automated translation tools were noted across studies. In conclusion Arabic medical terminology, when supported by structured processes and expert review, makes a meaningful contribution to education and communication. Further efforts should prioritise the development of validated terminology repositories, multidisciplinary collaboration, and the integration of Arabic-language resources into medical curricula to support inclusive and patient-centred care.

Keywords:
Arabic medical terminology, translation and cultural adaptation, medical education in Arabic, psychometric validation, healthcare communication

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INTRODUCTION

The integration of Arabic language and medical terminology has garnered increasing attention amid global efforts to promote inclusive medical education, linguistic accessibility, and culturally responsive health communication. As Arabic-speaking populations continue to expand across diverse regions, the demand for accurate, contextually appropriate, and pedagogically sound translations of medical terms has intensified.[1,2] This need extends beyond clinical settings to educational institutions where Arabic is the medium of instruction. However, medical terminology is inherently complex, being highly specialized and deeply rooted in Latin and Greek etymologies, which poses substantial challenges for direct translation into Arabic.[3,5] These linguistic difficulties are further compounded by sociocultural sensitivities and pedagogical limitations, necessitating strategies that strike a balance between linguistic precision and educational clarity. While academic interest in this area is growing, the existing literature remains fragmented, often confined to case studies or isolated issues such as lexical equivalence, transliteration accuracy, or classroom practices.[6,7] Moreover, there is limited agreement on standardised translation protocols, resulting in inconsistencies that may compromise both educational outcomes and healthcare communication.[8–10] Despite increased scholarly interest, there remains a paucity of systematic reviews that comprehensively address the translation, adaptation, and implementation of

Arabic medical terminology in education. The literature remains scattered and predominantly exploratory, lacking overarching frameworks or comparative insights.

This systematic literature review seeks to consolidate recent findings on how medical terminology has been translated and applied in Arabic contexts. It critically examines translation practices, psychometric validation of tools, and curriculum implementation strategies to address unresolved challenges. By synthesizing these insights, the review aims to propose a framework that supports the accurate, culturally relevant and educationally effective integration of medical terminology into Arabic language instruction.[11,13]

LITERATURE REVIEW

Recent efforts to integrate Arabic medical terminology into health education reflect the growing linguistic and cultural demands of Arabic-speaking communities. Numerous studies have addressed both student and faculty perceptions towards Arabic as a medium of instruction. While cultural motivations are strong, many educators and students report limited confidence in Arabic for medical education due to inconsistent terminology and the dominance of English in assessments.[14,15] Despite this, there is notable support for bilingual or dual-language instruction, particularly in enhancing communication with patients and reinforcing comprehension of complex concepts.[16]

One of the major challenges identified in the literature is the lack of consistency in Arabic translations of medical terminology. The variability in translations of abbreviations and acronyms across clinical documents poses potential risks in patient safety.[9] Other studies have similarly reported disagreement between translators and healthcare providers regarding terminology accuracy.[17] In the absence of established lexical norms, translators may resort to circumlocution, which can undermine clarity and standardisation.[18]

Beyond linguistic precision, the pedagogical dimension of Arabic medical terminology presents its own set of limitations. A widespread knowledge gap has been reported among pharmacy students regarding Arabic terms, particularly in institutions with outdated curricula.[19] Many students also felt unprepared for Arabic clinical communication, despite their confidence in patient interaction, largely due to minimal exposure to Arabic content during foundational training.[20,21] These findings suggest the need for curriculum reforms that prioritise Arabic instruction in early stages and include structured language support.

Retention of medical knowledge in Arabic is closely linked to contextual application. Long-term recall of biochemistry content was found to improve when students engaged with terminology in relevant settings.[22] Culturally adapted language in assessment tools has also been shown to support not only content understanding but also patient functionality.[23] However, Arabic-based digital health interventions often suffer from low engagement, especially among users with severe baseline conditions.[24] This implies that successful integration of Arabic in healthcare education must also consider psychological and technological adaptability.

Technological advances in Arabic health language processing show promise. AltibbiVec, a neural word embedding model trained on Arabic consultations, successfully captured the semantic nuances of medical terms.[25] AraBERT, a transformer-based model, has been effective in filtering misinformation in Arabic health discourse.[26] Nevertheless, the utility of such models is limited by the scarcity of Arabic corpora and regional dialect variation.[27] This further underscores the necessity of linguistically rich and standardised data for effective implementation. However, these technological initiatives remain underutilised in formal medical curricula, highlighting the disconnect between digital innovation and pedagogical practice.

Overall, the literature identifies three major gaps: the absence of standardised translation protocols, limited curricular alignment for Arabic instruction, and inadequate validation of technological tools. Addressing these gaps requires collaborative efforts between linguists, educators, and health professionals to build

comprehensive, culturally responsive and pedagogically effective frameworks for Arabic medical terminology in education.

RESEARCH QUESTIONS

To provide a structured foundation for this review, three research questions were formulated at the outset, guided by the Population Interest Context (PICo) framework. These questions were used to define the inclusion and exclusion criteria, develop the search strategy and structure the synthesis of findings. The thematic outcomes reported in the results section were not used to generate these questions but were instead derived from studies that addressed the pre-established research focus.

The PICo framework, a recognised tool for structuring qualitative evidence synthesis in health-related fields[28,29] was employed to frame the scope of this review. Each component was defined and operationalised as shown in Table I.

Table I: PICo framework for inclusion and exclusion criteria

Component	Definition In This Review	Application To Inclusion/ Exclusion
Population	Medical students, healthcare professionals, patients in Arabic-speaking contexts	Studies were included if they involved participants from medical or health-related education or services within Arabic-speaking populations
Interest	Arabic translation, adaptation, validation or use of medical terminology or tools	Studies must focus on the translation, cultural adaptation, psychometric evaluation or application of Arabic medical terminology in educational or clinical settings
Context	Educational and clinical environments using Arabic for instruction or communication	Only studies conducted in contexts where Arabic was a primary or secondary medium of communication in healthcare or education were included

No specific region or country within the Arabic-speaking world was prioritised or excluded if the study met the language and contextual relevance criteria. Studies were excluded if they focused solely on English-based tools without Arabic translation or on general linguistic topics unrelated to medical terminology.

The following research questions guided the entire review process:

1. How do Arabic-speaking healthcare professionals perceive the accuracy and cultural relevance of medical terminology translation and adaptation efforts in Arabic-speaking countries?
2. What is the psychometric validity of Arabic-translated medical education instruments among medical students and practitioners across Arabic-speaking regions?
3. How is Arabic medical terminology applied and integrated within educational curricula to enhance learning outcomes and clinical communication for students in Arabic-speaking contexts?

MATERIALS AND METHODS

This systematic literature review applied the PRISMA guidelines (Preferred Reporting Items for Systematic Reviews and Meta-Analyses), which are widely recognised for enhancing transparency and rigor in research reporting.[30] The framework includes four stages: identification, screening, eligibility, and inclusion. For this review, three reputable databases were selected: Scopus, Web of Science (WoS), and the Education Resources Information Center (ERIC). These databases were chosen for their interdisciplinary scope and comprehensive coverage of peer-reviewed literature.

Identification

A systematic search strategy was designed using combinations of keywords such as "Arabic," "medical," "health," "terminology," and "translate." These terms were refined using academic thesauri and tailored to suit the indexing formats of each database. The finalised search strings were applied across Scopus, WoS, and ERIC in May 2025, retrieving a total of 769 records. Table II presents the detailed search strings and access information used during the identification phase

Table II: The search string

Database	Search String
SCOPUS	TITLE-ABS-KEY (arab* AND (medic* OR doc* OR health) AND (term* OR language) AND translate) AND (LIMIT-TO (SRCTYPE , "j")) AND (LIMIT-TO (DOCTYPE , "ar")) AND (LIMIT-TO (PUBYEAR , 2021) OR LIMIT-TO (PUBYEAR , 2022) OR LIMIT-TO (PUBYEAR , 2023) OR LIMIT-TO (PUBYEAR , 2024) OR LIMIT-TO (PUBYEAR , 2025)) AND (LIMIT-TO (LANGUAGE , "English")) AND (LIMIT-TO (SUBJAREA , "SOCI") OR LIMIT-TO (SUBJAREA , "MULT") OR LIMIT-TO (SUBJAREA , "ARTS")) AND (LIMIT-TO (OA , "all")) Date of Access: May 2025
WoS	arab* AND (medic* OR doc* OR health) AND (term* OR language) AND translate (Topic) and 2025 or 2024 or 2023 or 2022 or 2021 (Publication Years) and Article (Document Types) and English (Languages) and Linguistics or Education Scientific Disciplines or Education Educational Research or Multidisciplinary Sciences or Language Linguistics (Web of Science Categories) and Linguistics or Language Linguistics or Multidisciplinary Sciences or Education Educational Research or Education Scientific Disciplines (Web of Science Categories) and All Open Access (Open Access) Date of Access: May 2025
ERIC	arab* AND (medic* OR doc* OR health) AND (term* OR language) AND translate naepubyearmin:2021 pubyearmax:2025 Date of Access: May 2025

Table III: Number and details of primary studies database

NO	AUTHOR	TITLE	YEAR	JOURNAL	SCOPUS	WoS	ERIC
1.	Alfakhry G.; Mustafa K.; Khwanda R.; Alhaffar M.; Alhomsi K.; Kodmani R.[31]	Translation, Cultural Adaptation and Linguistic Validation of The Postgraduate Hospital Educational Environment Measure into Arabic	2024	BMC Medical Education	/	/	
2.	Almahasees Z.; Meqdadi S.; Albudairi Y.[32]	Evaluation Of Google Translate in Rendering English Covid-19 Texts into Arabic	2021	Journal of Language and Linguistic Studies	/		
3.	Alaska Y.A.; Alqahtani N.M.; Al Zahrani A.K.; Alshahri R.; Malyani R.Z.; Alkutbe R.B.[33]	Evaluating The Content and Face Validity of Arabic-Translated Patient Measures of Safety Survey PMOS-30	2024	PLoS ONE	/		
4.	Mansour M.; Hasan A.A.; Alafafsheh A.[34]	Psychometric Evaluation of The Arabic Version of The Irish Assertiveness Scale Among Saudi Undergraduate Nursing Students and Interns	2021	PLoS ONE	/		
5.	Albabtain B.; Paudyal V.; Cheema E.; Bawazeer G.; Alqahtani A.; Bahatheq A.; Shuweihdi F.; Hadi M.A.[35]	Translation, Cultural Adaptation and Validation of Patient Satisfaction with Pharmacist Services Questionnaire (PSPSQ) 2.0 Into the Arabic Language Among People with Diabetes	2024	PLoS ONE	/	/	
6.	Alzain E.; Nagi K.A.; Algobaei F.[36]	The Quality of Google Translate and ChatGPT English to Arabic Translation: The Case of Scientific Text Translation	2024	Forum for Linguistic Studies	/		
7.	Alaqil A.I.; Gupta N.; Alothman S.A.; Al-Hazzaa H.M.; Stamatakis E.; del Pozo Cruz B.[37]	Arabic Translation and Cultural Adaptation of Sedentary Behavior, Dietary Habits and Preclinical Mobility Limitation Questionnaires: A Cognitive Interview Study	2023	PLoS ONE	/		
8.	Awwad O.; AlMuhaissen S.; Al-Nashwan A.; AbuRuz S.[38]	Translation And Validation of The Arabic Version of The Morisky, Green and Levine (MGL) Adherence Scale	2022	PLoS ONE	/	/	
9.	ElHafeez S.A.; Elbarazi I.; Shaaban R.; ElMakhzangy R.; Aly M.O.; Alnagar A.; Yacoub M.; El Saeh H.M.; Eltaweel N.; Alqutub S.T.; Ghazy R.M.[39]	Arabic Validation and Cross-Cultural Adaptation of the 5C Scale for Assessment Of COVID-19 Vaccines Psychological Antecedents	2021	PLoS ONE	/		
10.	Muller, R; Konecny, LT[40]	Patient Perceptions of The Readability and Helpfulness of Bilingual Clinical Forms: A Survey Study	2023	BMC Medical Education		/	
11.	Farag, HFM; Sultan, EA; Elrewany, E; Abdel-Aziz, BF[41]	Arabic Version of The Australian Type 2 Diabetes Risk Assessment Tool (AUSDRISK): Translation and Validation	2022	BMC Research Notes		/	
12.	Sharkas, H[42]	Nominalization In Arabic Translations of Patient Information Leaflets	2024	Translation & Interpreting-The International Journal of Translation and Interpreting		/	
13.	Alduhaim, A; Alkhaldy, M[43]	Medical Discourse Translation during COVID-19: A Case Study of Translating Medical Discourse into Arabic	2023	Jordan Journal of Modern Languages & Literature		/	
14.	Bahadi, A; Lagtarna, H; Benbria, S; Zajjari, Y; Elkabbaj, D; Zemraoui, N[44]	Physical Activity in Sahara Moroccan Hemodialysis Patients	2021	BMC Research Notes		/	
15.	Alfakhry, G; Kodmani, R; Almasri, IA[45]	Psychometric Properties of The Arabic Version of PHEEM Applied on A Sample of Medical Residents in Syria	2024	BMC Medical Education		/	
16.	Mohamed, E; Sarwar, R; Mostafa, S[46]	Translator Attribution for Arabic Using Machine Learning	2023	Digital Scholarship in The Humanities		/	
17.	Alharbi, MT; Ateef, M; Alanazi, A; Alzhrani, M[47]	Cross-Cultural Adaptation and Validation of The Arabic Version of The Knee and Hip Health-Related Quality of Life (Mini-OAKHQOL) Questionnaire in Male Saudi Patients with Osteoarthritis: A Methodological Observational Design	2024	PEERJ		/	/
18.	Chbab, H[48]	Translation Procedures for Medical Neologisms and Their Contribution to The Enrichment of Arabic Medical Terminology	2024	Hermeneus		/	
19.	Temehy, B; Soundy, A; Sahely, A; Palejwala, Y; Heath, J; Rosewilliam, S[49]	Exploring The Needs of Stroke Patients After Discharge from Rehabilitation Centres in Saudi Arabian Communities: An IPA Qualitative Exploratory Study Design	2023	PLoS ONE		/	

Screening

To ensure consistency and relevance, a set of predefined inclusion and exclusion criteria was applied during the screening phase. Only full journal articles published in English between 2021 and 2025 were included in this review. Non-journal sources such as conference proceedings, book chapters, reviews, and unpublished materials were excluded. Additionally, studies that were still in press or not yet finalised were removed. After excluding 50 duplicates and filtering out 467 irrelevant records based on titles and abstracts, a total of 252 articles were retained for full-text assessment.

Eligibility

The remaining 252 records underwent full-text assessment to determine their alignment with the review's objectives. Each study was examined for thematic relevance based on its title, abstract, and complete content. Articles that did not address any of the core areas, such as Arabic medical terminology, translation practices, pedagogical integration or clinical communication, were excluded. The eligibility process focused on selecting studies that provided substantive data or analysis related to these domains.

As a result, 19 articles were deemed suitable and included in the final synthesis. The final pool of 19 articles was drawn from three primary databases selected during the identification phase. Table III summarises the distribution of these studies by database, providing an overview of their source and accessibility.

The overall screening process, from initial identification to final inclusion, is illustrated in the PRISMA flow diagram shown in Figure 1.

Data Abstraction and Analysis

Thematic analysis was conducted using a six-point quality assessment framework adapted from previously established models.28,50 This framework comprised six evaluation criteria: clarity of study aim, methodological strength, conceptual definition, relevance, comparative insight, and acknowledgement of limitations. Only studies that scored above 3.0 were retained for further synthesis. This procedure ensured consistency and methodological credibility across the selected literature before thematic classification.

Bibliographic management and citation formatting were conducted using Mendeley, which ensured consistency in referencing across the manuscript. Thematic grouping and extraction of key information were performed manually using Microsoft Excel, allowing for structured data abstraction aligned with the predefined research questions and quality appraisal framework.

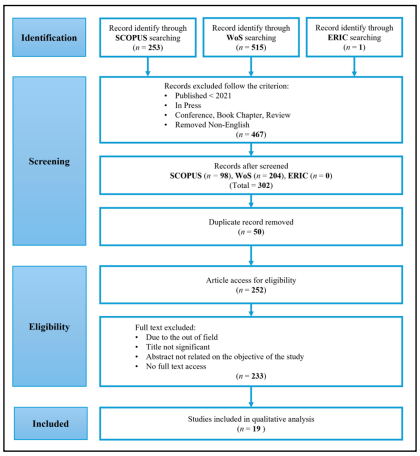

Figure 1: PRISMA 2020 flow diagram illustrating the identification, screening, eligibility and inclusion process.

RESULT AND FINDING

Thematic findings derived from the selected studies are structured using the PICo framework, followed by a quality appraisal to ensure methodological rigour.

Thematic Findings Based on PICo Framework

Based on the PICo structure applied in this review, three major themes were identified across the selected studies: (1) translation and cultural adaptation of Arabic medical terminology; (2) psychometric validation and application in Arabic-speaking healthcare and education; and (3) integration of Arabic medical terminology in educational and clinical settings.

Translation and Cultural Adaptation of Arabic Medical Terminology

The translation and cultural adaptation of medical terminology into Arabic has become a central focus in advancing healthcare communication and medical education within Arabic-speaking regions. Multiple studies emphasize the importance of rigorous translation protocols such as forward and backward translation, expert validation, and contextual adaptation to ensure medical tools remain semantically precise and culturally appropriate.[31,35,37] One notable example is the Arabic version of the Postgraduate Hospital Educational Environment Measure (PHEEM), which demonstrated strong content validity and contextual clarity after structured translation tailored to the Syrian context.[31] The Patient Satisfaction with Pharmacist Services Questionnaire (PSPSQ) also showed high internal consistency (Cronbach's alpha 0.99) following cultural and linguistic adaptation for diabetic patients.[35] Likewise, the Arabic version of the Morisky, Green and Levine (MGL) adherence scale exhibited strong construct validity, confirming its reliability in measuring patient behaviour and medication adherence.[38]

Further validation studies, including those involving the Mini Osteoarthritis Knee and Hip Quality of Life (Mini-OAKHQOL) instrument, ensured sociocultural alignment for Saudi osteoarthritis patients, reaffirming the importance of contextualised language in clinical assessments.[47] Tools measuring lifestyle and dietary behaviours also underwent cognitive interviewing to enhance regional comprehensibility and usability.[37] In addition to standard instruments, various translation strategies have been employed to generate Arabic medical neologisms. These include the use of calques, semantic extension, and lexical borrowing, particularly during health emergencies, to enrich terminology while preserving clarity and domain specificity.[47]

Overall, the findings confirm that effective translation in medical contexts requires more than lexical accuracy.

Cultural alignment and structured validation are essential to ensure that Arabic-translated tools serve both educational and healthcare objectives across diverse regional settings.

Psychometric Evaluation and Application in Arabic Medical Education

The psychometric evaluation of Arabic translated instruments is crucial for establishing their reliability and relevance in medical education and health research. Numerous studies have confirmed that these tools, when appropriately adapted, offer valid measurement constructs for Arabic-speaking populations. For instance, the Arabic version of the Irish Assertiveness Scale demonstrated strong internal consistency (Cronbach's alpha 0.80) and a content validity index of 0.93 among Saudi nursing students.[34] The 5C Scale of Psychological Antecedents to Vaccination, which measures five key dimensions (Confidence, Complacency, Constraints, Calculation and Collective responsibility) was validated in Egypt, Saudi Arabia and the United Arab Emirates. The scale showed strong internal reliability (alpha ≥0.7) and confirmed factor structure validity.[39]

The Arabic version of the MGL adherence scale also displayed solid psychometric properties, showing high internal consistency and construct validity when used among patients with chronic conditions.[38] Similarly, the PHEEM instrument, previously culturally adapted, underwent psychometric validation in Syria. The results confirmed excellent internal reliability (Cronbach's alpha 0.938) and a five-factor model explaining 43 percent of the variance, establishing its suitability for assessing clinical learning environments. Studies on lifestyle-related tools, such as sedentary behaviour and dietary habit questionnaires, highlighted the importance of cultural alignment and cognitive testing during the translation process. These tools were validated through cognitive interviews to ensure linguistic clarity and conceptual relevance in Saudi contexts.[37]

Moreover, evaluation of machine translation tools revealed limitations in accuracy. Although Google Translate outperformed ChatGPT in terms of technical precision, both platforms exhibited notable grammatical and semantic inconsistencies, thereby underscoring the critical need for human oversight in Arabic medical translation.[32,36] These findings underscore the importance of validation approaches that align psychometric rigour with regional sociolinguistic realities, ensuring practical utility across education and healthcare. Rigorous adaptation further enhances credibility and fosters broader acceptance of Arabic in medical and academic discourse.

Arabic Medical Education and Terminology Applications

Beyond validation, the application of Arabic-translated medical tools across educational and clinical contexts has yielded valuable insights regarding their usability, clarity and impact on both patient care and learning outcomes. Beyond enhancing patient–provider communication, these instruments also enrich the linguistic inclusiveness and pedagogical depth of Arabic medical education. One prominent example is the Arabic version of the Patient Measures of Safety Survey (PMOS-30), used to assess patient perceptions of healthcare safety. Following translation and testing, the tool demonstrated high face validity and clarity after cognitive revision, highlighting its value in improving patient engagement and institutional safety practices.[33] Similarly, the Arabic version of the MGL adherence scale proved reliable for evaluating medication adherence, reinforcing its role in clinical monitoring.[38]

Several studies have examined the effectiveness of patient-facing materials. Bilingual clinical forms were generally perceived by patients as helpful in enhancing understanding and communication within healthcare settings.[40] However, excessive nominalisation in Arabic translations of patient information leaflets was found to reduce readability, highlighting the need for simplification strategies when addressing non-specialist audiences.[42] Machine translation tools such as Google Translate and ChatGPT were also tested for rendering medical content into Arabic. Although Google Translate demonstrated superior technical accuracy, both tools produced notable grammatical and semantic errors.[32,36] These limitations

underscore the importance of human oversight and contextual refinement, particularly in the translation of sensitive medical materials.

In response to the COVID-19 pandemic, numerous Arabic medical neologisms emerged to address newly introduced health concepts. These terms were often constructed through descriptive translations or Arabicised forms, tailored to meet cultural and linguistic expectations.[43] Translation approaches such as calques and lexical borrowing have also been employed to expand the Arabic medical lexicon while preserving semantic clarity.[48] Collectively, these strategies affirm the value of Arabic in health communication and education, while highlighting the ongoing need for culturally sensitive adaptation, empirical validation and user-centred design in both clinical and educational materials.

Quality Appraisal of Selected Studies

The quality of the selected studies was assessed using a structured six-point appraisal framework adapted from Kitchenham and Charters[28] and Anas Abouzahra et al.[50] This framework provided a structured evaluation of methodological and conceptual rigour before inclusion into the thematic synthesis.

The six criteria used for appraisal were as follows:
QA1 – Clarity of the study's aim
QA2 – Methodological strength
QA3 – Conceptual definition
QA4 – Relevance to the field
QA5 – Comparative insight
QA6 – Acknowledgement of study limitations

The results of the quality appraisal for the 19 selected studies are summarised in Table IV. Eighteen studies attained the maximum score of 6.0 across all quality indicators, while one study (PS1) received a slightly lower score of 5.5 due to a limited discussion of its methodological limitations. Overall, the included literature demonstrates a high degree of methodological consistency, reinforcing the credibility and analytical soundness of the thematic synthesis. Table IV below provides a detailed breakdown of individual quality scores

Table IV: Quality assessment of selected studies based on systematic review criteria (QA1–QA6)

Primary Study	Title	QA1	QA2	QA3	QA4	QA5	QA6	Total Mark	Percentage (%)
PS1	Translation, Cultural Adaptation and Linguistic Validation of The Postgraduate Hospital Educational Environment Measure into Arabic	1	1	1	1	1	0.5	5.5	91.67
PS2	Evaluation Of Google Translate in Rendering English Covid-19 Texts into Arabic	1	1	1	1	1	1	6	100
PS3	Evaluating The Content and Face Validity of Arabic-Translated Patient Measures of Safety Survey PMOS-30	1	1	1	1	1	1	6	100
PS4	Psychometric Evaluation of The Arabic Version of The Irish Assertiveness Scale Among Saudi Undergraduate Nursing Students and Interns	1	1	1	1	1	1	6	100
PS5	Translation, Cultural Adaptation and Validation of Patient Satisfaction with Pharmacist Services Questionnaire (PSPSQ) 2.0 Into the Arabic Language Among People with Diabetes	1	1	1	1	1	1	6	100
PS6	The Quality of Google Translate and ChatGPT English to Arabic Translation: The Case of Scientific Text Translation	1	1	1	1	1	1	6	100
PS7	Arabic Translation and Cultural Adaptation of Sedentary Behavior, Dietary Habits and Preclinical Mobility Limitation Questionnaires: A Cognitive Interview Study	1	1	1	1	1	1	6	100
PS8	Translation And Validation of The Arabic Version of The Morisky, Green and Levine (MGL) Adherence Scale	1	1	1	1	1	1	6	100
PS9	Arabic Validation and Cross-Cultural Adaptation of the 5C Scale for Assessment Of COVID-19 Vaccines Psychological Antecedents	1	1	1	1	1	1	6	100
PS10	Patient Perceptions of The Readability and Helpfulness of Bilingual Clinical Forms: A Survey Study	1	1	1	1	1	1	6	100
PS11	Arabic Version of The Australian Type 2 Diabetes Risk Assessment Tool (AUSDRISK): Translation and Validation	1	1	1	1	1	1	6	100
PS12	Nominalization In Arabic Translations of Patient Information Leaflets	1	1	1	1	1	1	6	100
PS13	Medical Discourse Translation during COVID-19: A Case Study of Translating Medical Discourse into Arabic	1	1	1	1	1	1	6	100
PS14	Physical Activity in Sahara Moroccan Hemodialysis Patients	1	1	1	1	1	1	6	100
PS15	Psychometric Properties of The Arabic Version of PHEEM Applied on A Sample of Medical Residents in Syria	1	1	1	1	1	1	6	100
PS16	Translator Attribution for Arabic Using Machine Learning	1	1	1	1	1	1	6	100
PS17	Cross-Cultural Adaptation and Validation of The Arabic Version of The Knee and Hip Health-Related Quality of Life (Mini-OAKHQOL) Questionnaire in Male Saudi Patients with Osteoarthritis: A Methodological Observational Design	1	1	1	1	1	1	6	100
PS18	Translation Procedures for Medical Neologisms and Their Contribution to The Enrichment of Arabic Medical Terminology	1	1	1	1	1	1	6	100
PS19	Exploring The Needs of Stroke Patients After Discharge from Rehabilitation Centres in Saudi Arabian Communities: An IPA Qualitative Exploratory Study Design	1	1	1	1	1	1	6	100

DISCUSSION

This systematic review identified three interrelated themes in the integration of Arabic medical terminology: structured translation and cultural adaptation, psychometric validation of educational tools, and the application of translated resources in clinical and instructional settings. These themes reflect both the advancement and the persistent challenges in positioning Arabic as a viable medium for medical education and healthcare communication. Translation studies consistently revealed that direct lexical conversion is inadequate. Instead, high-quality outcomes require expert validation, cultural contextualisation, and iterative testing to ensure semantic fidelity and functional usability. The Arabic versions of PHEEM and PSPSQ 2.0, for instance, demonstrated strong internal consistency and contextual relevance, confirming the effectiveness of structured adaptation.[31,35] Nonetheless, inconsistencies in translation quality across instruments signal the need for centralised protocols or standardised glossaries to enhance semantic coherence.

Psychometric validation findings reinforced the methodological credibility of Arabic-adapted tools. Instruments such as the MGL adherence scale, the Irish Assertiveness Scale, and the 5C vaccine scale exhibited robust reliability and construct validity, affirming the capacity of Arabic to serve as a stable language for clinical and behavioural assessments.[34,38,39] However, the requirement for substantial item-level modifications in several tools highlights the limitations of direct translation and underlines the critical role of localised validation. Cognitive interviews and factor analyses were instrumental in identifying latent conceptual discrepancies and improving item clarity.[37,45]

Several limitations should be acknowledged. Firstly, the review only included studies published in English, which may have excluded relevant Arabic-language research, introducing potential bias. Secondly, although three major databases (Scopus, WoS and ERIC) were searched, relevant studies indexed in repositories such as PubMed or regional Arabic databases may have been overlooked. Thirdly, the review was restricted to publications between 2021 and 2025, potentially omitting earlier foundational works or newer studies not yet indexed. Lastly, the focus was limited to full-text peer-reviewed journal articles, excluding conference proceedings, grey literature, and in-press publications, which may contain additional insights.

The real-world application of Arabic medical terminology produced varied outcomes. Patient-facing materials such as bilingual forms and Arabic-language leaflets were generally well-received,[40] although readability challenges, particularly those caused by nominalisation, remained.[42] Automated translation platforms like Google Translate and ChatGPT demonstrated some utility, yet continued to exhibit semantic and grammatical shortcomings, reaffirming the irreplaceable role of human oversight in sensitive medical contexts.[32,36]

During the COVID-19 pandemic, the creation of Arabic medical neologisms illustrated the language's adaptability in responding to emergent health discourse.[43,48] While such innovation enhances relevance, it also necessitates systematic regulation and institutional alignment to prevent fragmented usage. Overall, the findings suggest that Arabic can function effectively in medical domains when supported by empirical validation, professional collaboration, and sustained curricular integration. Future research and policy efforts should prioritise the development of centralised term banks, cross-national studies, and hybrid strategies that blend technology with expert linguistic review.

CONCLUSION

This review highlights the growing significance of integrating Arabic language and medical terminology into health education and clinical communication. Across diverse studies, three core areas emerged: translation and cultural adaptation, psychometric validation, and real-world application. The findings confirm that accurate translation alone is insufficient without cultural alignment and empirical validation. Tools such as PHEEM, PSPSQ, and the MGL scale, once adapted and tested, proved effective in Arabic-speaking contexts, supporting their broader adoption in both educational and clinical

environments. Despite progress, notable challenges remain, including inconsistency in terminology, limited standardization protocols, and the continued reliance on English in assessments. Machine translation tools show potential but are not yet reliable enough to replace human oversight, especially in medical settings.

To strengthen the integration of Arabic in medical fields, collaborative initiatives between linguists, educators, and healthcare professionals are essential. Priority should be given to establishing national or regional repositories of validated Arabic medical terms to promote consistency and ease of reference. Educational institutions are also encouraged to embed Arabic-based tools and validated instruments into medical curricula through structured language planning. Furthermore, interdisciplinary training programmes should be introduced to equip educators and translators with the necessary linguistic and pedagogical skills, thereby supporting sustainable and context-appropriate medical communication in Arabic.

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CONFLICT OF INTEREST

The authors declare no conflict of interest in the conduct and reporting of this systematic review.

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ORIGINAL ARTICLE

Laboratory Biomarkers in Assessing the Severity of COVID-19 at Referral Hospital in Indonesia

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ABSTRACT

INTRODUCTION: The COVID-19 pandemic is challenging due to its high transmissibility and mortality rates. COVID-19 patients can rapidly deteriorate, underscoring the need to identify lab biomarkers for high-risk categorization. This study aims to explore the role and correlation of various laboratory parameters, including Neutrophil-to-Lymphocyte Ratio (NLR), Ferritin, Prothrombin (PT), D-Dimer, C-reactive protein (CRP), and Procalcitonin (PCT), in distinguishing between severe and non-severe cases of COVID-19. **MATERIALS AND METHODS**: This retrospective cross-sectional study was carried out at Sulianti Saroso Infectious Disease Hospital in Jakarta with approval from the ethics committee. The inclusion criteria for subjects consist of patients confirmed with COVID-19 through PCR test results, adults aged over 18 years, and those with relevant laboratory parameter results. The exclusion criteria include pregnant patients, patients who arrive in a state of death on arrival (DOA), and patients with incomplete data. A sample of 1,598 adult COVID-19 patients was analysed. Laboratory data were extracted from electronic medical records (SIMINTRO) from March 2020 to December 2022. The significance of the means was assessed through the independent Mann-Whitney test, with a p-value <0.05 regarded as statistically significant. After constructing the ROC (receiver-operating characteristic) curve, threshold values were identified based on Youden's index (J). **RESULT**: There are differences in the severe and non-severe groups based on age, gender, transmission risk factors, symptoms, and comorbidities ($p<0.05$). Severe COVID-19 patients show markedly elevated levels of (NLR, Ferritin, Prothrombin, D-Dimer, CRP, and Procalcitonin) compared to non-severe ones, and the statistical cut-off values between severe and non-severe groups according to parameters (NLR, Ferritin, PT, D-Dimer, CRP, and PCT) are significant ($p<0.001$). **CONCLUSION**: Besides clinical findings, biochemical parameters are valuable predictors for assessing COVID-19 severity.

Keywords:
COVID-19, C-reactive protein, ferritin, procalcitonin, severity

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INTRODUCTION

The initial recorded instance of Coronavirus Disease 2019 (COVID-19), attributed to the newly discovered virus Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2), was reported in December 2019.[1-3] Since that time, the virus has swiftly spread across almost every country, affecting millions of people worldwide.[3] Because of its rapid spread and a mortality rate exceeding 2%, in March 2020, COVID-19 was officially classified as a pandemic by the World Health Organization (WHO).[4,5] Although most coronavirus infections are generally mild and self-limiting, two specific coronaviruses have previously led to global pandemics: The Middle East Respiratory Syndrome (MERS) emerged in 2012 following the emergence of Severe Acute Respiratory Syndrome (SARS) in 2002.[5,6] As indicated by its name, this virus primarily targets the respiratory tract, potentially causing lung infections such as pneumonia and, in severe cases, Acute Respiratory Distress Syndrome (ARDS).[3]

The disease burden of COVID-19 is significant, with far-reaching impacts on public health and the global health system. According to reports from the World Health Organisation (WHO), COVID-19 has caused more than 6 million deaths worldwide and infected more

than 600 million people to date. This demonstrates the urgency to understand and effectively address this disease burden.[7] However, studies indicate that mortality rates are notably higher among individuals with underlying conditions, including hypertension, diabetes mellitus, pre-existing heart or kidney conditions, and obesity.[8,9] In addition to respiratory issues, conditions such as myositis, kidney failure, and disseminated intravascular coagulation (DIC) have also played a role in the mortality of COVID-19 patients.[10-12] Thus, the early detection and identification of COVID-19 patients who are severely affected is crucial for timely intervention and lowering mortality rates. At present, the severity of COVID-19 is assessed by the existence of lung infiltrates, declining oxygen saturation, and examining clinical symptoms since there are no validated biomarkers available to forecast the disease's severity.[13,14] However, reports indicate sudden exacerbation of symptoms and rapid deterioration in approximately 6.5% of COVID-19 patients, leading to death in about half of these cases.[15,16] Therefore, it is essential to identify serum biomarkers that can act as early indicators for predicting and managing potentially severe cases of COVID-19.

In severe cases of COVID-19, a condition called a cytokine storm, also known as cytokine release syndrome (CRS), often occurs.[17] This syndrome is mainly mediated by interleukin-6 (IL-6), which plays a role in increasing the morbidity and mortality of COVID-19 patients. The characteristics of CRS are high IL-6 levels, high fever, and hypoxic pneumonitis, which often requires mechanical ventilation. Therefore, an increase in the number of inflammatory cells is always accompanied by an increase in IL-6 levels. However, IL-6 testing is relatively expensive, and not all hospitals can do it, especially regional hospitals, so substitute markers are needed. Some haematological parameters are reported to be associated with disease severity.[18]

Therefore, this study aimed to delineate the epidemiology, laboratory findings, radiological manifestations in confirmed COVID-19 patients, clinical features, and explore the correlation of laboratory parameters (such as Neutrophil-to-Lymphocyte Ratio (NLR), Ferritin, Prothrombin Time (PT), D-Dimer, C-reactive protein (CRP), Procalcitonin (PCT)) between severe and non-severe COVID-19 patients. This study represents the first publication concerning biomarkers and COVID-19 severity from the Sulianti Saroso Infectious Disease Hospital. Because there's a lack of reliable treatments for COVID-19, it's crucial to pinpoint precise biomarkers that can identify, early on and before the condition worsens, those individuals most prone to severe or critical illness. This identification can pave the way for early interventions to prevent disease progression.

MATERIALS AND METHODS

Study design

This retrospective cross-sectional research was carried out at Sulianti Saroso Infectious Disease Hospital (SSIDH), a tertiary care facility for infectious diseases in Indonesia that was designated as a COVID-19 management center during the ongoing pandemic.

Sample/Participants

Selection of patients and sample size

In this study, the inclusion criteria for subjects consist of patients confirmed with COVID-19 through PCR test results, adults aged over 18 years, and those with relevant laboratory parameter results, including Ferritin, C-Reactive Protein (CRP), D-Dimer, Prothrombin Time (PT), Procalcitonin (PCT), and Neutrophil-to-Lymphocyte Ratio (NLR). The exclusion criteria include pregnant patients, patients who arrive in a state of death on arrival (DOA), and patients with incomplete data. This study did not apply a sample size based on statistical calculations because all COVID-19 patients fulfilling the inclusion and exclusion criteria were selected as the study sample (consecutive sampling). However, the sample in this study has met ensuring the number of events per parameter (EPP) of ≥10.[19] Data from 1,598 adult COVID-19 patients were examined using the Hospital's electronic medical records (SIMINTRO) database covering the period from March 2020 to December 2022.

Classification of COVID-19 patients

Based on the symptoms and criteria set by the Ministry of Health of the Republic of Indonesia, patients were divided into two categories. The first group included those with non-severe illness, encompassing mild cases (symptomatic but without signs of viral pneumonia or hypoxia) and moderate cases (pneumonia with SpO2 ≥94% on room air). The second group consisted of patients with severe illness, defined by SpO2 levels below 94% on room air, a PaO2/FiO2 ratio of less than 300 mmHg, a respiratory rate exceeding 30 breaths per minute, or lung infiltrates covering more than 50% of the lungs. Patients classified as having critical illness included those experiencing Acute Respiratory Distress Syndrome (ARDS), sepsis, septic shock, or multiple organ failure.

Sample and Data Collection

Patient records were reviewed to gather COVID-19 related data, including comorbid conditions, modes of infection, symptoms, complications, and patient outcomes (discharge or death). Hematological assessments, such as the Neutrophil-to-Lymphocyte Ratio (NLR), were performed on whole blood samples collected in EDTA tubes using the fully automated Beckman DXS Coulter-800 analyser.

Blood samples for D-dimer measurement were drawn into tubes containing 3.2% sodium citrate and analysed with a Stago-Compact Max automated analyser. Biochemical assessments were conducted on serum samples obtained from blood collected in clot activator tubes. C-reactive protein (CRP) and serum procalcitonin levels were measured using the COBAS-6000 automated system, while serum ferritin levels were determined with the Abbott Architect I-2000. Before testing, all analysers underwent calibration, and quality control checks were performed at two levels to ensure accuracy.

Instrument

In this study, we used a case report form for collecting secondary data.

Statistical analysis

All collected data were carefully verified for completeness and accuracy. Pre-coded data were entered into a computer and analysed using Statistical Package for Social Sciences (SPSS) version 25.0 (SPSS/PC; SPSS-25.0, Chicago, USA). Quantitative variables were summarized using medians and interquartile ranges (IQR), while qualitative variables were expressed as numbers and percentages. To compare qualitative variables, the chi-square test was utilized. The normality of quantitative data was assessed, and for non-normally distributed variables, comparisons were conducted using the nonparametric Mann-Whitney test. The receiver operating characteristic (ROC) curve was employed to assess the discriminatory power of clinical biomarkers in predicting disease severity. Additionally, Spearman's correlation test was performed to analyze the relationship between NLR, CRP, D-dimer, and serum ferritin levels. A p-value of ≤ 0.05 was considered statistically significant.

INSTITUTIONAL REVIEW BOARD (ETHIC COMMITTEE)

This study was carried out in compliance with the guidelines outlined in the Declaration of Helsinki and received approval from the Health Research Ethics Committee of Sulianti Saroso Infectious Disease Hospital (No. 26/XXXVIII.10/V/2023).

RESULT

Of the 315 COVID-19 patients with severe degrees. Both severe and non-severe COVID-19 patients were predominantly aged 18-59 years and male. In both groups, the majority had no history of travel-related risk factors or close contact exposure. Sociodemographic and transmission risk factors were significantly associated with severity ($p<0.05$). The most commonly occurring symptom is cough. Shortness of breath is mostly experienced by patients with severe illness. All of these symptoms were significantly associated ($p<0.05$) with severity, except fever, diarrhea, and nausea/vomiting ($p>0.05$). Hypertension and Diabetes mellitus comorbidities were significantly to the degree of COVID-19 pain ($p<0.001$). (Table 1).

Table I: Characteristics of the Study Patients (n=1598), According to Disease Severity

Variable	Severity			p-value	RR (95%CI)
	Severe (n=315; 19.7%)	Non-Severe (n=1283; 80.3%)	Total		
Age, mean (SD)	54.9 (12.9)	45.4 (15.2)			
≥ 60 years	123 (39.0%)	245 (19.1%)		0.000	2.71 (2.08-3.54)
18-59 years	192 (61.0%)	1038 (80.9%)			
Sex					
Male	194 (61.6%)	653 (50.9%)	847 (53.0%)	0.001	1.55 (1.20-1.99)
Female	121 (38.4%)	630 (49.1%)	751 (47.0%)		
Risk Factor					
Contact History					
Yes	42 (13.3%)	457 (35.6%)	499 (31.2%)	0.000	0.28 (0.19-0.39)
Travel History					
Yes	10 (3.2%)	109 (8.5%)		0.002	0.41 (0.22-0.74)
Symptoms					
Fever					
Yes	147 (46.7%)	572 (44.6%)	719 (45.0%)	0.547	1.07 (0.88-1.30)
Cough					
Yes	240 (76.2%)	1064 (82.9%)	1304 (81.6%)	0.007	0.72 (0.58-0.91)
Runny Nose					
Yes	17 (5.4%)	291 (22.7%)	308 (19.3%)	0.000	0.24 (0.15-0.38)
Sore Throat					
Yes	22 (7.0%)	158 (12.3%)	180 (11.3)	0.010	0.59 (0.40-0.89)
Headache					
Yes	34 (10.8%)	297 (23.1%)		0.000	0.46 (0.33-0.65)
Myalgia					
Yes	8 (2.5%)	78 (6.1%)		0.019	0.46 (0.24-0.89)
Diarrhoea					
Yes	11 (3.5%)	62 (4.8%)		0.384	0.76 (0.43-1.32)
Nausea&/or Vomiting					
Yes	71 (22.5%)	275 (21.4%)		0.726	1.05 (0.83-1.33)
Shortness of breath					
Yes	263 (83.5%)	417 (32.5%)		0.000	6.83 (5.16-9.04)
Underlying Illnesses					
Heart Disease					
Yes	1 (0.3%)	1 (0.1%)		0.355*	4.08 (0.26-65.45)
Hypertension					
Yes	18 (5.7%)	16 (1.2%)		0.000	4.80 (2.42-9.52)
Diabetes					
Yes	18 (5.7%)	14 (1.1%)		0.000	5.49 (2.70-11.17)
Asthma					
Yes	0 (0%)	1 (0,1%)		1.000	-
HIV/AIDS					
Yes	2 (0.6%)	2 (0.2%)		0.176*	4.09 (0.57-29.17)
Tuberculosis					
Yes	2 (0.6%)	1 (0.1%)		0.101	8.19 (0.74-90.63)
Obesity					
Yes	1 (0.3%)	0 (0%)		0.197*	-
Stroke					
Yes	2 (0.6%)	2 (0.2%)		0.176*	4.09 (0.57-29.17)

There was a statistically significant difference ($p<0.001$) between the two groups in terms of NLR, Ferritin, PT, D-Dimer, CRP, and PCT levels when compared according to the severity of the infection. Patients with severe infection had elevated values for these markers compared to those with non-severe infection (Table II).

Table II: Concentration of Haematological Markers in Study Patients (n=1598) Based on Disease Severity

Haematological markers (unit)	Severity of COVID-19		p-value
	Severe (n=315) (Median/Q1-Q3)	Non-severe (n=1283) (Median/Q1-Q3)	
NLR	9.44 (4.63-15.00)	2.75 (1.81-4.41)	0.000
Ferritin (ng/ml)	1494 (841.00-2001.00)	273 (103.00-733.00)	0.000
PT (seconds)	11.00 (10.40-12.00)	10.60 (10.00-11.20)	0.000
D-Dimer (ng/mL)	1.20 (0.70-3.40)	0.50 (0.30-0.90)	0.000
CRP (mg/L)	89.77 (41.77-155.77)	10.59 (4.00-41.47)	0.000
PCT (ng/mL)	0.20 (0.09-0.92)	0.04 (0.02-0.08)	0.000

Cut-off values for diagnosing COVID-19 were determined using Youden's index (J) and a Receiver-Operating Characteristic Curve (ROC). The area under the curve (AUC) was calculated. ROC analysis was used to further evaluate the statistically significant differences between the severe and non-severe groups for measures such NLR, Ferritin, PT, D-Dimer, CRP, and PCT. In connection with the findings from the ROC analyses, Table III and Figure 1 demonstrated the observations that have been made about patients with severe COVID-19 infection in this study.

Table III: Suggested threshold values for key markers in predicting severe cases of Coronavirus infection

Parameters	AUC*	95% CI	p-value	Recommended cut off	Sensitivity	Specificity
NLR	0.834	0.81-0.86	<0.001	4.62	0.756	0.776
Ferritin	0.825	0.80-0.85	<0.001	582.50	0.857	0.698
PT	0.613	0.58-0.65	<0.001	10.85	0.610	0.604
D-Dimer	0.755	0.73-0.79	<0.001	0.750	0.717	0.680
CRP	0.824	0.80-0.85	<0.001	37.22	0.806	0.732
PCT	0.816	0.79-0.84	<0.001	0.085	0.759	0.755

*Area Under the ROC (full term) curve

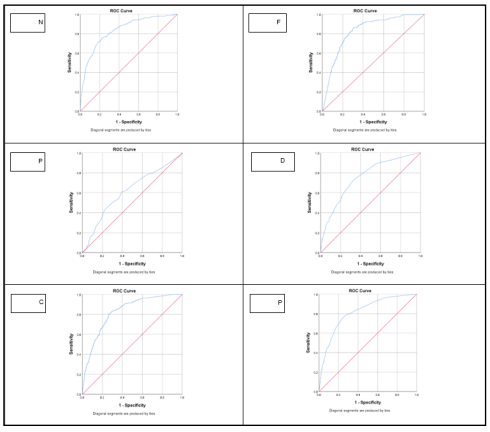

Figure 1: ROC (full term) for key markers in predicting severe cases of COVID-19

DISCUSSION

Male patients and individuals aged 50 years or older are at a greater risk of experiencing severe COVID-19. Additionally, comorbidities and clinical symptoms play

a crucial role in determining disease severity and prognosis. Our study found that men are more susceptible to COVID-19 infection and are more likely to develop severe conditions compared to women (OR=2.41, p<0.00001). Similar results have been reported in previous studies.[20,21] Research from Spain suggests that men may be more vulnerable due to a lack of caution regarding the risks of the pandemic. Another study from Spain also indicated that severity and case fatality rates (CFR) are higher among men and older individuals.[22] Furthermore, women appear to have greater resistance to the virus, which may be linked to female sex hormones, whereas men exhibit lower resistance due to the higher expression of ACE2 receptors, which facilitate viral entry.[23]

The main clinical symptoms are fever (temperature >38℃), cough, and difficulty breathing. ARDS, septic shock, metabolic acidosis, and haemorrhage are found in severe cases with rapid and progressive worsening.[24] More than 40% of fevers in COVID-19 patients have peak temperatures between 38.1-39°C, while 34% have fevers over 39°C.[17] The main clinical symptoms described by the Indonesian Lung Doctors Association (PDPI) are in line with the research journal report of Huang et al. (2020), by defines mild symptoms. According to the journal, mild symptoms in patients with Coronavirus Disease 2019 (COVID-19) are defined as patients with uncomplicated acute upper respiratory tract infections, which can be accompanied by fever, fatigue, cough (with or without sputum, anorexia, malaise, throat pain, nasal congestion, or headache.[25]

Comorbid diseases are health conditions that already exist before a person is infected with COVID-19, such as diabetes, hypertension, heart disease, obesity, chronic lung disease, and cancer.[26,27] The relationship between comorbid diseases and COVID-19 severity is significant, as comorbidities can worsen the body's immune response[27,28] and increase the risk of complications.[26,29] Comorbid diseases worsen COVID-19 infection by various mechanisms, such as weakening the immune system, increasing inflammation, and aggravating the work of vital organs. Therefore, people with comorbid diseases are advised to be more vigilant, implement strict health protocols, and get complete vaccinations to reduce the risk of severity.

Ferritin is a protein in the body that binds iron. According to Zhou et al. (2020), patients with severe COVID-19 exhibited elevated levels of hepcidin and serum ferritin compared to other groups [30]. Iron homeostasis is closely linked to the development of severe COVID-19. Its assessment is both specific and sensitive in predicting disease severity at an early stage in COVID-19 patients. In these patients, cytokine storm syndrome is driven by pro-inflammatory cytokines, leading to acute lung injury and multiorgan failure [31,32]. Increased serum ferritin needs to be observed in COVID-19 patients. Patients with COVID-19 may experience increased ferritin levels due to the inflammatory process. Hyperferritinaemia serves as a parameter for acute phase reactions, aiding clinicians in evaluating therapeutic responses. Meanwhile, recent studies indicate that elevated ferritin levels are not only observed during acute phase reactions but may also contribute significantly to inflammation, particularly in the progression of cytokine storms.[33]

The study conducted by Bozkurt et al. l showed that ferritin levels were the only significant predictor of COVID-19 disease severity (p = 0.004), with the results of ROC curve analysis obtained an AUC of 88%. [34,35] The study of Ahmed et al. (2020) showed a statistically significant difference in ferritin in the two severity categories.[36] Binary logistic regression showed ferritin to be an independent predictor of all causes of death completed with an AUC value of 69% on the ROC chart analysis. Research by Rajanna et al. (2020) shows that ferritin correlates with clinical outcomes. Based on ROC analysis obtained an AUC value of 80.08% with a cut-off point of 352 ng/ml, with a specificity and sensitivity of 76.32% and 74.6% respectively.

Ferritin is a protein found within cells that functions as an iron storage unit and plays a crucial role in various inflammatory conditions, including infections, cancer, and neurodegenerative diseases. As a characteristic of "hyperferritinaemia syndrome," elevated circulating ferritin levels are associated with four severe conditions:

macrophage activation syndrome (MAS), adult-onset Still's disease (AOSD), catastrophic antiphospholipid syndrome (CAPS), and septic shock. Several studies have indicated that ferritin serves as an independent risk factor for disease severity in COVID-19 patients.[37]

C-reactive protein (CRP) is an acute protein synthesized in the liver in response to the cytokines IL-1 and IL-6 and inflamed damaged tissue. CRP can be detected in blood, CSF, synovial, amniotic, and pleural fluid; its level increases 12 hours after inflammation and will peak at 2-3 days. The greater the stimulus, the longer CRP will persist, and once the inflammatory stimulus is removed, CRP levels will fall rapidly.[38] CRP serves as a marker of systemic inflammatory response by directly attaching to microorganisms as an opsonin, aiding the complement system, stimulating neutrophil activity, preventing platelet aggregation, facilitating the removal of necrotic tissue, and activating natural killer cells.[39]

Elevated D-dimer levels in COVID-19 can rapidly identify disease severity, pulmonary complications, and the risk of venous thromboembolism in pro-thrombotic states. This can help risk stratification and therapy selection to reduce COVID-19 morbidity and mortality. COVID-19 patients admitted to the ICU showed significantly elevated D-dimer levels. Special attention should be given to venous thromboembolism (VTE), particularly in severe cases, as these patients are often bedridden and have impaired coagulation function. A rapid decline in their condition was observed, accompanied by a notable increase in D-dimer levels. Especially if the patient presents with clinical symptoms such as rapid hypotension, sudden exacerbation of oxygenation, dyspnoea, post-DVT pulmonary embolism should be considered and treated immediately. Besides being associated with thrombosis and pulmonary embolism, D-dimer levels can also indicate the progression of severe viral infections.[40]

Elevated D-dimer is often found in severe COVID-19 patients and is a predictor of ARDS, the need for intensive care unit treatment, and death. The study by Zhou et al. showed that elevated D-dimer >1.0 µl/mL was the strongest predictor of mortality in COVID-19 patients.[41] The study by Cui et al. found that a D-dimer level exceeding 1.5 µg/mL served as a predictor of venous thromboembolism in COVID-19 patients, demonstrating a sensitivity of 85% and a specificity of 88.5%.

Research 55 found that D-dimer levels exceeding the cutoff (>788.5 ng/mL) are strongly linked to the progression and severity of COVID-19 infection. The risk of severe COVID-19 infection was more than five times higher, both before and after taking into account the influential factors of age and comorbid DM. D-dimer levels above the cut-off can predict the severity of COVID-19 infection with moderate accuracy.[42]

Prothrombin time (PT) is one of the frequently used coagulation parameters. Several studies have reported associations between PT and the severity of COVID-19. Study 56 in its review systematics reported that PT was higher in the severe degree group compared to the mild degree ($p<0.05$).[43] In the ICU and non-ICU groups, PT was also found to be higher in the ICU group than in the non-ICU group. These results are also supported by research, which states that prolongation of PT is an early prognostic indicator of severe COVID-19 events that require ICU treatment.[44] The study also compared survival rates between groups of patients with normal PT and prolonged PT.[12]

The presence of inflammatory indications in COVID-19 patients can be known using the PCT examination.[45] Patients with COVID-19 are always accompanied by a bacterial infection. In COVID-19 patients, PCT examination can help distinguish between severe bacterial pneumonia and mild viral pneumonia. However, the PCT parameter has several disadvantages, namely the high cost of the examination and the long examination time, so it is not widely available in health facilities in Indonesia[46]. High PCT levels suggest that COVID-19 patients with severe symptoms may develop bacterial superinfections that contribute to complications in the clinical picture.

While the initial PCT level can aid in assessing disease severity, it is not always a dependable prognostic marker.

Pre-existing comorbidities, such as CKD and congestive heart failure, can influence PCT levels, potentially causing them to be elevated from the start. However, when interpreted within the clinical context, PCT remains a highly valuable source of information.[47]

The neutrophil-lymphocyte ratio reflects the balance between distinct yet complementary immune pathways, combining the role of neutrophils in non-specific inflammatory responses with lymphopenia, which indicates severe physiological stress and weakened health. Therefore, this ratio represents two crucial immune mechanisms and serves as a predictive marker rather than just an isolated parameter.

In severe systemic inflammation, the immune system responds with an increased neutrophil-lymphocyte ratio. The rise in the neutrophil-lymphocyte ratio (NLR) results from direct or indirect stimulation of the bone marrow, leading to an increased number of neutrophils in the bloodstream.[48] The rise in neutrophil levels is driven by proinflammatory cytokines like IL-6, IL-1, and TNF-α, which are released by macrophages, while the reduction in lymphocyte count is due to elevated secretion of glucocorticoid hormones that inhibit lymphocyte production.[49]

COVID-19 is a pathological infection that attacks the respiratory tract, in some cases patients admitted to the ICU are accompanied by sepsis. An increase in NLR can be used as a warning sign for early signs of severe or worsening COVID-19 symptoms and as an independent prognosis in COVID-19 patients. Evaluating the neutrophil -lymphocyte ratio can enhance the assessment of COVID -19 patients. Therefore, incorporating this ratio along with age considerations is recommended for prognosis determination, assessing disease severity based on clinical symptoms, and guiding appropriate treatment for COVID-19 patients.[50]

The limitation of this study is that no analysis was performed on other biomarkers. For example, IL-6 testing is relatively expensive and not all hospitals, especially regional hospitals, can perform it, making the need for an alternative marker essential. Several haematological parameters have been reported to be associated with the severity of the disease.

CONCLUSION

This study highlights the significant role and correlation of various laboratory parameters, including Neutrophil-to -Lymphocyte Ratio (NLR), Ferritin, Prothrombin (PT), D -Dimer, C-Reactive Protein (CRP), and Procalcitonin (PCT), in distinguishing between severe and non-severe cases of COVID-19. Findings indicate that severe cases, particularly among elderly males, exhibit markedly elevated levels of these biomarkers, which are associated with a higher risk of cytokine storm and complications. Specifically, increased D-dimer levels serve as a critical indicator of lung tissue damage, reinforcing the importance of these biochemical parameters as valuable predictors for assessing the severity of COVID-19 and guiding clinical management.

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CONFLICT OF INTEREST

The author states that no conflicts of interest exist.

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AUTHOR CONTRIBUTIONS

Conceptualization was contributed by H, DWT, and FM; Design was carried out by ADW, SM, MLT, and DWT; Supervision was provided by MLT; Data collection was conducted by KW, R, and TZP; Data analysis was performed by SM; Literature search was conducted by ADW, FM, SM, and NM; Manuscript writing was carried out by H, DWT, ADW, and SM. All authors have read and agreed to the published version of the manuscript.

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ORIGINAL ARTICLE

Effect of Stromal Vascular Fraction and Platelet-Rich Plasma on Epithelialization in Anal Trauma Healing in Wistar Rats

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ABSTRACT

INTRODUCTION: Anal trauma should be treated immediately; early debridement should be performed to prevent infection and sepsis. The use of stromal vascular fraction (SVFs) and platelet-rich plasma (PRP) have now become an alternative surgical alternative. The aim of this study was to determine the effect of stromal vascular fraction (SVFs) and platelet-rich plasma (PRP) on morphological scores for epithelialization during induced anal trauma healing in Wistar rats. **MATERIAL and METHODS:** This experimental study assessed 32 male Wistar rats over a 2-month period. The rats were randomly allocated into four groups: Group A (negative control), Group B (anal trauma treated with PRP + SVF), Group C (anal trauma without PRP + SVF), and Group D (donor rats for PRP and SVF preparation; excluded from outcome analysis). **RESULT:** Although Group B (PRP+SVF) demonstrated higher epithelialization scores compared with Group C (control), the differences were not statistically significant on Day 1 (p=0.083), Day 7 (p=0.157), or Day 14 (p=0.317). However, a significant improvement in morphological scores was observed in the within-group comparison of the PRP+SVF treatment group between Day 1 and Day 14 (p=0.049). **CONCLUSION**: The combination of PRP and SVFs led to a significant improvement in morphological scores for epithelialization within the treatment group (PRP+SVF group) over 14 days. However, this combination therapy did not demonstrate a statistically significant acceleration of wound healing when compared to the untreated control group at the observed time points.

Keywords:
Wistar rat, stromal vascular fraction, anal trauma, platelet-rich plasma, histopathology.

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INTRODUCTION

Anal trauma is a medical term for tissue damage in the form of excoriation and/or laceration in anal layers. Even though it is not a life-threatening disease, anal trauma causes discomfort due to pain.[1,2] The causes of anal trauma are blunt trauma (e.g., due to traffic accident), trauma during birth delivery, trauma due to ingested sharp objects, sharp trauma, anal sexual activity, or pneumatic trauma.[2]

Currently, transanal ultrasonography, both 2D and 3D, are the primary methods for diagnosing anal trauma. Endoanal Magnetic Resonance Imaging (MRI) can detect anal trauma as well as visualize muscle fibre and fibrotic tissue better.[3] Traumatic injuries may be isolated and/or low-energy, involving only the anal sphincter or part of a high-energy polytrauma injury. Perineal injury alone is identified in 5.4% of patients and the injury occurs mostly in the urogenital tract.[4]

Anal trauma should be treated after a physician has evaluated the perineum and assessed the sphincter function by asking the patient to contract the muscle. Early debridement should be performed to prevent infection and sepsis. Repair or approximation of the

internal and external anal sphincters with absorbable sutures should be performed immediately.[5] For large perineal trauma, surgical debridement and exsanguination prevention must be done immediately.[6] Other than the surgical approach, certain non-invasive treatments are being researched. One such approach involves the combined use of Platelet-Rich Plasma (PRP) and Stromal Vascular Fraction (SVFs), both derived from autologous sources.[7] PRP is an autologous plasma concentrate characterized by a platelet concentration significantly above baseline. The therapeutic principle of PRP lies in its role as a reservoir of growth factors essential for tissue repair. Following application to a wound, platelet activation and degranulation occur, which releases a high concentration of bioactive proteins directly into the local microenvironment. These signalling molecules are critical for orchestrating the healing cascade and include key mediators such as platelet-derived growth factor (PDGF)-AB, transforming growth factor-β (TGF-β), insulin-like growth factor (IGF), epidermal growth factor (EGF), and fibroblast growth factor (FGF)-2.[8] Concurrently, SVFs enhance tissue regeneration by promoting angiogenesis. This mechanism is driven by the secretion of key growth factors, such as Vascular Endothelial Growth Factor (VEGF), and by its heterogeneous population of regenerative cells, which includes endothelial progenitor cells (EPCs) and adipose-derived stem cells (ASCs).[9]

Recent systematic reviews have highlighted the synergistic potential of this combination therapy in diverse clinical applications, particularly in accelerating the healing of complex wounds. These reviews conclude that the interaction between the mitogenic signals from PRP and the cellular regenerative capacity of SVFs creates a robust pro-healing microenvironment.[10,11] This evidence reinforces the hypothesis that their combined application could offer superior outcomes compared to the use of either agent alone, thus providing a strong impetus for further investigation in specific wound models, such as anal trauma. This study aimed to evaluate the effects of PRP and SVFs on epithelial histopathological parameters in induced anal trauma in Wistar rats. The histopathological features of anal epithelial tissue were assessed over a two-week period. Findings from this research may provide clinically relevant insights into the potential role of PRP and SVF in enhancing wound healing.

MATERIAL AND METHODS

Study Design

This experimental study used 32 healthy adult male Wistar rats (Rattus novergicus), aged 16-24 weeks and weighing 170-260 grams. The animals were randomly allocated into four groups. Group A (Negative Control, n=4) comprised healthy rats without any intervention to serve as a baseline for normal tissue histology. Group B (Treatment Group, n=12) consisted of rats subjected to induced anal trauma followed by treatment with PRP+SVF. Group C (Trauma Control, n=12) also underwent induced anal trauma but did not receive the PRP+SVF treatment, thus serving as the control for the natural healing process. Lastly, Group D (Donor Group, n=4) was designated for the sole purpose of harvesting blood and adipose tissue to prepare the PRP and SVFs administered to Group B.

Sample Size

The minimum sample size was determined using the Federer formula $(t-1)(n-1)\geq 15$, a standard guideline to ensure sufficient statistical power in animal experimental studies.[12,13] In this formula, t represents the number of experimental groups, and n denotes the number of subjects per group. In the present study, six experimental subgroups were included: Group B (treatment) and Group C (control), each evaluated at three distinct time points (Day 1, Day 7, and Day 14). With six groups ($t= 6$), the calculation yields $(6-1)(n-1)\geq 15(6-1)(n-1)\geq 15(6-1)(n-1)\geq 15$. Therefore, the minimum sample size required for each group was four rats. Exclusion criteria were the following: (i) biopsy tissue could not be analysed, (ii) infection occurred, and (iii) death before being sacrificed.

Study Protocol

All rats underwent a 2-week adaptation period with a 12-hour-long light-dark cycle in a 40 x 20 x 20 cm^2 cage, with each cage consisting of 4-5 rats. Environment temperature was controlled at 28 ± 2ºC with 5–60%

humidity. A standard diet using AD2 diet (20 grams for each rat) was given and the rats had free access to water.

Wistar rats were shaved on the back and anesthetized using ether. Thoracotomy was performed in 4 donor rats and blood was taken from the apex cordis using a 25G needle. Fat tissue was taken from both thighs. After blood and fat tissue were taken, the 4 donor rats (Group D) were sacrificed.

Group A (n=4, negative control group) were sacrificed after the adaptation period (Day-0). Groups B and C both underwent induced anal trauma, each had 12 rats (n=12) were sacrificed on Day-1 (n=4), Day-7 (n=4), and Day-14 (n=4). A biopsy was carried out on each rat at the induced anal trauma site.

On Day-0, anal injury was induced on to rats in Groups B and C Rats were anesthetized with ether, placed in supine position, the perineal and anal areas were sterilized. The anal canal was emptied and 6Fr catheter was inserted as a marker. Anterior perianal incision was made at 10-15 mm and adipose tissue was identified and dissected. A 5 mm incision was made in the muscular layers until the submucous was visualized. Mucous layers should not be injured (a catheter was used as a marker). If perforation occurred, an interrupted suture was made in the mucosal layer. The submucosal and muscularis layers were sutured using a 6.0 absorbable surgical suture. PRP+SVF were injected after induced anal trauma in Group B rats in their surgical wounds. Moreover, on the cutaneous layer, an interrupted suture was made using an absorbable surgical suture.

The surgical wound was then washed using sterile normal saline and covered with gauze. Postoperatively, rats received ceftriaxone 50 mg/kg intravenous once daily for 3 days as antibiotic prophylaxis and ketorolac 2 mg/kg intravenous once daily for 3 days for analgesia.

On Day-1, Day-7, and Day-14, rats were sacrificed using ether as an anaesthetic agent. The wound area was documented using a Nikon D5600 digital camera (Nikon Corporation, Tokyo, Japan) under standardized lighting conditions prior to biopsy collection. Epithelialization during wound healing was subsequently analysed by histopathological examination.

Preparation of Platelet-Rich Plasma and Stromal Vascular Fraction

To prepare the Platelet-Rich Plasma (PRP), blood collected from donor rats was placed in EDTA tubes and subjected to a first centrifugation at 2400 rpm (450 G) for 10 minutes. Supernatant plasma with buffy coat was then put into the second centrifugation with 3600 rpm (850 G) for 15 minutes. Infranatant buffy coat was stored for preparing the final product of PRP.[14] For the SVF, fat tissue from the thigh fold of donor rats was washed with phosphate buffer, minced, and digested with 0.15% collagenase in a 37 ℃ incubator for 30 minutes. The collagenase activity was neutralized by adding Dulbecco Modified Eagle Media (DMEM) with 10% FBS and 1% antibiotic-antimycotic. The sample was centrifuged at 1500 rpm for 5 minutes. The resulting cell pellet was resuspended, and the SVF cell count was measured using Trypan blue with a Neubauer counting chamber. The final PRP+SVF product was prepared by adding a 50,000 SVF cell preparation to 0.5 ml of PRP.[1,14,15]

Histopathological Assessment

Quantitative assessments of epithelialization score were performed on H&E-stained histopathological sections from the excised wound tissue. The degree of epithelialization was graded on an ordinal scale with four steps: (i) whole skin, if no new epithelialization was present; (ii) discrete, if new epithelium covered at least 1/3 of the wound area; (iii) moderate, if new epithelium covered more than 1/3 of the wound area; and (iv) complete epithelialization, if the new epithelium covered the entire wound area.

Statistical Analysis

All data were analysed using SPSS for Windows version 21.0 (IBM Corp., Armonk, NY, USA). Descriptive statistics were expressed as median (interquartile range [IQR]) for ordinal data. The Mann-Whitney U test was used to compare differences between Group B (PRP+SVF) and Group C (control) at each observation day and for the

pooled analysis. The Wilcoxon signed-rank test was applied for within-group comparisons across time points (e.g., Day 1 vs. Day 14 in Group B). A *p*-value <0.05 was considered statistically significant.

RESULT

Epithelialization scores were analysed across observation days for all groups (Table I). On Day 0, Group A (negative control) demonstrated early epithelial coverage with a mean ± SD score of 0.57±0.31, representing baseline histological characteristics before any treatment.

On Day 1, epithelialization remained limited in both experimental groups. In Group B (treatment group), discrete epithelialization was observed in three samples (75%) and whole-skin coverage in one sample (25%), with a median score of 1.0 (1.0-1.0). In contrast, Group C (control) exhibited whole-skin epithelialization in all samples (100%), with a median score of 0.0 (IQR 0.0-0.0). The between-group comparison showed no statistically significant difference (p=0.083, Mann-Whitney U test).

By Day 7, Group B (PRP+SVF) showed a higher proportion of complete epithelialization (50%) compared to Group C (0%). The median epithelialization score was 2.5 (2-3) in Group B and 2.5 (2-3) in Group C, indicating a statistically significant difference (p=0.157) between groups.

On Day 14, all rats in Group B (100%) achieved complete epithelialization, while in Group C, complete epithelialization was found in 75.0% of samples. The median score in Group B was 3 (3-3) compared to 3 (2-3) in Group C. This between-group difference was statistically significant (p=0.317).

Overall, the results indicate that epithelialization progressed over time in all groups, with a more consistent and complete healing pattern observed in the PRP+SVF group, although not all between-group comparisons reached statistical significance.

When cumulative epithelialization morphology across all observation days (Day 1, 7, and 14) was analysed using the Wilcoxon rank-sum test, a significant difference was found between groups (p=0.026) (Table II). The group B demonstrated a higher proportion of complete epithelialization (50 %) and a lower incidence of unhealed whole-skin areas (8.3 %) compared with the group C (25 % and 50 %, respectively).

Table I. Epithelialization Stages by Observation Day and Treatment Group

Day	Group	Whole Skin n (%)	Discrete n (%)	Moderate n (%)	Complete Healing	Epithelialization Score, Median	p value
0	Group A (Negative Control)	1 (25.0)	3 (75.0)	0 (0.0)	0 (0.0)	1 (1–1)#	—
1	Group B (Treatment	1 (25.0)	3 (75.0)	0 (0.0)	0 (0.0)	1 (1–1)	—
	Group C (Trauma Control)	4 (100.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0–0)	0.083*
7	Group B (Treatment	0 (0.0)	1 (25.0)	1 (25.0)	2 (50.0)	2.5 (2–3)	—
	Group C (Trauma Control)	2 (50.0)	1 (25.0)	1 (25.0)	0 (0.0)	1 (0–2)	0.157*
14	Group B (Treatment	0 (0.0)	0 (0.0)	0 (0.0)	4 (100.0)	3 (3–3)	—
	Group C (Trauma Control)	0 (0.0)	0 (0.0)	1 (25.0)	3 (75.0)	3 (2–3)	0.317*

Notes: Data are presented as frequency (percentage) and median (interquartile range [IQR]) for ordinal epithelialization scores, except for Group A, where mean ± SD is shown due to single baseline measurement. *Mann-Whitney U test (between groups at the same day). #Mean ± SD=0.57 ± 0.31 for Group A (Day 0 baseline). PRP: Platelet-Rich Plasma; SVF: Stromal Vascular Fraction.

Table II: Comparison of cumulative epithelialization morphology across all observation days between Group B and Group C

Epithelialization Category	B (Treatment) (n = 12)	C (Trauma Control) (n = 12)	p-value
Whole Skin (no epithelializa-tion)	1 (8.3 %)	6 (50.0 %)	0.026
Discrete Epithelialization	4 (33.3 %)	1 (8.3 %)	
Moderate Epithelialization	1 (8.3 %)	2 (16.7 %)	
Complete Healing	6 (50.0 %)	3 (25.0 %)	

Note: Data represent the cumulative distribution of epithelialization morphology assessed at Day 1, Day 7, and Day 14 following experimentally induced anal trauma in rats. Statistical comparison between group B and group C was performed using the **Wilcoxon rank-sum test**. PRP = Platelet-Rich Plasma; SVF = Stromal Vascular Fraction.

A within-group comparison in the group B showed a significant improvement over time. On Day 1, most animals exhibited discrete epithelialization, whereas by Day 14, all demonstrated complete epithelial healing. Statistical analysis using the Wilcoxon signed-rank test confirmed a significant temporal difference between Day 1 and Day 14 (p = 0.049) (Table III).

Table III: Comparison of Epithelialization Morphology between Day 1 and Day 14 in the PRP + SVF Group

Epithelialization Category	Day 1 (n = 4)	Day 14 (n = 4)	p-value
Whole Skin	1 (25%)	0 (0%)	0.049
Discrete Epithelialization	3 (75%)	0 (0%)	
Moderate Epithelialization	0 (0%)	0 (0%)	
Complete Healing	0 (0%)	4 (100%)	

Note: Data represent morphological changes in epithelialization from Day 1 to Day 14 following PRP + SVF treatment. Statistical analysis was performed using the **Wilcoxon signed-rank test**. PRP = Platelet-Rich Plasma; SVF = Stromal Vascular Fraction.

DISCUSSION

In this study, the application of PRP+SVF to induced anal trauma in Wistar rats was associated with a positive trend in epithelialization. While a direct comparison between the treatment and control groups did not yield statistically significant differences on Day 1, Day 7, or Day 14, a significant improvement in morphological scores was observed within the treatment group between Day 1 and Day 14 ($p=0.049$). This suggests that the combination therapy contributes to the progression of healing over time. The lack of statistical significance in between-group comparisons may be attributable to the small sample size or the relatively short observation period, which might have been insufficient to capture the full extent of the therapeutic effect.

The observed acceleration of epithelialization in the group B is biologically plausible. PRP provides a concentrated source of autologous growth factors such as PDGF, TGF-β, IGF, EGF, and FGF which act as potent chemotactic and mitogenic signals for keratinocytes, fibroblasts, and endothelial cells. Meanwhile, SVF contains a heterogeneous population of regenerative cells, including MSCs, pericytes, EPCs, and immune-modulatory macrophages. Together, these components contribute to angiogenesis, extracellular matrix remodelling, and re-epithelialization, thereby establishing a pro-healing microenvironment at the wound site.

Several biological factors may explain the lack of a statistically significant difference between the treatment and control groups at the specified time points, despite the positive trend observed with PRP+SVF administration. First, healthy Wistar rats possess a robust and efficient intrinsic wound healing capacity. The perianal region is highly vascularized,[16] which naturally promotes rapid cell migration and proliferation. This potent endogenous healing in the control group could have narrowed the observable therapeutic window, making a statistically significant advantage for the treatment group difficult to detect within a 14-day period.

Second, the mechanisms of PRP and SVFs operate on different timelines. PRP provides an immediate, potent bolus of growth factors (such as PDGF and TGF-β) that primarily accelerates the initial inflammatory and proliferative phases of healing.[17,18] In contrast, the regenerative contribution of SVFs which contain a population of mesenchymal stem cells, endothelial progenitors, and fibroblasts[19] is often more gradual, involving longer-term processes such as cell differentiation, sustained paracrine signalling, and tissue remodelling. Our 14-day endpoint may have been sufficient to capture the initial surge driven by PRP but too short to fully appreciate the more profound, structural contributions of the SVF cellular components that manifest in later stages of wound maturation. This could explain why a significant improvement was seen *within* the treatment group over time, but a significant advantage *over* the control group was not yet established

In our study, we also examined the effects of PRP+SVF on anal wound healing and compared it to healing without these treatments, specifically focusing on epithelialization. Our findings align with previous research that demonstrated faster wound healing with SVF+PRP compared to controls, SVF alone, or PRP alone.[20] A study found that SVF+PRP is beneficial in lower extremities with diabetic ulcer.[21]

Comparison of epithelialization between Group B (anal trauma treated with PRP+SVF) and Group C (anal trauma without PRP+SVF), on Day-1, Day-7, and Day-14 did not show a significant difference (Table I). Nevertheless, we found a significant difference in epithelialization on Day-1 and Day-14 only in the Group B (Table III). A study showed that average duration for wound healing in diabetic ulcer in lower extremities was 71.75 ± 29.57 days.[21] Hence, wound healing still required several days to be completed even after intervention. A study also showed effective wound healing in PRP+SVF in rats with a radiation wound one month, two months, and three months after intervention.[22] Another study found that EGF was significantly higher on Day-14 of anal trauma rats compared to Day-1.[15]

Our findings, which demonstrate a positive trend and a significant within-group improvement, are broadly

consistent with the conclusions of recent systematic reviews. For example, a study on regenerative therapies for skin quality found that combination therapies including PRP and a cellular component like SVFs offer a statistically significant advantage in improving skin quality over conventional treatments.[23] While our study did not demonstrate a significant between-group difference likely due to limitations such as sample size and the potent intrinsic healing of the animal model the observed pro-healing trend aligns with the direction of effect reported in this higher-level evidence. This suggests that our results are biologically plausible and contribute to the growing body of literature supporting this therapeutic strategy.

Our study has several important limitations that must be acknowledged. First, the study was conducted with a small sample size (n=4 per subgroup), which significantly limited its statistical power. This increases the likelihood of a Type II error, meaning a true therapeutic effect of the PRP+SVF intervention may exist but was not detected as statistically significant in our analysis. Second, the 14-day observation period was relatively short. This timeframe may have been insufficient to fully capture the later stages of wound maturation and tissue remodelling, where the regenerative contributions of SVFs might become more apparent.

For future studies, we recommend an experimental design that includes not only the combination therapy but also separate arms for PRP administration alone and SVF administration alone. Such a design is essential for several reasons. First, it would make it possible to distinguish the individual therapeutic contributions of each component. This would help determine whether the healing benefits are driven primarily by the immediate bolus of growth factors from PRP, the longer-term cellular regenerative capacity of SVFs, or a true synergistic interaction between the two. Second, understanding the efficacy of each component individually has significant clinical implications, potentially leading to more targeted, cost-effective treatment strategies for anal trauma and other complex wounds.

CONCLUSION

The combination therapy of PRP and SVFs was associated with a significant improvement in morphological scores for epithelialization within the treatment group over a 14-day period in this rat model. However, the therapy did not demonstrate a statistically significant acceleration of wound healing when compared directly to the control group at the observed time points. These findings suggest a potential benefit, but further studies with larger sample sizes and longer observation periods are warranted to fully elucidate the therapeutic efficacy of this combination for anal trauma healing.

FUNDING

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CONFLICTS OF INTEREST

None

INSTITUTIONAL REVIEW BOARD (ETHICS COMMITTEE)

This study obtained ethical approval from the Ethical Committee of Faculty of Medicine, Hasanuddin University (Number: 328/UN4.6.5.31/PP36/2021).

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ORIGINAL ARTICLE

Utilization of "*Skim Peduli Kesihatan untuk Kumpulan B40*" (PeKa B40) Programme among Felda Residence in Kedah, Malaysia: The Participation Rate and its Determinants

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ABSTRACT

INTRODUCTION: The Malaysian government has implemented the "Skim Peduli Kesihatan untuk Kumpulan B40" (PeKa B40) programme in response to the growing rate of non-communicable diseases (NCD) among the Malaysian population. This study aims to determine the participation rate of PeKa B40 programme utilization and its determinants. **MATERIALS AND METHODS:** This was a cross-sectional study designed at Felda Lubuk Merbau Kedah. A total of 237 participants were involved using the simple random sampling method from January-April 2023. Multiple logistic regression analysis was used to determine the determinants. **RESULTS:** The participation rate of PeKa B40 utilization among participants was 41.8%. The determinants of utilization of PeKa B40 programme were positively associated with age [Adj. OR: 1.06 (95%CI: 1.03, 1.10)], female gender [Adj. OR: 2.32 (95%CI: 1.15, 4.66)], presence of chronic disease [Adj. OR: 2.62 (95%CI: 1.28, 5.37)], persistent pain that mildly disturbs daily activities [Adj. OR: 3.30 (95%CI: 1.31, 8.33)], and high disturb daily activities [Adj. OR: 14.34 (95%CI: 2.18, 94.26)]. The determinants were also negatively associated with poor self-rate health [Adj. OR: 0.05 (95%CI: 0.01, 0.47)], intermediate self-rate [Adj. OR: 0.25 (95%CI: 0.06, 0.95)], and good self-rate [Adj. OR: 0.22 (95%CI: 0.06, 0.82)]. **CONCLUSION:** The participation rate of PeKa B40 among residents of Felda Lubuk Merbau was higher than at the national and state levels. Therefore, the government needs to advertise this programme and take more proactive steps to advertise the programme especially, to adult men. This programme needs to be extended to those respondents who are sick and need medical treatment.

Keywords:
Determinants, utilization PeKa B40 programme, Felda Residence

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INTRODUCTION

The Malaysian government has made significant efforts to address poverty and provide essential services to marginalized populations, in line with the Sustainable Development Goals (SDGs).[1] The government prioritizes delivering vital amenities, such as sanitation facilities, electricity, and clean drinking water, to improve living conditions and overall well-being. Additionally, the government recognizes the critical importance of healthcare in combating poverty and has implemented programmes and policies to ensure equitable access to healthcare services for low-income individuals and families.[2]

In 2015, Malaysia experienced an increase in fatalities caused by non-communicable diseases (NCDs). Out of 154,000 total deaths recorded that year, approximately 113,400 (73.64%) were attributed to NCDs.[3] The rise in NCDs in Malaysia can be linked to several factors, including an aging population, sedentary lifestyles, unhealthy eating habits, and a lack of awareness regarding preventive measures.[4]

To tackle the growing prevalence of NCDs, the Malaysian government in the Twelfth Malaysia Plan (2021) launched a plan prioritizing NCDs, focusing on cancer, diabetes, as well as mental health.[5] The plan known as *"Skim Peduli Kesihatan for the B40 group"* (PeKa B40) programme was specifically designed to address NCDs among the

economically disadvantaged B40 group, i.e. those at the bottom 40% of total households income distribution in Malaysia averaging RM5250.00 monthly. It aims to provide financial support and equitable access to healthcare.[6] This programme has four benefits, namely i) health screening, ii) eligible for health aid, iii) complete cancer treatment incentive, and iv) transport incentive.

Since it's launched in 2019, PeKa B40 was reported to have diagnosed many chronic diseases among the B40 community which was not diagnosed before such as diabetes mellitus (10.4%), hyperlipidaemia (29.8%), hypertension (13.8%) and mental health problem (1.6%).[7] Even though this programme had proven the success in diagnosing several chronic diseases among the targeted community, however during the implementation of this programme there were only 40,119 B40 participants who underwent health screening exercise out of 800,000 eligible persons.[8] Underutilization of health services was considered a significant factor that contributed to a substantial case of avoidable mortality and morbidity.

Although the programme began in 2019, the participation rate for health screening among eligible individuals was only 7.6%.[9] Therefore, this study seeks to assess the participation rate of PeKa B40 programme utilization and identify the factors that influence residents of Felda Lubuk Merbau, Kedah.

MATERIALS AND METHODS

Study population

This descriptive cross-sectional study was conducted in the Felda Lubuk Merbau community in Kedah, Malaysia, between January-April 2023. Among the 17 Felda areas in the northern region of Malaysia, Felda Lubuk Merbau was randomly selected using simple random sampling as the sampling area. Permission was obtained from the community head prior to the study. Based on sampling calculations, a total of 237 participants were required, and the simple random sampling approach was employed throughout the sample selection process.

Sample size determination

The sample size was calculated using a sample size calculator available in Epi Info Software. The population of Felda Lubuk Merbau includes 2,000 individuals aged 40 years old and older. As of December 2022, the proportion of Malaysians utilizing the PeKa B40 programme was 14%.[10] To achieve a statistical significance level of 5% and a statistical power of 80% while accounting for a non-response rate of 20%, a minimum sample size of 204 participants was required for this study.

Inclusion and exclusion criteria

Participants were selected based on specific eligibility criteria for the PeKa B40 programme. These included i) Malaysian citizen aged 40 years old, ii) receiving financial assistance from the government known locally as *"Sumbangan Tunai Rahmah (STR)"* (formerly *"Bantuan Sara Hidup (BSH)),* iii) within the bottom 40% of total household income, and iv) consented to participate in the research. Exclusion criteria consisted of individuals who were i) deaf, ii) mute, iii) illiterate, or iv) unable to communicate in the Malay language. The study population for utilization of PeKa B40 was identified when they fulfilled the inclusion and exclusion criteria.

Questionnaire

Data collection was conducted at various community venues, including health clinics, mosques, and public halls, using convenience sampling. A briefing was held to explain the study goals to the target group. Interested individuals were gathered, and a comprehensive description of the research process was provided. Prior consent was obtained before conducting the data collection. Participants were assured of confidentiality, and the survey forms did not contain any personal information that could identify them. The utilization of PeKa B40 is operationally defined as the proportion of eligible B40 individuals aged 40 years and above who have accessed at least one of the scheme's benefits (health screening, medical equipment assistance, cancer treatment incentive, or transport aid) within a specified period.

The sociodemographic and health status questionnaire was adapted from the PeKa B40 questionnaire. It was developed by Corporation Sdn. Bhd., a wholly owned subsidiary of ProtectHealth Malaysia, which operates under the Ministry of Health Malaysia (MOH).[6,11] The questionnaire consisted of three domains: i) sociodemographic (seven items, including age, gender, marital status, ethnicity, educational level, occupation, and partner's occupation), ii) health status (seven items, including the presence of chronic diseases, chronic diseases among family members, smoking status, body mass index, systolic blood pressure, diastolic blood pressure, and pulse rate), and iii) socio-economic status (two items regarding financial difficulties in buying necessities and means of paying for medical expenses).

The health-seeking behaviour questions were adapted from the Malaysian National Health Morbidity and Mortality Survey 2019 (Volume 2 – Healthcare Demand).[12] Structured questionnaires were used to collect data based on the healthcare demand module established by the Ministry of Health Malaysia. The assessment of health-seeking behaviour included nine items: i) self-rated health, ii) whether respondents felt ill, iii) if they received treatment from a healthcare practitioner, iv) they felt the need for treatment, v) whether they used medication without a healthcare practitioner's advice, vi) whether they received advice from non-healthcare sources, vii) if they sought alternative methods beyond the previous questions, viii) whether they experienced persistent pain for three months or more, and ix) whether pain disrupted their activities.

A trained research team member measured the blood pressure using a manual sphygmomanometer,[13] measured the pulse rate at the radial artery,[14] and tabulated the body mass index (BMI) by weighing the participants and measuring their heights.[15]

Self-rated health is a single-item indicator that predicts morbidity and mortality independently. In this study, respondents were asked to self-report their current general health status, with responses classified into a 5-point ordinal scale (excellent, good, fair, poor, and very poor). The variables assessed in the sociodemographic, health status, socio-economic, and health-seeking behaviour questionnaires were based on the Health Belief Model (HBM). Data collection was carried out through face-to-face interviews.

Analysis

The data was cleaned and verified before being transcribed into Microsoft Excel for analysis using SPSS version 26. Descriptive analysis showcased numerical data through the mean and standard deviation, while categorical data was presented using frequency (n) and percentage (%). To compare the distribution of sociodemographic factors, health status, and socioeconomic variables stratified by the utilization of the PeKa B40 programme, an independent t-test was used for numerical independent variables, and a chi-square test was utilized for categorical independent variables. A univariate analysis was conducted to identify parameters associated with the utilization of PeKa B40 among Felda residents. Simple logistic regression was employed to determine odds ratios (OR) along with their corresponding 95% confidence intervals (CI). Independent variables that showed a significance level of less than 0.25 in simple logistic regression were selected for further analysis using multiple logistic regression, which accounted for potential confounding effects. The threshold for statistical significance was set at a p-value of less than 0.05.

RESULTS

A total of 237 respondents participated in this study. The participation rate of the PeKa B40 programme was 41.8%. Table I presents the distribution of sociodemographic factors, health status, and socioeconomic status, stratified by the utilization of the PeKa B40 programme.

Most participants in the study were Malay females, with an average age of 56.23 years (± 12.52). Most participants were female (59.5%), married (88.2%), Malay (98.3%), and worked in the agriculture sector (49.4%). Regarding health status, 57.0% of participants had chronic diseases, and 54.4% reported that their family members also had

chronic diseases. On average, respondents were classified as overweight, and the percentage of smokers was only 15.6%. However, both blood pressure and pulse rate measurements were within the normal range. Socioeconomically, most respondents indicated that they did not have financial difficulties in purchasing necessities, and 76.8% were responsible for paying their own medical expenses.

Table I: The distribution of the sociodemographic, health status and socioeconomics stratified by the utilization of the PeKa B40 programme (N=237)

VARIABLES	UTILIZE PeKa B40 PROGRAMME		TOTAL (n=237), n(%)	p-value
	Yes (n=99), n(%)	No (n=138), n (%)		
a) Sociodemographic				
Age, years (mean ± SD)	61.86 ± 11.19	52.20 ± 11.89	56.23 ± 12.52	<0.001*[a]
Gender:				
Male	37 (37.4%)	66 (47.8%)	103 (43.5%)	0.109[b]
Female	62 (62.6%)	72 (52.2%)	134 (59.5%)	
Marital Status:				
Married	84 (84.8%)	125 (90.6%)	209 (88.2%)	0.178[b]
Divorcee/Unmarried/ Widowed	15 (15.2%)	13 (9.4%)	28 (11.8%)	
Ethnic:				
Malay	98 (99.0%)	135 (97.8%)	233 (98.3%)	0.493[b]
Non-Malay	1 (1.0%)	3 (2.2%)	4 (1.7%)	
Education Level:				
Primary Education	31 (31.3%)	35 (25.4%)	66 (27.8%)	0.332[b]
Secondary Education	63 (63.6%)	90 (65.2%)	153 (64.6%)	
Tertiary Education	5 (5.1%)	13 (9.4%)	18 (7.6%)	
Occupation:				
Self-business	10 (10.1%)	18 (13.0%)	28 (11.8%)	0.364[b]
Professional	6 (6.1%)	10 (7.2%)	16 (6.8%)	
Agriculture sector	45 (45.5%)	72 (52.2%)	117 (49.4%)	
Housewife	38 (38.4%)	38 (27.5%)	76 (32.1%)	
Partner Occupation:				
Self-business	9 (9.1%)	11 (8.0%)	20 (8.4%)	0.193[b]
Agriculture sector	2 (2.0%)	9 (6.5%)	11 (4.6%)	
Professional	49 (49.5%)	52 (37.7%)	101 (42.6%)	
Housewife	26 (26.3%)	49 (35.5%)	75 (31.6%)	
Other	13 (13.1%)	17 (12.3%)	30 (12.7%)	
b) Health Status				
Presence of Chronic Disease:	79 (79.8%)	56 (40.6%)	135 (57.0%)	<0.001*[a]
Yes	20 (20.2%)	82 (59.4%)	102 (43.0%)	
No				
Presence of Chronic Disease Among Family Members				
Yes	58 (58.6%)	71 (51.4%)	129 (54.4%)	0.277[b]
No	41 (41.4%)	67 (48.6%)	108 (45.6%)	
Smoking Status				
Yes	17 (17.2%)	20 (14.5%)	37 (15.6%)	0.575[b]
No	82 (82.8%)	118 (85.5%)	200 (84.4%)	
BMI, kg/m² (mean ± SD)	27.18 ± 4.49	27.39 ± 4.38	27.30 ± 4.38	0.713[a]
Systolic Blood Pressure, mmHg (mean ± SD)	134.03 ± 12.83	130.31 ± 11.99	131.86 ±12.46	0.023*[a]
Diastolic Blood Pressure, mmHg (mean ± SD)	76.26 ± 8.96	79.21 ± 7.04	77.98 ± 8.02	0.005*[a]
Pulse Rate, beat per minutes (mean ± SD)	77.98 ± 9.32	81.57 ± 6.99	80.07 ± 8.22	<0.001*[a]
c) Socioeconomic				
Financial Difficulties to Buy Basic Necessities				
Yes	16 (16.2%)	25 (18.1%)	41 (17.3%)	0.695[b]
No	83 (83.8%)	113 (81.9%)	196 (82.7%)	
Payers for Medical Expenses				
Self-paying	75 (75.8%)	107 (77.5%)	182 (76.8%)	<0.006*[b]
Employer	6 (6.1%)	21 (15.2%)	27 (11.4%)	
Family members	18 (18.2%)	10 (7.2%)	28 (11.8)	

Notes:
*significant (p<0.05)
[a] independent t-test; [b] Chi-square test

There were significant age differences (p<0.001), as well as notable differences in the presence of chronic disease (p<0.001), systolic blood pressure (p=0.023), diastolic blood pressure (p=0.005), and pulse rate (p<0.001) when comparing participants who utilized the PeKa B40 programme to those who did not. Additionally, there was a significant difference in self-payment of medical expenses (p=0.006) between these two groups. Table II shows the distribution of health-seeking behaviour stratified by the utilization of the PeKa B40 programme.

Table II: The distribution of health-seeking behaviour stratified by the utilization of the PeKa B40 programme (n=223)

VARIABLES	UTILIZE PeKa B40 PROGRAMME		Total (n=223), n(%)	p-value
	Yes (n=95), n(%)	No (n=128), n(%)		
Self-rate health:				
Poor	4 (4.2%)	4 (3.1%)	8 (3.6%)	0.055[b]
Intermediate	53 (55.8%)	52 (40.6%)	105 (47.1%)	
Good	30 (31.6%)	64 (50.0%)	94 (42.2%)	
Excellent	8 (8.4%)	8 (6.3%)	16 (7.2%)	
If you are ill, do you receive treatment from a healthcare practitioner?				
Yes	88 (92.6%)	121 (94.5%)	209 (93.7%)	0.563[b]
No	7 (7.4%)	7 (5.5%)	14 (6.3%)	
Do you feel the need to receive treatment from a healthcare practitioner?				
Yes	86 (90.5%)	114 (89.1%)	200 (89.7%)	0.722[b]
No	9 (9.5%)	14 (10.9%)	23 (10.3%)	
Have you used medication without the advice of a healthcare practitioner?				
Yes	11 (11.6%)	13 (10.2%)	24 (10.8%)	0.735[b]
No	84 (88.4%)	115 (89.8%)	199 (89.2%)	
Have you received advice from others, aside from healthcare practitioners?				
Yes	21 (22.1%)	32 (25.0%)	53 (23.8%)	0.616[b]
No	74 (77.9%)	96 (75.0%)	170 (76.2%)	
Have you received advice from other sources, such as the internet, TV, radio, applications, and others?				
Yes	45 (47.4%)	63 (49.2%)	108 (48.4%)	0.785[b]
No	50 (52.6%)	65 (50.8%)	115 (51.6%)	
Have you done something other than the above?				
Yes	3 (3.2%)	5 (3.9%)	8 (3.6%)	0.766[b]
No	92 (96.8%)	123 (96.1%)	215 (96.4%)	
Have you experienced persistent pain for 3 months or more?				
Yes	48 (50.5%)	6 (20.3%)	74 (33.2%)	<0.001*[b]
No	47 (49.5%)	102 (79.7%)	149 (66.8%)	
Does pain disrupt your activities?	11 (11.6%)	2 (1.6%)	13 (5.5%)	<0.001*[b]
Highly disturb	10 (10.5%)	11 (8.6%)	21 (8.9%)	
Moderately disturb	28 (29.5%)	13 (10.2%)	41 (18.4%)	
Mildly disturb	46 (48.4%)	102 (79.7%)	148 (66.4%)	
Not disturb				

Notes:
*Statistically significant at p less than 0.05
Statistical test: [a] independent t-test; [b]Chi-square test

Most respondents rated their health as intermediate (47.1%). However, half (50.0%) of those who did not utilize the PeKa B40 programme rated their health as good, while the majority of those who did utilize the programme rated their health as intermediate (55.8%).

In assessing health-seeking behaviour, significant differences were found in the experience of persistent pain for three months or more (p<0.001) and whether pain disrupted daily activities (p<0.001) between those

who utilized and those who did not utilize the programme. Among programme users, a higher percentage reported experiencing persistent pain for three months or more compared to non-users (50.5% versus 20.3%, respectively). The majority of both groups indicated that their pain did not interfere with their daily activities. Table III presents a univariate analysis to determine the factors influencing the utilization of the PeKa B40 programme through simple logistic regression.

Table III: The univariate analysis to determine the determinants of the utilization of the PeKa B40 programme

Variables	Crude OR (95% CI)	Wald stat (df)	P-value
a) Sociodemographic			
Age	1.07 (1.05, 1.10)	30.784 (1)	<0.001 *
Gender:			
Male	reference		
Female	0.65 (0.38, 1.10)	2.552 (1)	0.110
Marital Status:			
Married	0.58 (0.26, 1.29)	1.787 (1)	0.181
Divorcee/Unmarried/Widowed	reference		
Ethnic:			
Malay	2.18 (0.22, 21.25)	0.448 (1)	0.503
Non-Malay	reference		
Education Level:			
Tertiary Education	reference	2.154 (2)	0.341
Secondary Education	2.30 (0.74, 7.19)	2.060 (1)	
Primary Education	1.82 (0.62, 5.36)	1.180 (1)	
Occupation:			
Self-business	reference	3.162 (3)	0.367
Agriculture sector	1.80 (0.73, 4.40)	0.198 (1)	
Professional	1.13 (0.48, 2.65)	0.788 (1)	
Housewife	1.08 (0.30, 3.86)	0.906 (1)	
Partner Occupation:			
Housewife	reference	5.768 (4)	0.217
Agriculture sector	1.77 (0.96, 3.29)	3.348 (1)	
Professional	0.42 (0.08, 2.08)	1.131 (1)	
Self-business	1.54 (0.57, 4.20)	0.719 (1)	
Other	1.44 (0.61, 3.42)	0.686 (1)	
b) Health Status			
Presence of Chronic Disease:			
Yes	5.78 (3.19, 10.51)	33.225 (1)	<0.001 *
No	reference		
Presence of Chronic Disease Among Family Members:			
Yes	1.34 (0.79, 2.25)	1.181 (1)	0.277
No	reference		
Smoking Status:			
Yes	1.22 (0.60, 2.47)	0.313 (1)	0.576
No	reference		
BMI	0.99 (0.93, 1.05)	0.136 (1)	0.712
Systolic Blood Pressure	1.03 (1.01, 1.05)	4.986 (1)	0.026*
Diastolic Blood Pressure	0.95 (0.92, 0.99)	7.377 (1)	0.007*
Do you feel the need to receive treatment from a healthcare practitioner?			
Yes	1.17 (0.49, 2.84)	0.126 (1)	0.722
No	reference		
Have you used medication without the advice of a healthcare practitioner?			
Yes	1.16 (0.50, 2.71)	0.115 (1)	0.735
No	reference		
Have you received advice from others, aside from healthcare practitioners?			
Yes	0.85 (0.45, 1.60)	0.252 (1)	0.616
No	reference		
Have you received advice from other sources, such as the internet, TV, radio, applications, and others?			
Yes	0.93 (0.55, 1.58)	0.075 (1)	0.785
No	reference		
Have you done something other than the above?			
Yes	0.80 (0.19, 3.44)	0.088 (1)	0.767
No	reference		
Have you experienced persistent pain for 3 months or more?			
Yes	4.01 (2.22, 7.22)	21.315 (1)	<0.001 *
No	reference		
Does pain disrupt your activities?			
Highly disturb	12.20 (2.60, 57.25)	16.957 (1)	0.001*
Moderately disturb	4.78 (2.27, 10.06)	10.05 (1)	0.002*
Mildly disturb	2.02 (2.60, 57.25)	2.209 (1)	0.137
Not disturb	Reference	24.875 (3)	0.001

*significant (p<0.05)

In the univariate analysis, the study identified significant findings for several variables (p<0.05). The variables that showed strong significance included age (p<0.001), the presence of chronic disease (p<0.001), blood pressure (systolic: p<0.026; diastolic: p<0.007), and methods of medical expense coverage-self-pay (p=0.025) and employer coverage (p=0.002). Additionally, the presence of persistent pain lasting more than three months was significant (p<0.001), as well as mild activity disturbance (p=0.001) and severe activity disturbance (p=0.002). All significant variables listed in Table 3 and those with a p<0.25 were further analysed using multiple logistic regression with the backward likelihood ratio method. The multivariable analysis, which aims to identify the factors influencing the utilization of the PeKa B40 programme while adjusting for confounding variables, is presented in Table IV.

Table IV: The multivariable analysis to determine the determinants of the utilization of the PeKa B40 programme

Variables	Adj. OR (95%CI)	Wald test (df)	P-value
Age	1.06 (1.03, 1.10)	13.880 (1)	0.001*
Presence of Chronic Disease:			
Yes	2.62 (1.28, 5.37)	6.920 (1)	0.009*
No	reference		
Gender			
Female	2.32 (1.15, 4.66)	5.583 (1)	0.018*
Male	reference		
Self-rate health			
Poor	0.05 (0.01, 0.47)	6.688 (1)	0.010*
Intermediate	0.25 (0.06, 0.95)	4.172 (1)	0.041*
Good	0.22 (0.06, 0.82)	5.132 (1)	0.023*
Excellent	reference	7.995 (3)	0.046
Does pain disrupt your activities?			
Highly disturb	14.34 (2.18, 94.26)	7.668 (1)	0.006*
Moderately disturb	3.30 (1.31, 8.33)	6.364 (1)	0.012*
Mildly disturb	1.40 (0.47, 4.19)	0.352 (1)	0.533
Not disturb	reference	11.876 (3)	0.008

Adj. OR: Adjusted Odds Ratio
Hosmer–Lemeshow test: p = 0.976; Cox and Snell R^2: 28.6%; sensitivity was 62.1%, and the specificity was 84.4%.
Overall, 74.9% of the cases are classified correctly. AUROC: 81.9% [95%CI: 76.3, 87.4), p<0.01])
*significant (p<0.05)
Statistical test: Multiple logistic regression

The multivariable analysis included all significant findings from earlier analyses, particularly the influence of gender (p=0.018) and self-rated health (p=0.046). The research indicated that certain factors affected programme usage. Notably, older participants were 1.06 (95% CI: 1.03, 1.10) times higher odds to participate in the programme than younger participants (p<0.001). Individuals with chronic diseases were 2.62 (95% CI: 1.28, 5.37) times higher odds

to participate in the programme than those without chronic diseases (p<0.009). Additionally, females were 2.33 (95% CI: 1.15, 4.66) times higher odds to participate in the programme than males (p<0.018).

Those experiencing high levels of pain (p=0.006) and moderately disturbed of pain (p=0.012) showed significantly higher participating the PeKa B40 programme. The high levels and moderately disturbed of pain were 14.34 (95% CI: 1.31, 8.33) and 3.39 (95% CI: 2.18, 94.26) times higher odds to participate in the programme compared to those without disturbed in pain, respectively. Conversely, individuals who rated their health as good (adjusted OR: 0.22, 95% CI: 0.06, 0.82, p=0.023), intermediate (adjusted OR: 0.25, 95% CI: 0.06, 0.95, p=0.041), or poor (adjusted OR: 0.05, 95% CI: 0.01, 0.47, p=0.010) were less likely to participate in the programme compared to those without chronic diseases.

DISCUSSION

The participation rate of PeKa B40 utilization among residents of Felda Lubuk Merbau exceeds both national and state averages. Approximately 41.8% of the target population actively participated in the PeKa B40 health screening initiative, marking a significant increase compared to the findings from Protect Health Corporation post-2021. Nationally, PeKa B40 utilization is around 14.9%, while Kedah reported 20.67% according to Protect Health Corporation.[10] However, recent data from the Ministry of Health, Malaysia indicates a higher utilization rate in Kedah at 28.42%.[16] The close-knit community of Felda, along with its proximity to health centres, likely contributes to the increased participation in the health screening programme. Additionally, various outreach initiatives by public and private entities targeting rural areas could be influencing the enhanced coverage of the PeKa B40 programme.[17]

According to multivariable analysis, increasing age correlates with higher odds of utilizing the PeKa B40 health screening programme. This aligns with previous local research indicating that older adults are more likely to take advantage of available health services compared to younger generations.[18,19] Furthermore, a study conducted in neighbouring Singapore found similar results, suggesting that advanced age is a significant predictor of health screening service utilization.[20] However, in certain disease screenings, such as ovarian or breast cancer, some researchers have indicated that younger age groups are more interested in participating.[21,22] Introducing case finding in primary care could facilitate the early identification of non-communicable diseases (NCDs) and their related risk factors, potentially leading to reduce complications and mortality rates. Countries are advised to prioritize addressing modifiable risk factors and early detection of NCDs among the "young old" population (aged 60–75 years) as part of various public health measures.[23] The objective is to lower illness and death rates among older individuals, thereby minimizing the impact of long-term NCDs, improving the quality of life for the elderly, and promoting greater self-reliance.[24]

The gender of an individual significantly impacts the likelihood of utilizing healthcare screening services. Previous research conducted in Perak and Selangor indicates that Malay females are more inclined to take advantage of the health screening packages available to them compared to males.[25] Additional studies show that females tend to visit their primary care facilities and diagnostic services more frequently than males.[26] Public hospitals, offering both conventional healthcare and traditional complementary medicine, are often overwhelmed by female patients. [27] According to Gómez [28], females are more likely than males to utilize preventive and diagnostic treatments, while males tend to rely more on emergency services. Several hypotheses have been proposed to explain this trend. Notably, females generally have a greater need for healthcare due to poorer health conditions compared to their male counterparts. This is reflected in higher illness rates, lower health perceptions, reduced quality of life related to health, and greater levels of disability among females. Furthermore, societal perceptions and constructions of illness differ between males and females, influencing their roles, attitudes, beliefs, and behaviours regarding health concerns. These distinctions lead to varying approaches to seeking healthcare and result in differences in the services provided to males and females.[29,30]

The presence of chronic diseases increases the likelihood of utilizing health screening services compared to those without chronic conditions. This finding aligns with other studies that have also concluded that individuals with one or more chronic diseases are more inclined to seek comprehensive health screening services.[31] However, a study conducted in Taiwan indicated that a person's health status did not significantly influence their use of health screening services.[32]

Self-rated health refers to an individual's subjective assessment of their well-being, shaped by their perceptions and priorities. Research on self-rated health often examines health-related behaviours such as smoking, dietary habits, physical activity, body mass index (BMI), obesity rates, and alcohol consumption.[33] This study highlights that self-rated health plays a crucial role in determining the utilization of the PeKa B40 health screening programme. Individuals who perceive their health positively are more likely to engage in health screening services. This observation is further supported by another study indicating that individuals who lead healthier lifestyles tend to have more negative views about their health status compared to others.[34] Conversely, another study found that individuals with cardiovascular and cerebrovascular diseases, visual impairments, mental disorders, or poor blood test results are more likely to give negative ratings in self-assessed health.[35] The differences in findings may be attributed to variations in cultural perceptions and disparities in health literacy based on the location of the research studies.[36]

Health-seeking behaviour (HSB) strongly influences the respondents to utilize PeKa B40 programme which provides free screening , cancer support, medical device aid and health promotion.[16] Therefore, enhancing awareness, improving access and promoting preventive health behaviours are essential strategies to increase PeKa B40 utilization to reduce health inequalities in Malaysia. A comparable national study using data from the National Health and Morbidity Survey (NHMS) 2019 found that Malaysian adults who rated their health poorly or who had long-term conditions were actually more likely to seek treatment—indicating a reactive HSB pattern (i.e., seeking care only when ill) rather than preventive screening behaviour.[37]

Additionally, the presence of chronic pain that interferes with daily activities also influences the utilization of health screening services. This conclusion is supported by numerous publications from other researchers.[38,39] Chronic pain has been shown to lead to increase healthcare usage, with higher levels of pain intensity and impairment correlated with a greater likelihood of seeking medical attention. Patients with chronic pain often view healthcare utilization as a method of coping with their symptoms.[40]This group may seek health screenings with the expectation of understanding the causes of their ongoing discomfort, rather than recognizing that these tests are intended to detect diseases at an early, asymptomatic stage. Moreover, individuals may use health screening appointments as an opportunity to consult with healthcare practitioners about their current health status.[41]

LIMITATIONS

This study encountered several limitations during its execution. As a cross-sectional observational study, it cannot establish causal relationships, as its design does not allow for the assessment of temporality according to Bradford Hill's criteria. The study was conducted exclusively within a specific neighbourhood, resulting in a limited scope due to the unique characteristics of that locality. As a result, the generalizability of our findings to other local populations in Malaysia remains uncertain and requires further investigation.

CONCLUSION

Overall, the prevalence of PeKa B40 utilization among residents of Felda Lubuk Merbau is higher than the national and state averages. This indicates a significant increase in participation in health screenings compared to previous studies. The compact structure of Felda Lubuk Merbau and its proximity to healthcare facilities may contribute to the higher levels of participation. Furthermore, outreach initiatives specifically targeting rural areas might be enhancing the coverage of the PeKa B40 programme. To improve the programme's reach, the

government should actively promote it, particularly to adult men. It is also essential to extend the programme's benefits to healthy individuals, not just those who are sick and require medical treatment. Although this study has certain limitations, such as its cross-sectional design, the findings provide valuable insights for future research and public health initiatives.

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INSTITUTIONAL REVIEW BOARD (ETHICS COMMITTEE)

Ethical approval was obtained from both the Medical Research and Ethics Committee (MREC) (NMRR-20-30882-57796 (IIR)) and the Research Ethics Committee at Universiti Teknologi MARA (UiTM) (REC/08/2022 (PG/MR/176)).

CONFLICT OF INTEREST

All authors declare that there are no conflicts of interest.

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ORIGINAL ARTICLE

Translation and Validation of the Readiness to Change Questionnaire for Alcohol Drinkers into an Indigenous Language (Jakun Version) in Malaysia

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ABSTRACT

INTRODUCTION: Assessing readiness to change is crucial in managing alcohol-related conditions and can be achieved by using the Readiness to Change Questionnaire (RCQ), grounded in the Transtheoretical Model (TTM). This is especially important in Indigenous communities in Peninsular Malaysia, where alcohol use is intertwined with cultural practices. This study aimed to translate and validate the RCQ from English into the Jakun language spoken by an Indigenous tribe in Malaysia to ensure its linguistic and cultural relevance for assessing readiness to change among Jakun alcohol drinkers. **MATERIALS AND METHODS**: This cross-sectional study was conducted among current alcohol drinkers in three Indigenous Jakun settlements in Rompin, Pahang, Malaysia. The RCQ underwent translation into Jakun language, followed by validation (face and construct) and reliability assessment. Exploratory factor analysis (EFA), confirmatory factor analysis (CFA), and internal consistency were used to evaluate its construct validity and reliability. **RESULTS:** A total of 317 participants were recruited, most of whom were males, aged 18-29, married, had primary education, were self-employed, and had low household income. From the EFA, the original three factors were retained (pre-contemplation, contemplation, and action), but three items were removed, one from each construct. The final model demonstrated acceptable fit in CFA. Cronbach's alpha values ranged between 0.733 and 0.838, indicating good internal consistency. **CONCLUSION:** The RCQ (Jakun version) is a valid and reliable tool for assessing readiness to change among Indigenous Jakun alcohol drinkers. It can potentially assist healthcare professionals in tailoring interventions based on stages of change to address risky alcohol use in this population.

Keywords
Alcohol Drinking; Indigenous Peoples; Transtheoretical Model

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INTRODUCTION

Among the Indigenous communities in Peninsular Malaysia, alcohol consumption has traditionally been associated with cultural celebrations, such as festivals or post-harvest gatherings.[1] However, recent studies indicate that modernisation and outside influences have introduced unhealthy practices, including alcohol misuse.[2-4] This raises concerns about risky alcohol consumption within these communities, as it may contribute to alcohol-related health issues and social disparities.[5-8]

The Jakun tribe is a subgroup of the Proto-Malay, one of the three main Indigenous groups in Peninsular Malaysia, predominantly residing in rural areas. Their native language is Jakun, although most members are also able to understand and speak Malay - the national language - with varying levels of fluency depending on their educational background.[9-11] The use of English as a third language is minimal and generally uncommon.[12] Within this community, alcohol consumption has emerged as a growing concern due to its associated health risks.[3,4] Notably, in 2019, a cluster of methanol poisoning occurred in a Jakun settlement, resulting in three fatalities.[13]

When managing alcohol-related conditions, it is always crucial to assess an individual's readiness to change their drinking behaviour. This assessment is grounded in the Transtheoretical Model (TTM), which describes the temporal and intentional aspect of change, from pre-contemplation, contemplation, preparation, action, and maintenance.[14, 15] Tailoring interventions to aligns with an individual's level of readiness has been proven to improve the treatment outcome.[16]

The Readiness to Change Questionnaire (RCQ) was developed in 1992 to assess readiness to change among individuals who engage in excessive alcohol drinking but have not sought treatment.[17] This was later followed by the development of the RCQ (Treatment Version) [RCQ (TV)] in 1999, specifically designed for individuals already undergoing treatment for alcohol-related problems.[18] Subsequently, in 2008, a revised version of the RCQ (TV) was introduced, which refined the questionnaire by reducing the number of items from 15 to 12.[19]

This study focused on the original RCQ, specifically designed to complement brief and opportunistic interventions targeting excessive alcohol drinking,[17,20,21] which is suitable for Indigenous individuals, considering the potential problems of accessibility associated with this population. To date, this tool has been translated into four languages, namely Dutch, Spanish, Swedish, and Lithuanian,[22-25] and has been widely employed in various clinical studies across both inpatient[26-28] and outpatient settings.[29-31] This study aimed to translate and validate the RCQ from English into the Jakun language, ensuring its linguistical and cultural relevance and to facilitate in assessing readiness to change among the Indigenous Jakun community.

MATERIALS AND METHODS

Study Design, Settings, and Participants

This cross-sectional study was conducted among the Indigenous Jakun community in the state of Pahang, one of the states in Peninsular Malaysia. Participants were recruited from three Indigenous settlements in Rompin district – district with the largest Indigenous population in Pahang, at approximately 15,099 individuals.[32] One of these settlements had a history of cluster of methanol poisoning in 2019, resulting in three deaths.[13] The other two settlements were selected due to their proximity to the first, as well as their significant population size, being the second largest in the district, and their shared Jakun subdialect. Data collection was performed between October 2024 and February 2025.

The inclusion criteria were individuals in the selected settlement aged ≥ 18 years old who are current alcohol drinkers, irrespective of volume, have never sought treatment for alcohol-related issues, and voluntarily agreed to participate after receiving an explanation of the study. Current drinker is defined as individual who has consumed any alcoholic beverage in the past 12 months.[33] The exclusion criteria are those with history of serious mental illness or cognitive impairment and those who are unable to speak proficient Jakun language.

Readiness to Change Questionnaire (RCQ)

The RCQ was developed from a questionnaire based on Prochaska and DiClemente's stages of change model, specifically designed for drinkers who have not sought help for alcohol problems.[17,21] It consists of 12 items, categorised into three constructs: pre-contemplation, contemplation, and action stages, with four items for each construct and factor loadings ranging from 0.35 to 0.88. The maintenance stage was excluded due to unreliable responses to its items. The tool demonstrates good internal reliability, with Cronbach's alpha coefficients of 0.73, 0.80, and 0.85 for the pre-contemplation, contemplation, and action stages, respectively.[20]

For scoring the RCQ, each item is rated on a five-point scale: -2 (strongly disagree), -1 (disagree), 0 (unsure), +1 (agree), and +2 (strongly agree), reflecting the respondent's level of agreement with each statement. To calculate the score for each construct, the scores of the items within each construct are summed, resulting in a possible range of -8 to +8. The construct with the highest total score indicates the individual's stage of change, classifying them as being in pre-contemplation, contemplation, or action.[20]

Study Conduct

This study consisted of two parts: (1) the translation and face validation, and (2) the psychometric evaluation.

Part 1: Translation and Face Validation

Written permission was first obtained from the author of the original questionnaire, who recommended the use of the original 12-item RCQ for this study (N. Heather, personal communication, 17 January 2024). This version was deemed more appropriate than the originally intended RCQ (TV), as the target population have never received treatment for their alcohol-related condition.

The translation process followed the established guidelines,[34, 35] using forward translation conducted simultaneously by two bilingual individuals. One translator is a medical worker (a medical assistant at a government health clinic serving the target population), while the other is a non-medical individual (a community leader). This was followed by backward translation, also performed in parallel by two bilingual individuals – one a medical professional (a medical officer at a government clinic serving the Indigenous communities) and the other a non-medical professional (a primary school teacher at the study site). All translators are of Jakun descent and are fluent in the Jakun language. The back translators were not informed of the original English version of the questionnaire. The translated versions were then reviewed and discussed by the research team which included a medical officer with considerable experience serving the Jakun community and two public health medicine specialists. Through this process, a harmonised preliminary version of the RCQ (Jakun version) was produced.

This harmonised version was then subjected to a face validation process with 10 respondents at the study site.[36] These participants were asked to assess the clarity and comprehension of the questionnaire, rating each item on a scale from 1 (not clear and understandable) to 4 (very clear and understandable).[37] Additionally, they were invited to provide feedback on the structure and wording of each item. The feedback gathered was used to revise and polish the questionnaire, leading to the development of the refined RCQ (Jakun version).

Part 2: Psychometric Evaluation

For construct validity assessment, a sample size of at least seven times the number of items, with a minimum of 100 participants was recommended.[38] Given that this questionnaire contains 12 items, the ideal sample size would be 84. However, since 100 is the minimum requirement, this larger sample size was used for the exploratory factor analysis (EFA).

To further assess construct validity through confirmatory factor analysis (CFA), another sample size of 200 was employed. This was based on a recommendation suggesting a sample size between 100 and 200 when communalities are around 0.5.[39] For the assessment of reliability (internal consistency) a minimum sample size of 100 was proposed.[38] Since 200 participants were already included in the CFA, the same sample size was applied for reliability evaluation.

Initially, recruitment was planned to be random, based on house numbers from a spot map of the settlement. However, due to a potentially limited respondent pool and logistical challenges, recruitment was ultimately conducted universally through house-to-house visits. The timing of the house visits was adjusted to occur between 3 p.m. and sunset, considering that most potential respondents were typically at work in the morning and early afternoon.

During the informed consent process, individuals were provided with explanation in simple Malay language, using a patient information sheet available in Jakun language to ensure clarity and understanding. For individuals who were unable to read, a family member or friend who can read may assist. Once the individuals agreed to participate, they underwent screening to assess their eligibility based on the inclusion and exclusion criteria. Those who did not meet the criteria were thanked and not be enrolled in the study.

Tentatively, socio-demographic information and the refined RCQ (Jakun version) were administered to the respondents. The questionnaires were primarily self-

administered. However, for respondents who had reading problems, the content of the questionnaires was read aloud to them, word by word, and in the order indicated.

Statistical Analyses

Part 1: Face Validation

Data from the face validity assessment of the refined RCQ (Jakun version) were entered into Microsoft Excel, where the item-level face validity index (I-FVI) for each question was calculated. A cut-off score of 0.83 was considered acceptable for inclusion.[37]

Part 2: Psychometric Evaluation

The data from the psychometric evaluation were entered into SPSS version 28. For descriptive analysis, continuous data were presented as mean or median, while categorical data were presented in numbers and percentages.

Construct validity was first assessed using EFA. The suitability of the data for factor analysis was determined by a Kaiser-Meyer-Olkin (KMO) value >0.6 and a significant Bartlett's Test of Sphericity (p-value <0.05). Any pair of items with bivariate correlation scores exceeding 0.80 required the removal of one item.[40,41] For the EFA, principal component analysis (PCA) was employed as the extraction method. The number of factors to be retained was determined by Kaiser's eigenvalues >1.[40,41] The Varimax rotation method was applied, with a maximum of 25 iterations for convergence. A minimum factor loading of 0.60,[39, 42, 43] no or minimal item cross-loadings, and no factors containing fewer than three items were considered acceptable for an item to be retained and proceed to CFA.[44]

CFA was conducted using SPSS AMOS version 29. Model specification involved defining the latent variables (pre-contemplation, contemplation, and action) and their corresponding observed variables (items retained in EFA). Model estimation was performed using Maximum Likelihood estimation. Model fit was assessed based on Chi-Square statistics, Root Mean Square Error of Approximation (RMSEA), Standardised Root Mean Square Residual (SRMR), Bentler's Comparative Fit Index (CFI), and Parsimonious Normed Fit Index (PNFI).[45]

A good fit is defined by a Chi-square/df <5.0, SRMR <0.08, CFI >0.90, and PNFI >0.5.[45] For RMSEA, ideally a value of ≤ 0.08 is desirable, although ≤0.10 can still be acceptable, with >0.10 being considered poor fit.[46]

The internal consistency was analysed by employing Cronbach's alpha coefficient and corrected-total correlations. A Cronbach's alpha coefficient >0.70 indicated good reliability. At the same time, the corrected item-total correlations of r>0.30 were considered good.[41]

RESULTS

Part 1: Translation and Face Validation

After undergoing harmonisation following the forward and backward translation, face validation was conducted with 10 respondents at the study site, aged between 18 and 74 years. The I-FVI ratings for both clarity and comprehension were 1 for each of the 12 items.

Part 2: Psychometric Evaluation

Participants' Sociodemographic Characteristics

Overall, 317 respondents met the inclusion criteria. Of these, 108 took part in the EFA during the first round of data collection in the first settlement. The remaining 209 participants were included in the CFA, which incorporated data from the second and third settlements, as well as the second round of data collection across all three settlements.

As shown in Table I, majority of the participants were males (82.0%), aged 18 to 29 years (37.9%), married (71.0%), had primary education (51.4%), and were self-employed (64.7%). In terms of household income, 99.7% of participants fell into the Below 40 (low-income) category, based on the classification by the Department of Statistics Malaysia.[47] Beer was the most commonly consumed alcoholic beverage (93.1%).

Table I: Sociodemographic characteristics and types of alcohol consumed (N=317)

Participants	n (%)	Mean ± SD / median (IQR)
Sex		
Male	260 (82.0)	
Female	57 (18.0)	
Mean age (years)		36.69 ± 13.54
Age group (years)		
18-29	120 (37.9)	
30-39	79 (24.9)	
40-49	57 (18.0)	
50-59	40 (12.6)	
≥ 60	21 (6.6)	
Marital status		
Single	57 (18.0)	
Married	225 (71.0)	
Divorced	24 (7.6)	
Widowed	11 (3.5)	
Highest educational level		
No formal education	62 (19.6)	
Primary education	163 (51.4)	
Secondary education	85 (26.8)	
Tertiary education	7 (2.2)	
Occupation		
Self-employed	205 (64.7)	
Private employee	59 (18.6)	
Housewife	38 (12.0)	
Not working (unemployed, retired, or unable to work due to illness)	13 (4.1)	
Government employee	2 (0.6)	
Monthly household income (RM)		1,000 (700)[a]
Monthly household income group (RM)		
< 1,000	117 (36.9)	
1,000 – 1,999	161 (50.8)	
2,000 – 2,999	24 (7.6)	
3,000 – 3,999	11 (3.5)	
≥ 4,000	4 (1.2)	
Type of alcohol		
Beer	295 (93.1)	
Liquor	8 (2.5)	
Others (wine, whisky, rice wine, samsu)	14 (4.4)	

Note: [a]Median income

Exploratory Factor Analysis (EFA)

In the 12-item factor solution, the KMO value was 0.741, indicating that the sample size was adequate. Additionally, Bartlett's test of sphericity was significant (<0.001), suggesting that the item correlation matrix was not an identity matrix.[40] These results confirmed that the data were suitable for factor analysis. No pair of items had bivariate correlation scores exceeding 0.80, hence no item was removed at this stage. Three factors, with initial eigenvalues ≥1.0, were retained, explaining 62.13% of the variance. Most of the communality values were ≥0.40, except for item number 7 at 0.354.

Following an initial rotated component matrix, three items, one from each factor (item 4, 5, and 7) did not meet the requirement of having the factor loading ≥0.6, and therefore were removed. Subsequently, the resulting 9-item factor solution had a KMO value of 0.687 and significant Bartlett's test of sphericity (<0.001). The three factors with total initial eigenvalues > 1 explained 68.59% of the variance. All the communality values were ≥ 0.40. There was no cross-loading, and the rest of the items were retained within their priori factors. Individual factor loadings following rotated component matrix on the initial and final three-factor solution are shown in Table II.

Table II: Rotated component matrix on the initial (12 items) and final (9 items) three-factor solution (N = 108)

Item	Factor 1	Factor 2	Factor 3
Pre-contemplation			
Item 1: I don't think I drink too much *"Nyop lah, kap nyop maler banyak pon"*	0.063 (0.092)	-0.299 (-0.308)	**0.619 (0.624)**
Item 5: It's a waste of time thinking about my drinking *"Buang masa ja mikir tabiat bermaler kap"*	0.494 (dropped)	-0.573 (dropped)	0.243 (dropped)
Item 10: There is no need for me to think about changing my drinking *"Ghaip keperluan kap untuk berhenti bermaler"*	0.046 (0.037)	0.055 (0.060)	**0.892 (0.903)**
Item 12: Drinking less alcohol would be pointless for me *"Nyop guna kap kurok bermaler"*	-0.151 (-0.183)	-0.105 (-0.115)	**0.738 (0.766)**
Contemplation			
Item 3: I enjoy my drinking, but sometimes I think I drink too much *"Kap berkenan maler, tapi kadang-kadang kalau kap maler, mesti maler banyak"*	0.035 (-0.020)	**0.803 (0.854)**	0.017 (-0.014)
Item 4: Sometimes I think I should cut down on my drinking *"Kadang-kadang kap rasa kap patut kurokkan bermaler"*	0.379 (dropped)	0.544 (dropped)	-0.455 (dropped)
Item 8: I am at the stage where I should think about drinking less alcohol *"Kap berada de tahap memang kap kenak kurokkan tabiat bermaler"*	0.426 (0.395)	**0.630 (0.600)**	-0.337 (-0.319)
Item 9: My drinking is a problem sometimes *"Tabiat bermaler kap, kadang-kadang bagi masalah pada kap lah"*	0.220 (0.219)	**0.754 (0.797)**	-0.117 (-0.157)
Action			
Item 2: I am trying to drink less than I used too *"Kap cubak maler sikit nyop macam duluk"*	**0.601 (0.678)**	0.308 (0.321)	0.150 (0.110)
Item 6: I have just recently changed my drinking habits *"Kap baru ja mengubah tabiat minum kap"*	**0.845 (0.881)**	-0.049 (-0.044)	-0.106 (-0.111)
Item 7: Anyone can talk about wanting to do something about drinking, but I am actually doing something about it *"Orang lain boleh cakap menapa pun pasal tabiat maler kap, tapi sebenarnya kap memang tengah buat sesuatu untuk kurokkan bermaler"*	0.575 (dropped)	0.117 (dropped)	-0.099 (dropped)
Item 11: I am actually changing my drinking habits right now *"Kap sebenarnya, tengah mengubah tabiat minum kap"*	**0.882 (0.886)**	0.125 (0.132)	-0.035 (-0.030)

[a]Factor loading for the initial 12-item solution are shown outside parentheses. Values in parentheses indicate the final 9-item solution following item reduction.

Confirmatory factor analysis (CFA)

CFA was conducted to validate the factor structure of the RCQ (Jakun version).[41] Three latent factors were proposed: pre-contemplation, contemplation, and action. The model fit was evaluated using several fit indices: Chi-square/df=2.961, SRMR=0.073, CFI=0.942, and PNFI=0.585, all of which suggested a good model fit. The RMSEA value of 0.097, although not ideal, was still within the marginally acceptable range.[46] Modification indices indicated that correlating the error terms of items 2 and 6 slightly improved the model fit. Overall, these results supported the validity of the three-factor structure, with no significant concerns regarding model specification.

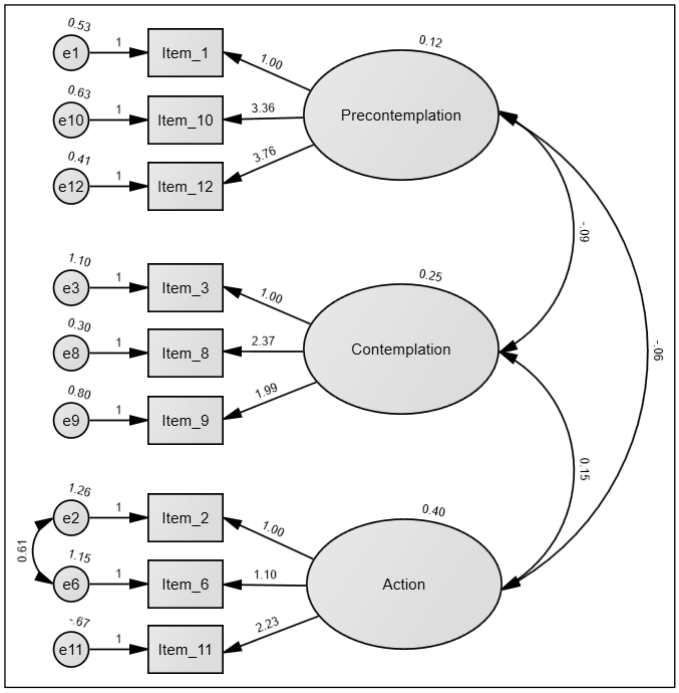

Figure 1: CFA of the RCQ (Jakun Version): three-factor model (N=209)

Reliability (Internal Consistency)

The Cronbach's alpha values for factor 1 (pre-contemplation), factor 2 (contemplation), and factor 3 (action) were 0.742, 0.733, and 0.838 respectively, indicating a high level of internal reliability. The corrected item-total correlation coefficients ranged from 0.378 to 0.740, which showed that all items correlate well with the overall scale.[41]

Table III: Cronbach's alpha value for each subscale in the finalised RCQ Jakun version) (N= 209)

Subscale	Number of items	Cronbach's alpha	Corrected item-total correlation	Cronbach's alpha if item deleted
Pre-contemplation	3	0.742		
Item 1			0.378	0.849
Item 10			0.697	0.489
Item 12			0.732	0.438
Contemplation	3	0.733		
Item 3			0.410	0.800
Item 8			0.648	0.530
Item 9			0.630	0.553
Action	3	0.838		
Item 2			0.668	0.810
Item 6			0.740	0.736
Item 11			0.701	0.779

DISCUSSION

I-FVI for each translated item was calculated to evaluate the face validity of the translated versions in terms of clarity and comprehensibility. Clarity refers to the absence of ambiguities in interpreting the items, while comprehensibility refers to whether the wording and structure of the items are easily understood. The I-FVI values of 1 for each translated item indicate universal agreement, ensuring that the items are clear and easily understood by prospective participants.[37]

EFA was conducted to assess whether the psychometric constructs of the translated questionnaires align with their original English versions. In this study, the three constructs (pre-contemplation, contemplation, and action) were retained, consistent with the original questionnaire. However, the number of items was reduced to three per domain. This reduction likely reflects cultural differences in the interpretation of certain statements in the dropped items.

For example, item number 5: *"Buang masa ja mikir tabiat bermaler kap"* (It's a waste of time thinking about my drinking), which was intended to assess the pre-contemplation construct, may suggest a lack of insight into one's drinking habits when agreed upon. However,

many respondents in this study expressed agreement with the statement, despite having a clear understanding of the negative effects of alcohol consumption. Some even remarked, *"Yes, it's a waste of time thinking about drinking."* This response highlighted a cultural difference in the interpretation of the item, where respondents viewed thinking about their drinking habit as something undesirable, rather than an indicator of self-awareness.

Another example is item number 7: *"Orang lain boleh cakap menapa pun pasal tabiat maler kap, tapi sebenarnya kap memang tengah buat sesuatu untuk kurokkan bermaler"* (Anyone can talk about wanting to do something about drinking, but I am actually doing something about it), which was designed to assess the action construct. This item is relatively long and linguistically dense. A notable number of respondents either paused for clarification or asked, "What?" after hearing or reading the statement. According to one of the forward translators, while long sentences are not necessarily grammatically incorrect in the Jakun language, they are not preferred in everyday conversation. The Jakun community generally favours shorter, more direct expressions in daily speech. As a result, the message of this item may become diluted or lost due to its length and complexity, hence affecting comprehension and consistency in responses.

Subsequently, the CFA results confirmed the three-factor structure of the RCQ (Jakun version), in line with the TTM. However, given the slightly elevated RMSEA value, a larger sample size might be needed to further improve model fit.[48] Alternatively, RMSEA could be omitted from the analysis, as it often misleadingly suggests poor fit in studies with smaller sample sizes.[49]

The final RCQ (Jakun version) demonstrated a good internal consistency with Cronbach's alpha values ranging from 0.733 to 0.838. This finding is consistent with the original study, which reported Cronbach's alpha values between 0.73 to 0.85,[20] as well as other cross-cultural studies showing values ranging from 0.58 to 0.83.[22, 23, 50] These indicate that this translated RCQ is reliable.

CONCLUSION

The RCQ (Jakun version) is a valid and reliable tool for assessing readiness to change among Indigenous Jakun alcohol drinkers. It can potentially serve as a brief and practical screening instrument, making it useful for identifying individuals at different stages of change. This tool can potentially assist healthcare professionals in tailoring interventions and developing targeted treatment strategies based on stages of change to address risky alcohol drinking behaviour within this population.

CONFLICT OF INTEREST

The authors declare no known competing financial interests or personal relationships that could have influenced the work reported in this study.

ETHICS APPROVAL

This study is part of a broader interventional research aimed at addressing risky drinking among the Indigenous community in Pahang, which has been approved by the Universiti Teknologi MARA (UiTM) Research Ethics Committee (REC/09/2024 (PG/MR/456)) and registered with the National Medical Research Register, Ministry of Health Malaysia (NMRR ID-24-02462-AQ6). Additionally, permission was obtained from the Department of Indigenous People Development (JAKOA) Malaysia (JAKOA.PP.R.004 JLD 8 (39)).

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ORIGINAL ARTICLE

Effectiveness of Solifenacin Monotherapy and Mirabegron and Solifenacin Combination Therapy in Patients with Stent-Related Symptoms

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ABSTRACT

INTRODUCTION: Ureteral stents are commonly used for internal urinary drainage but frequently cause stent-related symptoms (USRSs), which can negatively affect patient quality of life. This study aimed to evaluate the short-term effectiveness of solifenacin monotherapy compared to a solifenacin-mirabegron combination therapy in patients experiencing USRSs. **MATERIALS AND METHODS:** This double-blind, randomised controlled trial included 54 participants with USRSs. Patients were randomised to receive either solifenacin 5 mg/day (monotherapy) or a combination of solifenacin 5 mg/day and mirabegron 25 mg/day (combination therapy) for four weeks. The Ureteral Stent Symptom Questionnaire (USSQ) was used to compare treatment outcomes. Data were analysed using an independent t-test, with p-values <0.05 considered statistically significant. **RESULTS:** The solifenacin monotherapy group showed significantly lower (better) scores in the Urinary Symptoms domain compared to the combination group at week 1 (p=0.001) and week 2 (p=0.017), and in the General Health domain at week 1 (p=0.005), week 2 (p=0.027), and week 4 (p=0.045). The combination therapy group demonstrated significantly better scores for Sexual Function at week 2 (p=0.017). No statistically significant differences were observed between groups for Pain or Work domains. Both treatments were generally well-tolerated, with a low incidence of mild adverse events. **CONCLUSIONS:** Solifenacin monotherapy appears to provide superior short-term relief for urinary symptoms and general health issues compared to a low-dose solifenacin-mirabegron combination. The combination therapy demonstrated a targeted benefit in improving sexual function. Both treatment regimens were generally safe and well-tolerated.

Keywords
Stent-Related Symptoms, Mirabegron, Solifenacin, USSQ

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INTRODUCTION

The Double J (DJ) ureteral stent is a widely used surgical instrument for managing ureteral obstruction and facilitating postoperative drainage.[1] However, its use is frequently associated with stent-related symptoms (SRSs), also known as ureteral stent-related symptoms (USRSs). Conservative, non-invasive interventions for SRSs include alpha-blockers, anticholinergic medications, and analgesics.[2] To standardise the assessment of morbidity following DJ stent insertion, the Ureteral Stent Symptoms Questionnaire (USSQ) has been established as a validated, gold-standard instrument.[3]

The lower urinary tract symptoms (LUTS) caused by DJ stents are pathologically similar to symptoms of benign prostatic hyperplasia (BPH) and overactive bladder (OAB). Consequently, therapies effective for BPH and

OAB, such as alpha-blockers and antimuscarinics, are often used to manage SRSs.[4] Despite the high incidence of these symptoms, complications from DJ stent insertion remain under-investigated, particularly in Indonesia.

Current oral pharmacotherapies for OAB include the β3-adrenoceptor agonist mirabegron and antimuscarinics like solifenacin. Solifenacin is a competitive antagonist of cholinergic receptors with high selectivity for the M3 subtype, which plays a key role in bladder smooth muscle contraction. By inhibiting this pathway, solifenacin reduces bladder muscle tone, increasing its capacity and mitigating urinary urgency, frequency, and incontinence.[5] Mirabegron is a selective β3-adrenoceptor agonist; stimulating these receptors relaxes the detrusor muscle.[5] Studies have shown that combining mirabegron and solifenacin can increase treatment efficacy for OAB without reducing tolerability.[6]

This study aimed to investigate the effectiveness of solifenacin 5 mg/day monotherapy compared to a combination of solifenacin 5 mg/day and mirabegron 25 mg/day on USRSs after DJ stent insertion.

MATERIALS AND METHODS

This double-blind, randomised controlled trial included male and female patients with USRSs after DJ stent insertion. A complete medical history and physical examination were performed for all participants. Preoperative assessments included serum urea and creatinine levels, urinalysis, ultrasonography, and a kidney, ureter, and bladder (KUB) X-ray. All patients who underwent routine DJ stent insertion after endourological procedures (ureteroscopic lithotripsy or unilateral percutaneous nephrolithotomy) were eligible for inclusion. A 6 Fr DJ stent (Inlay Optima®, Bard Medical, Covington, GA, USA) was inserted under cystoscopic and fluoroscopic guidance.

After surgery, an abdominal X-ray was performed before hospital discharge to confirm stent positioning and the status of any residual stone fragments. The Foley catheter was removed on postoperative day one for all patients. Patients received a 5–7 day course of oral antibiotics (cefadroxil) and were prescribed mefenamic acid (500 mg every 6-8 hours as needed) for pain. The total analgesic consumption was recorded.

Treatment with either solifenacin 5 mg/day or the combination of solifenacin 5 mg/day and mirabegron 25 mg/day was initiated on day seven after DJ stent insertion. USSQ data were collected weekly on days 7, 14, 21, and 28 via direct interview or phone call. The study was conducted at four hospitals in Makassar, Indonesia (Ibnu Sina Hospital, Akademis Hospital, Hasanuddin University hospital, and Wahidin Sudirohusodo Hospital). It was conducted from September 2023 to February 2024. This research was approved by the local Ethics Committee (Reference No. 801/UN4.6.4.5.31/ PP36/2020).

Sampling Method and Sample Size

Patients were excluded if they met any of the following criteria: (1) under 18 years of age; (2) pregnancy; (3) bilateral stents; (4) previous ureteral stenting; (5) significant bladder pathology; (6) indication for long-term stenting; (7) symptomatic BPH; (8) active urinary tract infection; (9) severe systemic diseases; or (10) prior use of antimuscarinic or alpha-1 antagonist agents.

Participants were recruited using a consecutive sampling method. All eligible patients were then randomised into two treatment groups. A sample size calculation using a two-proportion test determined a minimum requirement of 25 patients per group. Group A received solifenacin 5 mg/day, and Group B received a combination of solifenacin 5 mg/day and mirabegron 25 mg/day.

Treatment Outcome Assessments

The USSQ was used to measure treatment outcomes over four weeks. This questionnaire evaluates stent-related morbidity across six domains: urinary symptoms, pain, general health, work performance, sexual matters, and other additional problems. A lower score in each domain indicates fewer symptoms and a better clinical outcome.

Data Collection

Participants were enrolled and received the USSQ forms during their follow-up visit at the outpatient clinic on day seven. Eligible patients were randomly assigned to receive either solifenacin monotherapy or combination therapy. The study was double-blinded. Participants took the medication orally for four weeks, and outcomes were recorded weekly from the completed questionnaires. All participants remained stented throughout the 4-week study period, and all data collection was completed prior to stent removal.

Safety and Adverse Event Assessment

At each weekly follow-up via phone or in-person visit, participants were systematically questioned about the occurrence of potential treatment-related adverse events. Specific inquiries were made regarding common side effects of antimuscarinics (e.g., dry mouth, constipation, blurred vision) and β3-agonists (e.g., headache, hypertension). All reported events were recorded, and their severity was graded as mild, moderate, or severe.

Data Processing and Analysis

Scores for each of the six USSQ domains were expressed as mean ± standard deviation. Demographic data, such as age, were categorised based on the standard classification from the Ministry of Health of the Republic of Indonesia.7 Comparisons between treatment groups were conducted using an independent t-test, and Fisher's exact test was used to analyze the incidence of adverse events. A p-value of <0.05 was considered statistically significant. Data were processed using Microsoft Excel 2010 (Redmond, WA: Microsoft Corporation) and SPSS version 24.0 (Armonk, NY: IBM Corp.).

RESULTS

A total of 54 participants were randomised: 28 to the combination therapy group and 26 to the solifenacin monotherapy group. Baseline demographic and clinical characteristics were generally similar between the two groups (Table I).

Table I: Participants' Characteristics.

Variables	Solifenacin + mira-begron n (%)	Solifenacin n (%)
Age		
Late adolescence (18–25 years)	2 (7.1)	2 (7.69)
Adulthood (26–45 years)	14 (50)	10 (38.46)
Early Elderly (46–55 years)	7 (25)	7 (26.92)
Late Elderly (56–65 years)	5 (17.9)	4 (15.38)
Very Elderly (>65 years)	0 (0)	3 (11.54)
Sex		
Male	21 (75)	14 (53.8)
Female	7 (25)	12 (46.2)
BMI (kg/m2)		
Mean ± SD	22.83 ± 3.47	24.43 ± 3.08
Weight (kg)		
Mean ± SD	60.92 ± 9.45	63.57 ± 10.91
Height (cm)		
Mean ± SD	163.35 ± 6.08	160. 84 ± 6.96
Marital Status		
Married	23 (82.1)	20 (76.9)
Double J stent insertion side		
Right	17 (60.74)	12 (46.15)
Left	11 (39.26)	14 (53.84)

Note: BMI, body mass index.

The treatment outcomes from the USSQ domains are presented in Table II. The solifenacin monotherapy group reported significantly lower (better) scores for Urinary Symptoms at week 1 (p=0.001) and week 2 (p=0.017).

Table II: Treatment outcomes by six USSQ index scores.

Observation time (week)	Mean score ± SD		p-value*
	Solifenacin	Solifenacin + Mirabegron	
Urinary symptoms index score			
1	15.19 ± 4.88	21.53 ± 5.79	0.001
2	10.88 ± 3.45	13.64 ± 4.63	0.017
3	8.11 ± 2.48	9.35 ± 3.71	0.158
4	5.61 ± 2.51	7.17 ± 3.62	0.073
Pain symptoms index score			
1	13.85 ± 3.51	12.21 ± 4.58	0.150
2	9.85 ± 3.90	8.36 ± 3.54	0.148
3	6.07 ± 3.37	4.82 ± 2.80	0.142
4	4.65 ± 3.06	3.96 ± 2.78	0.390
General health symptoms index score			
1	9.54 ± 3.63	12.53 ± 3.81	0.005
2	6.42 ± 3.06	8.53 ± 3.71	0.027
3	4.42 ± 3.02	6.14 ± 3.96	0.80
4	3.69 ± 2.54	5.32 ± 3.22	0.045
Working complaints index score			
1	6.96 ± 3.02	5.07 ± 5.12	0.114
2	4.84 ± 2.95	4.50 ± 3.76	0.718
3	3.36 ± 1.82	3.14 ± 2.60	0.730
4	2.12 ± 1.85	3.03 ± 2.51	0.142
Sexual dysfunction complaints index score			
1	2.33 ± 0.57	1.20 ± 2.02	0.350
2	3.57 ± 1.94	2.04 ± 1.76	0.017
3	2.67 ± 1.39	2.12 ± 1.33	0.225
4	1.93 ± 0.88	2.28 ± 0.97	0.269
Other symptoms score			
1	7.34 ± 2.99	8.46 ± 2.53	0.143
2	4.92 ± 2.41	5.03 ± 2.02	0.858
3	3.69 ± 1.78	3.42 ± 1.47	0.555
4	2.57 ± 1.87	3.25 ± 1.35	0.135

Note: *Independent t-test.

A similar trend favoring monotherapy was observed in the General Health domain at week 1 (p=0.005), week 2 (p=0.027), and week 4 (p=0.045). There were no significant differences between the groups in the domains of Pain, Work Performance, or Other Symptoms.

Both treatment regimens were well-tolerated by the participants. No serious adverse events or study discontinuations due to side effects were reported. The incidence of mild adverse events was low in both groups and is detailed in Table III. The most frequently reported adverse event was mild dry mouth, with no statistically significant difference in overall incidence between the two ams.

Table III: Incidence of Adverse Events.

Adverse Event	Solifenacin + Mirabegron (n=28) n (%)	Solifenacin (n=26) n (%)	p-value*
Dry Mouth	4 (14.3%)	4 (15.4%)	0.91
Constipation	3 (10.7%)	3 (11.5%)	0.93
Headache	2 (7.1%)	1 (3.8%)	0.60
Hypertension	1 (3.6%)	0 (0%)	0.48
Blurred Vision	1 (3.6%)	1 (3.8%)	0.99
Total Patients with any AE	8 (28.6%)	7 (26.9%)	0.90

Note: AE, Adverse Event. Total Patients with any AE represents the number of patients experiencing at least one adverse event. *P-values were calculated using Fisher's exact test.

Subgroup Analysis of Sexual Dysfunction

A subgroup analysis was performed to evaluate the impact of sex on the sexual dysfunction domain score. In the combination therapy group, the mean score at week 2 was 2.10 for males (n=21) and 1.86 for females (n=7), with a within-group comparison p-value of 0.42. In the monotherapy group, the mean score at week 2 was 3.86 for males (n=14) and 3.25 for females (n=12), with a within-group comparison p-value of 0.38. These results indicate no statistically significant difference between male and female participants within either treatment arm, supporting the statement that gender did not significantly affect sexual symptom outcomes.

DISCUSSION

This randomised controlled trial demonstrated the unexpected finding that solifenacin 5 mg monotherapy was superior to a combination of solifenacin 5 mg and mirabegron 25 mg in alleviating urinary symptoms and improving general health during the first few weeks following ureteral stent placement. Furthermore, both treatments demonstrated a favorable safety profile and were well-tolerated. This result challenges the assumption that combination therapy, which is often superior for OAB, would also be superior for USRSs.

The superiority of monotherapy in this context may be explained by the distinct pathophysiology of USRSs compared to OAB. USRs are primarily caused by the mechanical irritation of the bladder trigone by the stent's distal coil, which triggers involuntary detrusor contractions mediated by M3 cholinergic receptors.8 Our findings suggest that blocking this specific pathway with solifenacin is the most critical and sufficient intervention. The β3-adrenoceptor pathway, targeted by mirabegron, may play a less significant role in stent-induced irritation compared to its role in idiopathic detrusor overactivity seen in OAB.

Several factors might have affected treatment outcomes and USSQ data, including relationship status, older age, and sexual activity. The proportion of men to women was higher in the combination group, with a ratio of 3:1, whereas the proportions of men and women in the monotherapy group were almost equal (1:1.16). Notable age differences among the treated patients might have also affected therapy outcomes. Our findings are in line with a study that found LUTS impacted a significant proportion of individuals aged 40 years and older, and the incidence increases with advancing age.9 LUTS are associated with impaired quality of life (QOL) and mental health, but less than 50% of individuals in China experiencing LUTS pursue medical care for their conditions. A key point raised during the review process was the need for a more detailed analysis of the sexual dysfunction domain, and we have now incorporated a subgroup analysis by gender and data on marital status. Our study found no statistically significant difference in sexual symptom scores between male and female participants in either treatment arm. This finding contrasts with the literature suggesting a higher prevalence of sexual symptoms in female patients (up to 80%) compared to males (around 60%).10 The lack of a significant difference in our cohort is likely attributable to the limited statistical power of our subgroup analysis,

particularly the small number of female participants. Furthermore, a high proportion of participants in both groups were married, suggesting that sexual activity was a relevant quality-of-life factor for the majority of our sample. Future, larger-scale studies with a more balanced sex distribution are needed to properly elucidate the differential impact of USRSs on sexual function between the sexes.

Our study demonstrated that solifenacin monotherapy resulted in better outcomes for urinary symptoms and general health compared to the combination treatment group over four weeks, with a non-significant trend toward improvement in pain scores. This finding appears to contrast with the typical goals of therapy for LUTS, where combination treatments are often explored to maximise symptom improvement. An optimal therapeutic approach for LUTS aims to alleviate symptoms by understanding their underlying causes, making informed decisions, and systematically applying treatments. Interventions should not worsen a patient's condition; this includes avoiding aggressive or irreversible treatments for minor symptoms, reducing complications, and preserving sexual function where possible. Patients must also be informed about the potential adverse effects and realistic outcomes of their treatment options.[11]

Other studies in different patient populations have found success with combination therapy. For example, one trial demonstrated the safety and efficacy of a dual therapy (mirabegron 50 mg and solifenacin 5 mg) compared to solifenacin monotherapy (5 mg or 10 mg) in patients with OAB, with subgroup analyses by age.[12] It found that all treatments improved outcomes, but the combination therapy outperformed solifenacin monotherapy in managing OAB symptoms-such as urgency, frequent urination, and incontinence-particularly among older patients.

Similarly, a multinational, randomised, double-blind study that investigated different dosing regimens found that a higher dose of mirabegron (50 mg) in combination with solifenacin (5 mg) significantly decreased OAB symptoms compared to solifenacin monotherapy ($p<0.001$).[13] Another meta-analysis of 3,309 patients concluded that combining mirabegron and solifenacin was superior to solifenacin alone in reducing micturition, incontinence, and urgency episodes without increasing adverse effects.[14] Furthermore, a long-term study by Gratzke et al. found that combination therapy was well-tolerated for up to 12 months and was considerably more effective than monotherapy in reducing incontinence episodes in OAB patients, leading the authors to recommend it as a widely viable clinical option.[15]

The fact that our findings contrast with these major studies strongly suggests that USRS and OAB are distinct clinical entities driven by different primary mechanisms. While OAB often involves idiopathic detrusor overactivity, USRS are a direct consequence of mechanical irritation of the bladder trigone by the stent's distal coil. Our data suggest that for USRS, blocking M3 cholinergic receptors with solifenacin is the most critical and sufficient intervention. The addition of a low-dose β3 -agonist like mirabegron did not provide further benefit in our study and, in the early stages, was even associated with worse urinary symptom scores.

A meta-analysis demonstrated that the combination of mirabegron 50 mg with solifenacin 5 mg led to several significant improvements for patients with OAB. These included: a greater mean volume voided per micturition; a reduced incidence of urgency incontinence, micturitions, and urgency episodes; lower patient perception of bladder condition scores; and an increase in the number of patients achieving zero incontinence. These factors notably enhanced overall health-related QOL scores. 16 No significant difference was observed between the combination and monotherapy groups regarding treatment-emergent adverse events, such as QT prolongation on electrocardiograms, urinary tract infection, urinary retention, or dry mouth. The authors concluded that mirabegron and solifenacin combination therapy provides an adequate therapeutic outcome without increasing the risk of adverse effects, thereby improving QOL for patients with OAB. They recommended this combination to achieve a balance between efficacy and tolerability.[16,17]

Mirabegron as a monotherapy has also proven effective. Daily doses of 25 mg, 50 mg, and 100 mg showed notable improvements from baseline in mean volume voided, urgency incontinence, and micturition frequency, with effects sustained throughout treatment. The most common adverse events reported were nasopharyngitis, hypertension, and urinary tract infections. Importantly, the occurrence of dry mouth was similar to that of a placebo and significantly lower than with tolterodine. Given that dry mouth is a common and distressing side effect of antimuscarinics, mirabegron may be a beneficial alternative. The advantages of mirabegron were also observed in older adults and in patients who were either treatment-naïve or had previously discontinued antimuscarinic therapy. As it can be used concurrently with other medications like alpha-blockers, mirabegron is poised to become a standard treatment for OAB.[16,17]

In the context of ureteral stents, another study found that combining tamsulosin and solifenacin was an effective treatment option for reducing ureteral stent-related symptoms (USRSs). A separate analysis showed that mirabegron monotherapy was also effective for USRSs. Furthermore, based on OAB questionnaire scores, mirabegron monotherapy was superior to both a combination therapy group and oral hydration alone for treating OAB symptoms associated with DJ stents.[18]

Our study has several limitations. First, there was a sex imbalance between the groups. Second, as a multi-center study within a single city, our population may not be fully representative of the broader national demographic. Finally, the four-week duration was sufficient to assess short-term efficacy and safety but not long-term outcomes. Despite these limitations, the study provides a general comparison of combination therapy mirabegron and solifenacin with monotherapy solifenacin for further studies.

CONCLUSIONS

Solifenacin monotherapy appears to provide superior short-term relief for urinary symptoms and general health issues compared to a low-dose solifenacin-mirabegron combination, based on the findings of this small-sample, single-city study. The combination therapy, however, demonstrated a targeted benefit in improving symptoms related to sexual function. Both regimens were generally safe and well-tolerated, with only mild adverse events reported. These results highlight the potential utility of solifenacin monotherapy in managing ureteral stent-related symptoms, while emphasizing the need for larger, multi-center, and longer-term studies to confirm efficacy and safety.

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CONFLICTS OF INTEREST

The authors have no conflict of interest to declare

AUTHORS' CONTRIBUTIONS

All authors read and approved the final version of the manuscript.

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ORIGINAL ARTICLE

Prevalence and Factors of Fall-Risk Increasing Drug Prescribing for Older Patients in Medical Wards

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ABSTRACT

INTRODUCTION: Falls represent a major health concern among older adults, frequently compounded by the use of fall-risk-increasing drugs (FRIDs). This study aims to assess the prevalence of FRID prescribing among older patients admitted to medical wards and at discharge in a Malaysian hospital and investigates the associations between patient characteristics and the prescribing of FRIDs. **MATERIALS AND METHODS:** This is a retrospective cross-sectional study involving 455 older patients aged ≥60 years. FRIDs were identified using the Screening Tool of Older Persons' Prescriptions in older adults with high falls risk (STOPPFall) criteria. Sociodemographic and clinical data, including polypharmacy and comorbidities, were analyzed for associations with FRID prescribing during admission and discharge. **RESULTS**: FRIDs were prescribed to 60.7% of patients during admission and 48.4% at discharge. During admission, FRID prescribing demonstrated significant associations with polypharmacy, prolonged hospitalization, and the presence of multiple chronic conditions. At discharge, FRID prescribing was significantly associated with polypharmacy and multiple chronic conditions. The most prescribed FRIDs during admission included diuretics (28.4%), opioids (20.7%), and sedative antihistamines (8.1%), with these patterns persisting at discharge. **CONCLUSION**: FRID prescribing is prevalent during admission and at discharge, highlighting the need for targeted interventions. Pharmacists can play a critical role in mitigating fall risks through comprehensive medication reviews, deprescribing, and patient education.

Keywords
Aged, Drug Prescriptions, Accidental Falls, Polypharmacy, Pharmacists

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INTRODUCTION

The global population of older adults is projected to more than double by 2050, exceeding 1.5 billion, with one in six individuals worldwide aged 65 years or older. This demographic shift has heightened the public health burden of falls, a significant concern among older adults. According to the World Health Organization (WHO), 28–35% of individuals aged 65 and above experience at least one fall annually, and as life expectancy increases, the incidence of fall-related injuries continues to rise.[1]

In Malaysia, falls are prevalent among older adults, with rates varying across populations and settings. The National Health and Morbidity Survey 2018 reported that 14.1% of Malaysian older adults (aged ≥60 years) experienced at least one fall in the past year.[2] The Malaysian Elders Longitudinal Research (MELoR) study estimated a fall prevalence of 18.9% among urban older adults (aged ≥55 years).[3] These findings underscore the urgent need for targeted interventions that address modifiable fall risk factors.

One of the key modifiable risk factors for falls is the use of medication. Certain medications, commonly referred to as fall-risk-increasing drugs (FRIDs), including

antihypertensives, sedative-hypnotics, antipsychotics, antidepressants, antihistamines, and opioids, can significantly elevate the risk of falls. This risk is mainly due to central nervous system effects—sedation, dizziness, impaired balance, and orthostatic hypotension. Multiple studies have consistently demonstrated the association between FRIDs and an elevated risk of falls. For example, the adjusted relative risk (ARR) of falls among patients prescribed FRIDs was reported to be 1.35.[4] Additionally, another study found that the use of at least one FRID was associated with higher incidence rates of total, injurious, and recurrent falls.[5]

The prevalence of FRID prescribing during hospital admissions has been reported to be high in various studies. For instance, a retrospective study in Ireland involving 162 inpatients aged ≥65 years referred to a Falls and Syncope service reported that 74.1% were prescribed at least one FRID during admission.[6] A study in Spain found an even higher prevalence, with 91.3% of 252 older adults admitted for fall-related fractures prescribed at least one FRID.[7] Additionally, a multicentre study of 1,147 patients hospitalized for heart failure revealed that 94% were taking at least one FRID at admission.[8]

The World Guidelines for Fall Prevention emphasize the importance of structured screening and assessment tools to identify FRIDs.[9] Tools such as the Screening Tool of Older Persons' Prescriptions (STOPP)[10] and the Screening Tool of Older Persons' Prescriptions in older adults with high falls risk (STOPPFall)[11] have been specifically recommended for this purpose. STOPPFall, developed by Seppala et al. using the Delphi technique, provides a comprehensive and explicit list of drug classes associated with an increased risk of falls in older adults. This tool supports deprescribing by offering practical guidance and decision trees to assist healthcare professionals in discontinuing potentially inappropriate medications in patients with a history of falls.[11] Studies utilizing STOPPFall in both inpatient and outpatient settings have reported the prevalence of FRIDs identified by this tool to range from 33% to 74.1%.[4,6,12]

To date, the applicability of the STOPPFall criteria in Malaysian healthcare settings remains unknown, and there is a lack of studies examining FRIDs in both inpatient and outpatient contexts within Malaysia. This study primarily aims to determine the prevalence of FRID prescriptions among older adults admitted to the medical wards of a Malaysian tertiary teaching hospital, both during admission and at discharge and to explore the characteristics associated with FRID use during admission and at discharge.

MATERIAL AND METHODS

Study design and setting

This retrospective cross-sectional study was conducted among older patients admitted to the four medical wards of Hospital Al-Sultan Abdullah (HASA) in Puncak Alam, Malaysia. HASA is a 400-bed public teaching hospital that provides comprehensive multidisciplinary healthcare services, including specialist outpatient clinics, diagnostic centres, an emergency department, and pharmacy services. The study procedures and findings were documented in accordance with the STrengthening the Reporting of OBservational studies in Epidemiology (STROBE) Checklist.[13]

Study population

This study analyzed data from eligible older individuals aged ≥60 years who were admitted to the general medical wards between January 2022 and December 2023. The inclusion criteria were patients admitted during the specified period, prescribed at least one medication during their hospital stay, and discharged with at least one medication. Individuals with incomplete or missing data were excluded from the analysis.

The sample size was determined using the formula: $n = [Z^2P(1 - P)]/d^2$, where n represents the sample size, Z is the Z statistic corresponding to a 95% confidence level (1.96), P is the expected prevalence of FRID use (60%, or 0.6)[12], and d is the margin of error (0.05).[14] Based on this formula, the minimum required sample size was determined to be approximately 369 participants. To account for the potential issue of incomplete medical records, a 25% adjustment was applied, resulting in a final target sample size of 460 participants for recruitment.

Sampling

A list of patients aged 60 years and older admitted to all medical wards between the study period was retrieved using the electronic hospital healthcare information system, UniMEDS. A total of 1035 older patients were identified, and each was assigned a unique identification number to ensure anonymity and facilitate data management. From this cohort, 460 patients were randomly selected for inclusion in the study using a random selection software tool. Following further evaluation, 455 patients were confirmed to meet the inclusion criteria.

Study tool

A self-designed standardized data collection form was developed to systematically gather relevant information. It comprised two sections: Section 1 included sociodemographic details and clinical information (e.g., number of chronic medical conditions, number of prescriptions, Morse Fall Scale [MFS] score at admission and fall history). Section 2 documented medications classified as FRIDs based on the STOPPFall criteria.[11]

MFS scores were extracted from medical records, as it is routinely assessed for all admitted patients at HASA. The MFS is a validated tool that evaluates fall risk on a numeric scale, incorporating factors such as fall history, medical conditions, use of ambulatory aids, intravenous therapy, gait patterns, and mental status. Scores range from 0 to 125, with standardized categories indicating low risk (0–24), medium risk (25–44), and high risk (≥45). Higher scores reflect a greater risk of falling.[15]

The STOPPFall is a structured tool designed to identify FRIDs. Developed via a Delphi consensus by European experts, it integrates evidence from meta-analyses and national fall prevention guidelines. The tool covers medication classes such as diuretics, alpha-blockers for hypertension, opioids, antidepressants, antipsychotics, antiepileptics, benzodiazepines, centrally acting antihypertensives, alpha-blockers for prostate hyperplasia, sedative antihistamines, vasodilators for cardiac conditions, and drugs for overactive bladder and urge incontinence.[11]

Study procedure

Upon identifying eligible patients, their sociodemographic information, clinical details, and medication usage were retrieved from the UniMEDS. The MFS scores were extracted from physical medical record files. Additionally, physical nursing charts and medication administration records were referred when necessary to supplement the electronic data. From each patient's medication list, FRIDs were identified and recorded based on the medication classes outlined in the STOPPFall tool. Data was extracted by the primary researcher (FMR), and where there was doubt, the cases were discussed with the co-researchers, and consensus was reached.

Data analysis

Data analysis was performed using the Statistical Package for the Social Sciences (SPSS, Version 29, IBM Corp). Descriptive analysis was conducted, with categorical variables presented as frequencies and percentages, and continuous variables expressed as mean ± standard deviation (SD). Patients were categorized into two groups: those prescribed no FRIDs and those prescribed one or more FRIDs. Inferential statistical analysis was conducted to examine the associations between FRID prescribing and patient characteristics, including sociodemographic and clinical factors, using the chi-square test (χ^2). A p-value <0.05 was considered statistically significant.

RESULTS

Sociodemographic and clinical characteristics of patients

Table 1 provides an overview of the demographic and clinical characteristics of the 455 patients included in this study. Most patients were females (53.4%), with a mean ± SD age of 70.91 ± 7.65 years (range: 60–97 years). The largest age subgroup comprised individuals aged 60–69 years (49.2%), followed by those aged 70–79 years (37.6%) and those aged 80 years or older (13.2%). Additionally, majority of the patients (86.6%) had two or more chronic medical conditions.

During hospitalization, a total of 4,897 medications were prescribed, with a mean ± SD of 10.76±5.09 medications per patient (range: 1–29). Polypharmacy was highly prevalent, as 90.3% of patients were prescribed five or more medications. At discharge, 3,229 medications were documented, with a mean ± SD of 7.10±3.79 medications per patient (range: 1–19), and 71.6% of patients continued to receive five or more medications.

The mean ± SD MFS score was 41.90±18.4, with patients categorized as low risk (14.1%), moderate risk (54.7%), and high risk (31.2%). A history of falls within the preceding three months was reported by 10.1% of patients, while 19.6% required the use of ambulatory aids. The mean ± SD length of hospital stay was 7.2±6.49 days.

Prevalence of FRID prescribing to study participants and its association with sociodemographic and clinical characteristics of study participants

Among patients admitted to the medical wards, 60.7% were prescribed FRIDs during their hospital stay, accounting for a total of 381 prescriptions. FRID prescribing during admission was significantly associated with the number of prescriptions (p<0.001), length of hospitalization (p=0.029), and the number of chronic medical conditions (p=0.007). At discharge, 48.4% of patients were prescribed FRIDs, amounting to 275 prescriptions. Significant associations were observed between FRID prescribing at discharge and both the number of prescriptions (p<0.001) and the number of chronic medical conditions (p=0.014).

Table 1. Sociodemographic and clinical characteristics of patients and their association with the prescribing of FRIDs

Characteristics	Total (n = 455)	During admission		p[a]	At discharge		p[a]
		No FRID (n = 179)	≥ 1 FRID(s) (n = 276)		No FRID (n = 235)	≥ 1 FRID(s) (n = 220)	
Gender							
Male	212 (46.6)	84 (46.9)	128 (46.4)	0.908	108 (46)	104 (47.3)	0.779
Female	243 (53.4)	95 (53.1)	148 (53.6)		127 (54)	116 (52.7)	
Age group (years)							
60 – 69	224 (49.2)	83 (46.4)	141 (51)	0.504	112 (47.7)	112 (50.9)	0.532
70 – 79	171 (37.6)	69 (38.5)	102 (37)		94 (40)	77 (35)	
≥ 80	60 (13.2)	27 (15.1)	33 (12)		29 (12.3)	31 (14.1)	
Ethnicity							
Malays	400 (87.9)	159 (88.8)	241 (87.3)	0.630	208 (88.5)	192 (87.3)	0.686
Non-Malays	55 (12.1)	20 (11.2)	35 (12.7)		27 (11.5)	28 (12.7)	
Number of chronic medical condition							
None	21 (4.6)	15 (8.4)	6 (2.2)	***0.007***	17 (7.2)	4 (1.8)	***0.014***
1	40 (8.8)	17 (9.5)	23 (8.3)		23 (9.8)	17 (7.7)	
≥ 2	394 (86.6)	147 (82.1)	247 (89.5)		195 (83)	199 (90.5)	
Number of prescriptions (during admission)[b]							
< 5	-	37 (20.7)	7 (2.5)	***<0.001***	-	-	-
≥ 5	-	142 (79.3)	269 (97.5)		-	-	
Number of prescriptions (at discharge)[b]							
< 5	-	-	-	-	87 (37)	42 (19.1)	***<0.001***
≥ 5	-	-	-		148 (63)	178 (80.9)	
Morse Fall Scale Score during admission							
Low risk (≤ 24)	64 (14.1)	28 (15.6)	36 (13)	0.613	40 (17)	24 (10.9)	0.161
Medium risk (25 – 44)	249 (54.7)	99 (55.3)	150 (54.3)		126 (53.6)	123 (55.9)	
High risk (≥ 45)	142 (31.2)	52 (29.1)	90 (32.6)		69 (29.4)	73 (33.2)	
History of a fall within the last 3 months							
No	409 (89.9)	158 (88.3)	251(90.9)	0.355	208 (88.5)	201 (91.4)	0.313
Yes	46 (10.1)	21 (11.7)	25 (9.1)		27 (11.5)	19 (8.6)	
Use of ambulatory aid(s)							
No	366 (80.4)	150 (83.8)	216 (78.3)	0.146	195 (83)	171 (77.7)	0.158
Yes	89 (19.6)	29 (16.2)	60 (21.7)		40 (17)	49 (22.3)	
Duration of hospitalization (days)							
≤ 7	318 (69.9)	136 (76)	182 (65.9)	***0.029***	169 (71.9)	149 (67.7)	0.318
8 - 14	104 (22.9)	36 (20.1)	68 (24.6)		53 (22.6)	51 (23.2)	
≥ 15	33 (7.3)	7 (3.9)	26 (9.4)		13 (5.5)	20 (9.1)	

[a] Chi-square test used.
b Total number was not reported since frequency during admission and discharge are different

Type of FRIDs prescribed during admission and at discharge

Table 2 presents the types of FRIDs prescribed during admission and at discharge, categorized based on the STOPPFall criteria. During admission, the most frequently prescribed FRID classes were diuretics (28.4%), opioids (20.7%), and sedative antihistamines (8.1%). At discharge, diuretics remained the most commonly prescribed class (24.6%), followed by opioids (11.2%) and sedative antihistamines (4.6%).

Table 2. Type of FRIDs prescribed during admission and at discharge (n=455)

FRID classes	n (%)	
	During admission	At discharge
Diuretics	129 (28.4)	112 (24.6)
Opioids	94 (20.7)	51 (11.2)
Sedative antihistamines	37 (8.1)	21 (4.6)
Alpha-blockers as antihypertensive	29 (6.4)	20 (4.4)
Antiepileptics	18 (4)	18 (4)
Vasodilators used in cardiac diseases	18 (4)	16 (3.5)
Antipsychotics	19 (4.2)	10 (2.2)
Alpha-blockers for benign prostate hyperplasia	14 (3.1)	15 (3.3)
Benzodiazepines and related drugs	18 (4)	9 (2)
Antidepressants	3 (0.7)	2 (0.4)
Centrally acting antihypertensive	1 (0.2)	1 (0.2)
Medication for overactive bladder	1 (0.2)	0 (0)

DISCUSSION

This study is the first in Malaysia to utilize the STOPPFall criteria to identify FRIDs prescribed during admission and discharge among older adults in medical wards of a tertiary teaching hospital. At admission, 60.7% of older patients received FRIDs, which were significantly associated with polypharmacy, hospitalization duration, and the number of chronic medical conditions. At discharge, 48.4% of patients were prescribed FRIDs, which were significantly associated with polypharmacy and the number of chronic medical conditions. Diuretics, opioids, and sedative antihistamines were the most prescribed FRIDs at admission and discharge.

In this study, the prevalence of patients with a history of falls within the previous three months was 10.1%, which is considerably lower than the prevalence reported in previous Malaysian studies involving larger samples of older people (14.1%–18.9%).[2,3] This disparity could be attributed to differences in the study periods: while the previous studies examined fall prevalence over the past 12 months, the current study focused on a shorter timeframe of three months. Notably, the use of FRIDs was not associated with a history of falls in this study, contrary to findings from earlier studies,[5,16] likely due to the limited sample size of patients with a history of falls in the present study.

The high prevalence of polypharmacy observed is noteworthy, with 90.3% of patients experiencing polypharmacy during admission and 71.6% at discharge. The average number of medications was 10.76 ± 5.09 during admission and 7.10 ± 3.79 at discharge. Freeland et al. reported that in patients aged ≥65 years with a history of falls, the risk of falls increases by 14% with each additional medication beyond four.[17] In this study, the prescribing of FRIDs was significantly associated with polypharmacy, consistent with prior findings.[8,18] This association is particularly concerning, as previous research has consistently demonstrated that the risk of falls escalates with the total number of medications, especially when FRIDs are included in the regimen.[19,20]

The high prevalence of FRID prescribing observed in this study aligns with previous reports, which range from 74.1% to 94% during admission [6-8] and 33% to 95.2% in outpatient settings.[4,12,18,21,22] Variations are influenced by care settings, patient characteristics, and criteria used to identify FRIDs. For instance, higher prescribing rates are frequently reported in orthopaedic and psychiatric settings due to the prevalent use of opioids and psychotropics.[23] Similarly, studies focusing on specific populations, such as patients with cardiovascular diseases or cancer, often report elevated use of cardiovascular-related FRIDs or opioids and antidepressants for symptom management.[8,22] Differences in the tools used to identify FRIDs further contribute to variability across studies.

A notable finding of this study is the concerning prevalence of FRIDs prescribed to high fall-risk patients and those with a history of falls, despite the absence of a significant association between FRID prescriptions and

these patient groups. Specifically, a substantial proportion of high fall-risk patients (63.4%, 90/142 during admission; 51.4%, 73/142 at discharge) and those with a history of falls (54.3%, 25/46 during admission; 41.3%, 19/46 at discharge) were prescribed FRIDs.

The widespread use of diuretics, opioids, and sedative antihistamines among our patients is consistent with findings from previous studies.[7,8,23,24] Diuretics, in particular, have been strongly associated with an increased risk of falls.[25,26] In our cohort, diuretic use showed a modest reduction from 28.4% during admission to 24.6% at discharge. A similar trend of slight reductions in prescribing rates was observed for other cardiovascular medications, such as alpha-blockers and vasodilators used for cardiac diseases. The limited decline may reflect deprescribing barriers, including concerns about symptom recurrence and lack of clear guidelines for managing cardiovascular disease in older populations.[27,28]

Opioids, the second most frequently prescribed FRIDs during admission and discharge, have been strongly associated with an increased risk of falls, particularly within the early days of initiation.[29] In this study, opioid prescribing decreased by approximately 45% from admission to discharge—a reassuring finding considering the well-documented challenges of deprescribing opioids, such as clinicians' time constraints and patients' concerns about pain recurrence.[30,31] This reduction may reflect the short-term use of opioids for managing acute pain and could also indicate an increasing awareness among clinicians of the risks associated with opioid use in older adults. Sedative antihistamines, though prescribed to only approximately 8% of patients at admission and 5% at discharge, should be used with caution due to their contribution to fall risk through adverse drug events (ADEs) such as light-headedness and somnolence.[32]

Our findings underscore the critical role of pharmacists in managing FRIDs. Pharmacists are well-equipped to conduct comprehensive medication reviews to identify and address FRIDs.[33] Significant associations between FRID prescribing at admission and patient characteristics such as polypharmacy, prolonged hospitalization, and multiple comorbidities highlight the importance of prioritizing these groups for medication reviews. Similarly, patients with a history of falls or identified as having a high fall risk should also be prioritized for such evaluations.

Pharmacists should minimize medications, ensuring that only essential ones are included, while reducing FRIDs to prevent falls in older patients.[20] The STOPPFall tool offers a systematic approach to identifying FRIDs and guiding deprescribing efforts.[11] In a study involving inpatients referred to a Falls and Syncope service where STOPPFall was utilized, nearly half of the patients prescribed a FRID had at least one FRID discontinued, and over a quarter of all FRIDs were deprescribed following review.[6] Furthermore, it is essential for pharmacists to adopt a more proactive role in patient education. Educating patients and caregivers about the potential risks associated with FRIDs and providing practical strategies to mitigate these risks remains a critical component of care.[34,35]

To improve clinical integration, the implementation of STOPPFall can be embedded into hospital workflows through pharmacist-led ward rounds and digital clinical decision support systems integrated within electronic health records. Furthermore, hospitals should consider incorporating STOPPFall assessments into discharge planning protocols to ensure safer medication regimens upon transition to home or community care settings.

The study has several limitations. Its generalizability is constrained by the small sample size and single-centre design. The focus on medical ward patients may underestimate FRID use in higher-risk populations, such as those with dementia or hip fractures. Moreover, the study primarily involved patients with low to moderate fall risk, with only 10% reporting a history of falls. Importantly, the data did not capture FRID dosages during admission, limiting the understanding of prescribing patterns. While a slight to moderate decline in FRID prescribing across most drug classes was observed, key details, such as dosage adjustments and treatment durations, were not documented. Future studies should address these limitations by including larger, more diverse

cohorts and accounting for dosage and treatment duration to provide a more comprehensive understanding of FRID prescribing practices.

CONCLUSION

The prevalence of FRID prescribing among older patients admitted to medical wards and upon discharge from a Malaysian tertiary teaching hospital is high. FRID prescribing during admission was significantly associated with polypharmacy, prolonged hospitalization, and the presence of multiple chronic conditions, while FRID prescribing at discharge was associated with polypharmacy and multiple chronic conditions. Diuretics, opioids, and sedative antihistamines were frequently prescribed during both admission and discharge. Prescribing rates showed a modest reduction at discharge for most drug classes. STOPPFall criteria application provides insights into FRID prescribing patterns. However, the single-center design limits the generalizability of the findings. Nevertheless, the findings of this study emphasizes the critical role of pharmacists in mitigating medication-related fall risks through comprehensive medication reviews and targeted patient education.

INSTITUTIONAL REVIEW BOARD (ETHICS COMMITTEE)

Ethical approval for the study was obtained from the Research Ethics Committee (REC) of Universiti Teknologi MARA (UiTM) ([PH]/PG/135/2024 [MR]), and authorization to conduct the research at HASA was granted (500-HUiTM [PJI.18/4/45]).

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ORIGINAL ARTICLE

Therapeutic Effect of Thymoquinone on MAPK Signalling Pathway in K562 Chronic Myeloid Leukaemia Cells

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ABSTRACT

INTRODUCTION: Chronic Myeloid Leukaemia (CML) is a hematopoietic malignancy caused by the BCR-ABL1 fusion oncoprotein, originating from Philadelphia chromosome translocation that enhances leukemic cell survival and therapeutic resistance. Aberrant MAPK pathway activation promotes proliferation and inhibits apoptosis, contributing to CML progression and resistance to tyrosine kinase inhibitors like imatinib with prolonged use. Thymoquinone (TQ), a bioactive molecule, has attracted considerable interest Zfor its anticancer characteristics that are worth investigating. **MATERIALS AND METHODS**: K562 CML cells were divided into an untreated group and a TQ-treated group and observed for 24 and 48 hours. Different TQ concentrations were administered to the TQ-treated group. Dose and time dependent effects on cell growth were assessed to evaluate cytotoxicity and determine the IC50 value in both groups. RNA was extracted from K562 CML cells based on the IC50 value and proceeded with RT-qPCR analysis on 7 genes involved, assigned as *Raf1, B-Raf, ERK1, ERK2, K-Ras, H-Ras,* and *N-Ras* genes, while the *beta-actin* gene was used as a housekeeping gene. Protein was extracted for the determination of protein and phosphorylation levels of Raf, MEK1/2, and ERK1/2, and assessed using the Jess Simple Western protocol. The Wilcoxon Signed-Rank test was performed using IBM SPSS, with $p<0.05$ considered statistically significant. **RESULTS**: TQ treatment significantly reduced the expression of all genes analysed in K562 cells. It also decreased protein and phosphorylation levels of Raf, MEK1/2, and ERK1/2. **CONCLUSION**: These findings suggest that TQ effectively inhibits MAPK signalling in K562 CML cells, highlighting its potential as a future treatment for CML.

Keywords
leukaemia, signalling pathways, thymoquinone, K562 CML, MAPK

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INTRODUCTION

Chronic myeloid leukaemia (CML), commonly referred to as CML, is a type of myeloproliferative neoplasm that arises from the stem cells of the haematopoietic system.[1] The onset of CML is driven by the BCR-ABL chimeric oncogene, which arises from a translocation between the breakpoint cluster region (BCR) gene on chromosome 22 and the Abelson murine leukaemia (ABL) gene on chromosome 9.[1] This translocation results in the formation of a fusion oncogene.[2,3] The constitutive activation of the BCR-ABL kinase plays a central role in CML pathogenesis by overactivating several downstream signalling pathways, including the MAPK pathway, ultimately causing uncontrolled proliferation of myeloid cells.[4,5]

Patients diagnosed with CML have shown a favourable response to tyrosine kinase inhibitors (TKIs) that target BCR-ABL, including imatinib (IM). Conversely, the overwhelming majority of individuals with CML do not achieve remission and may acquire resistance to TKIs

during prolonged treatment.[6,7] Consequently, diverse therapeutic strategies are necessary to address aberrant signalling pathways, which are crucial for the progression of chronic myelogenous leukaemia.[8]

The MAPK signalling pathway involves key protein kinases, including MEK, ERK, Raf and Ras which belong to multigene families. The Ras family consists of K-Ras, H-Ras, and N-Ras, while the Raf family includes Raf1, A-Raf, and B-Raf. MEK has five members (MEK1, MEK2, MEK3, MEK4, and MEK5), and ERK comprises four gene members (p38 MAPK, ERK1/2, ERK5 and JNK1/2/3). Among the ERK family, only ERK1/2 serve as the downstream target of MEK1/2 kinase activity.[9,10]

The MAPK signalling pathway plays a crucial role in regulating cell growth, apoptosis, and differentiation.[9,11] Its hyperactivation is a key contributor to the development of various cancers, including CML.[5,12] Consequently, targeting and inhibiting this pathway represents a vital therapeutic approach for managing CML.[13]

Natural phytochemicals have demonstrated potential epigenetic activity and may offer supportive benefits in leukaemia management, though further clinical validation is required to establish their efficacy and safety.[14] Among these, thymoquinone (TQ) a monoterpene quinone and the principal bioactive compound of Nigella sativa seeds has garnered attention for its broad pharmacological profile.[15,16] Biochemically, TQ exhibits antioxidant, anti-inflammatory, and anticancer properties. It exerts anticancer effects by modulating multiple molecular targets, including NF-κB, STAT3, PI3K/Akt, and MAPK pathways, and by inducing apoptosis, cell cycle arrest, and autophagy in various cancer cell lines.[17,18,19] In haematological malignancies, TQ has been shown to suppress proliferation and promote apoptosis in leukaemia cells by downregulating anti-apoptotic proteins (e.g., Bcl-2) and upregulating pro-apoptotic markers (e.g., Bax, caspases).[20] Although recent studies have explored its anticancer effects, the specific anti-leukaemia properties of TQ and the underlying mechanisms remain insufficiently understood. This study aimed to examine the effects of TQ in K562 CML cells, focusing on its impact on the expression of genes such as *K-Ras, H-Ras, N-Ras, Raf1, B-Raf, ERK1,* and *ERK2.*

MATERIAL AND METHODS

Cell Culture of K562 CML cell line

The BCR-ABL-positive K562 CML cell line, acquired from ATCC (American Type Culture Collection), was cultivated in a growth medium. This medium consisted of Roswell Park Memorial Institute (RPMI) 1640 medium (Nacalai Tesque, Kyoto, Japan), supplemented with 10% foetal bovine serum (FBS) (Tico Europe, Netherland) and 1% penicillin/streptomycin (Gibco, Thermo Fisher Scientific, United State America). Cells were incubated in T-25 and T-75 culture flasks at 37°C with 5% CO2 in a humidified environment. The culture medium was refreshed every 3-4 days to ensure adequate cell nourishment. For subsequent experiments, cells were subcultured and allowed to grow until they reached approximately 80% of their maximum capacity. To ensure reproducibility and account for biological variability, all experiments were conducted using three independent biological replicates derived from separate culture batches.

TQ Treatment of K562 CML cell line

TQ was purchased from Sigma Aldrich (Sigma Aldrich, USA). Stock solution was prepared by adding dimethyl sulfoxide (DMSO) in TQ as 39 mM and stored at -80°c for not more than a month. Appropriate working solutions were prepared by diluting the stock solution with complete RPMI-1640 culture medium. Final concentration of DMSO in culture media was less than 1%. A series of TQ concentrations (5, 10, 15, 20, 25 μM) were prepared from a stock solution diluted with RPMI-1640 culture medium and incubated with BCR-ABL-positive K562 CML for 24 and 48 hours for IC50 determination.

RNA Extraction of Treated and Untreated K562 CML cell line

Total RNA of cultured cells was extracted from both untreated cells and TQ-treated cells (16μm for 48 hours) by using miniprep kit from Zymo Quick-RNA (Zymo Research Coorporation, Murphy Avenue, Irvine, USA)

according to the protocol from manufacturer. Extracted RNA was evaluated the purity and concentration by using Nanodrop photometer (Thermo Fisher Scientific, USA).

Quantitative Reverse Transcription PCR (RT-qPCR)

cDNA was synthesized from 100 ng of total RNA using GoScript™ Reverse Transcriptase. Quantitative PCR (qPCR) was then performed using GoTaq® qPCR Master Mix and gene-specific primers. PCR reactions were carried out in a 20µL volume and included 2µL of cDNA template, 2µL primers (1µL forward, 1µL reverse), 5.8µL of RNase free water, 0.2µL of CXR dye and 10µL of qPCR MasterMix. Cycling settings consisted of an initial denaturation step (95℃, 2 minutes) followed by 40 cycles of denaturation (95℃, 15 seconds) and lastly annealing and extension (60℃, 1 minute). Data analysis was performed using StepOne Software v2.3 (Applied Biosystems, USA). β-actin serving as the housekeeping gene. All samples were measured in triplicate manner. Primer sequences were designed and verified using database in NCBI (Table I).

Table I: Sequence of primers employed in RT-qPCR for gene expression studies

Genes name	Primer sequence (5' – 3')	References
K-RAS	Forward: CTAGAACAGTAGACACAAAACAGG Reverse: CGAACTAATGTATAGAAGGCATC	(18)
H-RAS	Forward: TACGGCATCCCCTACATCGAGAC Reverse: CACCAACGTGTAGAAGGCATCCTC	(18)
N-RAS	Forward: GAGTTACGGGATTCCATTCATTGAAAC Reverse: TGGCGTATTTCTCTTACCAGTGTGTAAAA	(18)
B-Raf	Forward: AGAAAGCACTGATGATGAGAGG Reverse: TGGCGTATTTCTCTTACCAGTGTGTAAAA	(19)
RAF1	Forward: TATTGGGAAATAGAAGCCAGTGAAGTGA Reverse: AACATCTCCGTGCCATTTACCCTTATA	(18)
ERK1	Forward: CGCTTCCGCCATGAGAATGTC Reverse: CAGGTCAGTCTCCATCAGGTCCTG	(18)
ERK2	Forward: CGTGTTGCAGATCCAGACCATGAT Reverse: TGGACTTGGTGTAGCCCTTGGAA	(18)
β-actin	Forward: GAGCGCGGCTACAGCTT Reverse: TCCTTAATGTCACGCACGATTT	(20)

Protein Extraction

Total Protein was extracted from both untreated cells and TQ-treated (16µm for 48 hours) by using Macherey-Nagel NucleoSpin® RNA/Protein extraction kit (Macherey-Nagel, Germany) accordance to the guidelines from company. In this study, both treated and untreated K562 leukaemia cells were collected in 15mL Falcon tubes and centrifuged at 10,000 x g for 5 minutes. Following centrifugation, cell lysis was initiated by adding 350µL of Buffer RP1 supplemented with 3.5µL of β-mercaptoethanol to the cell pellet. The mixture was thoroughly vortexed to ensure proper mixing. The lysate was then transferred into a NucleoSpin® Filter within a collection tube and centrifuged at 11,000 x g for 1 minute. After centrifugation, the NucleoSpin® Filter was discarded, and 350µL of 70% ethanol was added to the flow-through. This mixture was then transferred into a NucleoSpin® RNA/Protein Column in a new collection tube and centrifuged again at 11,000 x g for 30 seconds. Next, the flow-through was moved to a fresh collection tube, where one volume of Protein Precipitation (PP) solution was added. For about 10 minutes, the sample was incubated at room temperature, allowing for adequate protein precipitation. After incubation, the sample was centrifuged at 11,000 x g for 5 minutes, and the supernatant was completely removed. To wash the pellet, 500µL of 50% ethanol was added, which was later centrifuged at 11,000 x g for about 1 minute. Following centrifugation, the supernatant was again discarded. The pellet was air-dried for 10 minutes at room temperature with the lid open to ensure complete drying. Subsequently, 50µL of PSB-TCEP was added to the pellet, and the sample was incubated at 96°C for 3 minutes. Finally, the sample underwent a final centrifugation for 1 minute, allowing for the recovery of the supernatant, which was then prepared for further analysis. This method provides a reliable process for extracting RNA and proteins from K562 leukaemia cells, with the use of specific buffers and centrifugation steps ensuring purity and efficiency in sample preparation.

Jess Simple Western Analysis for Protein Analysis

Protein analysis was conducted using the Jess Simple Western system (Biomed Global, Wilayah Persekutuan Kuala Lumpur, Malaysia), following the protocol from manufacturer. Preparation of cell lysate that achieves final concentration of 1mg/mL start by diluting 0.1X sample buffer and heating to 95°C for five minutes. Fluorescent 5X mastermix were then mixed with the prepared lysate at 4:1 ratio. The plate was then filled with a total of 3µL of each sample. The analysis utilized a cartridge with a size range of 12-230 kDa. Primary antibodies, including anti-Raf 1, anti-pMEK1/2, anti-ERK1/2, and anti-pERK1/2 (R&D Systems, Minneapolis, MN, USA), were diluted at a 1:10 ratio in antibody buffer to probe the target proteins. HRP-conjugated secondary antibodies were applied afterward. In each sample, primary and secondary antibodies were added with total volume of

both antibodies 10 µL. To remove bubbles, the plate was centrifuged at 1000x g for five minutes. The plate and capillaries were then placed into Jess analyser. In this analyser, process of automated protein separation, blocking, incubation of antibody, and signal detection occurs. Data analysis was conducted using the manufacturer -supplied Compass software. Protein normalization was achieved using a reagent that binds biomolecules to amine groups, eliminating variability due to inconsistent housekeeping protein expression. No control was required for the Jess system during this experiment.

SPSS Statistical Analysis

Wilcoxon Signed Ranked statistical test was performed to analyse Ras/Raf/MEK/ERK gene expression analysis and protein phosphorylation analysis in both TQ-treated and untreated K562 leukaemia cells. Data were presented as medians with interquartile ranges (IQRs), based on three independent biological replicates (n=3). Statistical analysis was conducted by using International Business Machines® (IBM) Statistical Package for Social Sciences® (SPSS) software, version 21 (IBM, USA) in which p-value <0.05 was considered significant.

RESULTS

TQ inhibits K562 CML cell proliferation

K562 leukaemia cells were treated with varying concentrations of TQ (5,10,15,20,25 µM) with incubation period for 24 and 48 hours in order to identify cytotoxicity of TQ on the cells. Based on the result, TQ concentration that inhibited 50% of cell viability (IC50) were 21.5µM after treatment for 24 hours and 16 µM after incubation for 48hours (Figure 1).

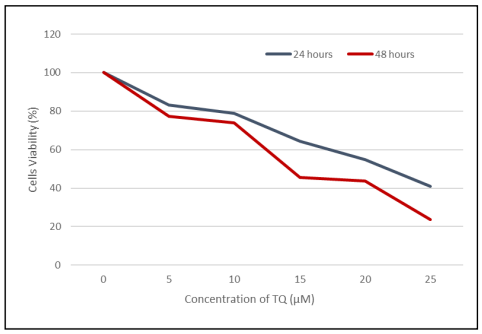

Figure 1: The suppression of K562 cell proliferation by thymoquinone in a dose and time dependent manner. Various concentrations of TQ (5 µM, 10 µM, 15 µM, 20 µM, 25 µM) were tested to evaluate its cytotoxic effects after 24 and 48 hours of treatment. The calculated IC50 values were 21.5 µM at 24 hours and 16 µM at 48 hours, respectively.

TQ reduces the expression of genes involved in MAPK Signalling pathway in K562 CML cells

Gene expression that involved in MAPK signalling pathway in both treated and untreated K562 CML cells was assessed with RT-qPCR. The results demonstrated a significant decrease in the expression of *Raf1, B-Raf, ERK1, ERK2, K-Ras, H-Ras,* and *N-Ras* genes in TQ-treated cells ($p<0.05$) compared to the untreated cells (**Figure 2, Table II, Table III**).

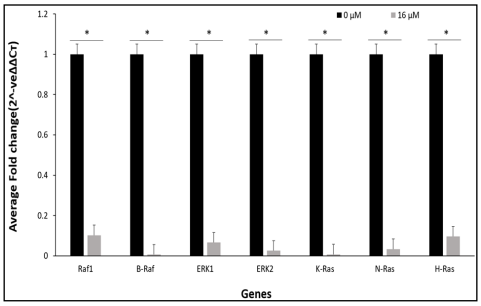

Figure 2: Gene expression analysis of genes participating in MAPK Signalling Pathway in K562 Leukaemia Cells. Analysis was done by using RT-qPCR. The graph shows the downregulation of all genes after 48 hours of incubation with 16 µM TQ in K562 leukaemia cells. Wilcoxon signed-rank test was used to determine the statistical significance. Data are presented as medians with interquartile ranges (error bars), based on three independent measurements (n=3) conducted on separate days to ensure reproducibility. *Denotes statistical significance when p is < 0.05.

Table II: Expression level of Untreated and Treated K562 Leukaemia Cells

Gene	Sample Type	ΔCr	ΔΔCr	Fold change(2^-veΔΔCr)
Raf1	Untreated	-4.04921659	0	1
	TQ Treated	-0.76298714	3.286229451	0.10250531
B-Raf	Untreated	-7.84446971	0	1
	TQ Treated	2.701745987	10.54621569	0.000668764
ERK1	Untreated	-3.92287572	0	1
	TQ Treated	0.004199982	3.927075704	0.065740411
ERK2	Untreated	-5.27196955	0	1
	TQ Treated	0.004199982	5.276169535	0.025805644
K-Ras	Untreated	-7.36014951	0	1
	TQ Treated	-0.25081126	7.109338252	0.00724229
N-Ras	Untreated	-3.96586545	0	1
	TQ Treated	0.937428157	4.90329361	0.033416545
H-Ras	Untreated	-3.18306732	0	1
	TQ Treated	0.190394592	3.373461914	0.096490993

Table III: Median Gene Expression Data for Untreated and Treated K562 Leukaemia Cells

Gene	n	Z-statistic[a]	Medians (IqR)		p-value [a]
			Untreated K562 cells	TQ-treated K562 cells	
Raf 1	3	-2.27	25.48 (0.28)	22.87 (0.34)	0.023
B-RAF	3	-2.27	21.96 (0.58)	26.22 (0.22)	0.023
ERK1	3	-2.22	22.25 (0.22)	23.20 (0.21)	0.026
ERK2	3	-2.22	24.33 (0.07)	23.46 (0.21)	0.026
K-RAS	3	-2.22	22.25 (0.22)	23.20 (0.21)	0.026
N-RAS	3	-2.22	26.58 (0.44)	23.70 (0.17)	0.026
H-RAS	3	-2.27	25.68 (0.09)	24.40 (0.18)	0.023

[a]Wilcoxon signed-rank test was employed in evaluate differences of statistical significance in gene expression between treated K562 leukaemia and untreated K562 leukaemia cells.

TQ inhibits MAPK Signalling pathway in K562 CML cells

Phosphorylation of protein that involved in MAPK signalling pathway in both untreated and TQ-treated K562 CML cells were evaluated by using Jess Simple Western Analysis. Results demonstrated reduction of the protein level of *Raf-1, p-Raf-1, MEK1/2, p-MEK1/2, ERK1/2* and *p-ERK1/2* in K562 cells after being treated

with TQ ($p<0.05$) as opposed to untreated K562 CML cells (**Figure 3 and Table II**).

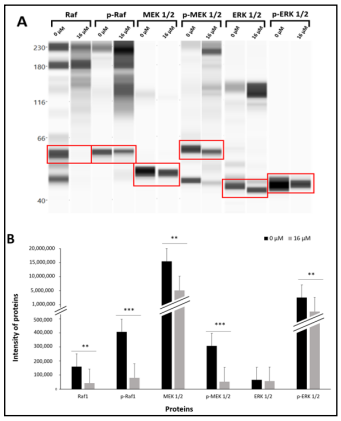

Figure 3: The effect of thymoquinone on the activation of MAPK signalling in K562 leukaemia cells. The cells were treated for 48 hours in incubator with 16µM TQ. Protein levels were quantified using Jess Simple Western protocol. Figure A illustrates the results of this analysis on target proteins before and upon treatment with TQ. Figure B presents the bar graph demonstrating a significant reduction in the protein levels of Raf-1, p-Raf-1, MEK1/2, p-MEK1/2, ERK1/2, and p-ERK1/2 in K562 cells upon TQ treatment. Statistical significance was determined using the Wilcoxon signed-rank test. Data are presented as medians with interquartile ranges (error bars) and are based on three independent measurements (n=3).
**Denotes statistical significance at $p<0.01$, while ***Denotes statistical significance at $p<0.001$ compared with untreated control cells.

Table IV: Medians of The Protein Expression levels within K562 Leukaemia Cells.

Proteins	n	M.Ws (kDa)	Protein Expression Levels medians (IqR)		p-value [a]
			Untreated K562 CML	TQ-treated K562 CML	
Raf 1	3	60	150,600 (31,413)	42,470 (29,423)	0.004
p-Raf 1	3	60	3,760,087 (450,379)	75,592 (11,561)	< 0.001
MEK 1/2	3	50	14,086,331 (614,461)	5,445,683 (153,350)	0.006
p-MEK 1/2	3	59	288,079 (67,531)	44,117 (12,621)	< 0.001
ERK1/2	3	45	61,216 (47,923)	53,680 (30,745)	0.037
p-ERK1/2	3	46	2,591,558 (202,440)	1,493,945 (112,560)	0.004

[a]Wilcoxon signed-rank test was employed in evaluate differences of statistical significance in protein intensities between treated and untreated K562 leukaemia cells.

DISCUSSION

CML is a type of myeloproliferative neoplasm marked by excessive granulocyte proliferation and the presence of the Philadelphia chromosome translocation, t(9;22)(q34;q11.2).[1,8] CML is characterized by the presence of the BCR-ABL1 fusion gene. The aberrant activation of MAPK signalling plays a critical role in the development of CML.[4] BCR-ABL induces the hyperactivation of numerous pro-oncogenic molecules involved in cellular signalling pathways including Ras, Raf, MEK, and ERK. Consequently, cell proliferation is augmented, while apoptosis and cell differentiation are markedly suppressed.[4,25]

TKIs have emerged as the most effective first-line therapy for CML.[26] TKIs are effective medications for treating patients with CML. Nonetheless, not all patients achieve long-term disease-free survival with TKIs, as some individuals exhibit intolerance to the medication.[27,28] Consequently, addressing abnormal signalling pathways critical to the progression of CML necessitates alternative therapeutic strategies.

Previous studies have shown that Thymoquinone (TQ) exhibits anti-cancer properties in various malignancies both in vitro and in vivo.[28] However, the specific mechanisms underlying TQ's anti-leukemic effects remain unclear. This study aimed to investigate the anti-leukemic effects of TQ in CML by examining its impact on the MAPK signalling pathway. This involved analysing the phosphorylation status and protein levels of key molecules in this pathway, as well as assessing the expression of genes involved in this signalling cascade.

MAPK signalling is known for its involvement to play crucial role in complex cellular programs such as differentiation, transformation, proliferation, development and apoptosis. The pathophysiology of CML is significantly influenced by the aberrant activation of the MAPK pathway.[12] Given the crucial role of genes within the MAPK signalling pathway in CML, this study aimed to elucidate the mechanisms underlying the anti-proliferative and apoptotic effects of TQ by assessing its ability to regulate the expression of *K-Ras, H-Ras, N-Ras, Raf1, B-Raf, ERK1,* and *ERK2* genes in K562 CML cells. The findings from this investigation revealed a significant decrease in the mRNA levels of *K-Ras, H-Ras, N-Ras, Raf1, B-Raf, ERK1,* and *ERK2* in K562 cells after treatment with TQ (Figure 3.2). These findings align with a previous study that demonstrated significant suppression of *BCR-ABL, JAK2, STAT3, STAT5A*, and *STAT5B* genes in K562 CML cells following TQ treatment.[29] The findings of the current study also agree with previously reported findings in which revealed TQ significantly inhibited *STAT3, STAT5a, STAT5b* and *JAK2* gene in HL60 cells after treatment with TQ.[30]

The constitutive activation of the MAPK pathway is significant in the progression of haematological malignancies, including CML. The BCR-ABL oncoprotein exhibits abnormal tyrosine kinase activity, which enhances the function of signal transduction pathways, such as MAPK signaling.[4,5,31] In turn, the suppression of activated proteins implicated in MAPK signalling is one possible therapeutic strategy for CML

treatment. This research evaluated the influence of TQ on the activation of the MAPK signalling pathway in K562 cells. The results indicated that TQ treatment caused a substantial decrease in the phosphorylation and protein levels of Raf, MEK1/2, and ERK1/2 in K562 CML cells (Figure 3.3 and Table 3.2).

These findings align with earlier research that demonstrated TQ-mediated suppression of STAT5, JAK2, Akt, STAT3, and PI3K phosphorylation and protein levels in K562 CML cells, HL60 AML cells and MV4-11.[16,31,33,34] While the reduction in total ERK1/2 protein levels was relatively modest, this is consistent with the established understanding that ERK1/2 activity is predominantly regulated through phosphorylation rather than total protein abundance. According to previous studies, ERK1/2 activation occurs via dual phosphorylation on threonine and tyrosine residues (Thr202/Tyr204 for ERK1 and Thr185/Tyr187 for ERK2), which induces conformational changes essential for downstream signalling functions such as proliferation, differentiation, and apoptosis.[34] Therefore, the observed decrease in phosphorylated ERK1/2 (p-ERK1/2) in this study is of greater functional relevance, indicating suppression of ERK activation rather than degradation of the protein itself. This distinction underscores the importance of evaluating both total and phosphorylated forms of signalling proteins when assessing pathway modulation.

Another study on other natural compound which is Asperuloside shows a significant reduction in the protein levels of RAF, RAS, MEK, p-MEK, p-ERK and ERK in K562 CML cells as opposed to the control group.[5] Similarly, a previous study using the phytochemical compound Hinokiflavone also demonstrated inhibition of ERK phosphorylation within the MAPK/NF-κB pathway in K562 cells, supporting the broader potential of phytochemicals in modulating this signalling axis.[35] In the present study, TQ at 16μM reduced cell viability by approximately 50% in K562 cells. While this concentration was effective in vitro, further investigation is required to determine its selectivity and safety profile in vivo, particularly regarding potential cytotoxicity toward non-malignant cells. Although TQ was dissolved in DMSO in this study, the final concentration was maintained below 1%, which is generally considered non-toxic and unlikely to interfere with MAPK signalling. Supporting this, a recent study reported that DMSO concentrations ranging from 0.25% to 1.5% did not result in cellular toxicity and produced results comparable to DMSO-free controls.[36] Nonetheless, the absence of a DMSO-only control in the present study limits definitive attribution of MAPK modulation solely to TQ. Future studies should incorporate vehicle controls to fully exclude solvent-related influences.

Although TQ's anticancer effects have been previously reported, its direct impact on MAPK gene and protein expression in CML remains incompletely explored. This study addresses this gap by demonstrating that TQ significantly downregulates key MAPK pathway genes and proteins in K562 cells, suggesting a mechanistic role in modulating oncogenic signalling. By providing evidence of TQ's ability to suppress both transcriptional and post-translational activation of MAPK components, this research offers new insights into its potential as a targeted therapeutic agent for CML. However, the study is limited by its use of a single cell line (K562), the absence of apoptosis assays to confirm downstream functional effects, and lack of in vivo validation. Future investigations should include multiple CML cell lines, assess apoptotic markers, and explore the efficacy and safety of TQ in animal models. Additionally, evaluating TQ in combination with TKIs may reveal synergistic effects and provide a basis for integrative therapeutic strategies.

CONCLUSION

The findings of this study demonstrate that TQ exhibits promising anti-leukemic activity against K562 CML cells. TQ treatment significantly inhibited cell proliferation, as evidenced by reduced cell viability. Gene expression analysis revealed that TQ downregulated key components of the MAPK signalling pathway, including *K-Ras, H-Ras, N-Ras, Raf1, B-Raf, ERK1*, and *ERK2*, indicating its potential to suppress oncogenic transcriptional activity. Furthermore, protein analysis using the Jess assay confirmed that TQ effectively reduced the phosphorylation

and protein levels of Raf, MEK1/2, and ERK1/2, suggesting inhibition of MAPK pathway hyperactivation a hallmark of CML pathogenesis. Collectively, these results support the hypothesis that TQ exerts its anti-proliferative effects through modulation of MAPK signalling. Given its ability to target critical molecular mechanisms involved in CML progression, TQ may serve as a potential therapeutic candidate, particularly for patients who exhibit resistance or intolerance to conventional tyrosine kinase inhibitors. However, given the limitations of the current study including the use of a single cell line, a single phytochemical agent, and short-term exposure, further investigations are warranted. Future studies should incorporate additional CML models, extended treatment durations, and in vivo validation to comprehensively assess the therapeutic potential and mechanistic specificity of TQ in targeting MAPK signalling in leukaemia. Further in vivo studies and clinical validation are necessary to establish its safety, selectivity, and translational applicability.

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ORIGINAL ARTICLE

Development and Validation of A Weight Management Program Model for Overweight and Obese Individuals

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ABSTRACT

INTRODUCTION: Developing a structured and theory-based model can enhance the effectiveness of weight management program. However, the underlying model requires validation to ensure its accuracy and applicability. Therefore, this study aimed to demonstrate the development and validation process of a weight management program model for overweight and obese individuals. **MATERIALS AND METHODS:** A weight management program model was previously developed using thematic analysis and scoping review by utilizing Theory of Change as the framework. Seven experts were invited to evaluated using the content validity index through online questionnaire, comprising 42 items, and provided feedback on its features for face validity. **RESULTS:** The developed model structured with 6 domains, 6 constructs, and 23 sub-constructs. More than 85.71% of the experts agreed on the face validity of the developed model. The content validity result indicated that the experts reached a consensus on all 6 domains. The modified kappa represented excellence in all 6 domains, with a k* value of 0.97-1.00. Meanwhile, the S-CVI/Ave and S-CVI/UA values ranged from 0.97-1.00 and 0.80-1.00, respectively. Several experts suggested ideas and recommendations for improvement. The validation led to all items being accepted without elimination. **CONCLUSION**: The weight management program model has been validated, and all 6 domains were retained following expert validation. This model is expected to provide insightful and valuable guidance for future practitioners in planning and executing weight management programs among individuals with overweight and obesity issues

Keywords
Content validity index, validation, model, obesity, weight management program

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INTRODUCTION

The increasing prevalence of overweight and obesity has become a worldwide concern. In 2022, approximately 43.0% and 16.0% of adults aged 18 years old and above were classified as overweight and obese, respectively.[1] This figure has doubled since 1990.[1] In Malaysia, the latest findings by the National Health and Morbidity Survey (NHMS) in 2024 revealed that 32.6% of the population was overweight, while 21.8% were obese. There has been a significant rise of almost 10.0% in the rates of overweight and obesity between 2011 and 2023.[2] Being overweight and obese may increase the likelihood of having various types of non-communicable diseases such as high blood pressure,[3,4] diabetes,[5] cardiovascular disease,[6,7] colorectal cancer,[7,8] and dementia.[9] Being overweight and obese also impacts the individual's social life and well-being[10] as well as affects the individuals' cost of living and socioeconomic status.[11]

Many strategies can be used to overcome this issue, such as implementing a weight management program. A weight management program serves as a strategy to assist individuals in losing weight, maintaining ideal weight, and avoiding unnecessary or uncontrollable weight gain.[12] This program generally promotes physical activity and healthy dietary practices to empower participants to manage their health. Organizations have been encouraged to collaborate and produce health interventions and activities to promote a healthy lifestyle.[13]

Theoretical frameworks provide an important foundation for behavioural changes within participants in weight management programs.[14] Previous studies have demonstrated that the theoretical and model-based weight management programs provide a reliable structure in achieving desired weight loss outcomes.[15,16] Additionally, participants also showed improvement in healthier dietary intake,[17] increased physical activity,[18] and reduced high calorie food consumption.[16] Therefore, developing a well-structured program to enhance its effectiveness to the target population is crucial, such as incorporating the program development with theory and model.

In this study, a weight management program model was constructed by incorporating several theories and models, such as i) Theory of Change (TOC), ii) Transtheoretical Model (TTM), iii) Social Cognitive Theory (SCT), and iv) Health Belief Model (HBM). Previous studies have demonstrated that integrating theories and models into a health-related program can increase the probability of positive outcomes.[18,19] In addition, a qualitative approach was utilized, which involved an in-depth interview with practitioners experienced in managing weight management programs.[20] This model was developed after combining themes from interviews and constructs of the existing theories and models. It comprised 6 domains, with 6 constructs and 22 sub-constructs and was written in the Malay language. However, a comprehensive evaluation was conducted for validation.

Validity refers to how a method accurately evaluates a variable to its intended measurement. It is advisable to conduct a validation test before data collection to ensure that the scales or scores can measure and reflect the variables precisely.[21] Among the types of validity are face validity and content validity. Some studies categorized these two as translation validity due to their primary purpose of determining the meaning of the construct by translating it into operationalization through subjective judgement (face validity) and assessment of the content domain (content validity).[22] The content validity index (CVI) and modified kappa can be applied as quantifiable methods to assess and evaluate consensus from experts.[23] Therefore, this study aimed to demonstrate the development and validation process of a weight management program model for overweight and obese individuals by obtaining expert agreement through the face and content validity scores.

MATERIALS AND METHODS

Study Design

This study employed a quantitative survey approach through an online questionnaire. The questionnaire validation process was conducted from July-August 2023. The research received ethical approval from the Secretariat of Research and Innovation at Universiti Kebangsaan Malaysia, with the reference number UKM PPI/111/8/JEP-2020-516. Before beginning the data collection, participants were provided with information sheets and signed a formal consent form.

Weight Management Program Model Development Process

To develop the model, the researchers used Theory of Change as a framework and its components, such as domain, construct, and sub-construct due to its suitability as guidance in planning a program and intervention.[24] Several suggestions from previous studies were used to determine the domains and constructs of this model, such as the input, activity, output, outcome, impact, and assumption.[25-27] Meanwhile, a sub-construct was built based on the themes acquired during the in-depth interview[20] and scoping review[28] from the previous phase of the study. Thematic analysis was obtained after conducting in-depth interview with practitioners who were experienced in handling the weight management program. Semi-structured questions were used as guidance to probe information such as activities that were helpful to modify behaviour and the outcomes of the program.[20] In the meantime, a scoping review was employed to identify health program in the existing literature which were guided by models and theories.[29] The scoping review synthesis found that the components from Transtheoretical Model (TTM), Social Cognitive Theory (SCT), and Health Belief Model (HBM) were frequently utilised in a successful health program especially among adults with overweight and obese. By

gathering information from the Theory of Change, thematic analysis and scoping review, the weight management program model comprised 6 domains, 6 constructs, and 23 sub-constructs was drafted, as shown in Figure 1.

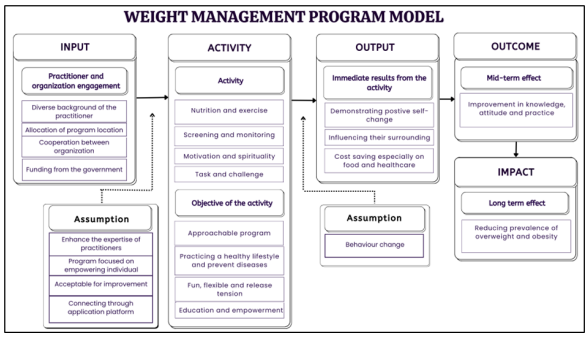

Figure 1: The weight management program model before validation

Face and Content Validity Assessment

A questionnaire was prepared based on the constructs and sub-constructs to validate the model. The questions were derived and elaborated based on the previous interview sessions with the practitioners, which came from coding, sub-themes, and themes. Two academicians specializing in health education and statistical analysis reviewed and refined the questionnaire before proceeding with face and content validity assessment. The face validity was assessed descriptively on various aspects, including the size and formatting, the ease of understanding the model, the appropriateness of the graphic used, overall neatness and layout of the model. Next, several questions were utilized to evaluate the content validity. Table I provides information regarding the domain, construct, and number of questions or items in the weight management program model.

Table I: Domain, construct, and number of questions or item in the model

Domain	Construct	Number of questions or items
A (Input)	Practitioner and organization engagement	5
B (Activity)	Activity Objective of the activity	23
C (Output)	Immediate results from the activity	4
D (Outcome)	Mid-term effect e.g., behavioural change effect from the output	3
E (Impact)	Long term effect	1
F (Assumption)	Condition that may affect the result	6
Total		42

The face validity and content validity data were collected from a panel of 7 experts. The term constructs the specific concept, properties, attributes, and variables being measured or studied.[28] Content validity can be used to measure the degree of agreement of an item that represents the content to validate the relevance of the constructs.[23] Hence, the constructs established in this study were measured using content validity according to the following six steps recommended by several studies[28,30] as shown in Figure 2.

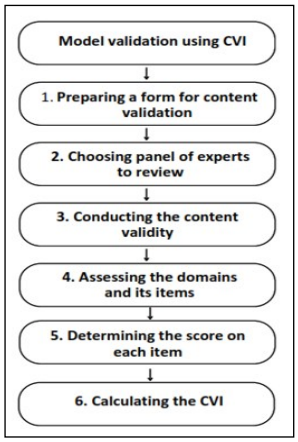

Figure 2: Validation steps using content validity index CVI

Preparation for content validation

The form was prepared with clear instructions and a summary of the model. The experts were provided with detailed explanations about the model's domain, construction, and items to ensure their understanding. Past research has recommended four main criteria be included in the measuring scores: i) Relevance, ii) Clarity, iii) Simplicity, and iv) Ambiguity (Table II).

Table II: Score for content validity evaluation[24,25]

Score	1	2	3	4
Relevance	Not relevant	Item need some revision	Relevant but need minor revision	Very relevant
Clarity	Not clear	Item need some revision	Clear but need minor revision	Very clear
Simplicity	Not simple	Item need some revision	Simple but need minor revision	Very simple
Ambiguity	Doubtful	Item need some revision	No doubt but need minor revision	Meaning is clear

Panel of experts to review

Experts were usually selected based on their positions in the public health system,[31] academic qualifications, years of experience, and field of expertise.[30] The experts in this study were chosen based on several criteria, such as having more than five years of working experience,[32] having qualifications and knowledge in the respective field, and having excellent communication skills.[33] Therefore, purposive sampling was used to seek experts from relevant fields, such as nutrition, sports science, and models or statistics. Recruiting six to ten experts was recommended to achieve a CVI value of 0.78.[34]

Hence, 7 experts were invited to participate in this study. They were; nutritionist (n=3), sports science (n=3), and statistician (n=1).

Conducting the content validity

Content validity can be conducted face-to-face or online.[28] The face-to-face approach involves scheduling a physical meeting with the expert, while an online approach requires the researcher to provide explicit instruction to facilitate the validation process.[28] Both methods have advantages and disadvantages, such as cost, response rate, and evaluation duration. The primary factor for using the online approach in this study was the duration of the evaluation. Coordinating the experts simultaneously due to their work schedule was a challenge. Therefore, an email containing information regarding the model, the model's figure, and the validation form was sent to all 7 experts. The experts were asked to evaluate the model using a Google Form provided in the email. They were given approximately one week to validate the model. However, some experts responded more than a week due to work and research commitments.

Assessing the domains and its items

The fourth step involved providing comprehensive information about the domains and items to facilitate the expert's comprehension of the model. Experts were encouraged to submit verbal and written opinions to improve the relevancy of the domains and their items.[28] In this study, all opinions were attentively considered for improvement.

Determining the score on each item

After reviewing the domains and items, the experts were asked to rate each item independently based on the scoring scale provided in Table II. They were also asked to complete the form in order to compute the CVI.[28] Hence, the validation form was created using Google Forms to ensure the experts rated all items.

Calculating the CVI

The obtained data were analysed by using Microsoft Excel. The calculation of content validity was divided into two categories, i) CVI for item (I-CVI) and ii) CVI for scale (S-CVI).[30] The I-CVI was the item-level validity index, which was the proportion of experts giving evaluation scores between 3-4.[30] A rating score of 3-4 by an expert will be converted into 1 mark. While a rating of 1-2 will be converted into a 0 mark. The formula for calculating I-CVI is to divide the items agreed by the experts by the number of experts (items agreed by the experts/number of experts).[28] Usually, the I-CVI value will exceed 0.78 if the number of experts exceeds 5 persons.[34]

The S-CVI is a scale level for the content validity index, which refers to the average of the I-CVI for all items on the scale.[30] There are two calculation methods for S-CVI, i.e. i) the average I-CVI score for all items in the scale (S-CVI/Ave) and ii) the proportion of items that received a rating score of 3-4 by all experts (S-CVI/UA).[30] S-CVI/UA is more sensitive to the number of experts, where there is a probability that S-CVI will be low when many experts are involved.[35] Therefore, this study follows the recommendation of at least 6 and no more than 10 experts for validation.[28,36] A greater CVI value indicates a greater level of consensus among experts. As suggested by previous studies,[37,38] CVI value of 1.00 indicate excellent, 0.90-0.99 is good, 0.80-0.89 has adequate indicator, and 0.70-0.79 showed an average validity acceptance.

Next, the modified kappa calculation was used to determine the probability of agreement among experts by calculating the index of agreement between experts.[23,39] In addition to CVI, previous studies suggest using kappa statistics because it will provide additional information on the agreement among experts.[39,40] Thus, the calculation of modified kappa is started by determining the probability of agreement (Pc) as follows:

$$Pc = [N! / A! (N-A)!] * .5^N$$

N = number of experts, A = number of experts that agree on the items

Subsequently, the modified kappa value was determined by the following formula:

$$k^* = \frac{(\text{I-CVI} - p_c)}{(1 - p_c)}$$

The results from the calculation will be interpreted using the following indicators: excellent (0.78-1.00), good (0.60-0.74), fair (0.40-0.59) and poor (below 0.39).[23,40,41]

RESULTS

Table III shows the background information of the experts (n=7), with 4 of them being males. The findings show that most of the experts have expertise in sports science (n=3) and nutrition (n=3). While only 1 have more than 11-15 years of experience, the majority have experience ranging between 5–10 years (n=3) and 16-20 years (n=3). Subsequently, the study findings were categorized into two main aspects, i) face validity and ii) content validity.

Table III: Expert's years of experience and field of expertise

Expert ID	Gender	Field of expertise	Experience
Expert 01	Male	Sport science	16 – 20 years
Expert 02	Female	Statistic	5 – 10 years
Expert 03	Male	Sport science	16 – 20 years
Expert 04	Male	Sport science	5 – 10 years
Expert 05	Female	Nutrition	5 – 10 years
Expert 06	Male	Nutrition	16 – 20 years
Expert 07	Female	Nutrition	11 – 15 years

Face validity

For face validity, the percentage of agreement among expert is 100% for two items which are *'the appropriateness of the graphics used'* and *'the layout and arrangement in the model'*; while *'the appropriateness of size and font type',* and *'the ease of understanding'* are 85.71%. Based on the comment and suggestions, Expert 02 and Expert 03 mentioned that the model has a clear flow and suitable, respectively. Meanwhile Expert 07 noticed a spelling error of the word 'positive', thus it was corrected by the research team.

Content validity

Altogether, 6 domains with 42 items in the weight management program model were scored by relevancy, clarity, simplicity, and ambiguity (Table IV). The S-CVI/Ave results were between 0.97-1.00, followed by S-CVI/UA between 0.80-1.00, and modified kappa between 0.96 -1.00. All 42 items were retained with none of the items were eliminated. However, some experts provided feedback regarding grammar mistakes and advised adding certain words to improve the construct.

Domain A (input) comprised 5 items describing the practitioner and organization's involvement in the weight management program. These items emphasized the practitioner's diverse skills, the importance of cooperation, and funding given by various organizations. The S-CVI results ranged from 0.8-1.00, while the modified kappa was 1.00. One of the sub-constructs, "*funding from the government*", was commented on by Expert 06. Expert 06 mentioned that the funding may come from various organizations, not necessarily from the government. Hence, this construct was edited and changed into "*funding from various organizations*".

Domain B (activity) comprised 23 items that outlined the activities and objectives needed in the program. This domain consisted of 2 constructs with 8 sub-constructs. The values of S-CVI and modified kappa were between 0.96-0.99. Expert 05 suggested adjusting 2 sub-constructs in the model. For the sub-construct *"approachable program,"* Expert 05 recommended adding the term "*doable program*" in the sub-construct due to the possibility that an approachable and doable program can enhance respondent participation. For the sub-construct "*fun, flexible, and relieving stress*," the expert suggested changing the phrase "*relieving stress*" to "*reducing stress*" because it is a more achievable objective in the context of a weight management program. Thus, the terms were added and changed as suggested.

Domain C (output) explained the direct outcomes of the activity from the program, such as positive self-changes, how participants can positively impact their surroundings, and how to reduce treatment costs and daily expenses. The results of the CVI value modified kappa were between 0.97-1.00. The word "positive," was wrongly spelt but has been amended.

Domain D (outcome) contained a single construct representing medium-term changes after following the program. Domain E (impact) also comprised a single construct representing long-term changes, such as decreasing the prevalence of overweight and obese adults. The results of both domains were similar, i.e., 1.00 for the CVI and modified kappa values. There was no

Table IV: Results of CVI

Domain	Relevancy			Clarity			Simplicity			Ambiguity			Indicator
	S-CVI / Ave	S-CVI / UA	k*	S-CVI / Ave	S-CVI / UA	k*	S-CVI / Ave	S-CVI / UA	k*	S-CVI / Ave	S-CVI / UA	k*	k*
A (Input)	1	1	1	0.97	0.80	0.97	1	1	1	1	1	1	Excellent
B (Activity)	0.99	0.96	0.99	0.99	0.96	0.98	0.99	0.96	0.99	0.99	0.96	0.98	Excellent
C (Output)	1	1	1	0.97	1	0.96	0.97	1	0.96	0.97	1	0.96	Excellent
D (Outcome)	1	1	1	1	1	1	1	1	1	1	1	1	Excellent
E (Impact)	1	1	1	1	1	1	1	1	1	1	1	1	Excellent
F (Assumption)	1	1	1	1	1	1	0.98	0.83	0.97	1	1	1	Excellent

additional comment for Domain D (outcome). Meanwhile, Expert 05 provided remarks on the sub-construct for Domain E, "*Reducing prevalence of overweight and obesity*." One term was missing, i.e., "*adults*." It was added to the sub-construct of "*Reducing prevalence of overweight and obese adult*." Another expert suggested adding one sub-construct in the domain, i.e., "*Reducing the government's burden in addressing obesity and its associated diseases*." The two comments were considered and added to the model. Lastly, Domain F (assumptions) comprised 5 constructs and was evaluated by 6 items. The CVI and modified kappa results were 0.83–0.98 for simplicity, while the others scored 1.00.

DISCUSSION

This study demonstrated the development and validation process of a weight management program model for overweight and obese individuals by obtaining expert agreement through the face and content validity scores. The weight management program model was previously developed by integrating several sources, such as thematic analysis from in-depth interviews, scoping reviews, and the Theory of Change. The primary purpose of developing this model was to facilitate the practitioners to create, manage, and implement a comprehensive and efficient weight management program. By using this model as guidance, practitioners will be able to cater to all the items needed in a weight management program. Based on recommendations from previous studies, it is crucial to carry out a validation process to validate the product.[42] While CVI is not commonly used for model evaluation, it can still provide a reliable and validated model.[42-44] Prior studies used CVI to evaluate various types of models and frameworks, such as the adoption model for wearable Continuous Glucose Monitoring System (CGMs) device adoption,[43] learning model for basketball passing,[42] pain and disability driver's management model,[44] nursing professionalism evaluation model,[45] and medical education e-professionalism framework.[46] Hence, CVI is deemed appropriate for evaluating a model's domains.

A model plays a vital role in offering a structured framework to establish the relationship between variables,[47] which also assists researchers in various phases of research. The weight management program model for overweight and obese individuals was developed to offer a guideline for the practitioner or program developers to follow while implementing a weight management program. The program model comprises 6 domains that could assist the practitioner in preparing a plan before or during the program. Additionally, some domains, such as output, outcome, and impact, are anticipated results after the program has been completed. A model serves as the foundation for identifying the variables, factors, and their relationship that affect the phenomena.[47] Thus, this model is hoped to serve as a reference in identifying essential variables and relationships that may influence the development of a weight management program.

The minimum score of 0.97 that was achieved for S-CVI/ Ave is classified as 'excellent'. It indicates that the item was well understood, the construct was accurately measured, and there was a significant consensus among experts. Hence, no revisions or deletions were needed, as the score exceeded 0.78 by more than 6 experts, as suggested by previous studies.[23,39] Additionally, kappa statistics are a valuable component of CVI because they offer information about the level of consensus. Therefore, the kappa statistic is an indicator of agreement among the inter-raters.[39] The comparison of the kappa statistics results with the indicators in all domains of the weight management program model achieved consensus

among the experts, and all the domains were in the 'excellent' categories.

This study has identified several strengths, such as developing a weight management program model with the foundation by several sources. Using Theory of Change as framework to described the relationship between the domain, construct and sub-construct has significantly increased the model's interpretability. Next, the content validity index (CVI) is a valuable method for obtaining expert validation of the research product, such as a model. The result demonstrated that the expert's agreement is significant for validating the model. Despite thorough validation, this research has some limitation. The number of experts involved was relatively small, which may limit the generalizability of the findings. Although including more experts could improve representativeness, it may also reduce consensus and lower content validity value. Furthermore, gathering the experts was challenging due to their tight schedules. Hence, utilizing a panel of 6-10 experts is reasonably appropriate for validating the model.

CONCLUSION

The weight management program model for overweight and obese individuals was developed, validated, and reached a consensus among 7 experts from various backgrounds. The model was developed as a strategy and guideline for weight management programs, mainly focusing on overweight and obese adults. Upon validation, the model is ready for the next step, such as an acceptance test among practitioners and participants or to be developed into a module that can be used for the weight management program or intervention.

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ORIGINAL ARTICLE

Knowledge and Awareness of Jordanian Speech and Language Pathologists on Radiation Protection while Performing Videofluoroscopic Swallowing Study

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ABSTRACT

INTRODUCTION: Videofluoroscopic swallowing studies (VFSS) are essential diagnostic tools used by speech-language pathologists (SLPs) to assess dysphagia. However, VFSS involves ionising radiation exposure, making comprehensive radiation safety knowledge critical for minimising risks to both patients and healthcare providers. This study evaluates radiation protection knowledge and practices among Jordanian SLPs performing VFSS. **MATERIALS AND METHODS:** A cross-sectional survey was conducted among 115 Jordanian SLPs who perform VFSS. The validated 22-item questionnaire assessed demographics, professional experience, radiation safety knowledge, and current practices. Data was analysed using descriptive statistics and cross-tabulations to examine relationships between education level, experience, and radiation exposure practices. **RESULTS:** Significant knowledge gaps were identified in fundamental radiation safety principles. Only 20.9% of respondents accurately estimated the appropriate exposure times (≤5 minutes), while 38.3% overestimated the VFSS duration to 16-20 minutes. Although 35.7% reported using pulsed fluoroscopy, 34.8% were uncertain about their equipment type. Only 27.8% correctly identified scattered radiation as the primary exposure hazard. While basic protective equipment knowledge was adequate comprehensive shielding understanding was limited. Educational background influenced duration estimates, with master's degree holders reporting longer procedures compared to bachelor's degree practitioners. On-the-job training was the predominant source of radiation safety knowledge (46.1%), yet overall self-rated competency remained moderate to low. **CONCLUSION:** Jordanian SLPs demonstrate inconsistent radiation safety knowledge with significant gaps in exposure time limits, equipment specifications, and comprehensive protective practices. These findings highlight urgent needs for structured radiation safety education, standardised national guidelines, and interprofessional training programs to ensure safe VFSS procedures.

Keywords
Videofluoroscopic Swallowing Study, Dysphagia, Radiation protection, Speech-language Pathologists, Jordan, Patient safety

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INTRODUCTION

Videofluoroscopic swallowing study (VFSS) is a critical instrumental assessment routinely performed by speech-language pathologists (SLPs) to evaluate dysphagia.[1] In this procedure, various food and liquid consistencies mixed with contrast material are presented to patients during an X-ray to assess swallowing anatomy and physiology. This procedure also facilitates the evaluation of swallowing manoeuvres and therapeutic strategies for managing dysphagia.[2] However, VFSS exposes both patients and healthcare professionals, including SLPs and radiologists, to ionising radiation. While the primary X-ray beam is directed toward the patient, scattered radiation resulting from deflection off the patient and surrounding surfaces poses potential exposure risks to

nearby healthcare workers.[3] Although scattered radiation doses typically remain below the annual occupational exposure limit of 20 millisieverts (mSv), cumulative exposure over time can heighten the risk of radiation-induced cancers.[4] Minimising radiation exposure during VFSS primarily involves three strategies: limiting exposure time, maximising distance from the radiation source, and employing appropriate protective shielding such as lead aprons and thyroid guards.[5-7] Additionally, radiologists must undergo specialised radiation safety training and use dosimetry badges to ensure they remain within safe exposure limits.[8]

While the American College of Radiology has provided radiology-focused guidelines,[9] a systematic review of VFSS protocols highlighted the lack of input from multidisciplinary teams and the insufficient evidence supporting many of the recommendations.[10] In response, the American Speech-Language-Hearing Association (ASHA) has delineated clear responsibilities for SLPs to enhance radiation safety during VFSS, emphasising collaboration with radiology teams, adherence to the As Low As Reasonably Achievable (ALARA) principle, and regular safety training.[11] Despite the availability of these robust international guidelines and recommendations, their implementation varies considerably across different healthcare settings and geographical regions. This variability is particularly pronounced in resource-limited contexts where institutional infrastructure, professional training frameworks, and regulatory oversight may differ substantially from settings where these guidelines were originally developed. Consequently, even with strong international guidance, translating these standards into consistent local practice remains challenging and highly context-dependent. In Jordan, the absence of standardised guidelines for VFSS conducted by SLPs has resulted in substantial practice variability, shaped more by individual clinician preferences than evidence-based standards.[12] This inconsistency is concerning as it can compromise assessment accuracy and increase unnecessary radiation exposure risks. Furthermore, limited hospital integration and variability in professional training exacerbate this issue, creating significant disparities in radiological knowledge and safety practices among SLPs.[13] Therefore, this study assesses the current knowledge and practices of Jordanian SLPs regarding radiation protection during VFSS. The aim is to identify gaps in awareness and promote safer, more standardised VFSS procedures, ultimately minimising radiation risks for both patients and practitioners.

MATERIAL AND METHODS

A cross-sectional survey was conducted among Jordanian SLPs who perform VFSS. Ethical approval was obtained from the University of Kebangsaan Malaysia ethics committee (JEP-2023-785). Participants had to meet the inclusion criteria, which included holding a recognised qualification in speech-language pathology, certification from the Jordan Ministry of Health (MOH), and actively managing dysphagia cases. Technicians, defined as bachelor-level SLPs licensed by MOH to treat dysphagia under specialist supervision, were also included. Exclusion criteria included academic professionals without clinical caseloads, SLPs working abroad, and those without active dysphagia caseloads for the past three months. A simple random sampling method was employed, with an estimated sample size of 114 participants calculated using Andrew Fisher's Formula.

The questionnaire was distributed openly between February and April 2024 through multiple online platforms, and a total of 115 SLPs who met the inclusion criteria completed the survey. While a specific response rate could not be calculated due to the open distribution method, the sample size exceeded the calculated requirement and represents a substantial proportion of practising SLPs in Jordan who perform VFSS procedures. To minimise selection bias, several strategies were implemented: (1) distribution of the questionnaire through multiple channels including various social media platforms and WhatsApp groups to maximise reach across different practice settings; (2) clear specification of inclusion and exclusion criteria to ensure only eligible participants completed the survey; (3) recruitment of participants from diverse work environments and (4) voluntary participation with informed consent to reduce coercion bias. The survey consisted of 22 multiple-choice questions covering demographics, professional experience, frequency of

radiological exposure, safety practices, and competency levels regarding VFSS radiation exposure. Originally developed by Russell and System,[14] it was administered online in the English language.

Data Analysis

Survey responses were analysed descriptively using Microsoft Excel, with cross-tabulations conducted using the Statistical Package for the Social Sciences (SPSS) version 29.0 (IBM Corp., Armonk, NY, USA) to examine associations between education level, VFSS experience, and radiation exposure time.

RESULTS

A total of 115 participants who graduated and trained in Jordan completed the survey (Table I). Most respondents (71.3%) work at speech and language pathology centres and hold a technician license (53.2%). The educational backgrounds varied, with 37.6% holding bachelor's degrees, 15.6% holding master's degrees, and 4.4% holding PhDs. On-the-job training was the primary source of radiation safety knowledge for nearly half (46.1%) of the respondents. Dysphagia services were primarily provided to adults and geriatrics (65.2%), with fewer services offered to paediatrics (29.0%) and infants (6.2%). Most respondents had between 2 to 5 years of VFSS experience (33.9%), followed by those with over five years of experience (30.4%). However, overall, they reported performing fewer than five procedures per month, indicating limited clinical exposure despite years in practice.

Table II presents key findings on radiation knowledge and practices. Only 20.9% of respondents correctly estimated the radiation exposure time as ≤5 minutes, which aligns with ASHA guidelines, while the majority (38.3%) overestimated the exposure time at 15-20 minutes. Compliance with ASHA's recommended exposure limit (≤5 minutes) was low, with only 23.5% adhering and 41.7% unaware of the guideline. The understanding of radiation sources was limited, with 34.8% of respondents uncertain about the primary source of exposure.

Table I: Demographic Information Questions of Jordanian SLPs (N = 115)

	Frequency	Percentages
What is your primary work setting?		
Speech and Language Pathology Centre	82	71.3
Special Education Pathology Centre	4	3.5
School or nursery	6	5.2
Hospital	12	10.4
Private (Home services)	11	9.6
What occupational licenses do you hold? (multiple answers allowed)		
Jordanian Ministry of Health Specialist	50	32.0
Jordanian Ministry of Health technician	83	53.2
Certificate of Clinical Competence in Speech-Language Pathology (CCC-SLP)	6	3.8
Clinical fellowship-SLP	3	1.9
Member of Special Interest Group	1	.6
Board-Recognised Swallowing Specialist	1	.6
What is/are the age groups you provide dysphagia services for? (multiple answers allowed)		
Provide dysphagia services for infants	10	6.2
Provide dysphagia services for paediatrics	47	29.0
Provide dysphagia series for adults/geriatrics	91	65.2
I am in an administration position / supervisory role	4	2.5
None applies to me	10	6.2
Please select the following that apply to you.		
Completed a bachelor's degree in SLP	77	37.7
Completed master's degree in SLP	32	15.6
Completed PhD in SLP	9	4.4
Completed a medical internship with VFSS exposure	3	1.4
Mentored graduate interns applying for VFSS	8	3.9
Mentored colleagues applying VFSS	21	10.3
Taught a graduate-level course on dysphagia	12	5.9
Lectured at the Jordan or national level on dysphagia.	39	19.1
It does not apply to me.	3	1.4
Where did you receive your radiation safety knowledge?		
Academic setting	49	29.3
Medical practicum	18	10.8
On-the-job training / in-services	77	46.1
Conferences and Seminars	2	1.2
Journals / Independent Study	21	12.6
How many years of experience performing VFSS?		
Less than one year	27	23.5
Between 1-2 years	14	12.2
Between 2-5 years	39	33.9
More than five years	35	30.4
What is the average number of VFSS completed per month?		
Less than two per month	54	47.0
3-5 patients per month	36	31.3
6-10 patients per month	18	15.7
More than ten patients per month	7	6.1

Competency in distance as a protective factor was low (M=2.02, SD=0.95), and understanding of exposure time management was similarly limited (M= 1.95, SD=0.93). Regarding collimation, only 20% correctly associated it with exposure reduction, while 32.2% erroneously believed it compromised image quality.

Table II: Levels of Radiation Knowledge and Practices Among Jordanian SLPs Questions.

	Frequency	Percentages
What is the estimated average radiation exposure time for the VFSS that you complete?		
< 5 minutes	24	20.9
6 - 10 minutes	9	7.8
11 - 15 minutes	25	21.7
16 - 20 minutes	44	38.3
> 21 minutes	1	0.9
I do not know	12	10.4
What is the type of fluoroscopy used?		
Pulsed Fluoroscopy	41	35.7
Continuous fluoroscopy	33	28.7
High-dose fluoroscopy	1	0.9
Unsure	40	34.8
Where does most of the radiation come from (excluding radiation beams)?		
Scattered Radiation	32	27.8
Sensitive Radiation	27	23.5
Termination Radiation	16	13.9
I do not know	40	34.8
Increasing the distance between the SLPs and the patient will		
Reduces radiation exposure to SLP	33	28.7
Increases the SLP's exposure to radiation	18	15.7
Increases the patient's exposure to radiation	34	29.6
I do not know	30	26.1
How should the staff alter her position to reduce radiation exposure?		
Take two steps backwards	12	10.4
Turn 90 degrees to face the patient	33	28.7
Turn 90 degrees to face the patient and take 2 to 3 steps back	27	23.5
I do not know	41	35.7
What is/are the additional shielding that should be used by staff to reduce radiation exposure?		
Staff 1-apron	2	1.7
Staff 3 Apron	3	2.6
Staff 2-Lead Gloves	3	2.6
Staff 1-Lead Gloves	31	27.0
Staff 3-Thyroid Shield	29	25.2
Staff 2-Thyroid Shield	6	5.2
Current shielding is sufficient	28	24.3
I do not know	13	11.3
When should the grid be removed?		
a 4-year-old patient	11	9.6
19-year-old-patient	19	16.5
a 40-year-old patient	32	27.8
I do not know	46	40.0
an 88-year-old patient	6	5.2
Collimation decreases radiation to the patient by?		
Increasing the field of exposure	14	12.2
It has no bearing on the field of exposure	23	20.0
Narrowing the field of exposure	23	20.0
Reduces the quality of the image	37	32.2
I do not know	18	15.7
What impact does magnification have on radiation exposure during the VFSS?		
Magnification increases the radiation dose to the patients	23	20.0
Magnification reduces the radiation dose to the patient	20	17.4
Magnification does not impact the radiation dose	28	24.3
I do not know	43	37.4
What will occur if lead-lined gloves are placed in the path of the primary X-ray beam?		
The fluoroscopy unit will automatically decrease the intensity of radiation	17	14.8
The fluoroscopy unit's intensity of radiation remains the same	32	27.8
The fluoroscopy unit will automatically increase the intensity of radiation	19	16.5
I do not know	47	40.9
Which of the following could be used to limit radiation exposure during the VFSS?		
Implement magnification mode to decrease exposure; SLP and Radiologist / Tech coordinate when to do the fluoroscopy	35	30.4
Use an intermittent beam-on/off during imaging	33	28.7
I do not know	47	40.9
Per ASHA's recommendation, the fluoroscopy time should not exceed how many minutes?		
≤ 5	27	23.5
> 5	40	34.8
I do not know	48	41.7
When positioning patients in the field of interest, the operator should?		
Approximate placement of the fluoroscope and intermittently exposing the patient to radiation	31	27.0
Continuously exposing patients to radiation to fine-tune their fields of interest	17	14.8
Increase the patient's distance from the fluoroscope and use continuous exposure to fine-tune the field of interest	9	7.8
Use magnification to ensure the appropriate field of interest is obtained in a timely manner	21	18.3
I do not know.	37	32.2

Table III presents the mean scores and standard deviations for the knowledge and practices of Jordanian SLPs related to radiation safety and dosimetry tools. Knowledge of dosimetry tools was highest for fetal badges but lower for wrist badges. Primary shielding, such as lead aprons and thyroid shields, was widely used, while the use of lead gloves and eye protection needs improvement. Collaboration with radiology technologists was strong; however, knowledge of collimation and magnification was lacking, with 12.2% and 37.4% of respondents being unaware of their effects. Confidence in discussing radiation risks with families was low. The primary sources of knowledge included radiology technologists and SLP co-workers, while radiation safety officers and medical physicists were less frequently cited.

Table III: Knowledge and Practices Regarding Radiation Safety and Dosimetry Tools

Dosimetry tool	Mean	SD	Rank	Importance Level
Fetal dosimetry badge (during pregnancy)	1.98	0.91	1	High
Dosimetry badge (outside lead)	1.90	0.86	2	Medium
Dosimetry badge (under lead)	1.77	0.79	3	low
Ring or wrist dosimetry	1.48	0.79	4	low
Shielding options use				
Piece lead vest/apron	3.06	1.07	1	High
Thyroid shield	2.94	1.00	2	Medium
Piece lead vest / pelvic apron (not pregnant)	2.46	1.04	3	Medium
Piece lead vest / pelvic apron (during pregnancy, if applicable).	2.38	0.97	4	Medium
Remain in the control booth / behind other glass lead barriers.	2.09	0.83	5	Medium
Lead eye shields or lead glasses	1.39	0.72	6	Low
Lead gloves.	1.35	0.73	8	Low
Team members who operate during VFSS.				
SLPs	3.22	1.03	1	High
Radiology Technologist	3.17	1.03	2	High
Radiologist	2.68	0.94	3	Medium
Radiation exposure limits				
SLP requests continuation of VFSS beyond radiation exposure.	1.88	0.88	1	Low
SLP ends VFSS before satisfaction because radiation exposure limits exceed ASHA / institutions' radiation exposure guidelines.	1.77	0.85	2	Low
The level of time families express their concerns regarding radiation associated with the VFSS and SLPs' confidence in responding in descending order.				
SLP's confidence in responding to families	2.36	0.96	1	Medium
Families express their concerns regarding radiation associated with the VFSS	1.98	0.78	2	Low
Indicate the percentage of radiation safety and knowledge you received from the following professionals.				
Radiology technologist	3.24	1.39	1	Medium
SLP co-worker and SLP supervisor	3.18	1.17	2	Medium
Graduate school dysphagia faculty	2.60	1.07	3	Medium
SLP internship supervisor	2.24	0.89	4	Low
Radiation safety officer	1.90	0.94	5	Low
Medical physicist radiologist	1.51	0.79	6	Low
Other physician	1.39	0.72	7	Low
Rate your competency with the following topic.				
Shielding the SLP	2.81	1.10	1	Medium
Shielding patients	2.49	1.05	2	Medium
Patient education regarding radiation	2.37	1.14	3	Medium
Distance	2.02	0.95	4	Low
Radiation exposure time	1.95	0.93	5	Low
Dosimetry tools and report interpretation	1.83	0.95	6	Low
ALARA	1.57	0.93	7	Low
Principle/Inverse square law.	1.51	0.91	8	Low

Table IV: The relationship between different levels of education and VFSS experience to the average radiation exposure time (N = 115)

	average radiation exposure time during VFSS (minutes)					
	<5	6 - 10	11 - 15	16 - 20	> 21	I do not know
Bachelor's degree in SLP	24 (32.4%)	8 (10.8%)	12 (16.2%)	18 (24.3%)	1 (1.4%)	11 (14.9%)
Master's degree in SLP	0	1 (3.1%)	8 (25%)	23 (71.9%)	0	0
PhD in SLP	0	0	5 (55.6%)	3 (33.3%)	0	1 (11.1%)
Total	24 (20.9%)	9 (7.8%)	25 (21.7%)	44 (38.3%)	1 (0.9%)	12 (10.4%)
Less than one year of experience	12 (44.4%)	4 (14.8%)	0	0	0	11(40.7%)
Between 1-2 years of experience	7 (50%)	0	5 (35.7%)	2 (14.3%)	0	0
Between 2-5 years of experience	1 (2.6%)	1 (2.6%)	13 (33.3%)	24 (61.5%)	0	0
More than five years of experience	4 (11.4%)	4 (11.4%)	7 (20%)	18 (51.4%)	1 (2.9%)	1 (2.9%)
Total	24 (20.9%)	9 (7.8%)	25 (21.7%)	44 (38.3%)	1 (0.9%)	12 (10.4%)

Table IV shows the relationship between education level, VFSS experience, and average radiation exposure time during VFSS assessments. Chi-square analysis revealed a statistically significant association between education level and estimated exposure time (χ^2=40.23, p<0.001, Cramer's V=0.418). Bachelor's degree holders more frequently reported shorter exposure times (32.4% estimated to be ≤5 minutes), whereas master's degree holders predominantly reported longer durations (71.9% estimated to be 16-20 minutes). PhD holders primarily estimated 11-15 minutes (55.6%). A significant association was also found between years of VFSS experience and exposure time estimates (χ^2=88.02, p<0.001, Cramer's V=0.505). Participants with less than 1 year of experience showed the highest uncertainty (40.7% unsure of the appropriate duration), while those with 2-5 years of experience predominantly reported 10-20 minutes (94.8%), and those with over 5 years of experience reported 15-20 minutes (51.4%).

DISCUSSION

This study represents the first exploration of radiological safety knowledge and practices among SLPs in Jordan. In this country, the field of speech-language pathology has been developing since 1992, offering both Bachelor's and Master's Programs.[15] Despite the longstanding role of SLPs in managing dysphagia across diverse populations, limited data exist on their adherence to radiological safety guidelines in VFSS, which is a procedure that is integral to the assessment and intervention planning for swallowing disorders. The findings reveal significant inconsistencies in radiation safety knowledge among Jordanian SLPs, with significant gaps in fundamental concepts despite moderate clinical experience. Approximately one-third of participants were unaware of the specific fluoroscopy equipment they utilised, while only 27.8% correctly identified scattered radiation as the primary exposure hazard during VFSS procedures. Furthermore, the uncertainty regarding pulsed versus continuous fluoroscopy is particularly concerning, given that pulsed fluoroscopy can reduce radiation dose by up to 64% compared to continuous fluoroscopy when appropriately implemented. The misconception held by 32.2% of participants that X-ray beam collimation reduces image quality represents a fundamental misunderstanding of dose reduction techniques that improve image quality while minimising radiation exposure. These deficiencies align with international concerns regarding inadequate radiation safety education in allied health curricula. Similar knowledge gaps have been documented in Australia, Canada, and the United States, where radiographers frequently assume primary responsibility for advising SLPs on safety protocols.[16]

While participants demonstrated relatively strong knowledge of basic protective equipment, such as lead aprons and thyroid shields, which are likely attributable to visible departmental protocols and warning signage, only 27% could accurately identify comprehensive shielding requirements. This selective knowledge pattern mirrors findings in Jordanian dental radiography practices, where protective equipment awareness varied significantly despite widespread recognition of radiation safety importance.[17] Additionally, family concerns about radiation exposure tend to be low, as most prioritise the diagnostic benefits of VFSS over exposure risks; a trend also observed in studies where parents showed moderate concern levels regarding radiation exposure relative to diagnostic accuracy.[3] Current clinical practice guidelines

emphasise the importance of informed consent and patient education for VFSS procedures.[18] However, effective patient counselling about radiation exposure requires a comprehensive understanding of radiation safety principles by the clinician. The knowledge gaps identified in this study may compromise SLPs' ability to provide accurate information during the informed consent process, particularly regarding radiation risks and safety measures. This finding aligns with recent emphasis on effective interprofessional teamwork and clear communication protocols as prerequisites for safe radiological practice.[19]

Misconceptions were particularly significant regarding procedural timing. While ASHA recommends ≤5 minutes of exposure, 38% of SLPs believed VFSS takes 15-20 minutes. Educational background significantly influenced procedural duration estimates, with master's degree holders reporting longer assessment times (71.9% estimated 15-20 minutes) compared to bachelor's degree practitioners (32.4% completed assessments under 5 minutes). While extended procedures may indicate thoroughness, they also increase cumulative radiation exposure for both patients and clinicians. The discrepancy between reported procedural times and recommended guidelines suggests inadequate familiarity with efficient assessment protocols and time-saving techniques. Less experienced SLPs (<1 year) demonstrated the greatest uncertainty regarding appropriate exposure times, highlighting the critical importance of structured mentorship and competency-based training during early career development. Without consistent reinforcement through standardised protocols, even experienced clinicians may develop suboptimal practices that compromise both safety and diagnostic efficacy. This misjudgment, exacerbated by logistical issues including team availability, patient preparation, and institutional workflow, indicates a lack of familiarity with time-efficient practices.[16]

Although many SLPs possess strong clinical knowledge, there is a pressing need to improve their understanding of procedural logistics and radiation safety, as gaps remain in awareness of exposure limits and adherence to established guidelines.[20] This knowledge gap not only raises safety concerns for SLPs during VFSS but also highlights the necessity of aligning SLP training with the standards provided to radiologists and other healthcare professionals to reduce radiation risks and improve patient care coordination. To address these gaps, integrating VFSS-focused radiation protection training into graduate programs and continuing professional development is recommended. National guidelines co-developed by SLPs, radiologists, and medical physicists could standardise practice. Future research should explore the impact of such interventions through longitudinal outcomes and simulation-based training assessments.

The findings of this study, however, are limited by its reliance on self-reported survey data, which may introduce response bias and limit causal inference regarding the relationship between education, experience, and safety practices. The sample size of 115 participants, although representative of active practitioners, may not fully capture the diversity of practice settings and institutional policies across Jordan. Future research should incorporate longitudinal designs and objective competency assessments to evaluate the effectiveness of training interventions.

CONCLUSION

Jordanian SLPs exhibit variable and often limited application of radiation safety principles in VFSS. Although protective equipment, such as lead aprons and thyroid shields, is commonly used, uncertainty persists regarding the optimal procedure duration, fluoroscopy settings, and shielding placement. These findings underscore the urgent need for structured, interdisciplinary training and national VFSS safety guidelines tailored to the Jordanian context. Ongoing professional education, grounded in international best practices and supported by regulatory frameworks, can help ensure that VFSS procedures are both diagnostically effective and radiologically safe for all stakeholders.

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ORIGINAL ARTICLE

The Prevalence of Malnutrition and Its Relationship with Clinical Outcomes Among Critically Ill Children in PICU and PHDU

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ABSTRACT

INTRODUCTION: Malnutrition is a significant concern in critically ill pediatric patients, as it is associated with increased morbidity, prolonged hospital stays, and higher mortality rates. The aim of this study is to determine the nutritional status of patients in the Pediatric Intensive Care Unit (PICU) and the Paediatric High Dependency Unit (PHDU) of a teaching hospital, and to investigate the effects of malnutrition on clinical outcomes. **MATERIALS AND METHODS:** A retrospective cohort study was conducted, and patients admitted between March 2024 and September 2024 to the Pediatric Intensive Care Unit (PICU) and Pediatric High Dependency Unit (PHDU) were screened based on inclusion and exclusion criteria. The prevalence of malnutrition and its association with clinical outcomes, including length of hospital stay, duration of mechanical ventilation, and mortality risk, were evaluated and assessed. **RESULTS:** This study involved 51 patients aged between 6 months to 17 years old. The prevalence of malnutrition was 54.9%, with the most common forms being underweight (31.4%), followed by stunting (25.5%), wasting (17.6%), and thinness (7.8%). Moreover, this study identified a significant association between undernutrition and longer hospitalisation (P=0.051). However, no significant association was found between undernutrition and the duration of mechanical ventilation (P=0.154) or the risk of mortality (P=0.866). **CONCLUSION:** The prevalence of undernutrition remains high among critically ill children, with the most prevalent forms being underweight, stunting, wasting and thinness. Undernourished children had prolonged hospitalisation, but there was no association with duration of mechanical ventilation and mortality risk.

Keywords
malnutrition, nutrition, intensive care unit, critically ill, children

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INTRODUCTION

Malnourished patients often experience delayed wound healing, impaired immune function, and increased susceptibility to infections, all of which contribute to prolonged recovery periods and extended hospital stays.[1] It is reported that underweight children in PICU exhibited significantly longer hospital stay, highlighting the variability in outcomes based on nutritional status.[2] Nutritional care plays a pivotal role in the management of critically ill children in the PICU, as these patients are at a heightened risk of malnutrition, which can profoundly impact their recovery and overall clinical outcomes.[3] Effective nutritional interventions, such as enteral or parenteral feeding, have been shown to prevent malnutrition, enhance nutritional tolerance, improve calorie and protein intake, and ultimately reduce mortality rates.[4-6]

Numerous studies conducted globally have explored the prevalence, risk factors and the effect of malnutrition on clinical outcomes among critically ill children.[7-10] However, to date, only one study in Malaysia has examined the prevalence of malnutrition in this population. The

study in Malaysia reported that 43.2% of critically ill children were moderately or severely malnourished.[5] Notably, this study did not classify malnutrition based on World Health Organisation (WHO) criteria and primarily focused on enteral nutrition delivery. Furthermore, two studies conducted in Malaysia only involved hospitalised children.[11-12] One of the studies reported that the prevalence of acute and chronic malnutrition was 11% and 14% respectively.[12] Meanwhile, 25.4% of patients in the other study were at high risk of undernutrition.[11]

Consequently, there is a lack of research on the effect of malnutrition on clinical outcomes in Malaysia. Findings from other countries cannot be generalised to Malaysia, as malnutrition is influenced by a country's unique cultural background, economic status and healthcare protocols. Additionally, variations in hospital environments, nutritional support practices, and anthropometric measurement protocols among critically ill children further limit the applicability of these findings across different regions.

This study aims to address these gaps by determining the nutritional status of patients admitted to the PICU and Paediatrics High Dependency Unit (PHDU) and investigating the effects of malnutrition on clinical outcomes, including length of hospital stay, duration on mechanical ventilation and mortality risk.

MATERIALS AND METHODS

The study was conducted at Hospital Tunku Ampuan Besar Tuanku Aishah Rohani (HPKK-UKM). A total of 273 admissions to the PICU and PHDU between March 2024 and September 2024 were screened. Convenience sampling was applied, and patients were evaluated based on inclusion and exclusion criteria outlined in Figure 1.

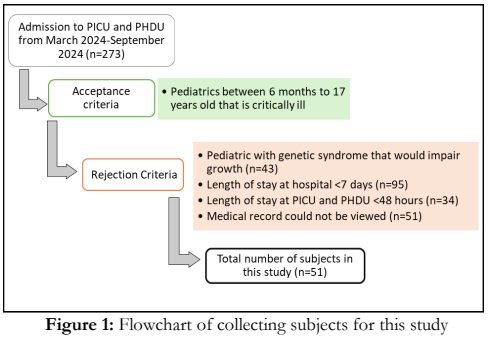

Figure 1: Flowchart of collecting subjects for this study

Data collection was conducted by using the electronic medical record system, IQVIA Hospital Information System (Aircus Air). The collected data included patients' demographics, pre-existing comorbidities, anthropometric measurements during admission, biochemical data, nutritional supports received during hospitalisation and duration of mechanical ventilation. The Pediatric Risk of Mortality III (PRISM III) is a validated mortality prediction tool with ROC curve of 0.927 and highly sensitive in PICU patients with acute respiratory distress syndrome (ARDS) who represented majority of subjects in this study.[13] PRISM III score predicts mortality based on the combination of physiological parameters, including vital signs, Glasgow Comma Scale (GCS), laboratory values (full blood count and kidney function) and arterial blood gas (ABG) during first 24 hours of PICU admission score was calculated using biochemical data and vital signs to assess the severity of illness. [13-14]

The Screening Tool for the Assessment of Malnutrition in Paediatrics (STAMP) tool was used to evaluate patients' current diagnoses that may impact nutritional intake, changes in nutritional intake and anthropometric measurements based on nutritional status criteria established by the WHO.[15] It is also a validated screening tool for hospitalised children, demonstrating high diagnostic accuracy with a Receiver Operating Characteristics (ROC) curve of 0.952, 0.846 and 0.818 for detecting underweight, stunting and wasting.[16] Finally, the relationship between these parameters and clinical outcomes was examined.

Terminology and definition

The classification of nutritional status in this study was based on the criteria established by WHO.[15,16] According to WHO guidelines, malnutrition is classified into six categories, which are stunting, wasting, underweight, thinness, overweight and obesity. These categories were determined using the WHO Child Growth Standards (0-5 years old) published in 2006 and the WHO Growth Reference 2007 (5-19 years old). Weight and height measurements taken during hospital admission were plotted against the WHO growth charts. Stunting, which

reflects chronic malnutrition and impaired growth, is defined as a length-for-age or height-for-age z-score of less than -2 standard deviations (SD). Underweight is classified as a weight-for-age z-score below -2 SD, indicating a deficit in overall body mass relative to age. Wasting, indicative of acute malnutrition, is determined by a weight-for-length or weight-for-height z-score below -2 SD for children under five years of age. Thinness is characterised by a body mass index (BMI) z-score below -2 SD in children aged 5-19 years. Overweight and obesity are identified based on age-specific growth references. In children aged 0-5 years, overweight is defined by a weight-for-length or weight-for-height z-score above +2 SD, while a z-score above +3 SD signifies obesity. In children aged 5-19 years, a BMI z-score above +1 SD is classified as overweight, and a z-score above +2 SD indicates obesity.

Statistical analysis

The data collected for this study were analysed using the Statistical Package for the Social Sciences (SPSS) version 27. A p-value <0.05 was considered statistically significant. The normality of quantitative data was assessed using the Shapiro-Wilk test and visual inspections, including histograms and probability plots. For normally distributed data, results were expressed as mean ± SD. For non-normally distributed data, results were presented as median with interquartile range (IQR). Categorical data were summarised as frequencies and percentages. To evaluate the relationship between categorical variables, the Pearson chi-square test or Fisher's exact test was applied, depending on the data distribution and sample size, to assess the association between undernutrition and the clinical outcomes.

RESULTS

In this retrospective, single-centre study that was conducted in Malaysia, data from 273 patients admitted to the PICU and PHDU between March 2024 and September 2024 were screened. Eligible subjects included critically ill pediatric patients aged 6 months to 17 years. Patients were excluded based on exclusion criteria as presented in Figure 1. After applying these criteria, a total of 51 subjects were included in the study. Descriptive statistics for age groups, gender distribution and medical diagnoses are summarised in Table I**.** Medical diagnoses were classified according to the International Classification of Diseases, 11th Revision (ICD-11).

Table I: Patient characteristics, nutritional status and clinical parameters

Demographics	Frequency (n=51)	%
Age		
6 months-2 years	28	54.9
3-17 years	23	45.1
Gender		
Male	38	74.5
Female	13	25.5
Race		
Malay	49	96.1
Chinese	1	2.0
Indian	1	2.0
Medical diagnoses		
Neoplasm	6	11.7
Endocrine, nutritional or metabolic disease	2	3.9
Respiratory diseases	23	45.1
Digestive system diseases	1	2.0
Infectious or parasitic diseases	4	7.8
Nervous system diseases	2	3.9
Other diseases	13	25.5
Enteral Nutrition (EN)		
No	20	39.2
Yes	31	60.8
Mechanically ventilated		
No	8	15.7
Yes	43	84.3
Length of hospital stay		
Days (mean ± SD)	12.92 ± 10.32	
Duration of mechanical ventilation		
Days (mean ± SD)	13.06 ± 10.25	
PRISM III*		
Score (mean ± SD)	4.76±6.245	
STAMP**		
Score (median (IQR))	4.00 (3.00-5.00)	
Nutritional status		
Stunting	13	25.5
Underweight	16	31.4
Wasting (< 5 years of age)	9	17.6
Thinness (> 5 years of age)	3	5.9
Overweight	2	3.9
Obesity	1	2.0

* Pediatric Risk of Mortality III
**Screening Tool for the Assessment of Malnutrition in Pediatric

From the 273 medical records that were screened, only 51 patients met the inclusion criteria, of whom 74.5% (n=38) were male, and the majority were Malay (96.1%, n=49). 54.9% (n=28) of patients were aged between 6 months to 2 years, and 45.1% (n=23) were 3-17 years old. The most common medical conditions were respiratory diseases (45.1%, n=23), followed by other diseases (25.5%, n=13) and neoplasms (11.7%, n=6). In addition, 43.1% (n=22) of patients had underlying conditions such as cancer and chronic heart disease during admission to the PICU or PHDU. Among the 51 patients, 60.8% (n=31) were

initiated on enteral nutrition (EN) during hospitalisation. Of these, 64.5% (n=20) began EN within 24 hours of admission, and 35.5% (n=11) started after more than 48 hours. The median estimated calorie intake provided through EN alone was 624.00 (496.80-828.00) kcal/day. Only 58.1% (n=18) of patients received an estimated calorie intake above 65% from EN alone, while 41.9% (n=13) received an estimated calorie intake of less than 65%. Additionally, 84.3% (n=43) of patients required mechanical ventilation, with a mean duration of 13.06±10.25 days.

A malnutrition screening using the STAMP score was conducted, and a score of 0 to 1 is categorised as low risk, while a score of 2 to 3 indicates medium risk. The STAMP score above 4 signified a high risk of malnutrition. The median STAMP 4.00 (3.00-5.00), indicating that subjects in this study were at high risk of malnutrition. Furthermore, the PRISM III score averaged 4.76±6.245, signifying a low risk of mortality in this cohort. In general, a higher PRISM III score correlates with an increased risk of mortality. The PRISM III score of less than 10 is indicative of a low mortality risk, whereas a score between 11 and 20 signifies a moderate risk. A score exceeding 20 is associated with a significant risk of mortality.

In general, 54.9% (n=28) of patients admitted to the PICU and PHDU experienced malnutrition. Among these, the most prevalent forms were underweight (31.4%, n=16), followed by stunting (25.5%, n=13), wasting (17.6%, n=9), thinness (7.8%, n=4), overweight (3.9%, n=2), and obesity (2.0%, n=1). Furthermore, 19.6% (n=10) of patients were classified as having more than one form of malnutrition. Among these, 30% (n=3) were categorised as both stunted and underweight, 30% (n=3) as underweight and wasted, 20% (n=2) as stunted, underweight and thinness and 20% (n=2) were underweight, stunted and wasted.

Notably, the most prevalent form of malnutrition among patients aged 6 months to 2 years old was stunting (32.1%, n=9), followed by underweight (32.1%, n=9), and wasting (10.7%, n=3). Meanwhile, undernutrition remained the primary malnutrition issue among patients aged 3 to 17 years. The most prevalent form was underweight (30.4%, n=7), followed by wasting (26.1 %, n=6), stunting (17.4%, n=4) and thinness (13.0%, n=3). A comparison of the nutritional status of patients aged 6 months to 2 years and 3 to 17 years is presented in Figure 2.

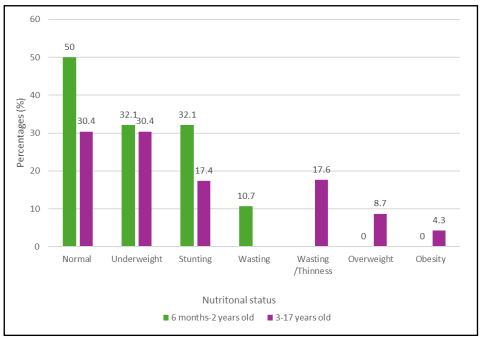

Figure 2: Comparison of nutritional status between children 6 months to 2 years and 3-17 years

This study also examined the effect of undernutrition on length of stay, duration of mechanical ventilation and mortality risk, as determined by PRISM III score. Pearson's chi-square test was used to assess the association between undernutrition and these outcome parameters. The test results are presented in Table II.

Table II: Effects of undernutrition towards clinical outcomes

Parameters	Value	P-value
Length of stay		
≤7 Days	3.803	0.051
≥8 Days		
Duration of mechanical ventilation		
≤8 Days	2.035	0.154
≥9 Days		
Risk of mortality (PRISM III score)		
≤4 scores	0.029	0.866
≥5 scores		

Undernutrition was found to be independently associated with a longer length of hospitalisation (P=0.051). However, no statistically significant association was observed between undernutrition and the duration of mechanical ventilation (P=0.154) or mortality risk (P=0.866).

DISCUSSION

The present study found that the prevalence of malnutrition among critically ill children admitted to the PICU and PHDU was 58.0% (n=29), whereas a systematic review reported a prevalence of 38.3% among critically ill children in middle-income countries.[19] The difference in prevalence in both studies is due to the fact that the current study is retrospective and relied on limited anthropometry assessments in the medical

records, whereas the systematic review involved previous prospective studies that allowed more comprehensive anthropometry measurements and a diverse sample size, which reduced bias. This study also revealed that the primary issue is undernutrition, with the most prevalent form being underweight (31.4%, n=16), followed by stunting (25.5%, n=13), wasting (17.6%, n=9) and thinness (7.8%, n=4). The high prevalence of malnutrition is due to the heightened stress response, which increases the cortisol levels, catecholamines and glucagon. This will cause hypercatabolism, resulting in elevated muscle protein breakdown and lipolysis.[1] Furthermore, the presence of comorbidities among patients in this study (43.1%, n=22) may exacerbate the stress response and further increase the severity of the malnutrition.[21,22]

Notably, the primary issue among patients aged 6 months to 2 years old and 3 to 17 years old is undernutrition. Between the ages of 6 months and 2 years, children undergo a transition to complementary feeding and develop their oral food processing skills. Feeding difficulties are common among 2-year-old children, particularly those who have acute or chronic illnesses, undergone multiple surgeries at an early age, require long-term enteral nutrition and parenteral nutrition or are mechanically ventilated. This can delay the maturity of mastication, swallowing and gag reflexes.[1,23] Moreover, undernutrition is likely due to muscle atrophy caused by a hypercatabolic state during critical illness, which can begin as early as 3 days of ICU admission.[23] Furthermore, the percentage of weight loss experienced by critically ill children who were diagnosed with respiratory diseases can be up to 18%.[24]

In addition, a significant association was identified between undernutrition and length of hospitalisation (P=0.051). Similar to the previous study, the majority of subjects in this cohort were diagnosed with respiratory diseases, a condition that frequently necessitates mechanical ventilation.[25] Notably, malnutrition in these patients is often attributed to loss of muscle mass, which can result from increased metabolic demands, prolonged immobilisation and inadequate nutritional intake.[24] The respiratory system relies on the diaphragm muscle for breathing. A reduction in diaphragm muscle strength weakens lung contraction and expansion, delaying weaning from ventilation and a slower transition to spontaneous breathing.[24,26] Prolonged weaning from mechanical ventilation results in a longer hospitalisation. Additionally, prolonged hospitalisation may also be due to the severity of the illnesses upon admission into the PICU and PHDU.

However, in this study, no significant association was found between undernutrition and the duration of mechanical ventilation (P=0.154). The lack of association could be attributed to the small sample size and the influence of confounding factors, such as disease diversity, management protocols, and comorbidities. Previous studies have shown an association between malnutrition and longer duration of mechanical ventilation.[25-27] In contrast, a study suggested that disease severity and underlying conditions may affect ventilation duration.[28] Critically ill children often require prolonged ventilation due to conditions like severe respiratory infections, neurological impairment or sepsis, which could obscure the impact of malnutrition.[29] ARDS or severe infections are often the primary reasons for a longer duration of mechanical ventilation.[25] Variability in ventilation weaning protocols, sedation practices, and intensive care management may also have contributed to the lack of association. Larger studies with controlled cofounders are necessary to investigate the relationship between nutritional status, ventilation duration and disease severity.

Although malnutrition is well known to be linked to mortality in critically ill children, this study found no significant association between undernutrition and mortality risk (P=0.866), based on the PRISM III score. A possible reason is the low mortality risk in this population, with an average PRISM III score of 4.76±6.245. Malnutrition can increase mortality risk through various mechanisms, including immune system suppression, which causes pneumonia and sepsis. Additionally, malnutrition disrupts metabolic balance and impairs organ function, particularly in the heart, liver and kidneys, potentially leading to fatal outcomes if not promptly managed. However, since mortality in critically

ill children is influenced by multiple factors, such as disease severity, comorbidities, and intensive care interventions, malnutrition alone may not have been the key determinant in this study.

The findings of previous studies also support the current research, which shows no association between malnutrition and mortality risk among critically ill children.[26-29] Additionally, the early initiation of enteral nutrition in more than 60% of patients in this study might have reduced the adverse effects of undernutrition, mitigating its direct impact on mortality.[5] However, a larger cohort might be necessary to confirm whether a true association exists. It is worth noting that mortality in critically ill children is multifactorial, influenced by underlying diagnoses, severity of illness and access to timely and adequate interventions.

One of the key strengths of this study is its focus on critically ill children in a tertiary referral centre, providing insights into a high-risk population that is often underrepresented. Additionally, this study utilised an electronic medical record for data collection, allowing for standardised retrieval of demographic, anthropometric and clinical information. This facilitated a comprehensive evaluation of malnutrition prevalence and potential contributing factors, even within the constraints of a retrospective design.

However, this study has several limitations. It was conducted in a single centre, which restricts the generalizability of the findings to other PICUs. The small sample size was another major limitation, reducing the statistical power to detect associations. Additionally, weight and height measurements were not routinely performed during hospitalisation due to heavy caseloads, which may have impacted the accuracy of the malnutrition assessment.

CONCLUSIONS

This study highlights that malnutrition remains a significant concern among critically ill children admitted to PICU and PHDU, with a prevalence of 58.0% (n=29). The predominant forms of malnutrition identified in this study were underweight, stunting, wasting, and thinness. Malnutrition also contributes to longer hospitalisation due to a hypercatabolic state that causes prolonged recovery during critical illness.

From a clinical perspective, the findings served as a primary source for prevalence data and nutritional status assessment, using criteria established by the WHO, due to a shift in pediatric nutritional status trends towards triple-burden malnutrition (where undernutrition, overnutrition, and micronutrient deficiencies coexist within the same population) in Malaysia. Furthermore, the use of the WHO criteria ensures comparability with the national annual report. In addition, the study also underscores the need for routine nutritional screening using validated tools such as STAMP in a clinical setting. Routine nutritional assessment allows for early detection of malnutrition risk and early nutritional intervention, further improving patients' clinical outcomes. This study is significant in providing essential guidance for large-scale and multicenter studies to strengthen the current evidence base in developing nutritional protocols for critically ill pediatric patients.

CONFLICT OF INTEREST

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

INSTITUTIONAL REVIEW BOARD (ETHICS COMMITTEE)

The study was approved by the Universiti Kebangsaan Malaysia Research Ethics Committee (JEPUKM), Kuala Lumpur, under the ethics application code JEP-2024-265.

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ORIGINAL ARTICLE

Cross-Sectional Study of Glycaemic Control Among Women with Type 2 Diabetes Mellitus in A Primary Health Care Clinic: A Call for Tailored Intervention

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ABSTRACT

INTRODUCTION: More than two thirds of the women attending primary health care (PHC) clinics have poor glycaemic control with lifestyle, stress, and attitudes as risk factors towards poor type 2 diabetes mellitus (T2DM) control. This study aims to determine the prevalence and identifying the factors associated with poor glycaemic control among the T2DM women in PHC clinic. **MATERIALS AND METHODS:** This is a cross-sectional study on women with T2DM in Seremban Health Clinic (HC), Negeri Sembilan, conducted over 3 months from January-April 2023. The women were recruited through systematic random sampling. Information was gathered using medical records and self-administered questionnaires. Factors associated with poor glycaemic controlled were determined using multiple logistic regression analysis. **RESULTS:** The prevalence of poor glycaemic control among women with T2DM in Seremban HC is 57.8%. Factors found to be associated with poor glycaemic control among these women included age ≥60 years old (aOR 2.26; 95% CI 1.35, 3.78), non-Malay ethnicity (aOR 1.89; 95% CI 1.05, 3.40), non-insulin treatment (aOR 7.56; 95% CI 4.13, 13.84), and perceived social support (aOR 1.47; 95% CI 1.12, 1.91). **CONCLUSION:** Over half of the women with T2DM attending Seremban HC have poor glycaemic control, associated with factors such as age ≥ 60 years, non-Malay ethnicity, non-insulin treatment, and perceived higher social support. This suggests a need for a women-centred primary care strategies to manage T2DM, potentially enabling the development of tailored interventions specifically for women. Collaboration with healthcare providers and policymakers could further enhance the glycaemic control among women with T2DM.

Keywords
Type 2 diabetes mellitus, women, glycaemic control

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INTRODUCTION

The global prevalence of diabetes mellitus (DM) is estimated at 10.5%, affecting approximately 537 million individuals worldwide and 90% of them are diagnosed with Type 2 Diabetes Mellitus (T2DM).[1] The rising prevalence of T2DM in Asia, now ranging from 8.1% to 9.6%, has emerged as a leading cause of death with varying impacts across regions.[2,3] Achieving optimal glycaemic control in T2DM individuals is crucial, assessed through multiple parameters, however HbA1c stands out as the most recommended metric, correlating with average plasma glucose over the past 8-12 weeks.[4] Optimal control based on HbA1c, typically within the range of 6.5%-8.0%, with a preferred target of 7.0%, aims to mitigate long-term complications.[1,5,6]

Diverse factors contribute to the susceptibility and outcomes of T2DM especially among women with T2DM as they often exhibit poorer glycaemic control than men, leading to increased risks of complications such as cardiovascular disease, mortality, and mental health

challenges.[7,8] A significant proportion of women with T2DM are treated in primary healthcare settings.[9] Studies conducted in Indonesia, Saudi, and Morrocco PHC settings revealed that 47.7%-71.4% of women with T2DM had poor glycaemic control.[10-12] Thus, identifying and addressing the factors related to glycaemic control is crucial for managing T2DM, especially among women.[2,4]

In Malaysia, DM has increased significantly over the past decades, with a prevalence of 14.3%, higher than that of its neighbouring countries.[13] Out of 1,698,683 active DM individuals, 99.33% are diagnosed with T2DM, with 57.02% being females.[14] Poor glycaemic control among T2DM women is reported to be between 66.1%-79.5% in PHC clinics.[14-16] Negeri Sembilan, a state with a substantial T2DM burden and the highest prevalence in Malaysia, may serve as a model for understanding and addressing these challenges, highlighting the urgency for comprehensive strategies.[14, 17] However, in the last 5 years, only 1 study on T2DM patients has been conducted in the state, focused on diabetes literacy and knowledge.[18] Therefore, the current study in Seremban seeks to bridge the gaps by determining the prevalence of poor glycaemic control and identifying the factors associated with glycaemic control among women with T2DM, offering actionable recommendations. Given the limited focus on gender-specific management methods for glycaemic control, this research aims to contribute significantly to the field.

MATERIALS AND METHODS

Study design and population

This study was a cross-sectional study involving women with T2DM under follow-up at the Non-Communicable Disease (NCD) Unit in Seremban Health Clinic, conducted over 3 months from 12 January-12 April 2023. Written informed consent was obtained from all participants prior to enrolment. Women with T2DM who met the study criteria (i) aged 18 years old and above (ii) diagnosed with T2DM for at least one year, (iii) able to write and read in Malay language *(Bahasa Melayu)* or English were included in the study. Those with (i) acute complaint/life threatening event during visit, (ii) cognitive impairment, (iii) hearing/vision impairment, (iv) psychiatric illness, or (v) physical disability were excluded from the study. Seremban Health Clinic, is one of the largest government primary health clinics in Negeri Sembilan, with approximately 800 women with T2DM attending the clinic monthly. The current study is part of bigger study on glycaemic controls, and the details of the study also have been published elsewhere. [19]

The sample size for the study was calculated using the OpenEpi Sample Size Calculation software with a two-sided confidence interval of 95%, power of 80%, and precision of ±0.05 based on a reference study by Ghouse et al., which assessed the effect of diabetes duration on the relationship with glycaemic control.[20] The authors reported that among those who has been diagnosed with T2DM for less than 5 years, 18.9% had poor glycaemic control, whereas 42.6% of those diagnosed with T2DM for 5 years or more had poor glycaemic control. From the calculated sample of 134 women, an additional 10% of the sample was added to compensate for missing data and non-response, resulting in a minimal final sample size of 148 women. A systematic sampling method was used in this study. All women with T2DM registered for follow-up at Seremban Health Clinic were identified and numbered. Those with even numbers (6th number) were chosen at a random point and invited to participate in the study.

Study Instruments

A bilingual (English and Malay language) self-administered structured questionnaires was used to collect data in this study.[19] The questionnaires were divided into six parts: (i) sociodemographic, clinical characteristics and medication, (ii) The Summary of Diabetes Self-care Activities (SDSCA), (iii) problem-focused coping strategies using the Brief Coping Orientation to Problems Experienced (Brief-COPE), (iv) The Scale for Measuring Role Strain in Women with Diabetes (SMRSWD), (v) The Multidimensional Scale of Perceived Social Support (MSPSS), (vi) perceiving diabetes as a priority. Permission to use the questionnaires from the original authors was obtained prior to the study.

The SDSCA by Toobert et. al., 1988, is used to assess the level of self-care among women.[21] The original version of consists of a 12-item scale, but the latest version of the SDSCA comprises of 10 items for self-care, organised into four key domains: (i) general diet (2 items) and specific diet (2 items), (ii) exercise (2 items), (iii) blood sugar (2 items), and (iv) foot care (2 items), utilising a 7-point Likert scale. General diet refers to overall healthy eating habit whereas specific diet is eating behaviours for diabetes control. Participants choose a score between 0-7 to indicate their self-care activities for the past seven days, with 0 representing 'none of the days' and 7 representing 'all seven days'. The total score ranges from 0-70, with higher scores indicating better performance of self-care activities for the past seven days. The Malay version of the SDSCA is used in this study. It has acceptable internal consistency (Cronbach's α=0.740).[22]

The assessment of problem-focused coping strategies was done using the Brief-COPE by Carver et al., 1989. The Brief-COPE comprises 14 scales with a total of 28 questions.[23] Three scales, each with 2 items, totalling 6 questions, are utilised in this study to measure problem-focused coping strategies. It has been established that effective problem-solving in individuals with T2DM, especially women, can lead to improved disease outcomes, including glycaemic control.[24] Participants rate themselves on a scale of 1 (I have not been doing this at all) to 4 (I have been doing this a lot). Scores for each question ranged from 2-8. The minimum total score is 12, and the maximum total score is 48. A higher total score indicates increased use of problem-focused coping strategies among the participants. In this study, the Malay Brief COPE is used, and it has high internal consistency (Cronbach's α=0.83); the test-retest reliability value was 0.69 ($p<0.001$).[25]

The Scale for Measuring Role Strain in Women with Diabetes (SMRSWD), was developed by Wang RH (2020) from Taiwan based on the Chinese version of the Women's Role Strain Inventory (WRSI) for working women, which originally contained 36 items.[26] The short-form scale developed contains 9 items with 2 subscales: (i) role guilt, and (ii) role conflict. Each statement is scored on a scale of 1 (strongly disagree) to 5 (strongly agree). The total score ranged from 9-45 with higher scores indicating a higher role strain experienced by the women. The original questionnaire of the SMRSWD is available in English; permission was sought to translate it into Malay. Following the guidelines outlined by Tsang et al., the translation of the SMRSWD was done proceeded with validation by panel of experts and pilot study involving 60 women with reliability assessment yielded a Cronbach's α value of 0.91 for the English version and 0.80 for the Malay version.[19,27]

The MSPSS instrument, developed by Zimet G et. al (1988), is used to assess participants' perception of social support.[28] The instrument contains 12 items with 3 subscales: (i) family (4 items), (ii) friends (4 items), and (iii) significant others (4 items). It is rated using 7-point Likert scale ranging from 1 (very strongly disagree) to 7 (very strongly agree). The total score of the 12 items is added together (ranged 12 to of 84) and then divided by 12. A higher score indicates greater perceived social support, or it can be scored according to its subscales [29]. In this study, the Malay version MSPSS will be used.[30]. The instrument has good internal consistency (Cronbach's α=0.89), parallel form reliability (0.94) and test-retest reliability (0.77). Perceiving diabetes as priority consisted of one question developed by the authors. The women were asked to rate a statement indicating the degree of their priority in managing diabetes by using a 10 -point Likert-scale ranging from 1 (strongly disagree) to 10 (strongly agree). A higher score indicates a higher priority in managing diabetes.

Outcome variable

The outcome variable for the study is the glycaemic control level, which is measured by glycated haemoglobin (HbA1c) level, with a cut-off point of >7.0% indicating poor glycaemic and ≤7.0% indicating good glycaemic control.[6] This cut-off point also recommended by Canadian guidelines.[31]

Independent variables

There are eight independent variables in this study: (i) sociodemographic (7 items), (ii) clinical characteristics (3 items), (iii) medication (1 item), (iv) self-care in diabetes

using the SDSCA (10 items), (v) problem-focused coping strategies using the Brief-COPE (6 items), (vi) role strain by using the SMRSWD (9 items), (vii) social support by using the MSPSS (12 items), and (viii) perceiving diabetes as priority (1 item). For the sociodemographic, women with T2DM were categorised into different age groups, marital statuses, ethnicities, educational levels, occupations, household income, living arrangements, clinical characteristics, and comorbidities accordingly. The further categories such as age and occupation group were adapted from National Health Morbidity Survey whereas household income was from Department of Statistic Malaysia.[17,32] Comorbidities were defined as the presence of namely high blood pressure, high cholesterol level or other chronic disease.

The SDSCA was measured with scores ranging from 0-70, with higher scores indicating better performance of self-care activities over the past seven days. Problem-focused coping strategies were recorded using the Brief-COPE, where higher total scores indicate increased use of problem-focused coping strategies among the women. The SMRSWD was assessed with total scores ranging from 9-45, with higher scores indicating a higher role strain experienced by the women. In the MSPSS used in this study, higher scores indicate greater perceived social support. Perceiving diabetes as a priority was measured by a statement rated on a Likert-scale from 1 (strongly disagree) to 10 (strongly agree).

Statistical analysis

Data analyses were carried out using Statistical Packages for the Social Sciences (SPSS) version 26. Categorical nominal and ordinal variables are described as frequencies (n) and percentages (%). Continuous numerical variable is described as mean with standard deviation (SD) or median with interquartile range (IQR), depending on the data distribution. Each of the variables was then re-categorised to facilitate further regression analysis such as age group, ethnicity, occupation and BMI.[33]

Ethnicity was further categorised into Malay vs. non-Malay with Malay used as reference category to represent the majority group in the Malaysian population and to be comparable with other studies locally. Apart from that, dichotomisation ensured stability of regression estimate and the full breakdown of ethnic subgroups is presented in the result for transparency. BMI categories were further collapsed into two categories based on a cohort study on glycaemic control and obesity by Newson et al. and utilised ≥27.5kg/m² as obese cut-off point by Malaysian Guidelines.[34,35]

Simple and multiple logistic regression analysis were used to demonstrate the association between independent variables and poor glycaemic control. The crude and adjusted odds ratio (OR) with their corresponding 95% confidence interval (CI) were reported. The significance level was set at $p<0.05$. Any interaction term that differs insignificantly and is not of important difference will be regarded as not significant and will not be included in the regression model.[36]

RESULTS

Study population and characteristics

From January to April 2023, a total of 346 women met the criteria and completed the study. This number exceed the minimal sample size of 148. Based on Table I, which outlines the sociodemographic characteristics, most of the women were aged ≥60 years old (61.0%) and married (66.5%). Almost half of the women were of Indian ethnicity (48.3%). More than half had completed secondary education (51.7%), and the majority were housewives (57.8%). The majority of the women had a household income of ≤RM5000 (78.9%) and lived with family or friends (94.8%).

Regarding clinical characteristics, the majority had T2DM for ≥5 years (82.1%) and had other comorbidities present (86.1%). Half of the women were obese (50.9%). The most common mode of therapy among T2DM women was oral antidiabetic drugs (OAD) only (66.8%).

For self-care, the scores are as follows: general diet, 5.11 (SD 1.33), specific diet, 4.33 (SD 1.20); exercise, 2.97 (SD 1.34); self-monitoring blood-glucose, 0.72 (1.29); and foot care, 0.93 (SD 1.67). The total mean score for

problem-focused coping strategies was 15.04 (SD 4.98). The mean score for social role strain was 21.31 (SD 6.07). Most of the women had higher perceived social support (62.7%) from family, friends, and significant others, with a total score mean 5.38 (SD 1.00). The women with T2DM perceived T2DM as a priority, with a mean score of 7.07 (SD 1.90).

Table I: Study population and characteristics (N=346)

No	Factor	n (%)	Mean (SD)
1	**Glycaemic control (HbA1c level)**		
	i. Poor (>7.0%)	200 (57.8)	7.83 (1.73)
	ii. Good (≤7.0%)	146 (42.2)	
2	**Sociodemographic**		
	a) Age (years)		
	i. <40	13 (3.8)	
	ii. 40-49	32 (9.2)	
	iii. 50-59	90 (26.0)	
	iv. ≤60	211 (61.0)	
	b) Marital status		
	i. Single	9 (2.6)	
	ii. Married	230 (66.5)	
	iii. Divorced	20 (5.8)	
	iv. Widowed	87 (25.1)	
	c) Ethnic		
	i. Malay	81 (23.4)	
	ii. Non-Malay	265 (76.6)	
	- Chinese	91 (26.3)	
	- Indian	167 (48.3)	
	- Others	7 (2.0)	
	d) Education level		
	i. No formal education	5 (1.4)	
	ii. Primary	95 (27.5)	
	iii. Secondary	179 (51.7)	
	iv. Tertiary	67 (19.4)	
	e) Working status		
	i. Housewife	200 (57.8)	
	ii. Office work	54 (15.6)	
	iii. Self-employed	46 (13.3)	
	iv. Others	46 (13.3)	
	f) Household income (RM)		
	i. ≤5000	273 (78.9)	
	ii. >5000	73 (21.1)	
	e) Living arrangement		
	i. Alone	18 (5.2)	
	ii. Lives with family/friends	328 (94.8)	
3	**Clinical characteristics**		
	a) Duration of T2DM		
	i. < 5 years	62 (17.9)	
	ii. ≥ 5 years	284 (82.1)	
	b) Comorbidities*		
	i. None	48 (13.9)	
	ii. Yes	298 (86.1)	
	c) Body Mass Index (BMI)		
	i. Underweight	8 (2.3)	
	ii. Normal	53 (15.3)	
	iii. Overweight	109 (31.5)	
	iv. Obese	176 (50.9)	
4	**Mode of therapy**		
	a) OAD only	231 (66.8)	
	b) Insulin only	9 (2.6)	
	c) Combination of OAD and insulin	103 (29.8)	
	d) Lifestyle modifications	3 (0.9)	
5	**Summary of Diabetes Self-care Activities (day in a week)**		
	a) General Diet		5.11 (1.33)
	b) Specific Diabetic Diet		4.33 (1.20)
	c) Exercise		2.97 (1.34)
	d) Self-monitoring blood glucose		34 0.72 (1.29)
	e) Foot care		0.93 (1.67)
	f) Total score (total score range: 0 to 70)		28.12 (8.03)
6	**Problem-focused coping** (total score range: 12-48)		15.04 (4.98)
7	**Social role strain** (total score range: 9-45)		8.3 (16.07)
8	**Perceived social support**** (total score range: 1-7)		5.38 (1.00)
9	**Perceived diabetes as priority** (total score range: 1-10)		7.07 (1.09)

*high blood pressure, high cholesterol level or other chronic disease.
**psychological sense of belonging, acceptance, and aid that improves individuals' ability to cope with difficult situations

For self-care, the scores are as follows: general diet, 5.11 (SD 1.33), specific diet, 4.33 (SD 1.20); exercise, 2.97 (SD 1.34); self-monitoring blood-glucose, 0.72 (1.29); and foot care, 0.93 (SD 1.67). The total mean score for problem-focused coping strategies was 15.04 (SD 4.98). The mean score for social role strain was 21.31 (SD 6.07). Most of the women had higher perceived social support (62.7%) from family, friends, and significant others, with a total score mean 5.38 (SD 1.00). The women with T2DM perceived T2DM as a priority, with a mean score of 7.07 (SD 1.90).

Table II: Preliminary factors associated with poor glycaemic control.

No	Factor	Glycaemic Control		Crude OR	95% CI	*P* value
		Poor (n:200)	Good (n:146)			
1	**Sociodemographic**					
	a) Age (years)					
	i. <60	91 (67.4)	44 (32.6)	1.94	(1.23, 3.04)	0.004
	ii. ≥60	109 (51.7)	102 (48.3)			
	b) Marital					
	i. Single	3 (33.3)	6 (66.7)	0.36	(0.09, 1.45)	0.148
	ii. Ever married	197 (58.5)	140 (41.5)			
	c) Ethnic					
	i. Malay	52 (64.2)	29 (35.8)	1.41	(0.85, 2.37)	0.184
	ii. Non-Malay	148 (55.8)	117 (44.2)			
	d) Education level					
	i. No formal education	4 (80.0)	1 (20.0)	2.96	(0.33, 26.76)	0.334
	ii. Had formal education	196 (57.5)	145 (42.5)			
	e) Working status					
	i. Non-employed	113 (56.5)	87 (43.5)	0.88	(0.57, 1.36)	0.566
	ii. Employed	87 (59.6)	59 (40.4)			
	f) Household income (RM)					
	i. ≤5000	161 (59.0)	112 (41.0)	1.25	(0.75, 2.11)	0.394
	ii. >5000	39 (53.4)	34 (46.6)			
	g) Living arrangement					
	i. Alone	14 (77.8)	4 (22.2)	2.67	(0.86, 8.29)	0.089
	ii. Lives with family/friend	186 (56.7)	142 (43.3)			
2	**Clinical characteristics**					
	a) Duration of T2DM					
	i. < 5 years	28 (45.2)	34 (54.8)	0.54	(0.31, 0.93)	0.027
	ii. ≥5 years	172 (60.6)	112 (39.4)			
	b) Comorbidities*					
	i. None	25 (52.1)	23 (47.9)	0.76	(0.42, 1.41)	0.388
	ii. Yes	175 (58.7)	123 (41.3)			
	c) BMI					
	i. Non-obese	93 (54.7)	77 (45.3)	0.78	(0.51, 1.19)	0.252
	ii. Obese	107 (60.8)	69 (39.2)			
3	**Mode of therapy**					
	i. Insulin	96 (85.7)	16 (14.3)	7.50	(4.16, 13.51)	<0.001
	ii. Non-insulin	104 (44.4)	130 (55.6)			
4	**Summary of Diabetes Self-care Activities**	27.73 (7.83)	28.66 (8.29)	1.02	(0.99, 1.04)	0.286
5	**Problem-focused coping**	14.82 (4.83)	15.36 (5.18)	1.02	(0.98, 1.07)	0.318
6	**Social role strain**	21.29	21.34 (5.57)	1.00	(0.97, 1.04)	0.937
7	**Perceived social support****	5.28 (1.07)	5.52 (0.89)	1.28	(1.03, 1.60)	0.029
8	**Perceived diabetes as priority**	6.94 (2.00)	7.25 (1.76)	1.09	(0.97, 1.22)	0.131

*high blood pressure, high cholesterol level or other chronic disease.
**psychological sense of belonging, acceptance, and aid that improves individuals' ability to cope with difficult situations

The prevalence of poor glycaemic control among women with T2DM is 57.8% with a mean HbA1c of 7.83% (SD 1.73%). Simple logistic regression analysis revealed four factors associated with poor glycaemic control: (i) age

≥60, (ii) diabetes duration of ≥5 years, (iii) non-insulin treatment and (iv) higher perceived social support as shown in Table II. The multiple logistic regression model in Table III shows four final factors associated with poor glycaemic control among women with T2DM: (i) age ≥60 years old (aOR 2.26; 95% CI 1.35, 3.78), (ii) non-Malay ethnicity (aOR 1.89; 95% CI 1.05, 3.40), (iii) treatment using non-insulin (aOR 7.56; 95% CI 4.13, 13.84) and (iv) higher perceived social support (aOR 1.47; 95% CI 1.12, 1.91). Women with T2DM who scored one point higher in social support had odds of 1.47 for poor glycaemic control. Model is based on forward logistic regression, dataset fit to logistic model (Hosmer-lemeshow 0.557), 68.2% of cases predicted correctly with specificity of 67.5% and sensitivity of 69.2%. R^2=0.263 and no outlier.

Table III: Final factors associated with poor glycaemic control

No	Factor	Adjusted OR (95% CI)	*p* value
	Age		
1	<60 years old	2.26 (1.35, 3.78)	0.002
	≥60 years old		
	Ethnicity		
2	Malay	1.89 (1.05, 3.40)	0.035
	Non-Malay		
	Treatment		
3	Insulin	7.56 (4.13, 13.84)	<0.001
	Non-insulin		
4	**Perceived social support**	1.47 (1.12, 1.91)	0.005

DISCUSSION

The current study reveals a recent prevalence of poor glycaemic control among women with T2DM in Seremban PHC settings, affecting approximately half of the women studied. This finding diverges from studies conducted in Korea and Brazil, where the prevalence of poor glycaemic control among women with T2DM was reported to be 61.1% and 74%, respectively.[7,37] The study from Korea, although conducted in PHC settings, may exhibit disparity from the present study due to its definition of poor glycaemic control as HbA1c >6.5%, focusing solely on women diagnosed with T2DM within a year and on OHA as medication. Conversely, the study from Brazil defined poor glycaemic control as HbA1c ≥7.0% and was conducted as a multicentre study in hospital settings. The cut-off points usage variation is due to stringent level of 6.5% suggested to younger and newly diagnosed individuals. For most other adult with T2DM, a target level of <7.0% is recommended however with caution to those prone for hypoglycaemia.[5]

Furthermore, this study reveals that women with T2DM aged ≥60 years have twice the odds of poor glycaemic control. This finding aligns with studies conducted in Thailand and locally.[38, 39] However, conflicting results emerge from another local study, indicating that elderly and retired individuals have more time to dedicate to their health, potentially leading to increased access to healthcare post-retirement.[40] Additionally, current study also highlighted that being non-Malay is associated with poor glycaemic control among women with T2DM, with twice the odds. It is reported that Indian has higher HbA1c levels compared to Malay and Chinese.[17] Such findings justify the inclusion of ethnicity as an important factors in glycaemic control studies. However, due to small subgroup and distribution of ethnicity in the current study, ethnicity was categorised with Malay as reference to facilitate analysis.[33] In contrast, a local study with an equally distributed ethnic groups reported that Malay ethnicity was also associated with higher HbA1c levels, which related to genetic variations between ethnics.[41] Notably, the majority of the women in the current study are non-Malay. Disparities in glycaemic control among ethnic groups may be attributed to the distribution of ethnicities in populations and their sociodemographic status as discovered in the current study.[7]

Non-insulin treatment is associated with an 8-fold increase in the odds of poor glycaemic control. This finding is consistent with recommendations advocating for early combination therapy and the use of insulin for long-term glycaemic control, while non-insulin treatment in T2DM is associated with poor glycaemic control.[1, 5, 6] A cohort study done in Malaysia involving 40 PHC clinics reported that high medication count in the management of T2DM associated with poor glycaemic control.[42] The possible explanation is T2DM individuals mostly had at least one other condition such as hypertension, hyperlipidaemia or cardiovascular disorders which contributed to the additional medications.[43] Conversely, other studies have also associated insulin usage with poor glycaemic control, possibly due to low adherence to insulin regimens. [43, 44] Additionally, local

findings indicate that nearly 40% of patients with high HbA1C levels did not receive insulin due to concerns about potential adverse effects.[43] In the current study, adherence to medication is not investigated.

T2DM is a complex, chronic condition that can be challenging to manage both physically and mentally; it can have an impact not just on patients' medical well-being, but also on their relationships with loved ones. A psychological sense of belonging, acceptance, and aid that improves individuals' ability to cope with difficult situations is defined as social support.[45] The current study reveals that higher perceived social support is associated with poor glycaemic control in women with T2DM. Similarly, a local study found that increased perceived social support is associated with poor glycaemic control especially in older patients with T2DM and a longer duration of diabetes.[46] As elderly who lives with T2DM for an extended period, they could experience physical limitations due to neuropathy and peripheral artery disease.[47] Physical limitations or functional decline may hinder their ability to engage in self-care activities even with higher social support.[47] Notably, many of the women in this study were elderly, which necessitates further assessment. In addition, living with families that provide strong social support can negatively impact a patient's ability to manage their diet healthily, as eating is often viewed as a social engagement.[48] However, these findings are contrary to other previous research emphasizing the positive impact of social support especially on glycaemic control.[49]

The study provides valuable insights into adopting a gender-based approach with women centred primary care strategies socially and biologically in managing T2DM among women, aligning with recommendations from the World Health Organization (WHO) and the Sustainable Development Goals (SDGs).[50] These goals are interconnected and necessitate addressing other objectives such as SDG 3 (promoting healthy lives) and SDG 4 (ensuring quality education). Therefore, enhancing health and its approach is paramount for attaining these objectives for example addressing the specific issues pertaining to women for effective health services.

Nonetheless, the study acknowledges limitations. Firstly, the findings may not be generalisable to all PHCs as the study was conducted in single PHC although the site was selected for having the highest monthly attendance of T2DM women. Secondly, we acknowledge the grouping ethnicity into Malay vs non-Malay may mask intra-group differences. However, this grouping was chosen to ensure statistical stability and to align with epidemiological practice in Malaysia which majority to minority comparisons are frequently used. Future studies with larger samples are suggested to examine ethnic-specific effects more closely.

CONCLUSION

Over half of the T2DM women attending Seremban Health Clinic have poor glycaemic control. Factors such as age ≥60 years, being non-Malay, receiving non-insulin treatment, and having higher perceived social support are associated with poor glycaemic control. These findings offer new insights into employing a gender-based approach with women centred primary care strategies to manage T2DM among women particularly in Seremban HC. Thus, it may provide baseline information for designing interventions specifically tailored to address the unique needs of women with T2DM. Interventional studies are recommended to test tailored approaches targeting these unique needs, such as focused education programs, lifestyle interventions, and social support initiatives involving both T2DM women and their family. Collaboration with healthcare providers and policymakers to implement evidence-based strategies aimed at improving glycaemic control among women with T2DM is recommended.

INSTITUTIONAL REVIEW BOARD (ETHIC COMMITTEE)

This study was approved by the Medical Research and Ethics Committee of the Ministry of Health Malaysia (NMRR ID-22-02611-8TX-11R) and the Research Ethics Committee of Universiti Kebangsaan Malaysia (UKM) (JEP-2022-632). Permission to perform the study in the Seremban Health Clinic was obtained from the District Health Office and the Family Medicine Specialist in charge. Confidentiality was maintained by anonymising data and not including participants' addresses. All data

were entered into a password-protected computer, and access to the data is restricted. All data were analysed anonymously using Statistical Packages for the Social Sciences (SPSS) version 26.

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ORIGINAL ARTICLE

Effects of Retinoic Acid on Liver Triglyceride Level and Diacylglycerol Acyltransferase-2 *(DGAT2)* Gene Expression in Rats with High-Cholesterol Diet-Induced Steatosis

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ABSTRACT

INTRODUCTION: Non-alcoholic fatty liver disease (NAFLD) incidence is rising globally without effective treatment available. Serum retinoic acid level was found to be low in NAFLD patients. Thus, this study investigated the effects of retinoic acid administration on liver triglyceride levels and Diacylglycerol acyltransferase 2 (*DGAT2*) gene expression in high-cholesterol diet-induced steatosis rats. **MATERIALS AND METHODS:** Forty male Sprague-Dawley rats were divided into five groups (n=8/group). Groups A and B received a normal diet, while groups C, D, and E were fed a high cholesterol diet (HCD) for four weeks to induce steatosis (Phase 1) and continued with the same diet for the next four weeks (Phase 2). In Phase 2, Group D received vehicle (Olive oil), while Groups B and E received retinoic acid (7.5mg/kg subcutaneously) twice weekly with their respective diet. Liver triglyceride levels were measured using the Bligh and Dyer's method, and hepatic *DGAT2* gene expression was quantified using Real-Time qPCR. Data was analysed using the One-Way Analysis of Variance (ANOVA) test. **RESULTS:** Retinoic acid-treated groups showed a reduced pattern in liver triglyceride levels, in which Group E level is 3.6±0.88 mg/g compared with Group C 4.12±1.5 mg/g, but statistically insignificant ($p>0.05$). The *DGAT2* expression was significantly reduced in Group E by 0.63-fold (63%) when compared to Group C. **CONCLUSION:** These findings suggest that retinoic acid administration might reduce the liver triglyceride level by down-regulating *DGAT2* gene expression. However, further studies are required to confirm retinoic acid as a potential candidate for improving NAFLD.

Keywords
Nonalcoholic fatty liver disease, High cholesterol diet, Retinoic acid, liver Triglyceride, *DGAT2* expression

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INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) is the hepatic manifestation of metabolic syndrome, characterised by the presence of more than 5% of macrovesicular steatosis in the liver cells, with the absence of secondary triggering factors.[1] The incidence and prevalence of the disease are rapidly growing.[2] It affects more than a quarter of the world's population,[3,4] with the highest 42% in Southeast Asian countries and specifically 37.4% in Malaysia.[5,6] NAFLD manifestations range from simple steatosis to non-alcoholic steatohepatitis (NASH). Then it progresses to cirrhosis and hepatocellular carcinoma (HCC), which is the leading cause of liver-related death.[7–10]

The liver processes a large number of fatty acids, but only a tiny quantity is stored as triglycerides in cytoplasmic lipid droplets, which account for less than 5% of liver weight.[11] Insulin resistance, metabolic syndrome, and type II diabetes disrupt fat metabolism, creating a build-up of free fatty acids in hepatocytes, resulting in lipotoxicity, inflammation, and fibrosis.[12] Many factors, like accelerated

modernisation, increased Western diet, aged population, physical inactivity, and genetics, are ascribed to be the contributing factors involved in the dramatic escalation of NAFLD worldwide.[13–15] However, to date, no Food and Drug Administration (FDA) approved prescription is available for NAFLD therapy.[13,16] Studies showed that liver storage ability was reduced in NAFLD, affecting vitamin A metabolism[17,18,] and serum retinol level was negatively associated with the severity of NAFLD.[19]

Carotenoids and retinyl esters are the dietary sources of vitamin A. Retinoids are vital transcriptional factors that regulate the expression of over 500 genes.[20] The hepatic stellate cells (HSCs) store retinoic acid (RA) in the form of retinyl ester-filled lipid droplets.[21] Lacking RA in both clinical and animal studies showed the development of NAFLD and NASH.[9] In mammals, structurally two unrelated diacylglycerol acyltransferases (DGAT) enzymes, DGAT1 and DGAT2, catalyse the terminal step of triglyceride (TG) biosynthesis.[22] DGAT1 is abundant and expressed in the intestine, while DGAT2 is found in the liver and adipose tissues. Many studies suggest that inhibition of DGAT2 led to the suppression of *de novo* lipogenesis.[23,24] However, the role of DGAT2 in causing lipid aggregation and the benefit of its inhibition in NAFLD is still unclear. While these studies highlight the roles of RA and DGAT2 in hepatic lipid metabolism, further research is necessary to directly establish RA's impact on *DGAT2* gene expression and its subsequent effects on lipolysis and lipogenesis. Therefore, this research aimed to evaluate the effects of RA on liver triglyceride levels and *DGAT2* gene expression in high cholesterol-induced steatosis rats.

MATERIALS AND METHODS

Animal model

Ethical approval for this study was obtained from Institutional Animal Care and Use Committee IACUC of the International Islamic University Malaysia (IIUM/IACUC/2019(14)). All the rats were treated following the institutional Guidelines for the Care and Use of Laboratory Animals.

This experimental study was conducted on adult male healthy Sprague-Dawley rats with an average body weight of 200 to 300 grams. They were individually housed with appropriate facilities in standard plastic cages and fed on a standard dry pellet diet and water *ad libitum*. They were kept under closely controlled environmental conditions with a 12:12-hour light-dark cycle. They were kept in a room at 21-22 °C temperature and 50-55% humidity. The animals were acclimatised for 2 weeks. The rats were randomly divided into 5 groups, with n=8 rats per group. The total sample size of 40 rats was determined using G*Power version 3.1 with a power of 95%. The calculation was performed using the mean and standard deviation of serum triglyceride levels in the control and treated animal groups, as reported by Geng et al. (2017).

For the first four weeks (Phase 1), rats in Groups A (control) and B received a normal diet (ND). While rats in Groups C to E received 12% cholesterol daily as a high cholesterol diet (HCD) to induce steatosis, based on the established method.[25] For the second 4 weeks (Phase 2), the rats in the control group continued receiving a normal diet. The rats in group B (ND+RA) were injected with RA (7.5mg/kg subcutaneous) twice weekly. The rats in group C received HCD throughout the experiment as a positive control. The rats in group D received HCD with vehicle (olive oil), and rats in group E received HCD and RA (7.5mg/kg subcutaneous) twice weekly to evaluate the effects of RA on the steatotic liver. The dose and duration of drug administration were determined based on the previous reports.[26,27]

At the end of the experimental study, all animals were fasted overnight and euthanised with an overdose mixture of anaesthesia. The abdomen was opened with a midline incision, and the liver tissues were removed *in toto*. Part of the central lobe of the liver was cut gently, directly to avoid *in vivo* lipolysis. The liver tissue was collected, washed with cold saline, and stored at -80°C for further analysis of liver triglyceride (TG) estimation and *DGAT2* gene expression.

Liver Triglyceride Measurement

Bligh and Dyer's method was used to measure liver triglyceride levels, with some minor changes.[28] Firstly, 75% ice-cold methanol (32µl/mg methanol and 10.6 µl/mg HPLC-graded water) was added to the samples and homogenised using SilentCrusher M homogenizer (Germany) for 5x60s at 7500 rpm. Each homogenate was transferred into 10 ml glass vials, and 16 µl/mg of chloroform was added. Samples were mixed using a vortex mixer for 3 minutes at 2000 rpm and then centrifuged at 18°C for 10 minutes at 2420xg using a refrigerated centrifuge (Eppendorf, Germany) to pellet the protein and tissue particles. The whole supernatant layer (5ml) was transferred to another clean 10ml glass vial. A total of 16 µl/mg of chloroform and 18.2 µl/mg of HPLC-graded water were added to induce phase separation. Samples were then mixed again on the vortex mixer for 1 minute at 2000 rpm and incubated at 18°C for 10 minutes to allow the partitioning of the solvent system, and then centrifuged at 18°C for 10 minutes at 2420xg. The non-polar parts of each tube were aliquoted into a clean 5ml glass tube and then dried down using a nitrogen blow-down evaporator (Mini-Vap 6-port evaporator). The dried lipid was resuspended in 250 µl of 2-propanol and measured by using an AU680 Beckman Coulter Chemistry analyzer. All samples were run in triplicate.

DGAT2 gene expression by reverse transcription quantitative polymerase chain reaction (RT-qPCR)

Reverse transcription quantitative polymerase chain reaction (RT-qPCR) was conducted to measure the relative *DGAT*2 gene expression. Total RNA was extracted from the rat's liver tissue using the SV Total RNA Isolation System kit (Promega, U.S.). Complementary DNA (cDNA) was synthesized from 100ng of RNA using the PROMEGA GoScript™ Reverse Transcription system according to the manufacturer's protocols. The cDNA samples were stored at -30°C before qPCR amplification. The synthesized cDNA was then subjected to qPCR using gene-specific primers and a TaqMan probe (as shown in Table I) and GoTaq® qPCR Master Mix, 2X (Promega, USA). Each PCR reaction was performed at a total volume of 13 µL per reaction. The mixture consisted of 6.5 µL of GoTaq® qPCR Master Mix, 2X (Promega, USA), 0.4 µL of the forward primer, 0.4 µL of the reverse primer, and 0.25 µL of the TaqMan probe. The reaction was completed with 2.45 µL of RNase-free water and 3 µL of the cDNA template, which was diluted 1:16 prior to use. All samples were run in triplicate and analysed on a 96-well plate. RNase-free water was used as a non-template control (NTC). The qPCR reaction was accomplished on a Bio-Rad CFX96 Maestro Real-time system. The thermal profile consisted of an initial single cycle for Taq DNA activation at 95◦C for 2 minutes. This was followed by 38 cycles of three steps: denaturation at 94◦C for 30 seconds, annealing at 53◦C, and extension at 72◦C, both for 30 seconds. The entire cycling program concluded with a single cycle of final extension at 72◦C for 1 minute.

Data was analysed using the relative quantitative expression method. The data were normalised to glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) and presented relative to the control. The C_T value for the target gene (*DGAT2*) was normalized to the endogenous control (*GAPDH*) to obtain the ΔC_T value using this formula:

$$\Delta C_T = C_T\,(DGAT2) - C_T\,(GAPDH)$$

The relative fold change in gene expression was then calculated using the comparative cycle threshold (C_T) method, also known as the $2^{-\Delta\Delta CT}$ method.

Table I: Sequences of primers and probes for Quantitative RT-PCR

Gene	Forward	Reverse	Probe
DGAT2	GCTGATGCTGCTCTC TACTTCACC	TGTGATCTCCTGC CACCTTTCT	TGGCATTTGACTGGA ACACGCCCA
GAPDH	GAACATCATCCCTGC ATCCA	CCAGTGAGCTTCC CGTTCA	CTTGCCCACAGCC TTGGCAGC

Statistical analysis

Data was analysed using IBM SPSS Statistics version 23. Comparison of liver triglycerides and *DGAT2* gene expression among the control and the high cholesterol diet-induced steatosis rats was analysed by conducting the One-Way Analysis of Variance (ANOVA) test. Post Hoc multiple testing was amended by using LSD correction to find out the significant differences among different treatment groups. The data were expressed as means ± standard deviation (SD) with a 95% confidence interval (95% CI). The P-value of <0.05 was considered statistically significant.

RESULTS

Liver triglyceride level

Results for the liver triglyceride levels are shown in Figure 1. The positive control group, Group C (HCD only), had the highest liver triglyceride (TG) level with a mean of 4.12±1.50 mg/g, demonstrating an increased pattern compared to Group A (ND), which had 2.42±1.78 mg/g, but not statistically significant. In the retinoic acid (RA)-treated groups, a reduced pattern in liver TG levels was observed. Group B (ND+RA) had the lowest TG level overall at 0.98±0.16 mg/g. Meanwhile, Group E (HCD+RA) had a mean TG level of 3.60±0.88 mg/g, which showed a reduced pattern in comparison to Group C (4.12±1.50 mg/g) and Group D (4.05±0.76 mg/g), but statistically insignificant (p>0.05).

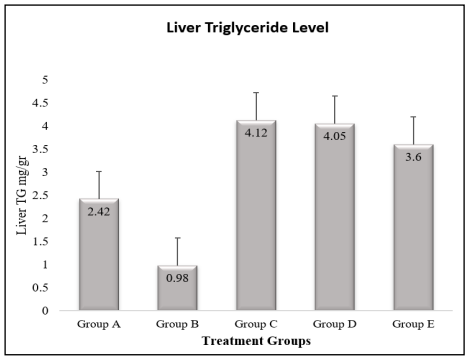

Figure 1: Liver Triglyceride levels in Normal Diet & HCD groups: Group A- Normal diet; Group B -Normal diet & Retinoic acid, Group C -High cholesterol diet, Group D -High cholesterol diet & Vehicle, Group E -High cholesterol diet & Retinoic acid. Data is expressed as mean ± standard deviation (n=8).

Gene Expression Analysis of Hepatic *DGAT2* by TaqMan qPCR

GAPDH was used as the reference gene for the analysis of *DGAT2* gene expression. The relative expression values were calculated using the $2^{-\Delta\Delta CT}$ method and are represented in Figure 2. In Group C, hepatic *DGAT2* gene expression was upregulated compared to Group A, but not statistically significant. *DGAT2* gene expression was downregulated in the RA-treated groups (B and E) compared to their respective control groups (A and C). In Group B (ND+RA), *DGAT2* expression was reduced by 0.15-fold (15% decrease) compared to Group A (ND), but not statistically significant. In Group E (HCD+RA), *DGAT2* gene expression was significantly ($p<0.05$) reduced when compared to the positive control group. Specifically, *DGAT2* expression was significantly reduced by 0.63-fold (63% decrease) when compared to Group C (HCD). The reduction was also 0.54-fold (54% decrease) when compared to Group D (HCD+vehicle), but statistically insignificant.

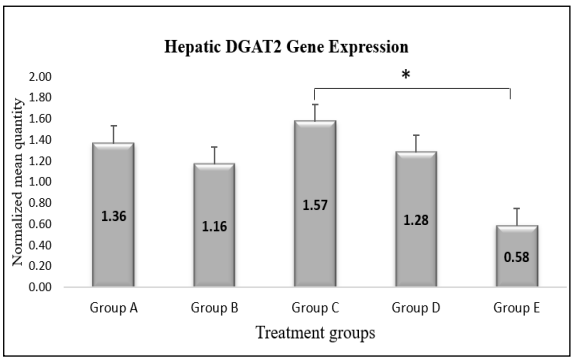

Figure 2: Hepatic *DGAT2* gene expression in normal diet and HCD groups. Group A-Normal diet; Group B- Normal diet & Retinoic acid; Group C- High cholesterol diet; Group D- High cholesterol diet & Vehicle; Group E- High cholesterol diet & Retinoic acid. Data is expressed as mean ± standard deviation (n=8). *P <0.05.

DISCUSSION

This study explored the effects of retinoic acid (RA) on liver triglyceride levels and the expression of the Diacylglycerol Acyltransferase 2 *(DGAT2)* gene in a high-cholesterol diet (HCD) induced steatosis rat model.

Liver triglyceride levels

This study revealed a trend toward reduced liver TG levels in RA-treated groups, both under normal dietary conditions and following HCD induction. However, these reductions did not reach statistical significance. The downward trend in TG levels suggests that RA may play a protective role in hepatic lipid homeostasis, particularly under lipotoxic dietary challenges. This observation is in agreement with recent reports indicating that RA can attenuate hepatic steatosis by modulating lipid metabolism pathways.[29–31] A study by Zarei L, et al. showed that rabbits lacking retinoic acid receptors (RARs) developed microvesicular hepatic steatosis and had poorer fatty acid oxidation capability. The study discovered that RA considerably reduces liver steatosis, comparable to atorvastatin. It also reported a decrease in liver oxidative stress marker-protein carbonyl, serum total antioxidant capacity, and serum lipid in high-fat diet-induced steatosis rabbits. It is stated that RA's antioxidant and anti-inflammatory characteristics might be the primary effective mechanisms for it.[31] Moreover, RA is known to influence hepatic lipid partitioning, not

only by suppressing lipogenesis but also by enhancing mitochondrial β-oxidation.[32,33]

The lowest TG levels were observed in Group B, which was fed a normal diet with RA, indicating that RA might have a triglyceride-lowering effect even under baseline conditions. This aligns with a previous report showing treatment of male mice with all-trans retinoic acid (AtRA) significantly reduced body weight, body fat content, and hepatic TG levels.[34]

Hepatic *DGAT2* gene expression

In this study, hepatic *DGAT2* gene expression was found to be upregulated in rats fed a high-cholesterol diet (HCD; Group C) compared to those on a normal diet (Group A). *DGAT2* encodes a key enzyme responsible for the final step in triglyceride (TG) synthesis, and its elevated expression has been implicated in the pathogenesis of nonalcoholic fatty liver disease (NAFLD) through promotion of hepatic lipid accumulation.[24,35]

Notably, administration of retinoic acid (RA) in HCD -fed rats (Group E) led to a statistically significant downregulation of *DGAT2* expression compared to Group C. This suggests that RA may suppress hepatic triglyceride synthesis by modulating *DGAT2* transcription. These findings support the hypothesis that RA influences hepatic lipid metabolism by targeting key enzymatic regulators of lipogenesis. Supporting this, a study demonstrated that knockout of *DGAT2* in C57BL/6J and ob/ob mice for six weeks resulted in significant reductions in liver triglyceride content and overall body weight, underscoring the central role of DGAT2 in lipid homeostasis.[36]

Interestingly, RA treatment also reduced *DGAT2* expression in rats on a normal diet (Group B), showing a 0.15-fold (15%) decrease relative to Group A. Furthermore, in Group E, *DGAT2* expression was significantly reduced by 0.63-fold (63%) and 0.54-fold (54%) when compared to Group C (HCD) and Group D (HCD+vehicle), respectively. These transcriptional findings are consistent with immunohistochemical data, which showed a pronounced decrease in hepatic DGAT2 protein expression following subcutaneous RA administration in HCD-fed rats.[37]

Overall, the significant suppression of *DGAT2* expression in RA-treated HCD rats (Group E) suggests a protective role for RA in ameliorating diet-induced hepatic lipid accumulation. While the precise molecular mechanisms remain to be fully elucidated, these results highlight that RA has a potential role as a modulator of liver triglyceride metabolism and a candidate for therapeutic intervention in NAFLD.

CONCLUSION

The administration of RA in this study led to a reduced pattern of liver TG levels and a significant downregulation of *DGAT2* gene expression in HCD-induced steatosis rats. This suggests that RA could be a potential therapeutic approach for treating fatty liver disease and preventing conditions like NAFLD and NASH. However, there are several limitations, primarily related to its scope and duration of the study. Specifically, while a beneficial trend toward reduced liver triglyceride levels was observed in the RA-treated groups, this reduction did not reach statistical significance, suggesting a lack of sufficient statistical power or treatment duration. Therefore, further investigation into a larger sample size and longer study durations, i.e., more than 4 weeks of RA treatment, would achieve a statistically significant reduction in liver TG levels. Furthermore, more rigorous molecular and mechanistic studies beyond *DGAT2* gene expression are necessary to fully elucidate the pathway through which RA modulates hepatic lipid metabolism. Such studies should target downstream factors like fatty acid oxidation (e.g., CPT-1), inflammatory markers, and the specific retinoic acid receptor (RAR) mediating the effect to confirm the exact beneficial mechanism of RA in NAFLD.

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CASE REPORT

Navigating the Diagnostic Challenges of Cervical Plexus Schwannoma: A Rare Entity

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Keywords
Schwannoma, Cervical Schwannoma, Posterior neck mass, Nerve sheath tumours, Cervical plexus

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ABSTRACT

Schwannomas are the most commonly occurring neurogenic tumours. However, cervical plexus schwannomas are rare, and they mostly present with an asymptomatic neck mass. An elderly male with multiple comorbidities presented with a painless posterior triangle neck mass for a year without any neurological symptoms. After undergoing a few diagnostic modalities, including ultrasound-guided biopsy and MRI, the mass was suggestive of a schwannoma. Post-excision biopsy, no neurological deficits or recurrence were seen. This case exemplifies the challenges associated with diagnosing cervical plexus schwannomas and highlights the necessity of integrating multiple diagnostic modalities for their accurate identification. The rarity of this tumour, coupled with its uncommon anatomical location, highlights the importance of considering cervical plexus schwannomas in the differential diagnosis of asymptomatic lateral neck masses.

INTRODUCTION

Schwannoma is a mesenchymatous type of tumour that develops from the Schwann cells. Schwannomas are part of the peripheral nerve sheath tumours. They are slow-growing and can originate from the cranial, peripheral, or autonomic nerves. Cervical schwannoma, in general, has a growth rate of 5.3% in a year.[1] Head and neck schwannomas commonly present with a painless, unilateral neck mass. Occurrences of neurological symptoms like paraesthesia and neuralgic pain are rare. However, excising schwannomas without knowing their possible nerve of origin can lead to various morbidities, especially when the tumour occurs at uncommon locations, and one such morbidity is a permanent nerve injury.[2] Based on a study that analysed the nerve of origin of schwannomas, it can be categorised into medial and lateral groups. Lower cranial nerves of IX, X, XI, XII, and the cervical sympathetic chain consist of the medial group. Meanwhile, the lateral group comprised the tumours seen originating from the cervical plexus, cervical neck trunk, and brachial plexus.[3] Hereby, we present a unique case of posterior neck schwannoma in an elderly patient. We emphasise the challenges experienced in the work-up of a neck mass.

Case report

Clinical Presentation

A 68-year-old male with multiple comorbidities presented with a painless left-sided neck swelling that had been progressively enlarging over the past year. He did not report any obstructive symptoms, upper aerodigestive tract complaints, or constitutional symptoms such as fever, weight loss, or night sweats. There were no symptoms suggestive of pulmonary tuberculosis, and no known tuberculosis contacts.

On examination, a solitary left-sided neck mass was noted in the posterior triangle at level V, measuring approximately 3×3 cm. The mass was firm, non-tender, and not attached to the overlying skin. It was mobile in the horizontal plane but fixed in the vertical axis (Figure 1). Examination of other systems revealed no additional abnormalities.

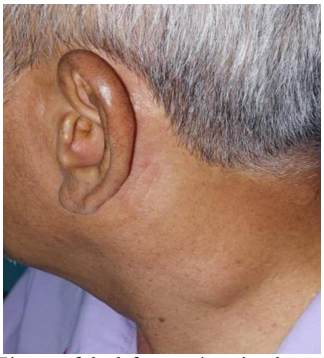

Figure 1 Picture of the left posterior triangle neck mass.

Investigations

Biopsy

An initial fine-needle aspiration (FNA) yielded unsatisfactory results. Subsequently, an ultrasound-guided core needle biopsy was performed, which was reported as consistent with a schwannoma.

Imaging

Contrast-enhanced computed tomography (CECT) of the neck demonstrated a well-defined hypodense lesion in the left posterior neck region, measuring 2.4 × 2.3 × 2.7 cm. Minimal central hypodensity was noted within the lesion, suggestive of cystic or necrotic changes. Anterolaterally, the mass was seen compressing the sternocleidomastoid (SCM) muscle and abutting the left internal jugular vein. Posteriorly and medially, it was indenting the scalene muscles and the longus capitis muscle, respectively.

Subsequent magnetic resonance imaging (MRI) revealed a T2-weighted hyperintense lesion located deep to the SCM muscle, which enhanced on post-contrast sequences. A beak-like configuration was observed pointing towards the left C2/C3 neural foramen; however, there was no widening of the foramen or intraspinal extension (Figure 2a and 2b). No brachial plexus involvement was identified. Collectively, the imaging features suggested a schwannoma arising from the cervical nerve root.

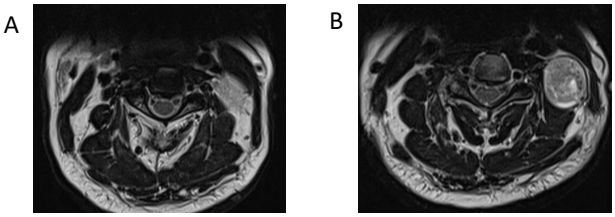

Figure 2 MRI neck axial view showing a hyperintense mass on T2-weighted, which was located deep to the SCM muscle (A), and a beak-like configuration was seen pointing towards the left C2/C3 neural foramen (B).

Surgical Findings

The patient underwent surgical excision under general anaesthesia. Intraoperatively, a well-encapsulated mass measuring 4×2.5 cm was visualised deep to the SCM muscle and anterior to the trapezius muscle. The greater auricular nerve and spinal accessory nerve were identified and preserved. The tumour was found to arise from the cervical plexus (Figure 3a and 3b).

Intracapsular dissection was carefully performed, and the tumour was successfully separated from the surrounding neural structures. The nerve of origin was preserved and left intact. There were no complications during the procedure.

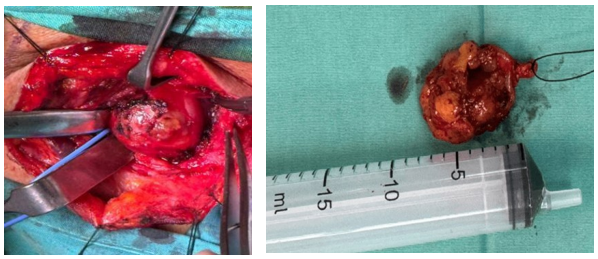

Figure 3 Intraoperative picture of the posterior neck mass (A). Gross specimen of the mass, which was excised as a whole (B).

Histopathological Findings

Histological examination confirmed the diagnosis of schwannoma, demonstrating classic Antoni A and Antoni B architectural patterns. Immunohistochemical staining showed diffuse positivity for S-100 protein, further confirming the diagnosis (Figure 4a–d).

Outcome

The postoperative course was uneventful, and the patient did not develop any neurological deficits. He remains well and under regular follow-up, with no evidence of recurrence observed over the past year.

Patient Consent

Written informed consent was obtained from the patient for publication of this case report and accompanying images. Efforts have been made to ensure anonymity and confidentiality.

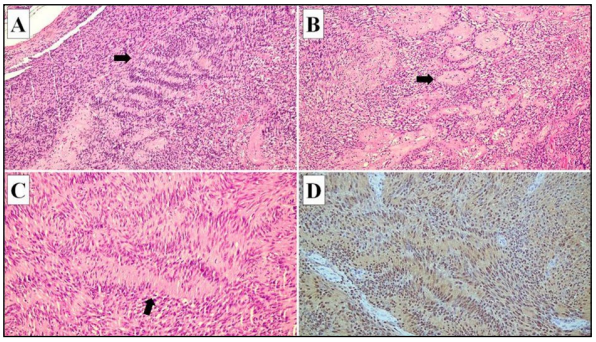

Figure 4 The tumour is composed of a biphasic pattern of compact hypercellular Antoni A area (arrow) and myxoid hypocellular Antoni B area (A, H&E, x20). Focally, there are many blood vessels with thickened hyalinised walls (arrow) (B, H&E, x20). The neoplastic cells demonstrate elongated and wavy nuclei with tapered ends, and form nuclear palisading called Verocay bodies (arrow) (C, H&E, x40). These cells are positive for S100 immunohistochemistry (D, x40).

DISCUSSION

Schwannomas are benign tumours that arise from Schwann cells, and they account for about 25%–45% of all tumours in the head and neck region.[1] The commonest of them are intracranial schwannomas.[1] However, cervical schwannomas and schwannomas originating from the cervical plexus are less commonly reported.[2] The cervical plexus, formed by the anterior rami of the upper four cervical nerves (C1–C4), is deeply situated beneath the sternocleidomastoid muscle. Among these, the C2 nerve root is the most frequent origin of schwannomas in this region.[1]

Many patients with schwannoma lack noticeable symptoms during their presentation.[3] Commonly, patients present with a unilateral neck mass, which is painless and slow-growing.[1] The challenge and difficulty in diagnosing these cases are experienced during this stage of the disease, as not many present with evidence of neurological deficit, although schwannomas are known as neurogenic tumors.[3-4] Occasionally, patients can have symptoms suggestive of Horner's syndrome, which points more towards origin from the cervical sympathetic chain.[2] Due to a lack of specific signs and symptoms, these benign tumours are frequently misidentified as cervical lymphadenopathies, branchial cysts, or other benign soft tissue tumours.[5] Zhang et al. and de Araujo et al. reported the annual tumour growing rates are 2.75mm and 3mm, respectively, supporting their indolent nature.[3]

Despite being a benign lesion, the risk of malignant transformation has been observed in about 8% and 13.9% of head and neck schwannomas.[3] Owing to their deep anatomical location topped with subtle symptoms and minimal to absent neurological deficits, clinical suspicion alone is insufficient for its diagnosis, necessitating other investigations like imaging and histopathological evaluation.

The primary investigation for unilateral neck mass is fine-needle aspiration cytology (FNAC). It is widely available and cost-effective for evaluating lymphadenopathy and distinguishing between benign and malignant lesions.[6] However, FNAC has a relatively low sensitivity (0%–40%) for diagnosing schwannomas. This is due to the tumour's heterogeneous nature, which frequently undergoes cystic degeneration. Thus, they contain a combination of densely cellular areas and sparsely cellular, myxoid regions, which are Antoni A and Antoni B, respectively.[7] This architectural complexity contributes to the high rate of unsatisfactory FNAC results in schwannomas. FNAC also lacks both the ability to report any nerve involvement nor to use immunohistochemical analysis, such as S-100 protein to aid in its diagnostic accuracy.[5,7] Thus, this further reduces the role of FNAC to diagnose tumours like cervical schwannomas.[8] A study that analysed 30 cases of extracranial head and neck schwannomas reported only four that were able to be correctly diagnosed via FNAC. Specificity of only about 20% was detected via FNAC, which was less than imaging modalities such as CT and MRI, which had a specificity of 38%.[8]

Another investigation of choice is the ultrasound-guided core needle biopsy, which has emerged as a superior alternative to FNAC. A retrospective study of 154 cases of peripheral nerve sheath tumours reported an overall diagnostic accuracy of 99.3% using ultrasound-guided core needle biopsy, with sensitivity and specificity rates of 90.9% and 100%, respectively.[9] Core needle biopsy allows for better preservation of tissue architecture, facilitating immunohistochemical staining for markers such as S-100, SOX10, and EMA, which are key in differentiating schwannomas from other spindle cell tumours.[7] In our case, both FNAC and ultrasound-guided core needle biopsy were performed, with the definitive diagnosis established through core needle biopsy.

Imaging, particularly MRI, is the preferred modality for diagnosing schwannomas due to its soft tissue contrast. MRIs are particularly useful in defining tumour boundaries, evaluating involvement with adjacent nerves and vessels, and characterizing internal structure variations that are significant in head and neck tumours.[1] Schwannomas typically appear as well-circumscribed, encapsulated masses and are hyperintense on T2-weighted images, reflecting their biphasic cellular architecture.[1] Besides, diffusion-weighted MRI can aid in distinguishing benign schwannomas from their malignant counterparts as the former shows a higher apparent diffusion coefficient value (ADC) due to their lower cellular density and abundant myxoid content.[10] Despite MRI's diagnostic advantages, imaging alone often cannot confirm schwannomas as these tumours can mimic other soft tissue masses, thus histopathological analysis remains essential.[11]

Management of cervical schwannomas is primarily surgical, with extracapsular resection being the preferred approach to preserve neural function, while ensuring complete tumour excision.[5] However, due to the intimate association with cervical nerves, conventional extracapsular excision, while effective, carries risks of nerve injury, leading to unwanted sensory or motor deficits.[11] To mitigate this, intracapsular excision with gentle dissection between the tumour capsule and normal fascicles has been advocated. The epineurium layer covering the capsule should be dissected akin to peeling an onion, allowing for safe removal of the tumour via its proximal and distal poles.[12] Notably, fascicles within the tumour are usually non-functional, and their excision typically does not result in postoperative deficits.[12]

The usage of intraoperative nerve monitoring is preferred, especially for tumours originating from functionally significant nerves. Besides, the usage of an operating microscope, microsurgical instruments, and techniques is pivotal.[2] Advances in nerve-sparing enucleation and intraoperative neuromonitoring have significantly reduced postoperative neurological complications.[5,13] In our case, meticulous surgical planning and intraoperative nerve preservation strategies were employed to avoid any neurological complications.

The choice of surgical approach should be guided by the tumour's size, location, proximity to great vessels, and any suspicion of malignancy. In large or infiltrative types of schwannomas, where nerve preservation is not feasible, a nerve sacrifice may be warranted, followed by its reconstruction and rehabilitation planning.[11] Thorough preoperative counselling is crucial to inform patients of potential neurological sequelae. Despite their benign nature, schwannomas often require timely surgical intervention due to the possibility of progressive compression symptoms. Nonetheless, long-term surveillance is generally not necessary post-operatively given their low recurrence rates and excellent prognosis following a complete excision.

CONCLUSION

This case exemplifies the challenges associated with diagnosing cervical plexus schwannomas and highlights the necessity of integrating multiple diagnostic modalities for an accurate diagnosis. Although neurogenic tumours like cervical plexus schwannomas are benign in nature, they should be considered as part of the differential diagnosis of any unilateral, slow-growing neck mass in adults.

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CASE REPORT

Ultrasound Guided Dextrose Prolotherapy For Chronic Medial Collateral Ligament Injuries In Young Footballers

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Keywords
dextrose prolotherapy, medial collateral ligament, knee injury, football, adolescent

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ABSTRACT

Dextrose prolotherapy (DPT) is increasingly recognized for its regenerative potential in managing ligamentous injuries. However, evidence supporting its use in medial collateral ligament (MCL) injuries among young athletes remains scarce. This case series describes four adolescent footballers (aged 14–16 years) with chronic medial knee pain unresponsive to rehabilitation. Three athletes with isolated MCL injuries achieved complete pain resolution within 4–6 weeks following ultrasound-guided 12.5% dextrose injections, combined with knee bracing and structured rehabilitation. They successfully returned to full training after passing functional assessments, with no reinjury at 3- and 6-month follow-ups. Conversely, the fourth athlete, with concomitant MCL, anterior cruciate ligament, and meniscal injuries, experienced only partial pain relief and sustained reinjury after premature return to play, ultimately requiring surgical intervention. These findings suggest that DPT may accelerate recovery in isolated chronic MCL injuries but has limited benefit in multi-ligament knee injuries.

INTRODUCTION

Medial collateral ligament (MCL) injuries are prevalent in young athletes, especially in sports such as football and skiing. In football, MCL sprains rank among the three most common knee injuries, often resulting from valgus forces that overload the ligament. While most cases respond well to activity modification, early mobilization, bracing, and structured physiotherapy, incomplete recovery may result in persistent pain, instability, and prolonged absence from sport. Surgical intervention is rarely indicated in young patients with partial-thickness MCL tears, prompting interest in minimally invasive treatment modalities.

Prolotherapy is a regenerative injection therapy aimed at stimulating tissue repair. Dextrose, owing to its safety, affordability, and accessibility, is the most commonly used agent. Dextrose prolotherapy (DPT) is postulated to induce localized inflammation via osmotic mechanisms, initiating the healing cascade, and to modulate pain perception by acting on nerve ion channels. Although promising results have been reported for various musculoskeletal disorders, there is limited evidence for its use in isolated MCL injuries. To date, only one published case report (2015) has described DPT for MCL injury. This paper reports four cases of adolescent footballers with chronic MCL injuries treated with ultrasound-guided DPT following suboptimal recovery from intensive rehabilitation

CASE SERIES

CASE 1

A 15-year-old male striker sustained a valgus injury to his right knee, presenting two months later with persistent medial knee pain (VAS 7/10) despite rehabilitation. Clinical assessment revealed proximal MCL tenderness and a positive valgus stress test at 30°. Ultrasound demonstrated a hypoechoic lesion over the proximal MCL. A diagnosis of chronic partial MCL tear was made. He underwent three ultrasound-guided 12.5% dextrose injections at two-week intervals. Two weeks after the final injection, his pain had resolved (VAS 0/10), with no tenderness and a negative valgus test. He resumed light

drills and returned fully to competition six weeks later after passing the Advanced Lower Extremity Sport Assessment (ALESA). No reinjury was reported at 3- and 6-month follow-ups.

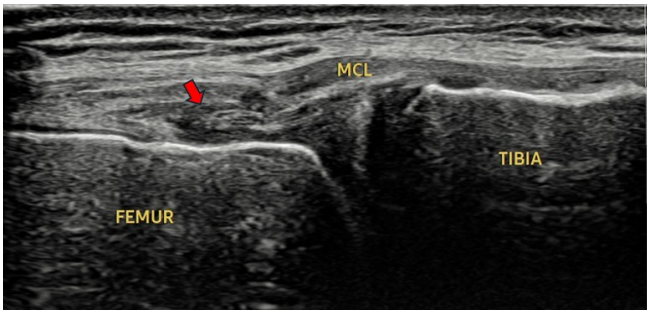

Figure 1: Ultrasound finding of right MCL, shows hypoechoic lesion over the proximal MCL (red arrow).

CASE 2

A 16-year-old male striker reported a two-month history of severe left medial knee pain (VAS 10/10) following a valgus stress injury. Examination showed proximal MCL tenderness and a positive valgus stress test at 30°, with ultrasound confirming a hypoechoic lesion. Chronic partial MCL tear was diagnosed. He received three ultrasound-guided 12.5% dextrose injections at two-week intervals. His pain resolved completely (VAS 0/10) within two weeks after the final injection, with a negative valgus test. He resumed sport-specific drills and passed ALESA testing at six weeks, returning to competitive play without reinjury at follow-up.

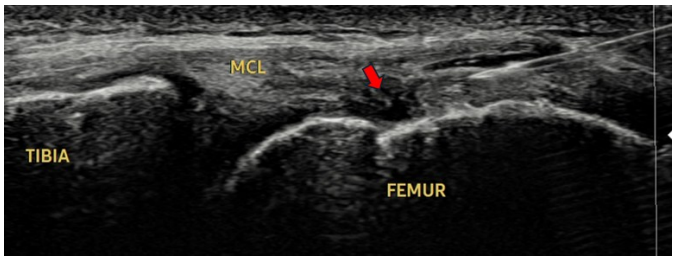

Figure 2: Ultrasound finding of left MCL, shows hypoechoic area at the proximal MCL (red arrow), with needle in situ during the guided procedure.

CASE 3

A 14-year-old male winger presented with a six-week history of left medial knee pain (VAS 8/10) after a missed tackle forced the knee into valgus. Proximal MCL tenderness and a positive valgus test at 30° were noted. Ultrasound demonstrated thickening of the proximal and mid-MCL. After a single ultrasound-guided 12.5% dextrose injection, his pain resolved completely (VAS 0/10) and valgus testing was negative. He returned to full training within three weeks after passing ALESA, with no recurrence at follow-up.

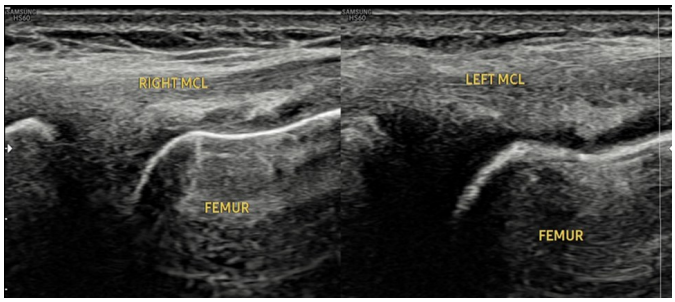

Figure 3: Ultrasound finding of thickened left MCL compared to the normal right MCL.

CASE 4

A 16-year-old female striker, with a four-year history of recurrent left MCL sprains, sustained a twisting injury causing pain (VAS 8/10), instability, and swelling. Imaging confirmed partial tears of the MCL, ACL, and medial meniscus. Despite surgical advice, she opted for conservative treatment to participate in an upcoming international tournament. She underwent three ultrasound-guided 12.5% dextrose injections at two-week intervals, achieving partial pain relief (VAS 4/10). However, premature return to play led to reinjury, and she subsequently required ACL reconstruction, MCL repair, and meniscus repair.

DISCUSSION

These four adolescent footballers presented with chronic medial knee pain that persisted despite out-of-training and intensive rehabilitation for at least 6 weeks. Clinical examination, supported by ultrasound confirmed the MCL injuries. They were treated with ultrasound-guided DPT using 12.5% dextrose solution, prepared by diluting 1 mL of 50% dextrose in 3 mL water for injection. Half of the mixture was injected within the MCL fibers and the other half just superficial to the ligament. NSAIDs were avoided during the treatment period to allow the desired inflammatory response. Patients were advised to apply ice compression after the injections. Paracetamol and Tramadol were permitted as analgesic, although none of the patients required them post-procedure. All patients followed structured rehabilitation protocols, including the use of hinged knee braces, under supervision of their respective team physiotherapists. Mild post-injection

soreness was reported by all patients but subsided within 48 hours after the injections.

Following treatment, the three footballers with isolated MCL injuries, achieved complete pain resolution within two to six weeks post, demonstrating the potential effectiveness of DPT. In contrast, the fourth case who sustained combined MCL, ACL and meniscus tears, demonstrated only partial pain relief before sustaining a re -injury after premature return to play, eventually requiring surgical intervention. The different outcomes highlight the importance of proper patient selection. Isolated MCL injury has excellent healing capacity due to its ability to increase blood supply through angiogenesis,8 making it suitable for non-surgical intervention like DPT. However, MCL injury with concomitant ACL and meniscus tears, may significantly compromise the knee stability. In such cases, surgical intervention is required for optimal recovery.[1] These findings indicate that DPT should not be used as universal solution for all knee injuries but rather as a targeted therapy for carefully selected cases.

DPT is believed to act via two main mechanisms: pain modulation and tissue regeneration. For pain modulation, dextrose opens potassium channels which lead to hyperpolarization of nerve fibers, as well as enhances inhibitory glycine receptors. These collectively suppress the nociceptive signals.[9] Regarding tissue regeneration, dextrose acts in a concentration-dependent manner. Dextrose solution below 10% concentration promotes cell proliferation and tissue repair without triggering inflammation, whereas dextrose with concentrations above 10% create an osmotic gradient that triggers cell lysis and draws inflammatory mediators, facilitating tissue healing.[10]

Our findings align with the existing evidence that DPT may be an effective therapy for musculoskeletal conditions.[4,5,6] When compared DPT to other regenerative therapies such as platelet-rich plasma (PRP) and stem cell, it is cost effective and easy-to-prepare, making it an accessible option for any clinical settings.

This case series highlight the diversity of patient outcomes in relation to the complexity of the injuries. While DPT may be a potential treatment for isolated partial MCL injury, its effect appears limited in multiligamentous injuries as Case 4.

Given the small sample size, case series design and absence of a control group, definitive clinical recommendations cannot be made. Future studies with larger samples, particularly randomized controlled trials comparing DPT to placebo and other regenerative therapies, are essential to validate these preliminary findings and refine treatment strategies for MCL injuries in athletes. Additionally, a key limitation of this case series lies in the reliance on clinical assessment and return -to-play ability as outcome measures. Incorporating radiological evaluations at 3 and 6 months would have provided more objective insights into tissue healing and efficacy of DPT.

CONCLUSION

This case series suggests that DPT is a promising adjunct in the treatment of isolated MCL injuries, providing effective pain relief and successful return to play in young athletes. However, its role is limited in complex knee injuries, where surgical intervention remains essential. Proper patient selection is crucial to achieving optimal outcomes.

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CASE REPORT

Prolonged Fever and Unremitting Back Pain in Elderly Patient: A Case of Disseminated Tuberculosis with Spondylodiscitis

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Keywords
back pain, disseminated tuberculosis, tuberculous spondylodiscitis, paravertebral abscess

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ABSTRACT

Disseminated tuberculosis (DTB) is a life-threatening condition caused by the hematogenous spread of Mycobacterium tuberculosis. As the clinical presentation is often nonspecific, it leads to late diagnosis. We reported a case of a 75-year-old man who presented with intermittent fever for one month and lower back pain for four months. Bronchoscopy with bronchoalveolar lavage revealed low-level of Mycobacterium tuberculosis, leading to a diagnosis of smear-negative pulmonary tuberculosis. Magnetic resonance imaging of the thoracolumbar spine showed multiple enhancing skip lesions involving thoracic and lumbar vertebrae bodies with a T8/T9 paravertebral abscess compressing onto the spinal cord, suggestive of tuberculous spondylodiscitis and confirming a diagnosis of DTB. The patient's condition improved with anti-tuberculosis therapy. This case emphasizes the need for high clinical suspicion in patients with multisystem involvement and the importance of timely, targeted investigations to confirm the diagnosis.

INTRODUCTION

Tuberculosis (TB) is endemic in Malaysia. Malaysia aims to reduce TB deaths to fewer than 85 deaths per year by year 2035.[1] The global incidence of disseminated tuberculosis (DTB) is unclear, but it accounts for less than 2% of all TB cases and up to 20% of extrapulmonary TB cases.[2] DTB is characterized by Mycobacterium tuberculosis infection at two or more non-contiguous sites due to lymphohematogenous spread.[3] While DTB is rare, its severity necessitates prompt diagnosis and treatment.[4]

There is a rising trend in disseminated tuberculosis (DTB) diagnoses, largely attributed to the increasing prevalence of immunocompromising conditions such as diabetes, malignancies, HIV/AIDS, and the use of immunosuppressive medications.[2] Diagnosing DTB remains highly challenging due to several factors, such as nonspecific symptoms, atypical clinical presentations, difficulties in obtaining appropriate samples, and the possibility of false-negative test results, of which can delay treatment and increase the risk of mortality.[4] Although untreated DTB is often fatal, an early intervention significantly improves outcomes.[4]

Case presentation

A 75-year-old gentleman with pre-fibrotic primary myelofibrosis (PMF), hypertension, and hyperlipidemia presented with a one-month history of intermittent fever associated with chills and rigors. He also complained of intermittent lower back pain for the past four months, with no cough, tuberculosis contact, seizures, or headaches. He also reported an unintentional weight loss of three kilograms due to poor appetite.

He was treated twice at our center for similar complaints within the past month. He was initially treated for acute pharyngitis with tablet augmentin 625mg three times per day and later for viral fever with supportive care. Three days prior to the current presentation, he sought emergency care for fever and left-sided pleuritic chest pain. His chest X-ray revealed patchy opacities at the right middle zone and consolidation at left lung apices (figure 1). He then was diagnosed with community-acquired pneumonia (CAP) and treated with tablet augmentin 625 mg three times per day and tablet azithromycin 500 mg once daily. Despite the complete courses of the prescribed antibiotics, his fever persisted.

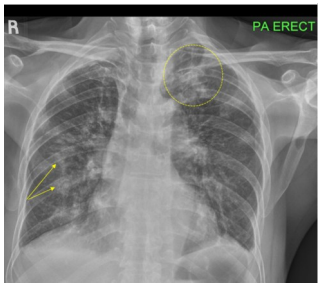

Figure 1: Chest radiograph shows patchy opacities at right middle and lower zone (yellow arrow) and consolidation at left lung apices (yellow circle).

On examination, he appeared cachectic but stable with a temperature of 37.3°C. Respiratory, cardiovascular, and abdominal examinations were unremarkable. His full blood count (FBC) revealed leucocytosis (total white cell (TWC) count of 13.45 x10^9/L), anaemia (haemoglobin level of 10.2 g/dL), and thrombocytosis (platelet count of 637 x10^9/L). He was scheduled for follow-up in one week for further investigation.

A week later, he remained febrile, with temperatures ranging from 38°C to 39°C. His sputum cultures and sensitivity (C&S), acid-fast bacilli (AFB), and urinalysis was all negative. Repeated FBC revealed normal TWC count (10 x10^9/L) and anaemia (haemoglobin of 10.4 g/d). His inflammatory markers were elevated with C-reactive protein (CRP) of 84.5 mg/L and erythrocyte sedimentation rate (ESR) of 101 mm/h. He was then was admitted for further evaluation.

In the ward, he remained febrile despite intravenous (IV) ceftazidime and IV meropenem. Repeated AFB sputum, sputum C&S, Sputum Mycobacterium tuberculosis C&S, and blood culture were negative. Further investigation with bronchoscopy with bronchoalveolar lavage by GeneXpert MTB/RIF test revealed low-level Mycobacterium tuberculosis with rifampicin resistance not detected, leading to a diagnosis of smear-negative pulmonary tuberculosis. He began anti-tuberculosis therapy, tablet Akurit 4 (isoniazid + rifampicin+ethambutol+ pyrazinamide) 3 tablets daily and tablet pyridoxine 20mg daily leading to fever resolution and improvement in thrombocytosis (615 x10^9/L to 217 x10^9/L). After 16 days of ward admission, he was discharged with follow-ups scheduled for orthopaedic and respiratory clinics including daily observed therapy (DOTS) at the primary clinic.

Figure 1 **Figure 2**

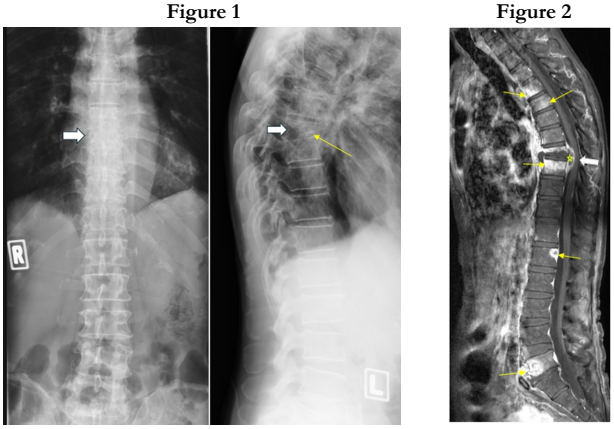

Figure 2: Thoracolumbar radiograph in AP and lateral view shows reduced height of T8 vertebrae body (white arrow) associated with irregularity and sclerosis at inferior end plate of T8 vertebrae (yellow arrow).

Figure 3: MRI thoracolumbar shows abnormal enhancement involving T4, T5, T9, L1 and L5 vertebrae bodies (yellow arrows) associated with T8 vertebrae plana and paravertebral collection at T8/T9 vertebrae body (yellow star). Noticed epidural cord compression at T8/T9 level (white arrow).

At the orthopedic clinic appointment, he reported intermittent back pain. Examination revealed mid-thoracic kyphosis with no neurological deficits. Thoracolumbar radiograph showed total T8 vertebrae collapse with irregularity and sclerosis (figure 2) , while MRI revealed enhancing skip lesions in thoracic and lumbar vertebrae bodies with T8/T9 paravertebral abscess compressing the spinal cord (figure 3), consistence with tuberculous spondylodiscitis. The current diagnosis is disseminated tuberculosis with tuberculous spondylodiscitis at T8-T9, with no neurological deficits. Conservative management was chosen.

At subsequent follow-up, the patient demonstrated marked clinical improvement. His appetite had returned to normal, his back pain had resolved, and he was afebrile. His haemoglobin level also improved, reaching 11.2 g/dL.

DISCUSSION

The World Health Organization (WHO) reports a steady decline in global TB incidence and mortality, aiming for a 90% reduction by 2035.[5] Despite this progress, cases of DTB, particularly in immunocompetent adults, remain rare but clinically significant.[2]

Its variable presentations like fever, weight loss, and night sweats, along with manifestations such as anorexia and, in severe cases, multiorgan failure, will occur, depending on the affected organs.[6] These symptoms mimic other infectious and non-infectious diseases, causing delays in diagnosis. In this case, the patient's advanced age and PMF on treatment complicate his clinical picture. He presented with persistent fever, weight loss, and cachexic with negative sputum tests for acid-fast bacilli, sputum culture, and sputum MTB C&S, further complicated the diagnosis.

Despite advancements in diagnostic techniques, clinicians must consider risk factors, clinical symptoms, systemic involvement, laboratory results, and imaging studies when evaluating DTB.[7] Chest radiography remains a key diagnostic tool for TB.[2] The initial chest radiograph of our patient revealed patchy opacities at the right middle zone with consolidation at left lung apices, which were initially interpreted as CAP.

DTB diagnosis is established if a patient has any of the following: (1) Isolation of Mycobacterium tuberculosis from bone marrow, liver biopsy or ≥ 2 non-contiguous organs; (2) Isolation of Mycobacterium tuberculosis from one organ and histopathological demonstration of caseating granulomas from the bone marrow, liver biopsy specimen or another non-contiguous organ; (3) Isolation of Mycobacterium tuberculosis or histopathological identification of caseating granulomas from one organ and radiographic finding of miliary lung lesions.[2]

In this case, the diagnosis of smear-negative pulmonary TB was confirmed by a bronchoalveolar lavage (BAL) specimen sent for GeneXpert MTB/RIF, which revealed low-level Mycobacterium tuberculosis with no rifampicin resistance.

Given the patient's persistent back pain, tuberculous spondylodiscitis should have been considered earlier in the diagnostic process. In any patient presenting with abnormal chest X-ray findings and back pain, clinicians should maintain a high index of suspicion for spinal tuberculosis, as early diagnosis and intervention are critical to prevent severe complications such as vertebral collapse, neurological deficits, or permanent spinal deformity.[10] Furthermore, establishing a diagnosis of spinal TB has significant implications for management. International guidelines recommend 6-9 months of treatment (2EHRZ/4-7HR) for bone and joint tuberculosis.[1] Extrapulmonary TB, including musculoskeletal involvement, can be overlooked if clinicians focus solely on pulmonary findings. Given that imaging plays a crucial role in diagnosis, prompt MRI should be performed in suspected cases to confirm spinal involvement and initiate appropriate treatment without delay. MTB typically affects the thoracic spine.[8] Radiographs often show anterior vertebral body destruction, loss of disk height, end plate erosion, vertebral geodes, bone sequestration, sclerosis, and paravertebral masses.[8] Imaging, in this case, revealed thoracolumbar involvement with T8 vertebral collapse, skip lesions, and a paravertebral abscess consistent with tuberculous spondylodiscitis. A previous study identified three key indicators of TB spine: subligamentous spread, vertebral collapse over 50%, and large thin-wall abscess, with subligamentous spread having the highest predictive value (97.5%). MRI is, therefore one of the best diagnostic methods for TB spondylodiscitis.[9]

In this case, the diagnosis of DTB was obtained after about 10 weeks of presentations. The delay in reaching the final diagnosis in this case highlights the complexities involved, including the need for more advanced investigations such as bronchoscopy and spine MRI when standard tests yield negative results.

Following the diagnosis, the patient was initiated on anti-tuberculosis. Treatment for DTB depends on the affected organ/system,[1] which in this case is musculoskeletal. Thus, the patient was planned for a 2-month intensive phase with tablet Akurit 4, 3 tablets daily, followed by a 4 -month maintenance phase with tablet isoniazid and rifampicin.

Despite advancements in supportive care, DTB mortality remains high, between 25% to 30%.[2] Predictors of poor outcomes include meningismus, liver cirrhosis, variations in white blood cell counts, older age, pre-existing health conditions, altered mental status, and persistent night sweats.[2] In this case, the elderly patient has multiple pre-existing health conditions, increasing the risk of poor outcomes. The spine was involved in our case, making early diagnosis and prompt treatment crucial to avoid complications.

CONCLUSION

Greater awareness can improve clinical suspicion and prompt the timely use of imaging and microbiological or histopathological tests. Immunosuppressed patients with prolonged fever, weight loss, and abnormal blood indices should be evaluated for DTB to enable prompt management and reduce mortality risk.

ACKNOWLEDGMENTS

The authors express their gratitude to the patient for his cooperation in allowing us to share this case report.

CONFLICT OF INTEREST

No potential conflict of interest relevant to this article was reported.

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CASE REPORT

Flare-Up in Full Bloom: Navigating Rosacea Fulminans During Pregnancy

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Keywords
Rosacea fulminans, Pregnancy, Severe acne.

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ABSTRACT

Rosacea fulminans (RF) is a rare and severe variant of rosacea, characterized by the sudden onset of extensive facial skin lesions. Hormonal changes, particularly during pregnancy have been proposed as potential contributing factors for RF. Management of RF in pregnant patients poses unique challenges, as standard therapeutic options may carry teratogenic risk. To date, reported cases of RF during pregnancy in the medical literature is very limited. We report a case of RF that developed during pregnancy, likely precipitated by hormonal fluctuations. This case underscores the importance of prompt recognition and timely intervention to prevent permanent facial scarring, which may have profound psychological consequences for affected patients.

INTRODUCTION

Rosacea fulminans (RF), also known as pyoderma faciale, is a rare and severe inflammatory dermatosis first described by O'Leary and Kierland in 1940.[1] RF predominantly affects women of reproductive age and is differentiated from typical rosacea by the abrupt onset and rapid progression of painful facial lesions.[2] Although its exact aetiology remains unknown, RF can lead to significant facial disfigurement, necessitating prompt diagnosis and appropriate management. Such dermatological manifestations may contribute to heightened emotional distress, particularly during pregnancy, a period when women are mostly physiologically and psychologically vulnerable.

CASE REPORT

A 35-year-old Malay primigravida at 9-weeks' period of amenorrhoea (POA) presented with 4-week history of progressively worsening, painful, erythematous papules and pustules on the face. The onset of eruption coincided with confirmation of pregnancy at approximately 5 weeks' POA. She denied systemic symptoms such as fever, photosensitivity, or musculoskeletal pain. Her regular skincare routine included a twice-daily cleanser, moisturizer, and sunscreen, with no recent changes in cosmetic or skincare. She had no history of allergies or prior acne.

Initial treatment at a primary care clinic with topical therapy for presumed mild acne yielded no improvement. Lesions rapidly enlarged, coalesced, and ruptured, forming scabs and nodules. A subsequent consultation provided similar reassurance. She trialed alternative remedies (turmeric, honey, lime juice) once, after which symptoms worsened. However, the lesion was not consistent with contact dermatitis, as it was non-itchy, with no vesicles or eczematous changes.

The facial lesions' pain was progressively intensified, necessitating daily paracetamol use. She reported nocturnal burning and pruritus over the affected area causing sleep disturbance. Otherwise, there was no lesions were noted elsewhere on the body. Psychosocially, the

disfiguring lesions caused self-consciousness and concerns about stigmatization, though she denied depressive symptoms.

Moreover, her facial condition was adversely affecting her self-esteem. She often felt self-conscious about going to work, fearing stigmatization due to perceived infectiousness. After she shared her concerns, her husband encouraged her to seek a second opinion at a health clinic. Despite these challenges, she denied experiencing any depressive symptoms.

On examination, vital signs were stable. Cutaneous assessment revealed multiple erythematous papules, pustules, nodules, and purulent cysts with overlying scabs, measuring 0.2–0.5 cm in diameter, distributed over the bilateral cheeks, nose, and chin. Otherwise, there was no comedones or telangiectasia were observed. There was no cervical lymphadenopathy or extrafacial involvement.

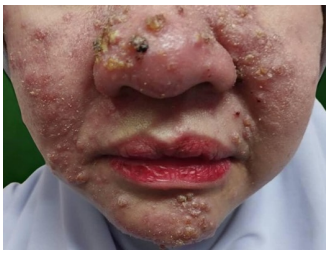

Figure 1: Close-up picture of the face upon presentation to our clinic.

Based on the patient's history and physical examination, a diagnosis of rosacea fulminans (RF) was established. The case was subsequently discussed with the Dermatology team. Initial management included oral amoxicillin, topical metronidazole, and symptomatic treatment with paracetamol and loratadine. Psychoeducation was provided to explain the nature of RF, its association with pregnancy, available treatment options, and to address psychosocial concerns with the aim of reducing distress and improving self-esteem.

After ten days, there was minimal improvement in her skin condition (Figure 2). She was counselled regarding a skin biopsy. However, she declined due to concerns about potential scarring and opted to continue with oral and topical therapy. Oral erythromycin was subsequently initiated, with topical metronidazole maintained. At the one-month follow-up, significant clinical improvement was noted, with resolution of pain, burning sensation, and no new pustular lesions (Figure 3). After three months of treatment, there was almost complete resolution of the facial lesions (Figure 4). The patient was advised to continue topical therapy to maintain remission.

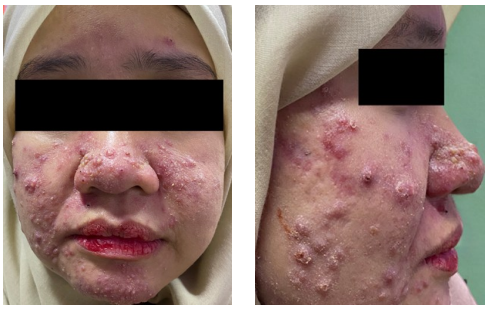

Figure 2. Ten days of treatment initiation (minimal improvement seen over the face)

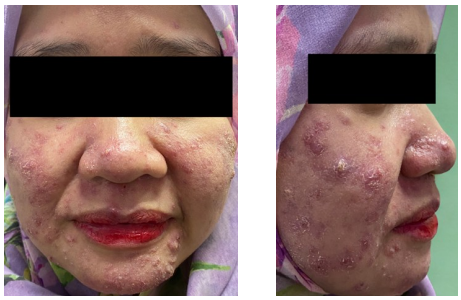

Figure 3. One-month post-treatment (resolution of the facial lesion)

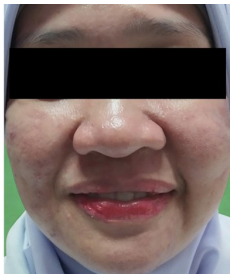

Figure 4: Three months post-treatment (significant improvement is seen over the face)

DISCUSSION

Rosacea fulminans (RF) is a severe and acute variant of rosacea that predominantly affects the face, typically involving the chin, cheeks, and forehead.[3] It is characterized by the sudden onset of painful and pruritic papulopustules, coalescing nodules, cyanotic erythema, and draining sinuses.[4,5]

The primary differential diagnosis is acne conglobata (AC). However, AC typically presents with comedones and acneiform lesions on the trunk and extremities, in which are absent in RF.[3] Due to relatively non-specific findings, histopathological examinations has limited

diagnostic value in RF, though it may help determine the stage of the disease and provide supportive diagnostic information when excluding alternative diagnosis.[1,2,3,,6] In this case, the patient's lesions were confined to the facial region, with no involvement of other body areas, supporting a diagnosis of RF.

Although the exact aetiology of RF remains unclear, interactions between hormonal changes and emotional stressors are thought to play a role in neurovascular dysregulation, triggering the abrupt onset and intense inflammatory response. This mechanism may explain the higher prevalence of RF among young women during pregnancy.[2,6,7]

A review of 135 cases of RF, rosacea conglobata, or pyoderma faciale reported between 1916 and 2016 identified potential triggering in 42% (57/135) of cases, with pregnancy being the most common, accounting for 42% (24/57) of these cases.[1] In the present case, our patient developed severe papulopustular eruptions shortly after pregnancy confirmation, with progressive worsening despite no prior dermatological history.

Given the severity of RF, early recognition and intervention are crucial to prevent complications, particularly permanent facial scarring.[3] However, managing RF during pregnancy poses unique challenges. Systemic therapy is often required, yet conventional treatments such as retinoids and tetracyclines are contraindicated due to teratogenicity.[8]

The recommended first-line treatment for RF during pregnancy typically consists of oral and topical antibiotics' combination.[9] Oral macrolides, such as erythromycin and azithromycin, are preferred due to their favourable safety profile and therapeutic efficacy, offering anti-inflammatory effects, reduction of bacterial colonisation, immunomodulatory benefits and rapid symptom relief. Topical agents, such as metronidazole, are also commonly prescribed for their anti-inflammatory properties.[8,9] Systemic corticosteroids may be considered when first-line therapy is ineffective.[9] In this case, the patient initially received oral amoxicillin with minimal clinical response. Subsequent treatment with oral erythromycin and topical metronidazole led to marked clinical improvement.

RF can also have a profound impact on psychological well-being, particularly in pregnant women who may already be sensitive to physical changes. The disfiguring nature of RF lesions can lead to diminished self-esteem, feelings of unattractiveness, and social withdrawal. This psychosocial distress may exacerbate stress during pregnancy and potentially affect pregnancy outcomes.[10] Therefore, both physical and emotional aspects should be addressed in managing RF.

Consequently, careful follow-up and strict adherence to treatment regimens are essential, with close consideration of the balance between therapeutic benefits and potential maternal–fetal risks.

The prognosis of RF in pregnancy is generally favourable, though clinical outcomes may vary.[2] To date, 27 cases of pregnancy-associated RF have been reported in the English literature, with one case of recurrence in the subsequent pregnancy.[1,11] Adverse obstetric outcomes, such as intrauterine growth restriction, have been reported, particularly in cases treated with systemic corticosteroid.[9] Consequently, careful follow-up and strict adherence to treatment regimens are essential, with close consideration of the balance between therapeutic benefits and potential maternal–fetal risks.

CONCLUSION

Navigating RF during pregnancy requires a holistic approach that incorporates understanding, patience, and individualised care. The complexity of this condition arises from the interplay between pregnancy-related hormonal changes, disease severity, and the need to balance treatment risks and benefits. Nevertheless, with timely diagnosis, adequate support, and personalised treatment strategies, effective management of this disfiguring condition is achievable, thereby minimising the associated psychological distress.

CONFLICT OF INTEREST

The authors have no conflict of interest to declare.

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CASE REPORT

A Case of Uncommon Hematemesis from Acute Esophageal Necrosis

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Keywords
Acute esophageal necrosis, upper gastrointestinal bleeding, endoscopic hemostasis

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ABSTRACT

Acute esophageal necrosis (AEN) is a rare and severe condition characterized by extensive tissue necrosis of the esophagus. This condition predominantly affects the distal part of the esophagus. Reports estimate the prevalence of AEN to be up to 0.2% in autopsy studies, while endoscopic series report a prevalence ranging from 0.01% to 0.28% of cases. It primarily affects patients with poor nutritional health and multiple comorbidities often as a result of underlying systemic condition. Patients present with upper gastrointestinal bleeding, such a hematemesis and melena. We report a case of AEN in a female patient who had presented with hematemesis.

INTRODUCTION

Acute esophageal necrosis (AEN) is a rare and severe condition characterized by extensive tissue death of the esophagus, which results in a striking, diffuse blackened appearance of the esophageal lining. This condition predominantly affects the distal part of the esophagus and is often associated with significant inflammation and potential for tissue destruction.[1] Reports estimate the prevalence of AEN to be up to 0.2% in autopsy studies, while endoscopic series report a prevalence ranging from 0.01% to 0.28% of cases.[2,3] Men are over four times more likely to develop this condition than women, with the average age of diagnosis being 68 years.[1,2,3] It primarily affects patients with poor nutritional health and multiple comorbidities often as a result of underlying systemic condition.[4,5] AEN is also linked to malignant tumors in about 10% of cases and has been reported in patients experiencing toxicity due to chemotherapy.[6] Around 70% of patients present with upper gastrointestinal bleeding, such a hematemesis and melena which can develop rapidly, often within 18 hours of the triggering event.[2,3,4] Other gastrointestinal symptoms may include dysphagia, epigastric pain, and chest pain. We report a case of acute esophageal necrosis in a female patient who had presented with hematemesis.

CASE REPORT

A 52-year-old woman diagnosed with a Myeloproliferative Syndrome was hospitalized in the Hematology Clinic referred to our Endoscopy unit for urgent Esophagogastroduodenoscopy (EGD) due to a primary complaint of hematemesis. Upon arrival, she was hypotensive with low SpO2 levels. Initial laboratory tests revealed a hemoglobin level of 8.0 g/dL, hematocrit of 24.2%, mean corpuscular volume (MCV) of 73.2, leukocytes at 116,000/μL, and thrombocytes at 64,000/μL. During the gastroscopic examination, circumferential necrosis of the esophageal mucosa was observed, presenting a darkened appearance extending to the distal esophagus. Three haemorrhagic lesions were identified in this region. The stomach mucosa appeared hyperaemic and edematous. with hemosiderin-stained blood, alongside two ulcerative lesions were noted without active bleeding (Figures 1 and 2). Haemostasis was achieved through infiltration using diluted adrenaline (1:10,000) and placement of hemoclips on all three haemorrhagic esophageal lesions. Biopsies were not taken due to active bleeding and unstable patient condition. The patient was transferred to the Gastroenterology Clinic for continued care, where she was administered parenteral proton pump

inhibitors (PPI) antibiotics (due to the risk of infection/ sepsis), mucoprotective agents, and received fresh blood and plasma as indicated by her hemogram and clinical status. Laboratory parameters, including acid-base status, were monitored regularly. Her medical history included treatment with Imatinib for her myeloproliferative syndrome, type 2 diabetes mellitus was managed with insulin therapy, palliative splenectomy six months prior, and hysterectomy with adnexectomy two years earlier. Over the following days, the patient showed no clinical or laboratory evidence of recurrent bleeding. Consequently, preparations were made for her transfer back to the Haematology Clinic for further management of her hematologic condition. A follow-up endoscopy was recommended to monitor for any potential late esophageal complications.

DISCUSSION

The management of AEN is guided primarily by clinical experience due to limited evidence-based recommendations.

Laboratory findings in AEN are typically nonspecific and reflect the underlying condition, such as lactic acidosis, hypoalbuminemia, anaemia, renal insufficiency, and hyperglycaemia.

Esophagogastroduodenoscopy (EGD) is the preferred diagnostic tool for AEN. It reveals characteristic findings such as circumferential black discoloration of the esophagus, predominantly in the distal segment, with clear demarcation from unaffected proximal tissue.[4] The endoscopic appearance can vary widely. In patients with an uncertain diagnosis, esophageal biopsies can be useful due to their characteristic histologic findings.[10] Findings may include active bleeding or blood clots in the esophagus, "coffee ground" material in the stomach, and esophageal or gastric ulcers may also be observed.[1] Submucosal adrenaline injections are commonly used to control bleeding.[7] In our case we decided to put also hemoclips, in absence of other modalities to archive haemostasis. Treatment focuses on addressing underlying clinical conditions, stabilizing hemodynamics, maintaining nil per os (NPO) status, and providing supportive care including blood transfusions and intravenous PPI to minimize further esophageal tissue damage. In most cases, patients respond well to conservative management, though a high mortality rate (approximately 32%) has been reported, primarily attributable to underlying comorbidities.[1,8] Prognostic factors for mortality include advanced age, elevated pulse rate, low hemoglobin, and hypoalbuminemia at presentation.[9] Acute esophageal necrosis is now increasingly recognized as a spectrum ranging from reversible superficial ischemia limited to the mucosa, as likely occurred in our case, to severe transmural necrosis associated with perforation, mediastinitis, and death. While no formal staging system currently exists, this case likely represents an earlier stage of AEN, with prompt endoscopic hemostasis and supportive care preventing progression to more fulminant forms. A classification system reflecting the graded nature of ischemic injury to the esophagus could help guide clinical decision-making and prognostication.

Further research is needed to refine diagnostic and therapeutic strategies and improve outcomes for patients with AEN.

CONCLUSION

AEN is a rare but significant condition that should be considered as a differential diagnosis in patients with multiple comorbidities who present with upper gastrointestinal bleeding. The condition spans a spectrum from superficial mucosal ischemia to full-thickness necrosis, and timely identification of its stage is essential. Early identification through endoscopy and timely intervention can improve patient outcomes.

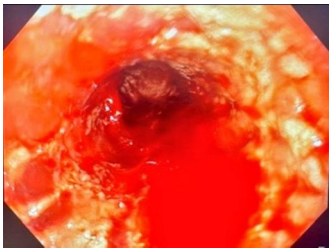

Figure 1 circumferential necrosis of the esophageal mucosa

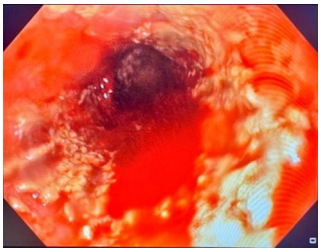

Figure 2 circumferential necrosis of the oesophageal mucosa

CONFLICT OF INTEREST

No conflict of interest from all authors!

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