IMJM

VOLUME 23 NO 4
OCTOBER 2024 ISSUE
IIUM MEDICAL JOURNAL MALAYSIA

ISSN : 1823-4631
E-ISSN : 2735-2285

Loneliness by Prof. Dr. Pakeer Oothuman

IMJM
IIUM MEDICAL JOURNAL MALAYSIA

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IIUM MEDICAL JOURNAL MALAYSIA

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IMJM
IIUM MEDICAL JOURNAL MALAYSIA
Volume 23, Issue 4, October 2024
https://doi.org/10.31436/imjm.v23i04

TABLE OF CONTENTS

IMJM
IIUM MEDICAL JOURNAL MALAYSIA
Volume 23, Issue 4, October 2O24
https://doi.org/10.31436/imjm.v23i04

EDITORIAL

Volume 23 Number 4, October 2024

Loneliness Among the Elderly in Malaysia: A Burgeoning Problem

Loneliness is a pervasive issue affecting people of all ages, particularly the elderly. It is a growing public health issue, particularly among the elderly population. As the proportion of older adults continues to rise globally, including in Malaysia, addressing this has become a priority. It can lead to various adverse effects, including mental health issues such as depression and anxiety, as well as physical health problems like cardiovascular diseases.

The prevalence of loneliness varies widely across different countries, including Malaysia. A meta-analysis by Chawla et al. (2021) estimated a pooled prevalence of loneliness among older adults at 28.5% across 29 countries.[1] In Malaysia, studies on loneliness among the elderly remain limited. A 2014 national survey reported that 34.2% of the elderly felt lonely, with 9.6% experiencing loneliness frequently and 24.6% sometimes feeling lonely.[2] Another local study by Teh et al. (2014) found that 32.5% of elderly respondents reported feeling lonely occasionally, while 20.9% indicated they felt lonely most of the time. These findings underscore the need for targeted interventions to address this phenomenon in Malaysia's aging population. The prevalence among the elderly varies by region, gender, and sociodemographic factors, with rural areas reporting higher rates of loneliness than urban areas.[3]

In general, loneliness can be divided into social, emotional and existential loneliness. Several sociodemographic and health-related factors have been identified as contributors to this among the elderly in Malaysia and worldwide.

The sociodemographic factors like age, living arrangement, and marital status are closely associated with loneliness. It tends to increase with age, with those over 70 years old reporting higher levels.[3,4] Gender differences also play a significant role in the prevalence among the elderly. While findings are mixed, women generally report higher loneliness levels than men. [5]

It is also notably higher among individuals who are single, separated, divorced, or widowed.[3] Living arrangements further influence this issue, with those living alone or without their children experiencing greater loneliness.[5] Higher education and income levels are protective factors, with financial satisfaction being particularly influential in reducing this problem.[3]

The presence of chronic diseases contributes significantly to loneliness among the elderly. Those suffering from such conditions are often burdened with physical limitations that restrict social mobility, thereby hindering their ability to form relationships and leading to increased levels.[3]

Engaging in hobbies, religious activities, and social interactions can mitigate feelings of loneliness.[3,5] Additionally, the use of digital technology, including the internet and social media, can reduce this phenomenon, especially among the more tech-savvy elderly.

In Malaysia, cultural norms emphasize familial ties and support, but the effects of modernization, migration, and shifting family structures have impacted elderly care and social interactions, leading to feelings of isolation and loneliness. Studies show that elderly Malaysians, particularly those living alone or without the support of children, are more vulnerable to it.[5] Furthermore, societal stigma surrounding aging and the elderly contributes to their isolation, as well as reluctance to engage in social networks or seek assistance. The COVID-19 pandemic has exacerbated the issue of among the elderly.

Religious involvement has been highlighted as a protective factor against loneliness in Malaysia, particularly among the Malay elderly, as religious gatherings provide opportunities for social interaction.[3,5] Religious participation not only fosters a sense of belonging and purpose but also connects individuals to a supportive community, reducing feelings of isolation.

Identifying and addressing loneliness in older adults requires the use of effective screening tools. Two widely used scales are:

- **De Jong Gierveld Loneliness Scale (DJGLS)**: This tool assesses both emotional and social loneliness, making it suitable for detecting different dimensions of loneliness.
- **UCLA Loneliness Scale**: Commonly used to assess subjective feelings of loneliness and social isolation. This scale has been validated in various populations, including the elderly.

These tools help healthcare providers identify individuals at risk of this problem and implement appropriate strategies.

Addressing loneliness among the elderly requires a multifaceted approach, combining individual, community, and governmental efforts.

Promoting social inclusion and facilitating community engagement are essential strategies for reducing loneliness. In Malaysia, programs that encourage the elderly to participate in social, recreational, and religious activities have proven beneficial. Community centres such as the Senior Citizens Club, religious institutions, and non-governmental organizations play a pivotal role in offering these opportunities.[5] In Malaysia, Kelab Warga Emas (Senior Citizens Club) supported by the Ministry of Health in health clinics provides opportunities for older adults to engage in social, recreational, and cultural activities, promoting active aging, social interaction, and overall well-being. These activities are often organized by local communities, non-governmental organizations (NGOs), or supported by government agencies.

Remarkable measures have been taken by the government aimed at improving the well-being of the elderly, such as the National Policy for Older Persons, which promotes social participation and economic security.[2]

Loneliness is often linked to physical and mental health issues such as depression and anxiety. Providing regular health screening and counseling services, both in-person and through telehealth, can be effective in addressing the medical health and emotional needs of the elderly. Even though many elderly individuals may be unfamiliar with technology, digital literacy programs targeted at the elderly population can enhance their ability to use online communication tools which will help them to engage with family and friends.

Loneliness is a prevalent issue among the elderly in Malaysia, with significant physical and mental health consequences. Sociodemographic factors, health status, and social engagement all play crucial roles in determining loneliness levels. By identifying at-risk individuals through appropriate screening tools, targeted interventions can be developed to alleviate this increasing problem and improve the quality of life for Malaysia's aging population.

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REVIEW ARTICLE

Updates in Microglial Research with Respect to Brain Cancer

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ABSTRACT

Microglia resides in the microenvironment of the central nervous system (CNS) and is thought to play a key role in the development and progression of brain cancer. This is because it was shown that microglia comprised a large portion of the total brain tumour mass. Besides, the origin of microglia cells in brain tumours is worth understanding as it is important to distinguish the resident macrophages from the circulating macrophages when discussing the pathology of brain tumours. Activated microgliosis has been linked to increased inflammatory mediators like cytokines, growth factors, matrix metalloproteinases (MMPs) and many more, which would facilitate tumourigenesis. Brain tumour cells also proliferate under the influence of signalling pathways, such as the toll-like receptor 2 signals. Vascular endothelial growth factor (VEGF) which is an angiogenic factor aids in the growth of tumour cells. Brain cancer cells rely on suppressing the effector arm of immune system to evade attacks by downregulating major histocompatibility complexes (MHC) class II molecules and inducing the conversion of microglia to an immunosuppressive phenotype. Glioblastoma stem cells (GSCs) that give rise to brain cancers communicate with microglia cells, which determines their growth and invasion potential. Understanding the molecular interactions of brain cancer and microglia cells would help unlock novel treatments via means of immunotherapy, immunosuppressants and utilising microglia cells to deliver nanoparticle drugs to effectively target and treat brain cancer.

Keywords
Microglia, Brain Tumour, Immune Evasion, Cancer Stem Cells, Brain Cancer Treatment

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Received: 7th February 2024; Accepted: 8th May 2024

Doi: https://doi.org/10.31436/imjm.v23i04

INTRODUCTION

The conception of microglial (small glue) cells was introduced by Pio del Rio-Horteg.[1] Microglia is an immune macrophage cell of the central nervous system (CNS) that supervises the microenvironment of the CNS in order to encounter injuries and foreign substances in the body.[2] During the early development of the brain of a human, microglial cells appear as ~~an~~ amoeboid-shaped. In a fully-fledged developed brain, these cells modify into a more branched and ramified phenotypic shaped. At this stage, these cells will be evenly distributed throughout the CNS and will be ready to face pathological events, as they are equipped with properties of migration, multiplication and phagocytic skills.[3] The number of microglial cells complements the number of neurons found in the CNS which is around 100–200 billion cells, the condition of the body whether healthy or diseased affects the amount too.[1]

Brain cancer, brain tumour or intracranial tumour is a disease where there is an emergence of abnormal growth of cells in the brain or spinal cord of the CNS, which can be either benign or malignant.[4] In a benign tumour, these cells proliferate slowly and compresses neighbouring areas of the brain and spinal cord. Whereas when the tumour proliferates at a high rate and spread to other immediate surrounding tissues, the tumour becomes malignant. The compression of the tumour on the brain region will inhibit the function and blood supply of that region leading to ischemia. If the tumour arises from the brain, then it is known as a primary brain tumour. As for secondary brain tumours, it originates from a different organ of the body and spreads to the brain causing metastatic brain tumour, where this is more common compared to primary brain tumours.[5] Globally, around

250,000 people are afflicted by primary brain tumour which is less than 2% of all cancers. In children under the age of 15, brain tumours are usually the second most common type of cancer affecting them, next to acute lymphoblastic leukaemia. It has been shown that primary malignant brain tumours (PMBT) most commonly affect people in the age group of 20–60 with a male to female ratio of 2:3 by as reported by Krishnatreya and associates.[6] Brain tumour occurs when the genes involved in the regulation of cell division, fixing of other malformed genes that fails in differentiating between self and non-self are defective. Environmental factors such as exposure to vinyl chloride, ionising radiation and Epstein-Barr Virus (EBV) can also contribute to the destruction of regulatory genes in humans.[7]

Besides tumour cells, non-neoplastic cells such as microglial cells are found abundantly in the microenvironment of brain tumours, where it accounts for 30-50% of the total brain tumour mass.[8] The tumour cells and microglial cells interact with each other in the formation of tumourigenesis and invading the tumour. The high population of microglial cells observed indicates that they play a crucial role in tumourigenesis and metastasis.[9] For this reason, this paper focusses on the current updates in microglial research regarding brain cancer.

Physiology and Origins of Microglia in Brain Tumour

A copious amount of microglia infiltration was consistently observed within the human brain tumour tissues while performing immune-histological studies. Hence, it is said that the extent of stimulation of microglia certainly influences the grade of the brain tumour.[10] This stimulation indicates that the neoplastic progression correlates with the microglia activation. Recent studies have shown that microglia and macrophages are acquired from two different myeloid groups of cells. Even though older reports show these cells have resemblances in the surface biomarkers and physiological functions, microglia and macrophages have distinct origination where microglia are the parenchymal resident and macrophages arises from hematopoietic stem cells.[11] The microglial cells will inhabit the brain continually in order to strengthen the population of local microglia. Latest research have proven that the surface antigens of microglia and macrophages are discrete and specific too, where microglial cells are positive for chemokine receptor CX3CR1 and negative for chemokine receptor CCR2 ($CXC3R1^{+}/CCR2^{-}$), while it is the opposite for macrophages, which are $CX3CR1^{-}/CCR2^{+}$.[12] This clearly points out the differences in origins of microglial cells and macrophage populations. In brain cancers, microglia and macrophages are elucidated as $CD45^{low}$ and $CD45^{high}$ cells individually.[13] Flow cytometry analysis revealed that microglial cells are abundantly prominent at the site of tumour, consisting about 13-34% tumour mass, whereas macrophages are less evident, comprising of around 4.2-12% of the tumour mass.[11] These findings demonstrate that microglial cells play a pivotal role in facilitating an inflammatory reaction specific to the tumour. Studies have also shown that microglia-derived enzymes, cytokines and growth factors contribute to tumour expansion and colonization, immunodeficiency and angiogenesis in primary brain cancer.[10] In addition, activated microglial cells are found at various phases of primary and metastatic brain tumours. Microglial induction frequently exists in the primary tumour mid-phase with instant microglia or macrophage response to metastatic brain tumour cells.[14]

Cross-Talk Between Microglia and Tumour Cells by Multiple Factors That Stimulate Brain Tumour Progression

Due to the role of microglia in neuronal DNA damage and neuron death via secretion of proinflammatory mediators or reactive oxygen species (ROS), activated microgliosis is a feature of neurodegenerative diseases, neuron destruction and brain cancer.[15,16] Severe microgliosis, by comparison, raises the release of cytokines and chemokines that boost tumour growth, providing a conducive brain microenvironment for tumour progression and results in self-sustaining autocrine inflammatory reaction in the brain.[17] The crucial molecules involved in microgliosis leading to the worsening of brain tumour are cytokines, chemokines, prostaglandins, transforming growth factor-β (TGF-β), growth factors and matrix metalloproteinases (MMPs).[18]

Chemokines and receptors, including monocyte chemoattractant protein-1 (MCP-1), granulocyte/ macrophage-colony stimulating factor (GM-CSF), CX3CL1 and CCLL, have been shown to be highly expressed in brain tumours.[19-21] MCP-1/CCL2 is thought to be a significant contributor to microglia recruitment in brain metastases of breast cancers, causing higher microglial proliferation in glioma. It demonstrates that MCP-1 expression links favourably with higher grades of malignant glioma.[22] In promoting microglial proliferation, GM-CSF has a similar impact to MCP-1. Also, in GM-CSF, elevated levels of the granulocyte colony stimulating factor (G-CSF) receptor are released by brain tumour tissues.[23] In comparison to GM-CSF, G-CSF does not support microglial proliferation. However, autocrine pathways have shown to facilitate the differentiation of brain tumours.[24] Besides that, it is understood that MMPs weaken the extracellular matrix, which facilitates the invasion and metastasis of tumours. Nearly 80 percent of brain metastases from lung adenocarcinomas and 50 percent of breast cancers that metastasise to the brain are positive in MT1-MMP immunostaining.[25] Moreover, when MMP-2 and MMP-9 is upregulated, it causes loss of function to astrocyte end-feet, which in turn makes the blood-brain barrier more permeable to cancer cells, macrophages and T cells in the brain.[26]

Signaling Pathways of Microglia for Brain Tumour Growth and Invasion

Mutual microglia-tumour cell interactions activate several key signalling pathways that play a vital role in the development and invasion of brain tumours such as toll-like receptor 2 signal, S100B-RAGE-STAT3 signal, angiogenic factor and signalling in other brain residential cells. Numerous Toll-like receptors (TLRs) on microglia cells can identify the soluble substances released from a glioma.[27] In a recent research, it was shown that versican produced from glioma was demonstrated as TLR 2 ligand, which is capable of activating the p38 MAPK signalling pathway that gives rise to membrane type 1 metalloproteinase (MT1-MMP) in microglial cells.[28] Interestingly, impeding the activity of microglia by the Wnt inhibitor substantially decreases overall tumourigenesis. As such, the WnT signal is vital in the interaction between microglia and neural-metastasis tumour cells. In patients with breast cancer with brain metastasis, Wnt signalling is elevated, compatible with the analysis.[29] Triggering of Wnt signalling in microglia thus facilitates brain metastasis in part through the increased expression microglial cytokines.[30] Other than that, the development of the vascular network is required for the metastatic spread of cancer cells where they can provide oxygenated blood to tumour cells to support their development and infiltration.[31] At vascular branching points, microglia are found to be positioned there and release vascular endothelial growth factor (VEGF) that aids the endothelial $VEGFR^+$ cells to form effective vascular channels.[32]

Immune Evasion of Brain Tumours with Relation to Microglia

A common hallmark of cancer is immune evasion by means of inducing local or systemic immunosuppression. In brain cancers, for example, gliomas employ a variety of immunosuppressive mechanisms, which include reducing the expression of major histocompatibility complex (MHC) class II antigens on microglia cells. Immunophenotyping of these microglial cells help in determining tumour grades in the case of astrocytic gliomas, as high-grade astrocytomas often contain microglial cells with diminished expressions of MHC class II antigens.[33]

Immunohistochemical markers like CR3/43 were used to study microglia cells in human glioma and revealed a surprising finding that these microglia cells and the tumour cells were neighbouring each other. Despite being present in large quantities and in close approximation with the tumour cells, microglia cells are somehow unable to eliminate these cancerous cells, which is explained by the compromised efferent arm of the immune response involving microglia cells.[34] Immunosuppressive factors have been found to be abundant within the microenvironment of glioblastomas, particularly the transforming growth factor β (TGF- β) produced by the glioblastoma cells, which aids in converting resident or infiltrating immune cells like the microglia cells into a state of immunosuppressive phenotype. As a result, the

cancerous cells create an immune barrier that these immune cells are unable to break through and attack.[35]

For the preservation of local immunosuppression, the amplification of M2 microglia cells is essential. Thus, tumours develop an immunosuppressive microenvironment to encourage their growth while attracting microglia.[36] Several recent studies have suggested that S100B-RAGE-STAT3 signalling initiation induces the polarisation of M2 microglia cells. S100 calcium binding protein B (S100B) secreted tumour stimulates an advanced glycation on microglia, which triggers the activation of STAT3, resulting in the repression of M1 microglial activity, which inhibits the secretion of TNF-α and IL-1β.[37]

Interaction Between Microglia Cells and Cancer Stem Cells in The Brain

It is abundantly clear that tumour formation, aggression, and virulence factors can be driven by cancer stem cells (CSCs). Brain tumours are thought to originate from glioblastoma stem cells (GSCs), specifically glioblastomas. A research showed that the tendency of GSCs to attract microglial cells was greater than that of other tumour cells.[38] GSCs expressed higher levels of CCL2, CCL5, and CCL7 in contrast with non-GSC glioma cells, and also higher levels of VEGF and neurotensin.[39] In addition, GSCs were discovered to positively affect microglia polarisation. GSC-secreted periostin has recently been found to serve as a new powerful cancer treatment to recruit macrophages by integrin alpha-vβ3 signal induction. The alpha-3 signal was observed to regulate the morphology of microglia in the M2 genotype, leading to GSC proliferation in brain tumours.[40] Additionally, IL-6 has indeed been described as a growth factor for GSC, meaning that IL-6 originating from microglia may enhance the growth of GSCs. A recent study showed that naïve microglial cells curb GSC invasion. Moreover, growth- and differentiation-related genes were significantly down-regulated in GSCs when they were treated with naïve microglial-conditioned medium.[41] These results suggest that the crosstalk between GSCs and microglia/macrophages promotes GSC growth and invasion. According to a latest study, GSC interference is hindered by naïve microglial cells. Besides, when treated with naïve microglial-conditioned medium, growth- and differentiation-related genes were substantially downrated in GSCs.[42] These findings indicate that GSC development and intrusion are supported by the crosstalk between GSCs and microglia.

Inhibiting Microglia Activation as Therapeutic Strategy for Brain Tumour

A possible antitumour-targeted treatment to suppress brain tumour development is thought to be the modulation of signals arising from microglia. Immunotherapy, immunosuppressants, antibiotic disruption and drug distribution by microglia are many techniques that regulate and alter the functionality of microglia. A recent research has shown that immunotherapy with natural killer (NK) cells combined with the mAb 9.2.27 antibody decreased tumour growth by blocking tumour replication and encouraging cell death.[43] It was reported that a recombinant immunotoxin drug blocks the β-folate receptor on microglia, inducing microglia degradation and reducing the emergence of glioma in nude mice.[44] Evidently, microglial cytotoxic effect and phagocytosis that removed cancer cell expansion were effectively improved by IL-12, LPS and INF-γ. By inhibiting the expression of MT1-MMP and p38 MAP kinase in microglial cells, antibiotic drugs such as minocycline hydrochloride have been shown to slow down tumour invasion.[45] Cyclosporine greatly lowered IL-10 and GM-CSF levels, which in turn decreased microglia accumulation and slowed the proliferation of glioma.[46] Recent experiments have used polymer nanoparticle (CDP -NP) to regulate the activity of microglial cells in tumours without poisoning, due to the benefits from the microglial phagocytic mechanisms. In malignant brain tumours, microglial cells bound with CDP-NP might theoretically be used as nanoparticle drug delivery system.[47]

CONCLUSION

It is now evident that signalling cascade between cancer and microglial cells have a significant effect on microglial pro- and anti-tumour functions, contributing to the advancement and repression of brain tumours respectively. Nevertheless, there are still several issues that

remain unanswered. The key factors and pathways that mediate microglia's association with cancer cells in the brain tumour, for example, remain largely unexplored. Microglia and astrocyte associations can also lead to tumour formation, but the precise processes involved in their contact remain uncertain. Consequently, brain metastasis is a complex process and it is unclear how metastatic cells survive microglia's immune threat to populate the internal environment of the brain. These lingering questions require more studies on the role of microglia in brain tumour which may lead to the development of new brain cancer therapeutic agents. And early halting of the activity of microglia could also provide a promising therapeutic path for metastasis of brain tumours.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this manuscript.

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REVIEW ARTICLE

Teachers' Knowledge, Attitude, and Practice on Schoolchildren's Visual and Eye Health: A Scoping Review

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ABSTRACT

Visual impairment in children has become a public health issue due to the increasing prevalence of myopia. Studies on knowledge, attitude, and practice (KAP) of teachers regarding visual and eye health of schoolchildren is an important step before implementing further collaboration between education and healthcare systems. Such input is limited as many developing countries are early in involving teachers in school health screening programmes. This review aims to understand the level of KAP among teachers on this matter and the methodology used to conduct these studies. This scoping review was conducted using Arksey and O'Malley's framework for scoping studies. A systematic search using electronic databases (Scopus, Web of Science, PubMed and Ebscohost) was conducted to identify relevant articles, imported into the Rayyan web application for review management. A total of 463 articles were identified during the selection process, and 17 articles were eventually included in this scoping review after eligibility screening. These studies were grouped into three designs: cross-sectional, intervention, and qualitative. This scoping review revealed that the level of knowledge and practice among teachers on schoolchildren's visual and eye health was low to moderate, while the level of attitude was good. Some misconceptions exist among teachers regarding children's visual and eye health. Training programmes for teachers are crucial to significantly improve their KAP levels. Most of the teachers lacked the knowledge and practice concerning children's visual and eye health. However, with a good attitude, their awareness level can be upgraded with proper training, eye screening facilities, and access to information.

Keywords
Knowledge, attitude, practice, teachers, eye health.

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Received: 8th January 2024; Accepted: 10th May 2024

Doi: https://doi.org/10.31436/imjm.v23i04

INTRODUCTION

Visual impairment in children has become a public health issue due to the increasing trends of myopia prevalence. It is estimated that by 2050, 49.8% of the global population will be myopic, a significant increase from 22.9% in 2000.[1] Myopia increases the risk of myopic macular degeneration, retinal detachment, glaucoma, and cataracts, which further leads to a reduced quality of life in children.[2] A study in Taiwan revealed that the cause of severity of myopia among schoolchildren was the early onset of myopia. That study also proposed that more consideration should be given to the eye care of preschool children to reduce the prevalence and severity of myopia.[3] Due to the lack of manpower to conduct large-scale screening programmes, school teachers served as vision screeners in some countries.[4–6]

The role of teachers are important and are well-positioned to support the healthcare system in reducing vision impairment among children.[7] Hence, creating awareness of visual and eye health of children among school teachers is an important measure to be considered. By having teachers with basic visual and eye health knowledge, they are in a position to identify whether the students need proper eye examination and treatment. Besides, teachers can also educate students to practice good visual habits and consistently encourage them to practice the habits whilst in school and at home. Therefore, data on the level of knowledge, attitude, and practice (KAP) regarding the vision and eye health of children and methodologies to conduct the studies will be helpful for researchers in the

field before involving teachers in vision screening programmes.

Knowledge, attitude and practice (KAP) studies have been applied in the investigation of health-related behaviours and health-seeking practices of a community.[8] KAP may also be done prior to implementing awareness programmes in a community to understand the environment in which the programmes are to take place. [9] Moreover, data gathered from KAP studies can be used to implement and evaluate the awareness programmes and identify factors that might contribute to particular behaviour.[10] Several studies have been conducted globally regarding the KAP of teachers on visual and eye health; hence, this review aims to understand the level of awareness and methodologies used to carry out such studies.

MATERIALS AND METHODS

This review was carried out following Arksey and O'Malley's methodological framework,[11] as follows: 1) identifying the research questions; 2) identifying relevant studies; 3) study selection; 4) charting the data; and 5) collating, summarising, and reporting the results. Data for this review are summarised and reported according to the PRISMA-ScR (Preferred Reporting Items for Systematic Reviews and Meta-Analyses Extension for Scoping Reviews) standard.[12]

Identifying the research question

Arksey and O'Malley's framework [11] recommends that a research question in scoping studies should be broad to get a broad description of the topic. The research question for this review was "What is known about teachers' knowledge, attitude, and practice on schoolchildren's visual and eye health?"

Identifying relevant studies

A search was conducted for published and unpublished studies between July 2023 and August 2023 using the following sources: electronic databases, reference lists, and hand-searching of the existing key journal network. The electronic databases used were: Scopus, Web of Science (WoS), PubMed, and EbsCohost. Studies containing keywords such as 'knowledge', 'attitude', 'practice', 'teachers', 'schoolchildren', 'visual', and 'eye health' were identified using Boolean operators such as 'OR' and 'AND'. Synonyms for all the keywords were also included in the search. The search strategy is presented in Appendix A. A hand search was conducted from the reference list and key journals. Mendeley desktop was used as the citation manager for this review, and the Rayyan web application was used to facilitate the reviewing process.

Study selection

Articles from the database and hand search were imported into the Rayyan web application for title and abstract screening. The screening was performed by two reviewers guided by the research question stated above and followed the inclusion criteria for this review. Any discrepancies were discussed with a third reviewer to finalise the study selection process. The inclusion criteria included:

- Government or private school teachers.
- With outcome assessing either knowledge, attitude, or practice of teachers on either visual or eye health of schoolchildren.
- Articles are written in English for feasibility.
- Both published and unpublished literature.

This review did not have a timeline restriction, and the articles were selected based on the above criteria. Articles that did not fall within these criteria were excluded from the review. Next, the full article of the refined search was retrieved and read by two reviewers to finalise the chosen articles to be reviewed.

Charting the data

Data charting was performed following the study selection process. During this stage, key information from the selected articles was extracted and organised into Microsoft Excel. The following variables were recorded: author(s), year of publication, country, title, study objectives, study participants, response rate,

sampling method, study design, data collection tools, language used for data collection, main findings, and study limitations.

Collating, summarising, and reporting the results

The collating, summarising, and reporting stage involves organising the relevant findings into themes. [11] The main findings of the data extracted were organised into the following themes: knowledge, attitude, and practice. For this study, all synonyms for knowledge (awareness), attitude (belief, perspective, and perception), and practice (action and habit) were reported as knowledge, attitude, and practice.

RESULTS

Selection of studies

A total of 455 articles were identified through online databases (Scopus, Web of Science [WoS], PubMed, and EbsCohost), while 8 were identified through other sources (reference lists and hand-searching of key journals). After removing duplicates, 401 articles were retained for abstract screening conducted by two reviewers. Screening of the abstract was conducted to exclude studies that do not fall into the inclusion criteria of this scoping review, resulting in 380 articles being excluded. Hence, the number of full-text articles assessed for eligibility was reduced to 21. The full-text articles were read by two reviewers to determine those complying with the study. Three articles were excluded as the outcomes did not report either knowledge, attitude, or practice but focussed on the experience of teachers having students with uncorrected poor vision and the benefits of spectacles. The other article was excluded as the outcome was not specific to teachers alone. Finally, 17 articles were included in this review for data extraction. The Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) flow diagram for the scoping review process is presented in Figure 1.

Characteristics of included studies

The 17 studies were conducted between 2006 and 2023 and involved various countries. Six studies were conducted in Africa (Ghana, Nigeria, Ethiopia, and South

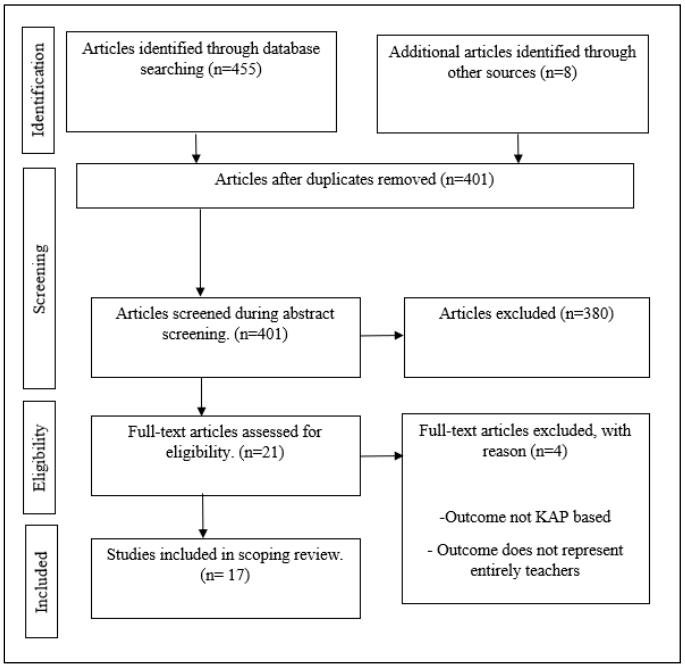

Figure 1: PRISMA flow-chart of the study selection process [13]

Africa), three in Pakistan, two in China, two in India, two in Northern Ireland, one in Malaysia, and one in the United States of America (USA)(Figure 2).

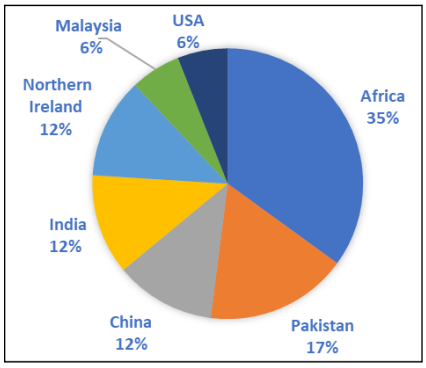

Figure 2: Distribution of included studies by country

Most of the study participants were primary school teachers,[14–21] followed by secondary teachers,[22,23] and pre-school teachers.[24] Some studies also involved preschool and primary school teachers[25,26] or primary and secondary school teachers,[27, 28] (Figure 3). However, two studies [29,30] did not describe the roles of the teachers in their study.

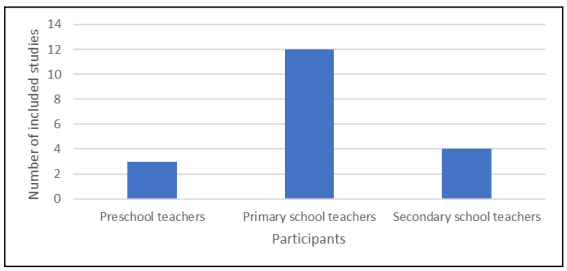

Figure 3: Distribution of included studies by participants

Methodology of included studies

Seven studies were cross-sectional, seven intervention studies, and three qualitative studies. Among the seven intervention studies, one was a cluster randomised controlled,[15] while another was a prospective study.[24] The qualitative studies were conducted as in-depth interviews [21,30] and focus group discussions.[22] Meanwhile, for the quantitative studies, ten studies used a self-administered paper questionnaire as a means of data collection,[14–16,19,20,23–26,28] while one study utilised a self-administered online questionnaire.[18] The other three studies[17,27,29] used a paper questionnaire; however, the method was not mentioned, i.e., whether self-administered or interviewer-administered. The language used in the data collection was mentioned in some studies, i.e., using the local language. Languages other than English were used, e.g., Urdu,[21,25,30] Amharic,[16] Bahasa Malaysia,[24] and Chaosan/Mandarin.[22]

Key variables from the data extraction table and main study findings are summarised in three separate tables, according to the study design. Table 1 summarises cross-sectional studies, Table 2 summarises intervention studies, and Table 3 summarises qualitative studies.

Main findings from the studies

The 17 studies identified three main themes, i.e., knowledge, attitude, and practice. Generally, most studies revolved around teachers' knowledge, attitude, or practice regarding vision screening, followed by visual and eye health among schoolchildren. For this review, studies that involved brief eye examinations were grouped under the heading of vision screening. Visual health encompasses visual impairment and treatments, whereas eye health focuses on eye diseases. The KAP levels of the studies are shown in Figure 4.

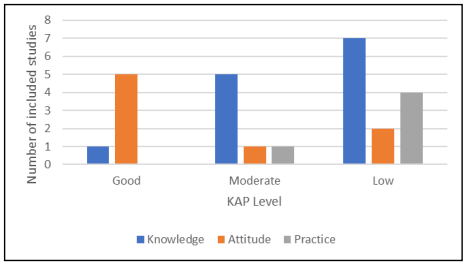

Figure 4: KAP level of included studies

Table I: Summary of the cross-sectional studies

Author, year	Country	Participants, Sampling method, Sample size, Response rate	Data collection tools	Main findings	Limitation
Habiba et al., 2017	Pakistan	Public and private preschool and primary teachers were selected via the simple random sampling technique. All preschool and primary (up to the 5th grade) teachers were invited to participate in the study, n = 443, 92.4%.	Self-administered paper questionnaire	Knowledge associated with eye health: 35.89% high, 49.89% moderate, and 14.22% low. Practice associated with students' eye health: 10.16% high, 23.02% moderate, and 66.82% low.	A limited study sample; a larger sample, including rural areas, would have made the study more generalised.
Alemayehu et al., 2018	Ethiopia	Primary school teachers. Simple random sampling was used to select the schools from Gondar City, involving 17 schools, and all teachers in the school were included in the study, n =5 65, 96.3%.	Self-administered paper questionnaire	Knowledge of refractive error: 55.9% had good knowledge, while others had poor knowledge. Attitude towards refractive error: 57.2% had a favourable attitude, while others had an unfavourable attitude.	Not mentioned
Ambika and Nair, 2013	India	Primary school teachers. A convenient sampling of primary school teachers in Mysore, n = 60, 100%.	Paper questionnaire	Knowledge of refractive error and its early identification: 80% had adequate knowledge	Not mentioned
Hinkley et al., 2011	USA	Primary school teachers, i.e., 370 elementary schools randomly selected throughout the state of Michigan. An online questionnaire was sent, n = 154, 6%.	Self-administered online questionnaire	Attitude towards vision examination referrals: 86% of teachers had recommended a student to obtain a comprehensive vision examination. Attitude towards vision therapy and school achievement: 18% noticed a dramatic change, 63.9% noted some improvement, and 18% noticed little or no improvement. Attitude towards vision screening: 93.4% believed that mandatory vision screening prior to kindergarten would benefit the students and school.	Low response rate. The survey did not reach preschool, pre-K, Head Start, or 7–12th grades.
Tchiakpe et al., 2016	Ghana	Secondary school teachers, selected through multistage clustered sampling, n= 346	Self-administered paper questionnaire	Knowledge on common eye problems: Adequate knowledge. Most (80.06 - 89.99%) of the teachers have knowledge regarding 'red eye', followed by refractive error and eye injury. More than half of the teachers can identify common signs and symptoms of eye problems in children such as blurred vision, red eye, frequent itching, and eye pain. Attitude towards eye health: Teachers have the right attitude. 93% responded that children with visual impairment should go to school, 90% had no objection to spectacle wear, and 84.39% recommended regular eye check-ups.	Not mentioned
Ramantsi, Rasengane, and Jita, 2023	South Africa	Preschool and primary school teachers. Convenience sampling was carried out with inclusion criteria being Grade R to Grade 3 teachers in Quintile 1 schools in Bloemfontein, n=36.	Self-administered paper questionnaire	Knowledge of children's vision disorder: 34 (94.44%) had good knowledge during the pre-test and 36 (100%) had good knowledge after the intervention.	small sample size, and limited time for educational sessions.
McConnell et al., 2020	Northern Ireland	Primary and secondary special school teachers. Purposive sampling was conducted in which the largest special education school in Northern Ireland was selected, n=23.	Self-administered paper questionnaire	Attitude on vision screening: 77.3% reported vision screening is useful. 100% reported having it in a school setting is convenient.	Not mentioned

Teachers' knowledge related to visual and eye health of schoolchildren

Thirteen studies reported knowledge as the outcome of research findings.[14–17,19,20,22,23,25–27,29,31] The knowledge assessment in the studies varied widely on different topics, which were further grouped into knowledge regarding vision screening, visual health, and eye health. The overall knowledge level was low to moderate. Five of these studies assessed teachers' knowledge regarding vision screening,[15,19,20,27,31] and all studies showed improvement in teachers' knowledge regarding vision screening after the intervention. The interventions involved lectures and training on vision screening.

Besides vision screening, ten studies reported teachers' knowledge regarding the visual health of schoolchildren.[14–17,19,20,22,26,27,29] These involved topics on refractive error, identifying signs and symptoms of visual disorders, vision care, and colour vision. A study in India that assessed teachers' knowledge in identifying visual problems in children showed that none of the teachers had any knowledge in that area.[17] This differs from a study in South Africa, where 63% of the participants had knowledge of the signs and symptoms of reduced vision.[15]

Two studies reported teachers' knowledge regarding eye health. A study in Pakistan showed that most teachers had moderate knowledge regarding children's eye health.[25] While another study conducted in Ghana showed that teachers had good knowledge, where 80.06 - 89.99% of the teachers had knowledge regarding children's eye health.[23] Some of these studies also reported a small sample size as the study limitation, rendering it less likely to be generalised to the overall population.[15,22,25–27]

Teachers' attitudes related to schoolchildren's visual and eye health

Eight studies reported teachers' attitudes toward visual and eye health. Among these, a study reported on teachers' attitudes towards vision screening and visual health,[18] three studies on visual health,[14,16,22] two studies on eye health[21,23] and two studies on vision screening.[28,30] Five studies showed that teachers had a good attitude toward vision screening and eye health.[18,21,23,28,30] Regarding teachers' attitudes towards visual health, a study showed good attitude,[18] a study showed moderate attitude[14] while another two studies showed low attitude.[16,22]

A study in the USA showed that the teachers had a good attitude towards vision screening and visual health.[18] The study reported that 93.4% of the participants believed that mandatory vision screening would benefit schoolchildren, and 86% of the teachers had recommended a student obtain a comprehensive vision examination. This is in contrast to a study in Ethiopia, which concluded that the attitudes of teachers were poor, with only 57.2% of the teachers having a favourable attitude towards refractive error.[16]

A study on attitudes of teachers towards glasses-wearing in China showed that teachers are highly motivated to prevent visual impairment among schoolchildren.[22] However, the lack of knowledge became a barrier that prevented them from taking action.[22] A study in China showed that attitudes of teachers toward the vision care for students were moderate but improved significantly after receiving training. [14]

A study on attitudes of teachers towards eye health in Pakistan revealed that the teachers had a good perception of the appearance of healthy and diseased eyes, factors that could damage the eyes, and the detection of children with eye problems.[21] However, in response to an eye injury, the teachers preferred treating minor eye injuries and only referred severe injuries to the doctor.[21] Most studies showed that teachers had a favourable attitude towards vision and eye health, with some misconceptions, especially in rural areas.

Practice of teachers related to visual and eye health of schoolchildren

Five studies assessed the practice of teachers related to visual and eye health of schoolchildren. Three studies reported practices on vision screening by teachers,[15,19,30] a study each on visual[20] and eye health.[25] Four studies

showed that teachers had poor practice in visual and eye health of schoolchildren, while a study showed moderate practice.

The practice on vision screening by teachers conducted in South Africa showed that none of the teachers could conduct vision screening before training was given.[15] After training, 79% of participants can conduct visual acuity screening correctly, and 73% can refer appropriately.[15] Another study on practice towards colour vision screening by teachers also showed similar results, whereby none of the teachers managed to perform the test prior to training.[19] However, after receiving training, 84.6% of participants managed to perform the colour vision test.

A study conducted in Pakistan regarding practice toward vision screening by teachers showed that most teachers (78.6%) followed the procedures for conducting vision screening. However, not all the teachers were able to make proper referrals to optometrists after vision screening.[30] This study also reported that some suggestions were given for improvements such as refresher training and supervisory visits from trainers.

DISCUSSION

This scoping review was carried out to gain input on the level of KAP among teachers regarding visual and eye health of schoolchildren and to identify the methods used to conduct the studies. The findings revealed some misconceptions among teachers regarding visual and eye health of children. In rural areas in China, teachers have the perception that schoolchildren should not get glasses before the age of 18.[22] Although they are aware of the dangers of uncorrected refractive error to children, the lack of knowledge and misinformation that prevails among teachers on how refractive error affects vision became a barrier for teachers to have good practice. Instilling good practice among teachers will positively contribute towards preventing further damage to the children's eyes.

Most of the studies in this review showed that teachers had good attitudes towards the visual and eye health of children. A study in the USA, a developed country, demonstrated that teachers had a good attitude toward visual health and vision screening.[18] The study indicated that Michigan state has a law that requires children enrolling in preschool to submit their eye examination evidence. Almost 95% of the schools participating in this study have a vision screening programme. This law might have affected teachers' awareness of children's visual health.

Studies that showed low to moderate levels of KAP towards visual and eye health of schoolchildren were from lower- and middle-income countries.[15–17,19,20,24–27] This could be due to the availability of facilities and access to healthcare services that are limited in those developing countries.[32] Overall, the levels of knowledge, attitude, and practice are similar in these countries. However, the factors that contribute to the level of knowledge could not be an indicator of the awareness level. A study in Ethiopia[16] reported that four factors were associated with the level of knowledge regarding refractive error. Teachers who wore spectacles, had their eyes examined, had undergone training on eye health, and had longer teaching experience had a higher level of knowledge than the rest. Surprisingly, the more experienced teachers had an unfavourable attitude towards refractive error. It indicates that experience affects the level of knowledge positively, although it might not portray a positive attitude. Other factors that contributed to the level of attitude towards refractive error are gender, i.e., being male, older age, and teachers working in the private sector. These factors instigate a more favourable attitude towards refractive error.

This review also revealed that training programmes introduced to teachers significantly improved their level of knowledge, attitude, and practice. The training programmes involved theory and practical sessions delivered to the teachers with the variation of training between 3 and 6 hours. Most of the studies were intervention programmes, whereby the baseline awareness was measured prior to a training programme, and then a post-assessment was given to identify the level of awareness. A study in China [14] investigated the impact of the teachers' knowledge and attitude on children's

behaviour. According to the study, students are more likely to be given an outdoor recess when teachers have better knowledge and attitudes toward vision care. Studies have shown that outdoor activities play an important role in minimising the progression of refractive error.[33–35] Thus, teachers need to have good knowledge, attitude, and practice towards visual and eye health, as these will significantly have an impact on their student's well-being.

CONCLUSION

This scoping review shows that the level of knowledge and practice among school teachers towards visual and eye health was low to moderate in many countries, while the attitude level was good. There was a vast difference in the research methodology of the studies. Most studies included intervention programmes, and the self-administered questionnaire was the most popular method used in data collection. The outcomes from qualitative studies were outstanding as they provided a more in-depth perception of this topic. Further exploration of the level of teachers' KAP and its effects on the behaviour of schoolchildren should be considered in future studies.

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REVIEW ARTICLE

Barriers Towards Healthcare Access and Services among People with Disabilities: A Scoping Review of Qualitative Studies

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ABSTRACT

Healthcare access and services is one of the challenges faced by people with physical disability (PWPD). PWPD also has at risk of early death and preventable chronic illness. Lack of access to healthcare services includes delay or failure to receive needed care, lack of continuity of care and financial burden will result in deterioration of health, wellbeing and functional status. The aim of this paper was to review barriers towards healthcare access and services among PWPD based on the existing qualitative studies. We conducted a scoping review of relevant qualitative articles from PubMed, Scopus and ProQuest. The article search was based on the available keyword in the title with the publication restricted within 10 years (between 2012 to 2021). The search strategy was conducted using MeSH terms of 'barriers, healthcare access, healthcare services and physical disability'. In this review, people with different kinds of physical disabilities were included. There were 2004 articles obtained from the initial search. 27 articles met the inclusion criteria for the final review. In each study, PWPD noted various barriers to access healthcare services. Findings from this review revealed five themes: personal; financial; attitudinal and communication; health system; structural and physical barriers. The findings showed that PWPD faces various barriers when accessing healthcare services. Addressing these barriers could help create a healthcare system that is inclusive and accessible for all.

Keywords
Barriers, Healthcare services/access, Physical disability, Qualitative research.

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Received: 4th December 2023; Accepted: 12th August 2024

Doi: https://doi.org/10.31436/imjm.v23i04

INTRODUCTION

According to the World Health Organization (WHO), disability can be defined as a restriction in terms of body function and structures, activities and participation which refers to the negative aspects of the interaction between an individual (with a health condition) and that individual's contextual factors (environmental and personal factors).1 People with disabilities (PWD) face unique health needs and may experience poorer health outcomes compared to those without disabilities. Access to healthcare services is a fundamental right for PWD, yet they encounter numerous barriers that impede their access to adequate and equitable care.

In Malaysia, PWPD accounts for 1.8% of the population, with a significant proportion experiencing disabilities above the age of 18.[2] PWPD have specific health requirements related to their impairments, as well as additional general health needs. However, inadequate access to healthcare can contribute to the development of chronic and secondary illnesses, worsening of existing conditions, and overall poorer health outcomes.[3] Ensuring access to healthcare for PWPD aligns with the principles outlined in the Convention on the Rights of Persons with Disabilities (CRPD), which emphasizes the right to the highest attainable standard of health without discrimination[4]. Addressing the gaps in healthcare access and services is a crucial priority for health systems to promote inclusivity and improve the health outcomes of PWPD.[4]

Accessing healthcare services poses numerous barriers for PWPD. Physical barriers, such as lack of accessible infrastructure and transportation, hinder their ability to reach healthcare facilities.[5] Attitudinal barriers, including stigma, biases, and discriminatory attitudes, create a negative healthcare environment that deters individuals with disabilities from seeking care.[6]

Aligned with the Sustainable Development Goals (SDGs), specifically the third goal, which aims to ensure access to quality healthcare for all individuals, regardless of disabilities, this review seeks to explore the barriers faced by PWPD towards healthcare access and services.[7] This review only included findings from qualitative studies since findings from qualitative studies will provide better understanding of the experiences and barriers of PWPD towards healthcare access and services. Our aim is to map the key findings and share them in this scoping review. The key findings of this review will be essential for developing inclusive and accessible healthcare systems that cater to their diverse needs.

METHODS

This scoping review follows five main steps as per the standard way of developing a scoping review. The steps include formulation of research questions, identification of relevant studies, selection of appropriate studies, organisation and mapping of data, and lastly; collection, summarizing, and reporting of results.[9] All these steps are essential to comprehensively map the existing scientific literature and identify areas of knowledge gaps. The last step (step number six) which is an optional consultation exercise was not done in this review.

Search strategy

The literature search was conducted in November and December 2022. Various articles were searched from three search engines: PubMed, Scopus and ProQuest. In this review, our search focused on the main keywords stated in the title: barriers, healthcare services/access and disability. The Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines were applied during the literature search.[10] (PRISMA flow diagram, Figure 1)

Selection studies

According to the aim of our review, only qualitative study design was eligible for inclusion. Thus, other studies such as observational studies (cross sectional, prospective and case control), experimental (randomized controlled and quasi) and review articles were excluded from this review. Other inclusion and exclusion criteria were also considered as stated below:

Inclusion criteria:

1. Qualitative studies
2. Published in English
3. Published between 2012 and 2021
4. Full-text articles and academic journals

Studies on barriers towards healthcare services or access

Exclusion criteria:

1. Quantitative studies
2. Abstracts, letter to editor, editorials and comments
3. Method articles or protocols
4. Grey literature (e.g., conference abstracts, research reports, dissertation, books, policy documents

Data extraction and management

In order to extract the data, we constructed a systematic table which gathered all the essential information about the included articles. The table consists of the titles, authors, country, sample participants, types of disabilities and main findings. Three members extracted data from included studies to guarantee the authenticity of the information acquired. Then, the corresponding author verified the accuracy of the data retrieved by the authors. If there were any issues, we would compare the findings in meetings and address any disputes through discussion.

RESULTS

Throughout the initial search, 2004 articles were found. The articles were selected from three main search engines which were PubMed, Scopus and ProQuest with the publication restricted within 10 years (between 2012 to 2021). Majority of articles were removed due to articles were not in English and also due to excluded study designs (quantitative studies, protocol, review, editorial

and grey literature). Secondly, we studied the abstract of these articles and only 41 were included. Articles that were not related to healthcare services/access for patients with disability were excluded. Finally, only 27 articles were chosen after studying the full text that met the inclusion criteria. All the findings were summarized in Table 1.

From the 27 articles selected, 15 studies were conducted in Africa, 15 in Asia, two from North America, two from South America, one from Europe and one from Australia. Majority of the studies were conducted among adults except for five studies were conducted among caregivers and three studies among children and teenagers. Mainly, the studies were conducted to identify the barriers in accessing the general healthcare facilities. However, several studies were more specific in which the studies involved access to rehabilitation centres, cancer services, pharmacy services, maternal care services and sexual and reproductive health services. Overall, a total of 1245 samples were included from 27 studies. From these studies, we identified five main key findings as shown in Figure 2.

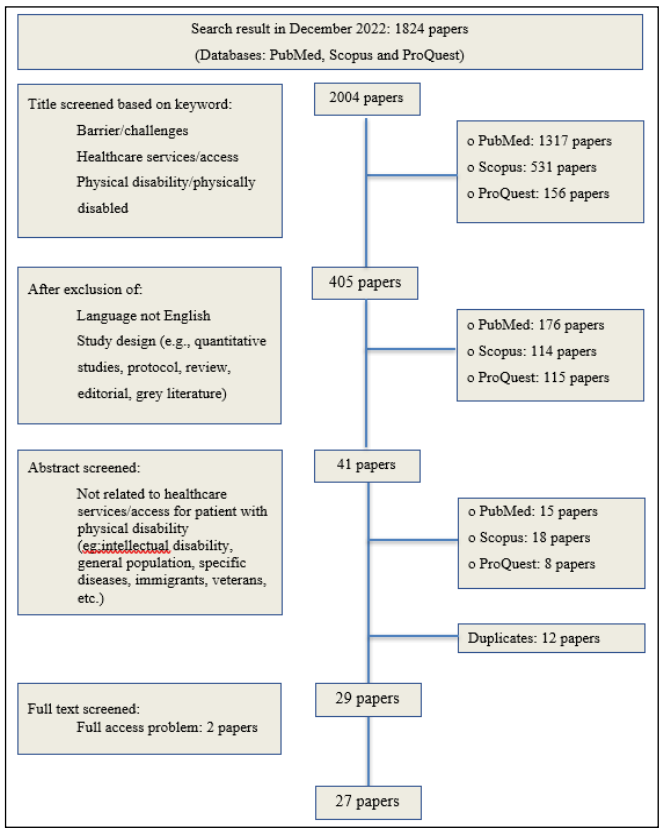

Figure 1: PRISMA flow diagram. The PRISMA diagram details the search and selection process applied during our systematic literature search for this scoping review. PRISMA, Preferred Reporting Items for Systematic Reviews and Meta-Analyses.

Table 1: Summary of articles

Title, Authors and Country	Sample and type of disability	Main findings
1. A path toward disability-inclusive health in Zimbabwe Part 1: A qualitative study on access to healthcare. Smythe et al. Zimbabwe	N: 24 PWD and 10 key informants Physical, sensory and intellectual	Barries: 1. Health literacy related to general health and impairment specific needs. 2. Out-of-pocket payments 3. Healthcare provider knowledge and capacity 4. Distance to healthcare clinic 5. Ability to engage
2. Understanding Barriers to the Access to Healthcare and Rehabilitation Services: A Qualitative Study with Mothers or Female Caregivers of Children with a Disability in Indonesia. Asa et al. Indonesia	N: 22 mothers/ caregivers of CWD (children with disability) Visual, hearing, speech, physical, cognitive	Barriers: 1. Lack of affordability of healthcare services 2. Religious or faith-based factors 3. Shortage of staff, distrust in the therapy skills of staff at rehabilitation centers, and unavailability of appropriately trained healthcare professionals
3. Cultural barriers in access to healthcare services for people with disability in Iran: A qualitative study. Soltani et al. Iran	N: 50 People with disability, healthcare services providers and policy makers Physical and intellectual disabilities	Cultural barriers: 1. Reluctance to provide health services and disrespect 2. Denial of disability, disproportionate expectation, shame and insufficient sociocultural supports 3. Lack of concern, little attention to the culture of disability and discrimination.
4. Financial Barriers to Access to Health Services for Adult People with Disability in Iran: The Challenges for Universal Health Coverage. Soltani et al. Iran	N:56 20 people with different disabilities, 14 health service providers and 22 policymakers Physical and intellectual disabilities	Financial barriers: 1. Health insurance (lack of insurance coverage for services like dentistry, occupational therapy and speech therapy) 2. Affordability (low income for PWD and their family) 3. Financial supports (low levels of pensions for people with disabilities) 4. Transportation costs (high cost of transportation to reach healthcare facilities for PWD)
5. Examining access to sexual and reproductive health services and information for young women with disabilities in Senegal: a qualitative study. Soule & Sonko Senegal	N:31 Physical disabilities	Barrier 1. Structural inaccessibility within health care establishments 2. Financial limitations 3. Inaccessible transportation and far-away health establishments 4. Long wait times in health care establishments 5. Prejudices and discrimination from health providers
6. Access to health care for people with stroke in South Africa: a qualitative study of community perspectives. Smythe et al. South Africa	N: 16 Physical and sensory disability post stroke	Barrier: 1. Complex pathways to care 2. Physical mobility related to stroke 3. Long travel distances 4. Limited transport options 5. Waiting times 6. Out of pocket expenses
7. Access to primary and secondary health care services for people living with diabetes and lower-limb amputation during the COVID-19 pandemic in Lebanon: a qualitative study. Chaiban et al. Lebanon	N:8 Physical disability with diabetes	Barrier 1. Economic barriers (increasing costs of food, health services and medications, transportation, shortage of medications, and limited income) 2. Structural barriers (availability of transportation, physical environment, and service quality and availability) 3. Cultural barriers (marginalization due to their physical disabilities; favoritism in service provision) 4. Personal barriers (lack of psychosocial support and limited knowledge about services) 5. Covid 19 barriers (fear of getting sick when visiting healthcare facilities, and heightened social isolation due to lockdowns and physical distancing)
8. Health care providers' and persons with disabilities' recommendations for improving access to primary health care services in rural northern Ghana: A qualitative study.[35] Dassah et al. Ghana	N: 33 Physical disability	Recommendation: 1. Making it more affordable 2. Increasing the availability of providers and services 3. Providing more education about system navigation 4. Improving access to disability friendly health facilities and equipment.

Con't

Title, Authors and Country	Sample and type of disability	Main findings
9. Access to health care for people with disabilities in rural Malawi: What are the barriers? Harrison et al. Malawi	N: 12 Physical and sensory disabilities	Barrier: Cost of transport, drugs and services 1. Insufficient health care resources 2. Dependence on others 3. Attitudinal barrier: unfavorable health seeking behavior
10. Perspectives of basic wheelchair users on improving their access to wheelchair services in Kenya and Philippines: a qualitative study. Williams et al. Philippines and Kenya	N: 48 Physical disability	Barrier: 1. Physical environment 2. The need for having multiple chairs to improve access 3. Perceived social stigma 4. Peer support
11. A qualitative exploration of barriers in accessing community pharmacy services for persons with disability in Addis Ababa, Ethiopia: a cross sectional phenomenological study. Dagnachew et al. Ethiopia	N: 15 Physical, visual and hearing disability	Barrier: Transportation 1. Physical layout 2. Communication 3. Medication price 4.
12. Barriers to accessing cancer services for adults with physical disabilities in England and Wales: an interview-based study. Sakellariou et al. England and Wales	N: 18 People with a diagnosis of cancer and a pre-existing physical disability.	Barrier: 1. Lack of acknowledgment of disability 2. Unseeing disability 3. Physical inaccessibility
13. Barriers and Facilitators to Accessing Health Services: A Qualitative Study Amongst People with Disabilities in Cameroon and India. Zuurmond et al. Cameroon and India	N:30 Vision, hearing or musculoskeletal impairments	Barrier: 1. Individual level: a. understanding and beliefs about an impairment b. the nature of the impairment and interaction with environmental factors. 2. Community level: a. family dynamics and attitudes b. economic factors c. social inclusion d. community participation
14. Missed Appointments at a Child Development Centre and Barriers to Access Special Needs Services for Children in Klang Valley, Malaysia: A Mixed Methods Study. Fadzil et al. Malaysia	Phase 1 N:197 Phase 2: N:23 Caregivers of children with developmental disability	Barrier: 1. Transportation factors: a. Personal transport: parking issues and traffic congestion b. Public transport: accessibility 2. Caregiver factors: dilemma between their commitment at work and attending appointments 3. Child factors: competing priorities between children's other obligations and the scheduled appointments 4. Healthcare services factors: inflexibility in setting the appointment slot
15. 'The world is not only for hearing people - It's for all people': The experiences of women who are deaf or hard of hearing in accessing healthcare services in Johannesburg, South Africa.[36] Masuku et al. South Africa	N:10 Deaf	Barrier: 1. Communication barrier 2. Accommodation barrier 3. Negative attitude of healthcare professionals
16. 'You must carry your wheelchair'--barriers to accessing healthcare in a South African rural area. Vergunst et al. South Africa	N: 26 Physical, psychosocial, sensory, cognitive and physical impairment	Barrier: 1. Geographical barriers 2. Transport barriers 3. Organizational barriers 4. Attitudinal barriers
17. "Nothing suitable for us" experiences of women with physical disabilities in accessing maternal healthcare services in Northern Vietnam Nguyen et al. Northern Vietnam	N: 27 women Physical disabilities	Barriers: 1. Attitudinal barriers from staff 2. Specialized information on pregnancy and childbirth was limited. 3. Long waiting times 4. Confusing referral system 5. Financial hardship 6. Facilities not disability friendly
18. Experiences with rehabilitation and impact on community participation among adults with physical disability in Colombia: Perspectives from stakeholders using a community based research approach Toro-Hernández et al. Colombia	N:32 Physical disabilities	1. Barrier: 2. Personal mobility 3. Perceptions and knowledge on disability 4. Navigating the system.

Con't

Title, Authors and Country	Sample and type of disability	Main findings
19. "This one will delay us": barriers to accessing health care services among persons with disabilities in Malawi Munthali et al. Malawi	N:52 Physical, visual, hearing, mental, epilepsy	Barrier: 1. Cost of accessing health care 2. Long distances to health facilities 3. Lack of transport 4. Hilly terrains and flooding of rivers during the rainy season 5. Communication challenges with the health providers 6. Poor attitude of health workers.
20. Access to Healthcare among People with Physical Disabilities in Rural Louisiana[37] N. Davidsson and B. Södergård Louisiana, US	N: 9 Physical disabilities	Barriers: 1. Insurance coverage 2. Financial resources 3. Guidance and knowledge about healthcare 4. Transportation 5. Accessibility within healthcare facilities 6. Quality and continuity of care
21. Addressing the barriers to accessing therapy services in rural and remote areas[38] Dew et al. Australia	N: 78 carers, 10 adult with physical disability	Barriers: 1. Travelling to access therapy 2. Waiting a long time to get therapy 3. Limited access to therapy past early childhood
22. Health care access and barriers for the physically disabled in rural Punjab, Pakistan[39] M. Ahmad Pakistan	N:245 Physical disabilities	Barriers: 1. Built environments 2. Healthcare delivery processes 3. Ceiling of health subsidies
23. Experiences of patients with a disability in receiving primary health care Walji et al. Toronto, US	N:18 Physical, sensory, learning, developmental disability, chronic illness, mental illness	Key findings: 1. Importance of relationship 2. Importance of multidirectional communication 3. Effects of disability 4. Effects of physical buildings issues
24. A qualitative study to explore the barriers and enablers for young people with disabilities to access sexual and reproductive health services in Senegal[40] E. Burke et al. Senegal	Focus group N: 128 Interviews N: 50 Physical, visual, or hearing impairment	Barriers: 1. Financial barriers 2. Provider attitudes 3. Accessibility (related to their disability)
25. Analysis of the impact of healthcare support initiatives for physically disabled people on their access to care in the city of SaintLouis, Senegal Senghor et al. Senegal	N: 105 Physical disabilities (motor, visual and albinism)	Barriers: 1. High cost of care 2. Ill-treatment by health workers 3. Limited human resources 4. Low levels of financial support 5. Logistical challenges
26. Accessing Healthcare in Ghana Challenges Encountered and Strategies Adopted by Persons with Disabilities in Accra Abrokwah et al. Ghana	N: 21 Visual, hearing and mobilities	Barriers: Physical, financial, communication, transportation, and attitudinal barriers, as well as healthcare professionals' lack of knowledge about disability issues, limited access of persons with disabilities to healthcare.
27. "Knocking on Doors that Don't Open" experiences of caregivers of children living with disabilities in Iquitos and Lima, Peru[41] Aguerre Et Al. Peru	N: 20 caregivers and 14 key informants Non specific disability	Barriers: 1. Emotional and Informational Support 2. Stigma and discrimination 3. Difficulty accessing services 4. Poor design of policy 5. regional and economic disparities

DISCUSSION

The purpose of this review is to identify barriers and experiences in accessing healthcare services among PWPD. With the results from qualitative studies selected for this review, we aimed to create a comprehensive and precise picture of the major barriers. In this review, PWPD reported several problems to get optimised healthcare access and services. The key findings were broken down into five main themes which were personal;

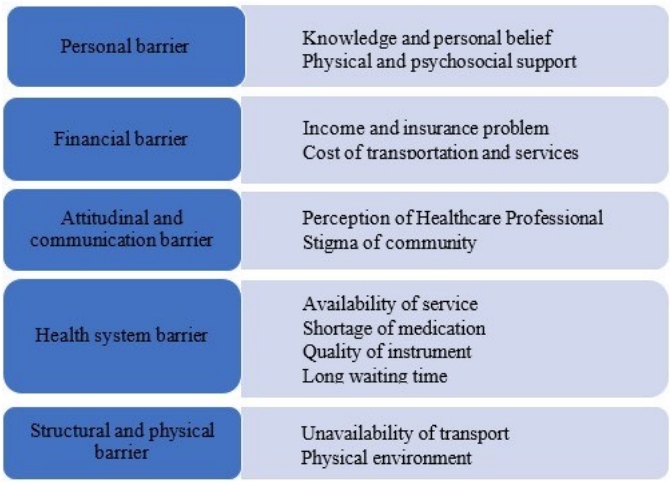

Figure 2: Summary of the main findings.

financial; attitudinal and communication; health system; structural and physical barriers.

Personal barrier

Most PWPD seem to have a lack of knowledge regarding healthcare services. The level of health literacy among PWPD has been found to significantly influence their ability to understand and accept their health needs.[10] This was also affected by unreliable beliefs that they uphold about healthcare.[11,14,15] The knowledge and beliefs among PWPD can vary depending on their personal experiences, education and exposure to healthcare systems. Limited health literacy can impact their understanding of healthcare information, treatment options, and the importance of preventive care.[10,11] This review also found that several negative experiences strengthened their negative perception towards healthcare services. Positive experiences with healthcare providers and services can foster trust and confidence among PWPD. Therefore, it is important to empower health education among PWPD and ensure clear communication takes place between healthcare providers and PWPD. Healthcare professionals can practice "teach back" technique by asking patients to repeat back information and instructions as well as using visual models to enhance the understanding and engagement of the patients.[31] By improving their knowledge and understanding of health-related matters, PWPD can make informed decisions and actively seek appropriate healthcare services.

Secondly, physical and psychosocial support for PWPD is essential to promote their overall well-being, independence, and inclusion in society. Such support plays a crucial role in addressing the challenges and barriers faced by people with disabilities, enabling them to lead fulfilling lives and participate fully in various aspects of society. From our findings, families, peers and surroundings become the core support system for PWPD.[14,16] Physical support for PWPD involves providing assistive devices, adaptive technologies, and accessibility modifications to their living environments. Assistive devices such as wheelchairs, crutches, hearing aids, and prosthetic limbs can enhance mobility and functional abilities, enabling individuals to engage in daily activities and participate in work, education, and social interactions. Not only physical support but psychosocial support is equally important for PWPD as it addresses their emotional, social, and mental well-being.[31] Psychosocial support is crucial for hindering the adverse effects of various stressors on disability, including the ongoing stress caused by physical disability. Psychosocial support may include counselling, peer support groups, and mental health services tailored to the unique needs of individuals with disabilities. To achieve this, organisations and associations can empower PWPD by facilitating their reintegration into society post-hospital discharge. Through recreational activities, vocational training, and workshops, opportunities for workforce participation and financial independence can be created, alleviating caregiver burdens.[20]

Financial barrier

Financial constraints posed a significant barrier to PWPD in accessing healthcare services. The issue of inadequate financial status was particularly prevalent among unemployed individuals, low-income families, and those living in poverty, making it challenging for them to afford health insurance.[18] Furthermore, certain crucial services like occupational therapy, technical orthopaedics, and speech therapy were not covered by insurance companies.[25] The financial status of PWPD can vary widely depending on factors such as the type and severity

of the disability, access to education and employment opportunities, social support systems, and the overall socioeconomic context. PWPD often face barriers in accessing employment opportunities due to discriminatory practices, lack of accommodations, and negative attitudes.[19,20] Consequently, they may experience higher unemployment or underemployment rates compared to the general population. This is primarily due to three factors: the perception that people with disabilities are unproductive, the belief that they incur high costs, and employers' limited understanding of disabilities.[32] On top of that, PWPD may require ongoing medical care, assistive devices, therapies, or medications, which can result in higher healthcare expenses.[18,25] These additional costs can put a strain on their financial resources, particularly if they lack adequate health insurance coverage or access to affordable healthcare services. Unfortunately, PWPD also often incur additional expenses related to their disability, such as accessibility modifications to their living spaces, transportation costs, specialized equipment, medications or personal assistance services.[10,13,18,20,21,22] In order to overcome this financial issue, a few solutions can be considered. Government should streamline the process for accessing disability benefits and social welfare programs. Simplifying application procedures, providing clear guidelines, and offering support in navigating the system can ensure that individuals with disabilities receive the financial assistance they are entitled to. Next, collaboration among government agencies, disability organizations, and advocacy groups is needed to address financial challenges faced by PWPD. This includes advocating for policies that protect the rights and financial well-being of individuals with disabilities, promoting disability-friendly regulations in various sectors, and ensuring the implementation of inclusive practices.

Attitudinal and communication barrier

The perception and attitudes of healthcare professionals play a crucial role in achieving equal access to healthcare services for individuals with disabilities. Unfortunately, our findings from previous studies show that healthcare providers and communities may have held negative stereotypes and biases towards PWPD.[12,19,23,24,25,26,27] These misconceptions could lead to assumptions about their capabilities, intelligence, or quality of life, potentially resulting in unequal treatment or lower expectations for their healthcare outcomes. On top of that, the stigma can contribute to social isolation and exclusion of PWPD.[6] They may face barriers in forming social connections, participating in community activities, or accessing public spaces. This exclusion can lead to feelings of loneliness, marginalization, and a sense of being different or unwanted.[6] Ideally, in order to improve this situation, continuous education and training are needed at different levels. This can involve school programs, community workshops, and media campaigns that highlight the capabilities and achievements of individuals with disabilities. Besides, ongoing advocacy efforts and active involvement of PWPD in shaping healthcare policies and practices are necessary to improve the perception and experience of healthcare.

Health system barrier

The healthcare system itself was also one of the significant issues that has been highlighted by PWPD from previous studies. These include unavailability of certain services, shortage of medication, poor quality of instruments and long waiting time.[11,12,13,14,15,16,17,19,20,23,24,28] These challenges can impact the effectiveness of delivering quality care towards PWPD. Addressing these healthcare system problems requires comprehensive strategies and collaboration among stakeholders. Partnership and cross collaboration between public and private sectors can help to expand healthcare services by establishment of clinics, diagnostic centers or specialty care facilities in underserved areas. In fact, this public-private partnership also can support healthcare companies to increase manufacturing capacity of medicine and good healthcare instruments. Long waiting times due to understaffing can be catered by investing in healthcare workforce development and addressing workforce shortages through training, recruitment, and retention strategies. By addressing these healthcare system problems, it is possible to enhance access to care, improve patient outcomes, and ensure that healthcare systems are efficient, equitable, and patient-centered.

Structural and physical barrier

We also found that accessing healthcare can be hindered by various physical barriers such as unavailability of transport and the physical environment of healthcare facilities. Buildings, clinics, and hospitals without proper ramps, elevators, or other accommodations can prevent PWPD from reaching healthcare services.[10,12,17,20,21,26,26] Furthermore, PWPD also experience a lack of public transportation and some of them live far from healthcare facilities which pose obstacles for them seeking healthcare.[15,16,20,27,28] Addressing these physical barriers requires a comprehensive approach involving various stakeholders. Governments and organizations should invest in building and maintaining healthcare facilities, particularly in underserved areas.[33] This includes constructing hospitals, clinics, and specialised medical centres, ensuring they are equipped with necessary medical equipment and staffed adequately. Governments also can enhance transportation infrastructure, especially in rural areas, to improve access to healthcare services. This may involve building or improving roads, bridges, and public transportation systems. Additionally, implementing mobile health clinics or telemedicine initiatives can bring healthcare services closer to communities that lack nearby facilities.[34]

CONCLUSION

This review identifies barriers to healthcare access and services for individuals with physical disabilities. The major barriers can be categorised into personal, financial, attitudinal and communication, health system, and structural and physical barriers. Limited knowledge and negative beliefs about healthcare, along with inadequate support systems, hinder access. Financial constraints and attitudinal biases further exacerbate the challenges. Improving health literacy, providing physical and psychosocial support, addressing financial barriers, promoting inclusivity, strengthening the healthcare system, and eliminating physical obstacles are essential to enhancing healthcare access for PWPD. By addressing these barriers, we can work towards creating a healthcare system that is highly accessible, inclusive and responsive to the needs of all individuals.

ACKNOWLEDGEMENT

This study was funded by "Geran Penyelidikan UMP-IIIUM Sustainable Research Collaboration 2022" with grant no; IUMP-SRCG22-012-0012. The authors would like to thank International Islamic University Malaysia (IIUM) and Universiti Malaysia Pahang (UMP) for providing funds for this study.

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ORIGINAL ARTICLE

ECG Risk Score Model to Predict SCD in HFrEF: Retrospective Review in a Tertiary Centre

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ABSTRACT

INTRODUCTION: Heart failure with reduced ejection fraction (HFrEF) patients need to be risk stratify as guidelines have shown that patients with left ventricular ejection fraction (LVEF) <35% could be prevented from sudden cardiac death (SCD) by insertion of prophylactic implantable cardioverter-defibrillator (ICD). Thus we conducted a retrospective single tertiary centre study to evaluate the used of electrocardiogram (ECG) risk score model in identifying the individuals who at higher risk of SCD. **MATERIALS AND METHODS:** A total of 356 heart failure with reduced ejection fraction (HFrEF) patients treated at University Malaya Medical Centre between January 2017 and December 2021 were enrolled into this study. The patients' demographics, types of heart failure, medications, and ECG parameters data were collected. The study outcomes were survivor or death in and the cause of death were subdivided into SCD or non-sudden cardiac death (non-SCD). **RESULTS:** A total of 156 study patients were survivor whereas another 120 had SCD and 70 had non-SCD. There were six ECG parameters that remained significant in the final model, namely the bundle branch block (BBB), abnormal P waves, QRS duration, QTc duration, TpTe interval and PR interval. The significant ECG parameters were combined into a risk score to enumerate prediction ability towards SCD. From our ECG risk score model, subject with ≥2 ECG abnormalities had more than 3-fold increased risk for SCD (HR 3.739, 95% CI 1.703-8.211, P 0.001) and the risk proportionately increased with increasing ECG abnormalities. **CONCLUSION:** Our findings suggested that the cumulative ECG risk score model was independently associated with SCD and particularly effective for LVEF <40% where risk stratification model remained scarce. So, we would like to propose for a prospective study to further evaluate our study outcome.

Keywords
Sudden Cardiac Death, Heart Failure, Electrocardiogram, Arrhythmia, Implantable Cardiac Device

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Received: 18th December 2023; Accepted: 11th June 2024

Doi: https://doi.org/10.31436/imjm.v23i04

INTRODUCTION

Cardiovascular diseases (CVD) contributed approximately 17 millions of deaths per year in the world, of which 25% were sudden cardiac death (SCD).[1] Patients with heart failure (HF) have higher rate of SCD compared to the general population as they experience number of changes in the electrical function of the heart that predispose to potentially lethal cardiac arrhythmias. Studies have shown that most patients with left ventricular ejection fraction (LVEF) <35% could benefit from prophylactic implantable cardioverter-defibrillator (ICD) insertion. However, the local data shown that prevalence of SCD in heart failure with reduced ejection fraction (HFrEF) patients were as high as 42% which may be explained by the underutilization of implantable cardioverter-defibrillator (ICD) insertion.

Framingham study shown that incidence of SCD were 62% in men aged 45-54 years and 58% and 42% for men aged 55-64 years and 65-74 years respectively.[2] Incidence of SCD was lower in women than men mainly because they are protected against coronary artery disease (CAD) during premenopausal period. Study by Di Zhao *et al*, shown that Whites had a lower risk for SCD than Blacks.[3]

A study of sudden natural deaths in 545 medico legal autopsies cases conducted over 5-years period in

University Malaya Medical Centre (UMMC), Kuala Lumpur shown that a SCD accounted for 65% of all sudden natural death.[4] A study on SCD revealed that the most prevalence aged for SCD in Malaysian population were 41 to 50 years of age.[5]

Study shown that 90% who succumbed from SCD had warning signs such as shortness of breath, giddiness, chest pain and syncope prior to the event. Most of the deceased sustained previous medical illness including coronary artery disease, valvular heart disease, cardiomyopathies, congenital heart disease or been taking drugs that are capable of provoking ventricular tachyarrhythmias.

Despite advancement in heart failure treatment for the past decade, various studies have shown high rt mortality rates in these patients. An observational study conducted among acute heart failure patients treated in Sungai Buluh Hospital shown an exceptionally high 1-year mortality rate (49.7%).[6] Another local study in Sarawak General Hospital reported all-cause mortality of 16.8% at 90 days.[7]

An implantable cardioverter-defibrillator (ICD) is remarkably effective in prevention of sudden cardiac arrhythmia. The advent of the ICD has revolutionized prevention of SCD in high-risk patients with underlying cardiac diseases. However, several challenges remain. Identification of patients at risk who should receive an ICD is suboptimal, and the sole criterion applied in clinical practice is a severely reduced left ventricular ejection fraction (LVEF) despite the fact that SCD occurs mostly in patients with preserved or mildly reduced ejection fraction.

In Malaysia, primary prevention for ICD insertion in HFrEF is limited by cost and resources. This amplify an urgent need to develop an assessment tool to further risk stratify our patients that will benefit the most from ICD.

MATERIALS AND METHODS

Heart Failure (HF) Registry

The primary study population were heart failure patients registered under University Malaya Medical Centre (UMMC) Heart Failure Registry (HF Registry). Our study enrolled a total of 356 patients of heart failure with reduced ejection fraction (HFrEF) treated in UMMC between January 2017 and December 2021. HF patients who were on cardiac resynchronisation therapy, ICD and pacemaker were excluded from this study.

During the study, we had collected information related to demographics, types of heart failure, medications, and electrocardiogram (ECG) parameters. We had divided the study outcomes into survivor or death, whereby the mortality was further subdivided into sudden cardiac death (SCD) or non-sudden cardiac death.

For patients who were lost to follow up during the study period, the patients and/or their family members were contacted for further information. Patients' cause of death was traced from National Registration Department (NRD).

The criteria used for SCD as the cause of death were as below.

1) In-hospital: within 1 hour of symptoms (chest pain or shortness of breath) onset
2) Outside hospital: within 24 hours of symptoms (chest pain or shortness of breath) onset

Electrocardiographic (ECG) Measurement

The patients' latest resting ECG with a paper speed of 50mm/s were analysed for the presence of:

1) Heart rate >75 beats per minute
2) Bundle branch block (BBB): left bundle branch block or right bundle branch block
3) QRS duration >120 milliseconds (ms)
4) PR interval: short PR <120ms or prolonged PR >220ms

5) Abnormal P waves morphology: atrial fibrillation, atrial flutter, retrograde P wave
6) QTC interval: QTc >450ms
7) T-peak to T-end interval, TpTe >90ms
8) Left ventricular hypertrophy (LVH)

Follow-up

The follow-up duration was limited to 5 years, to clarify the role of ECG in assessing risk of sudden cardiac death since the cardiovascular profile could ultimately change on longer follow-up period.

The primary endpoint was to identify ECG parameters that predict SCD whereas the secondary endpoint was to identify ECG parameters that predict non-SCD.

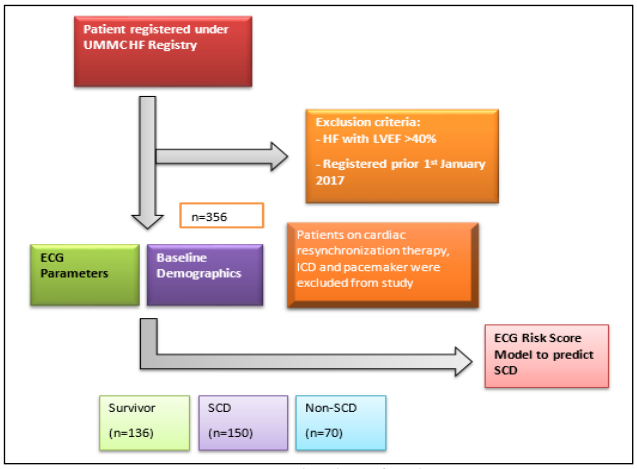

Figure 1. Flowchart of Study

Statistical Analysis

Initially one-way ANOVA and Pearson's Chi Square tests were used for bivariate case-control comparisons of continuous and categorical variables respectively. Secondly, a multivariate cox regression analysis was used to determine the independent predictors of sudden cardiac death and non-sudden cardiac death and the Crude Hazard Ratio (HR) with 95% confidence interval (95% CI) was obtained. Thirdly, the level of significance chosen was 2-tailed and considered at $P \leq 0.05$. Then, the significant ECG parameters and constructed an ECG risk score were identified. Next, the data were re-analysed with cox regression model to confirm the significance of newly postulated score. Finally, Kaplan-Meier survival analysis were used to compare the survival subjects with different ECG risk score. All statistical calculations were performed using SPSS version 26. For all analyses, value of $P \leq 0.05$ were considered statistically significant.

RESULTS

Baseline Characteristics

A total of 356 heart failure patients were included in this study. The enrolled patients were stratified into survivor (n=136), SCD (n=150) and non-SCD (n=70). The demographics and clinical characteristics of enrolled patients were summarized in Table I.

Our data shown no difference in age and race between cases. The survivor group (control) had a mean follow-up of 2.88±1.22 years whereas death cases had lower follow-up duration due to early mortality (SCD 1.83± 1.12 years vs non-SCD 1.74±1.11 years, P<0.001). SCD patients were predominantly male (P=0.028) and exhibited ischemic type of heart failure (P=0.017). Among patients with death end-point, smoking history was observed more in SCD group (P=0.004), while dyslipidaemia and chronic coronary syndrome were prevalent in non-SCD group (P=0.010 and P=0.017, respectively). Heart failure medications such as angiotensin receptor neprilysin inhibitor (ARNI), angiotensin converting enzyme inhibitor (ACEi) or angiotensin receptor blocker (ARB), beta blocker, sodium/glucose-cotransporter-inhibitor (SGLT2i) and mineralocorticoid receptor antagonist (MRA) were extensively used among survivor groups compared to SCD and non-SCD (P<0.001).

There were eight ECG parameters assessed across the group. An abnormal P wave morphology, bundle branch block, LVH pattern, short PR interval, long QTc and long TpTe, were all significantly prevalent in SCD group compared to control (P ranging 0<0.001 to 0.005). A similar pattern seen in non-SCD group (P ranging <0.001 to 0.002) except that PR interval and long QTc were not significantly different. The heart rate and QRS duration were similar across all three groups.

Table I: Baseline characteristics of heart failure patients according to survival outcomes

Demographics	Survivor n = 136	SCD n = 150	Non-SCD n = 70	P-value
Age (years)	62 ± 13	62 ± 11	65 ± 12	0.170
Follow up (years)	2.88 ± 1.22	1.83 ± 1.12	1.74 ± 1.11	<0.001
Races:				
Malay	69 (50.0)	73 (48.7)	39 (55.7)	0.192
Indian	40 (29.4)	53 (35.3)	14 (20.0)	
Chinese	28 (20.6)	24 (16.0)	17 (24.3)	
Gender:				
Male	94 (69.1)	122(82.0)	56(80.0)	0.028
Female	42 (30.9)	27 (18.0)	14 (20.0)	
LV Ejection Fraction (LVEF)	27.3 ± 7.6	27.0 ± 7.9	28.2 ± 7.3	0.538
HF Aetiology:				
Ischemic	99 (72.8)	104 (69.3)	61 (87.1)	0.017
Non-ischemic	37 (27.2)	46 (30.7)	9 (12.9)	
Risk factors:				
Smoking status	47 (34.6)	37 (24.7)	13 (18.6)	0.033
Hypertension	98 (72.1)	122 (81.3)	49 (70.0)	0.092
Diabetes Mellitus	92 (67.6)	103 (68.7)	57 (81.4)	0.090
Dyslipidaemia	113 (83.1)	125 (83.3)	67 (95.7)	0.028
History of CAD	102 (75.0)	101 (67.3)	58 (82.9)	0.045
Revascularization	86 (63.2)	81 (54.0)	45 (64.3)	0.189
LDL	2.19 ± 1.08	2.53 ± 1.31	2.17 ± 1.17	0.029
Medications:				
ARNI	57 (41.9)	34 (23.4)	4 (5.7)	<0.001
ACE-I / ARB	55 (40.4)	51 (34.0)	33 (47.1)	0.162
B-Blocker	125 (91.9)	103 (68.7)	40 (57.1)	<0.001
SGLT2i	95 (69.9)	52 (34.7)	3 (4.3)	< 0.001
MRA	97 (71.3)	66 (44.0)	16 (22.9)	<0.001
ECG Variables:				
Heart Rate > 75bpm	99 (72.8)	102 (68.0)	45 (64.3)	0.520
Abnormal P morphology	8 (5.9)	19 (12.7)	14 (20.0)	0.009
Bundle Branch Block	23 (16.9)	54 (36.0)	30 (42.9)	<0.001
LVH Pattern	38 (27.9)	68 (45.3)	39 (55.7)	<0.001
PR Interval (ms)	164.79 ±	148.06 ±	147.77 ±	0.030
- PR < 120ms	51.28	58.55	68.61	0.011
- PR > 220ms#	13 (10.2)	30 (23.6)	7 (12.5)	0.793
	1 (0.8)	2 (1.6)	1 (1.8)	
QRS duration (ms)	107.40 ±	111.36 ±	113.33 ±	0.179
- QRS > 120ms	21.66	25.20	24.61	0.058
	38 (27.9)	58 (38.7)	30 (42.9)	
QTc duration (ms)	464.74 ±	482.55 ±	484.57 ±	0.001
- QTc > 450ms*	44.22	44.76	49.32	0.004
	89 (65.4)	124 (82.7)	52 (74.3)	
TpTe Interval (ms)	76.91 ±	95.80 ±	89.86 ±	<0.001
- TpTe > 90ms	17.44	18.51	14.39	<0.001
	28 (20.6)	87 (58.0)	31 (44.3)	

The Prognostic Significance of Clinical and ECG Parameters in SCD: Primary Outcome

Cox regression model was created to determine association of clinical and EGG parameters that predict SCD. By using the univariate analysis, male gender, HF medications (beta blocker, SGLT2i, MRA) alongside with all ECG parameters were found to have association with SCD. In a multivariable analysis, all significant parameters from individual analysis were included in clinical and ECG models separately. For clinical parameters, beta blocker (HR 0.58; 95% CI 0.40-0.84; P=0.04), SGLT2i (HR 0.44; 95% CI 0.31-0.62; P<0.001) was associated with reduced risk of SCD. However, other clinical risk factors were not associated with SCD.

For ECG parameters, there were six variables which remained significantly associated with SCD, namely abnormal P morphology (HR 1.69; 95% CI 1.03-2.78; P=0.039), bundle branch block (HR 2.18; 95% CI 1.53-3.10; P<0.001), QRS duration (HR 1.01; 95% CI 1.003-1.02; P=0.018), QTc interval (HR 1.007; 95% CI 1.004-1.01; P<0.001), TpTe interval (HR 1.03; 95% CI 1.02-1.03; P<0.001) and PR interval (HR 0.995; 95% CI 0.993-0.998; P<0.001). The latter exhibited bidirectional increase risk of SCD, explaining paradoxical effect of risk reduction when analysing continuous variable of PR interval. All continuous ECG parameters were subcategorized into PR interval <120m (HR 1.87; 95% CI 1.23-2.86; P 0.004), PR interval >220ms (HR 7.27; 95% 1.67-31.62; P 0.008), QRS >120ms (HR 1.84; 95% CI 1.26 -2.77; P=0.002), QTc >450ms (>460ms for female) (HR 2.04; 95% CO 1.18-3.55; P=0.005), TpTe >90ms (HR 2.27; 95% CI 1.51-3.41; P<0.001; which all demonstrated association of increased SCD. Further details were summarized in Table II.

The Prognostic Significance of Clinical and ECG Parameters in Non-SCD: Secondary Outcome

The multivariable model of clinical characteristic was not significant in predicting non-SCD except for ARNI (HR 0.25, 95% CI 0.091-0.71; P=0.009) and SGLT2i (HR 0.057; CI 0.017-0.19; P<0.001) which both reduced risk of non-SCD. The multivariable cox of ECG parameters demonstrated bundle branch block (HR 2.89; 95% CI 1.64-5.086; P<0.001), LVH pattern (HR 2.54; 95% CI 1.48-4.35; P=0.001), QRS duration (HR 1.01; 95% CI 1.002-1.023; P=0.023) along with QRS > 120ms (HR 1.86; 95% CI 1.056-3.26; P=0.032), TpTe interval (HR 1.02; 95% CI; P=0.002-1.014P=0.007) particularly TpTe > 90ms (P=0.004) predicted higher risk of SCD. As opposed to SCD group, abnormal P morphology, PR interval and QTc interval were not associated with non-SCD occurrence, whereas the LVH pattern was distinctive predictor for non-SCD. The result details were summarized in Table III.

ECG Risk Score for SCD Prediction

All significant ECG parameters for SCD derived from Cox Proportional Hazard multivariable model were combined to enumerate SCD prediction ability based on cumulative ECG parameters. ECG risk score which

Table II: Univariable and Multivariable Predictors of Sudden Cardiac Death (SCD) in Cox Proportional Hazards Model

Variables	Univariable		Multivariable	
	Hazard ratio (95% CI)	**P-value**	**Hazard ratio (95% CI)**	**P-Value**
Clinical Variables				
Age	1.002 (0.989-1.102)	0.773		
Male	1.580 (1.040-2.401)	0.032	1.380 (0.906-2.103)	0.134
LVEF	0.991 (0.971-1.012)	0.093		
Ischaemic HF	0.840 (0.594-1.189)	0.326		
Smoking status	0.727 (0.502-1.054)	0.093		
Hypertension	1.378 (0.913-2.079)	1.378		
Diabetes Mellitus	0.877 (0.620-1.240)	0.456		
Dyslipidaemia	0.893 (0.581-1.372)	0.604		
History of CAD	0.729 0.518-1.026)	0.069		
Revascularization	0.781 (0.567-1.077)	0.132		
LDL	1.108 (0.974-1.261)	0.118		
ARNI	0.692 (0.474-1.011)	0.057		
ACE-I / ARB	0.724 (0.516-1.017)	0.063		
B-Blocker	0.513 (0.363-0.725)	<0.001	0.577 (0.398-0.837)	0.004
SGLT2i	0.405 (0.289-0.568)	<0.001	0.437 (0.309-0.620)	<0.001
MRA	0.519 (0.376-0.717)	<0.001	0.730 (0.513-1.039)	0.080
ECG Variables				
Heart Rate > 75bpm	0.677 (0.479-0.957)	0.027	0.929 (0.691-1.511)	0.913
Abnormal P morphology	2.008 (1.238-3.256)	0.005	1.690 (1.026-2.782)	0.039
Bundle Branch Block	2.490 (1.770-3.504)	<0.001	2.177 (1.531-3.096)	<0.001
LVH Pattern	1.809 (1.308-2.502)	<0.001	1.152 (0.815-1.628)	0.422
PR Interval (1-SD increase)	0.995 (0.993-0.998)	<0.001	0.997 (0.995-0.999)	0.017
PR Interval < 120ms	1.813 (1.203-2.734)	0.004	1.871 (1.225-2.856)	0.004
PR Interval > 220ms	4.273 (1.045-17.468)	0.043	7.272 (1.673-31.618)	0.008
QRS duration (1SD increase)	1.008 (1.001-1.016)	0.018	1.010 (1.003-1.017)	0.007
QRS > 120ms	1.616 (1.161-2.249)	0.004	1.837 (1.261-2.676)	0.002
QTc Interval (1-SD increase)	1.007 (1.004-1.010)	<0.001	1.005 (1.001-1.009)	0.011
QTc > 450ms*	2.057 (1.346-3.145)	0.001	2.044 (1.178-3.548)	0.005
TpTe Interval (1-SD increase)	1.025 (1.018-1.033)	<0.001	1.023 (1.015-1.031)	<0.001
TpTe > 90ms	2.429 (1.756-3.362)	<0.001	2.271 (1.512-3.410)	<0.001

Table III: Univariable and Multivariable Predictors of Non-Sudden Cardiac Death (Non-SCD) in Cox Proportional Hazards Model

Variables	Univariable		Multivariable	
	Hazard ratio (95% CI)	**P-value**	**Hazard ratio (95% CI)**	**P-Value**
Clinical Variables				
Age	1.014 (0.995-1.034)	0.145		
Male	1.520 (0.845-2.733)	0.162		
LVEF	1.011 (0.979-1.044)	0.518		
Ischaemic HF	1.896 (0.941-3.819)	0.073		
Smoking status	0.534 (0.292-0.976)	0.042	0.614 (0.330-1.140)	0.122
Hypertension	1.011 (0.605-1.688)	0.968		
Diabetes Mellitus	1.578 (0.863-2.887)	0.139		
Dyslipidaemia	3.043 (0.957-9.678)	0.059		
History of CAD	1.312 (0.704-2.443)	0.392		
Revascularization	1.046 (0.641-1.706)	0.857		
LDL	1.014 (0.814-1.263)	0.901		
ARNI	0.137 (0.050-0.375)	<0.001	0.253 (0.091-0.707)	0.009
ACE-I / ARB	1.173 (0.733-1.876)	0.507		
B-Blocker	0.305 (0.190-0.490)	<0.001	0.927 (0.534-1.611)	0.789
SGLT2i	0.035 (0.011-0.112)	<0.001	0.057 (0.017-0.187)	<0.001
MRA	0.203 (0.116-0.356)	<0.001	0.574 (0.301-1.092)	0.091
ECG Variables				
Heart Rate > 75bpm	0.729 (0.446-1.191)	0.207	0.696 (0.393-1.231)	0.213
Abnormal P morphology	2.830 (1.567-5.111)	0.001	1.293 (0.622-2.688)	0.492
Bundle Branch Block	3.865 (2.383-6.269)	<0.001	2.889 (1.637-5.086)	<0.001
LVH Pattern	2.770 (1.722-4.457)	<0.001	2.537 (1.480-4.349)	0.001
PR Interval (1-SD increase)	0.994 (0.991-0.998)	<0.001	0.999 (0.994-1.003)	0.500
PR Interval < 120ms	1.482 (0.334-1.482)	0.334	1.237 (0.550-2.784)	0.607
PR Interval > 220ms	4.168 (0.568-30.60)	0.160	4.405 (0.590-32.899)	0.148
QRS duration (1-SD increase)	1.014 (1.004-1.025)	0.007	1.012 (1.002-1.023)	0.023
QRS > 120ms	2.048 (1.273-3.294)	0.003	1.857 (1.056-3.263)	0.032
QTc duration (1-SD increase)	1.011 (1.005-1.016)	<0.001	1.008 (1.002-1.014)	0.013
QTc > 450ms*	1.613 (0.942-2.762)	0.082		
TpTe Interval (1-SD increase)	1.031 (1.019-1.044)	<0.001	1.024 (1.010-1.038)	0.007
TpTe > 90ms	2.641 (1.643-4.246)	<0.001	2.000 (1.118-3.579)	0.020

represented number of abnormal ECG parameters was used to demonstrate this effect and was independent of HR magnitude of individual parameters. Table IV provided HR and 95% CI for SCD according to ECG risk score. Our findings demonstrated that every additional ECG abnormalities were associated with increasing risk for SCD, and patients with ECG score ≥4 exhibited moderate risk of developing SCD (HR 5.99; 95% CI 2.65-13.59; P<0.001).

Kaplan-Meier Survival Plot for SCD According to ECG Score

Survival analysis using Kaplan Meier (Figure 1) was performed to investigate event-free survival following ECG abnormalities. The association between ECG risk score and SCD shown sustained effect throughout follow-up duration up until 5 years (mean survival 3.3 ± 0.14 years for SCD; 4.1 ± 0.2 years for non-SCD). Post hoc analysis using pairwise comparison demonstrated

a significant difference in SCD event-free between the two groups; no ECG abnormalities group and group with two or more ECG abnormalities (P<0.001).

Table IV: Risk of SCD Associated with ECG Risk Score Among Patients with HFrEF

ECG risk score	Survivor	Sudden Cardiac Death		
	N (%)	N (%)	HR (95% CI)	P-value
0	19 (14.0)	7 (4.7)	Ref	
1	68 (50.0)	19 (12.7)	0.842 (0.353-2.005)	0.697
2	25 (18.4)	59 (39.3)	3.739 (1.703-8.211)	0.001
3	14 (10.3)	30 (20.0)	4.070 (1.782-9.297)	0.001
≥4	10 (7.4)	35 (23.3)	5.994 (2.645-13.586)	<0.001

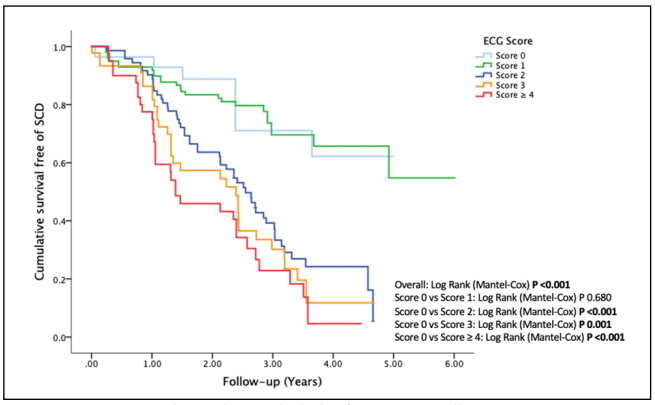

Figure 2. Kaplan-Meier Survival Plot for SCD according to ECG Score

DISCUSSION

Although our study was a retrospective single tertiary centre research involving a total of 356 HFrEF patients, we had managed to collect the risk variables that may predict SCD in HFrEF. The collected variables were the demographics, drug therapy and ECG parameters which were analysed revealed various important findings. Firstly, we managed to identify parameters that can estimate risk of SCD. Secondly, we manage to curate an ECG risk score model to predict risk of SCD.

Our study demonstrated that ischemic HFrEF have lowest association with SCD as compared to survivor and non-SCD group. An existing study of 3078 patients from Denmark showed that ischemic HFrEF predicts poorer prognosis.[8] Another study from China enrolled 873 patients which further divided into ischemic and non-ischemic HFrEF shown that HFrEF was associated with higher SCD and all-cause mortality.[9] Ischemic cardiomyopathy is the result of disturbance in between myocardial contractility and perfusion.

A permanent damage to myocardium following myocardial infarction will lead to gradual remodelling process and eventually myocardial fibrosis. In this situation, a revascularization therapy would not be beneficial as the tissues were no longer viable. As a result, the myocardial scarring may cause ventricular arrhythmia and sudden cardiac death. However, certain studies have found contrary result.

A study conducted in Portugal in 2011 enrolling a total of 286 heart failure patients with ischemic and non-ischemic aetiology shown that ischemic heart failure was not a predictor of mortality and the differences appears to fade along time.[10] The findings which contradicted previous studies indirectly highlighted that genetic variance and ethnicity could be a strong influencing factor for HF patients in different regions especially in multi-racial country such as Malaysia.

Our study also showed that male gender has higher association with sudden cardiac death. A previous study in Ireland which shown that incidence of SCD in male was 4.36 in 100,000 person-years which is higher than female which was 1.3 in 100,000 person-years.[11] Possible explanations for this result is the presence of coronary artery disease. Coronary artery disease is the commonest cause of SCD, contributing up to 80% of SCD. Male tend to be smoker and underwent more stress resulting in acceleration of cardiovascular risk such as diabetes mellitus, hypertension and dyslipidaemia, leading to the development of coronary artery disease which indirectly leading to SCD.

Our study revealed that several ECG parameters were proven to be significant in predicting SCD with bundle branch block proven to be one of the independent predictors. Our study included both right bundle branch (RBBB) and left bundle branch block (LBBB) in the analysis. A study conducted in Spain revealed that eight patients from different centres who contracted multiple aborted SCD shared similar ECG parameters. The patients had normal QTc interval but RBBB was persistent in all cases.[12] Another multi-centre study from seven countries (Denmark, Ireland, Finland, Germany, Norway, Sweden, and the United Kingdom), demonstrated that LBBB and RBBB which developed during follow-up was significantly associated with SCD.[13]

However, the mechanism of conduction problem leading to malignant arrhythmia remained speculative at present.

PR interval is another parameter that was shown to predict SCD in HFrEF patients. Our study divided PR interval into short PR <120ms and prolonged PR >220ms in which both were statistically significant to increase risk of developing SCD in our study population. However, the evidence for direct correlation between PR interval and SCD prediction is still lacking. A previous study conducted in Finland in 2014 revealed that prolonged PR interval was not associated with all-cause cardiovascular death.[14] However based on various studies, a wide QRS complex >120ms was widely approved as a risk of SCD. Another prospective study conducted in Finland in 2012 involving 2049 men aged 42 to 60 years were followed up for 19 years revealed 156 SCD among the enrolled patients. The study also demonstrated that the QRS duration was associated with 27% higher risk of developing SCD.[15] The potential mechanisms involved would be a delayed electrical conduction due to left ventricular dysfunction that lead to malignant arrhythmia.

Prolonged QTc had a long debate since a decade ago regarding its association with SCD. A study conducted in Netherland in 2006 enrolled 3,105 men and 4,878 women aged more than 55 years old. The study revealed that QTc >450ms in men and QTc >470ms in women was significantly associated with 3-fold increase in SCD risk.[16] Another study conducted in 2015, recruited a total of 195 clinical hypertrophic cardiomyopathy patients showed that QTC >460ms was associated with ventricular tachyarrhythmia or SCD.[17] However, in another study involving 254 initial ECGs of hypertrophic cardiomyopathy patients revealed no significant difference in QTc interval and SCD.[18]

Another ECG parameter related to SCD is the TpTe interval. Recently, studies are blooming gradually from various centre to prove that TpTe is a universal predictor of SCD. Mechanism leading to SCD is related to delay in repolarization phase from epi-myocardium to endo-myocardium which opens a probability of arrhythmia pre-excitation. Oregon Sudden Unexpected Death study conducted in Portland evaluated TpTe interval and other ECG parameters showed that TpTe interval is an independent predictor of SCD.[19]

Current guidelines, recommended that implantable cardioverter defibrillator (ICD) insertion in HFrEF should primarily be based on LVEF and NYHA classification. If this recommendation is to be applied in our local setting, a large number of HFrEF population would indirectly eligible for the ICD, Thus, the condition would give an impact to healthcare system expenses. MADIT-I trial showed ICD saves lives in high-risk patients with coronary heart disease whereas MADIT-II trial showed that prophylactic ICD therapy was associated with significantly improved survival in patients with ischemic cardiomyopathy. The study population in these trials was primarily confined to United States and Europe and the conventional treatment in non-ICD group was not optimal whereas the ICD group has better overall condition. Thus, there was significant difference between both groups.[20]

To date, evidence of such studies in Asian HFrEF population remains scarce and this open a wide realm for future study. To the best of our knowledge, this is the first study in Malaysia describing prevalence, demographics, and risk predictor model of SCD in HFrEF. From our ECG risk score model, subjects with ≥2 ECG abnormalities had more than 3-fold increased risk for SCD and the risk proportionately increased with an increased in ECG abnormalities. However, the predictive value remained relatively low, despite its significant. Thus, a larger sample size and a multi-center involvement is recommended to further strengthen the result. By application of this score, we managed to filter and prioritize our patient for ICD insertion, and in a long term it will be able to help in reducing healthcare expenses. Parameters listed in our risk score are relatively easily obtained from standard 12-lead ECG, making this risk score relevant for clinical use.

LIMITATIONS

Firstly, the optimization of medications in SCD group was very low, this may be the confounding factor for the

outcome. Secondly, both SCD and no SCD death group have very low intention to treat medications which may lead to selection bias. These two factors could be explained by poor insight and lack of awareness among our patients which need to be overcome in near future. Although including atrial fibrillation in abnormal P waves parameter may lead to significant confounding factors that affect mortality and morbidity rate, we would like to emphasize that our patients' selection processes were random. As the symptoms preceding the SCD occurred in patients outside hospital were clarified from the family or eye witness, this could potentially lead to overestimation of SCD as well.

CONCLUSION

This cumulative ECG risk score model was independently associated with SCD and particularly effective for LVEF <40% where risk stratification model remained scarce. These findings warrant further evaluation in prospective study to further clarify our outcome.

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ORIGINAL ARTICLE

The Management Outcome of Heart Failure Reduced Ejection Fraction with or without Angiotensin Receptor Neprilysin Inhibitor

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ABSTRACT

INTRODUCTION: Heart failure is associated with recurrent admission, higher mortality and low quality of life. Angiotensin receptor neprilysin inhibitor (ARNI) is a novel agent that has been used for treating heart failure reduced ejection fraction (HFrEF) patients. Thus, it is interesting to evaluate the effect of ARNI on the reverse cardiac remodelling, rehospitalization, cardiac biomarker and quality of life in HFrEF patients. **MATERIALS AND METHODS:** A case controlled study was conducted to assess the treatment outcome of HFrEF with or without ARNI. During the study, the patients' basic demography, co-morbidities, baseline echocardiography (ECHO) findings, NYHA classification, NT-pro BNP levels and KCCQ score were evaluated. The patients' admission history within 90 days from initiation of ARNI or non ARNI were obtained retrospectively. A follow up ECHO was obtained after at least 3 months of intervention. **RESULTS:** A total of 81 patients were recruited in which 54 patients were on ARNI and 27 were on non ARNI treatment. There was a statistically significant improvement of ejection fraction, left ventricular internal diameter end diastole and systole, and left ventricular end-systolic volume in ARNI group. The NYHA class was also noted to improve after ARNI treatment. The NT-proBNP value was lower whereas the KCCQ score was higher in ARNI group compared to non ARNI group. **CONCLUSION:** HFrEF patients with ARNI treatment had better reverse cardiac remodelling effect, cardiac biomarker and quality of life compared to non ARNI treatment. Furthermore, patient received ARNI demonstrated improved heart failure classification after treatment.

Keywords
Angiotensin Receptor Neprilysin Inhibitor (ARNI), Echocardiography, Heart Failure Reduced Ejection Fraction (HFrEF), N-terminal Pro-BNP, Quality of life.

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Received: 18th January 2024; Accepted: 11th June 2024

Doi: https://doi.org/10.31436/imjm.v23i04

INTRODUCTION

Heart failure (HF) is a clinical syndrome of the end stage of most cardiac disease in which the typical symptoms during the presentation are shortness of breath, ankle swelling and fatigue.[1] The prevalence of HF in Malaysia is 6-10%.[2,3] Acute decompensated heart failure (ADHF) leads to 11.1% mortality and 24% rehospitalization within 30 days.[4] The survivals from ADHF may experience 35% mortality within 1 year without treatment.[5] On the other hand, readmission for ADHF causes a heavy burden to our health care services and national economy, which costs about RM 194 Million directly and indirectly.[6,7]

Our understanding and management of HF improved over the years with more research, which translated into a lower mortality rate. However, 50% mortality within 5 years is still considered deadlier than certain notorious cancers such as colorectal cancer (35.5%) and breast cancer (10%).[8,9] Clinical history, clinical symptoms with

physical examination combined with natriuretic peptides or chest radiograph is necessary for making diagnosis of HF.[10] Echocardiography (ECHO) use may further classify HF into HF with preserved ejection fraction (HFpEF), HF with mildly reduced ejection fraction (HFmrEF) and HF with reduced ejection fraction (HFrEF).

According to the latest guideline of American College of Cardiology (ACC) and European Society of Cardiology (ESC), angiotensin converting enzyme inhibitors (ACEi), beta-blockers, mineralocorticoid receptor antagonists (MRA) were found to have mortality and morbidity benefits in HF patients and had been recognized as part of the standard management of HF. Angiotensin receptor neprilysin inhibitor (ARNI) is a new novel agent for HF treatment. It is a combination of Angiotensin II receptor blockers and Neprilysin inhibitor which targets the neurohormonal activation pathway (Renin-Angiotensin-Aldosterone system and Vasopressin) in HF. This drug showed additional mortality and morbidity benefits on top of conventional standard therapy in previous studies.

In PARADIGM-HF study for HFrEF patients, there was 21% relative risk reduction in HF admission and 20% relative risk reduction in all-cause mortality.[11] Meanwhile PIONEER-HF study reported greater reduction of NT-proBNP concentration with ARNI than enalapril.[12] EVALUATE-HF study demonstrated reverse cardiac remodelling seen at 12 weeks of ARNI treatment in left ventricular end-systolic and end-diastolic volume indexes (LVEDVI and LVESVI),[13] while PROVE-HF study observed increased LVEF from 28.2% to 37.% by 12 months of ARNI treatment.[14] Those mentioned NT-proBNP markers and ECHO parameters were important prognosticating factor in HFrEF patients.[15,16] With that, ARNI had been widely recognized as part of standard/important therapy for HFrEF by European Society of Cardiology (ESC), American Heart Association (AHA), Malaysia clinical practice guidelines (CPG) and some other guidelines.

In our clinical practice, ARNI usage was associated with marked improvement of patients' general condition, functional class, and even reduced readmission rate. However, the drug is a non-standard medication and has to be purchased by majority of patients. Besides, we also found that there was paucity of relevant real-world experience in our country. Therefore, we conducted a local case control study, to observe the impact of ARNI on HFrEF treatment. In addition, we also aimed to observe the impact on simple ECHO parameters from conventional ECHO as a full conventional ECHO may took 15-20 minutes per person. Routine conventional ECHO had been found to be difficult to implement in a busy heart failure clinic. Hence POC ECHO with capability of assessing simple ECHO parameters may be the solution on time management.

MATERIALS AND METHODS

Study Design and Patients Selection

This study was a case controlled study conducted to assess the effect of ARNI compared with ACEi or ARB, on top of HFrEF standard management. The study populations were patients under the Cardiology clinic and Heart Failure clinic in Hospital Universiti Sains Malaysia (HUSM). Patients who fulfilled the criteria were identified during clinic consultation and case note review, using the following inclusion criteria: age between 18 to 80 years, confirmed diagnosis of HFrEF (ejection fraction, EF<40%) and latest ECHO done were not more than 2 years, taking ARNI or ACEi/ARB for more than 3 months (on top of guideline directed medical therapy including beta blockers/mineralocorticoid/ivabradine/ sodium glucose co-transporter 2 inhibitor), compliance to medication (defined as more than 2 visits to pharmacy department for drug collection in 2 months). On the other hand, patient with device therapy for HF treatment, severe valvular lesion pending surgery intervention, post mechanical valve implantation for valvular heart disease, end stage renal disease and pregnancy were excluded.

Data Collection

Patients who fulfilled the above-mentioned criteria were contacted and stratified into ARNI group and non ARNI group in a 2:1 ratio. Patients' basic demography, co-morbidities, the New York Heart Association (NYHA) class upon HF diagnosis, quality of life, N-terminal pro b-type natriuretic peptide (NT-pro BNP) levels and

baseline echocardiography (ECHO) findings were evaluated. Patients' admission history within 90 days from initiation of ARNI or ACEi/ARB were obtained retrospectively from the electronic health records or paper records. All comorbidities were confirmed based on latest guidelines using data obtained from the patients' folders and laboratory results. The ischemic heart disease (IHD) were defined based on conventional coronary angiogram or computed tomography coronary artery. The NYHA was used to classify the extent of HF and the quality of life of these patients was assessed using the Kansas City Cardiomyopathy Questionnaire (KCCQ). The validated questionnaire in local language was used in this study. Serum NT-proBNP was measured using Cobas h 232 POC system (Roche Daignostics, Rotkreuz, Switzerland). Conventional ECHO were performed by certified cardiovascular technician using Philips EPIC or Philips Affiniti (Philips Healthcare, Selangor) with complete documentation of ejection fraction (EF) (simpson biplane), left ventricular internal diameter end diastole (LVIDd), left ventricular internal diameter end systole (LVIDs), end-diastolic volume (EDV) and end-systolic volume (ESV) were part of the selection criteria (ASE, updated 2018). The second ECHO was done at least 3 months apart from previous ECHO.

Statistical Analysis

All data was analysed using IBM SPPS software version 26 (Armonk, New York, USA). Data were presented in median (inter quartile range, IQR). The differences in median values between independent groups were assessed using MannWhitney Test and categorical variables were compared by chi-square test. The Wilcoxon Signed Ranks Test and McNemar Test were applied for two dependent variables analysis. A two tailed P-value of less than 0.05 was considered significant.

RESULTS

A total of 83 patients were screened but only 81 patients were recruited (2 patients in ARNI group were dropped out due to repetitive basic profile in study design). The recruited patients were divided into ARNI group (54 patients) and non ARNI group (27 patients). Majority of our study patients were male (81.4%) with the median age for ARNI group was 64.5 years (15.0) and non ARNI group was 62 years (13.0). The comorbidities were equally distributed in both ARNI group and non ARNI group and the concomitant HF medicines showed no significant difference between both groups. The systolic blood pressure (SBP) was not significantly differed between both groups while diastolic blood pressure (DBP) was higher in non ARNi group (Table I).

Table I: Baseline characteristics of study participants (n=81)

Parameters	ARNI (n=54)	Non ARNI (n=27)	P-value*
Age (years)	64.5 (15.0)	62.0 (13.0)	0.557^
Gender			
Male	44 (81.5%)	22 (81.5%)	1.000
Female	10 (18.5%)	5 (18.5%)	
Diabetes mellitus	35 (64.8%)	17 (63.0%)	0.870
Hypertension	36 (66.7%)	18 (66.7%)	1.000
Hyperlipidaemia	23 (42.6%)	11 (40.7%)	0.874
Ischemic heart disease	40 (74.1%)	19 (70.4%)	0.724
Atrial fibrillation	3 (5.6%)	1 (3.7%)	0.717
Chronic kidney disease	21 (38.9%)	11 (40.7%)	0.872
Beta blockers	41 (75.9%)	20 (74.1%)	0.855
Ivabradine	2 (3.7%)	1 (3.7%)	1.000
MRA	34 (63.0%)	48.0%)	0.203
SGLT2i	1 (1.9%)	1 (3.7%)	0.613
SBP, mmHg	110 (11.03)	111 (13.46)	0.070^
DBP, mmHg	58 (6.43)	62 (12.86)	0.005^

Data presented as Median (IQR) for continuous variables and Frequency (percentage) for categorical variables. *Chi-Square Test. ^Mann-Whitney U-Test. MRA=Mineralocorticoid receptor antagonists, SGLT2i=Sodium-glucose cotransporter 2 inhibitor, SBP=Systolic blood pressure, DBP=Diastolic blood pressure.

At the end of study, 9 patients (16.6%) received maximum dose of Entresto (200mg BD). 3 patients (5.6%) received 150mg BD, 15 patients (27.7%) were on 100mg BD, 17 patients (31.48%) remain on 50mg BD, and the last 10 patients (18.5%) only received 25mg BD. The median medication time upon enrolment in the study were 7 months. There were 13 patients (48.15%) received ACEi (individualized dose of Enalapril, Perindopril, Ramipril) whereas another 14 patients (51.85%) received ARB (individualized dose of Valsartan) in non ARNI group. Median exposure to non ARNI treatment were 9 months.

Second ECHO was done at 7.5 months in ARNI group and 9 months in non ARNI group after randomization. The repeated second EF at follow up was improved compared to baseline in ARNI group. However, there was no significant difference of EF changes in non ARNI treatment arm. The reduction of chamber size is statistically significant after ARNI treatment as indicated by the reduction of LVIDd. Cardiac volume was reduced in ARNI treatment group as showed in ESV reduction, albeit not statistically significant in EDV changes. However, the reverse cardiac remodelling changes was

not observed in non ARNI treatment arm as no changes in LVIDd. Furthermore, patient received non ARNI treatment did not showed significant cardiac volume reduction since no changes in EDV and ESV respectively (Table II).

Table II: Changes of echocardiographic parameters at baseline and follow up (n=81)

Parameters	ARNI (n=54)		P-value	Non ARNI (n=27)		P-value
	At Baseline	At Follow up		At Baseline	At Follow up	
EF	32.40 (9.25)	36.85 (10.10)	< 0.001	38.30 (8.80)	34.90 (11.30)	0.178
LVIDd	5.76 (1.14)	5.60 (0.96)	0.042	5.70 (0.96)	5.96 (1.23)	0.876
LVIDs	4.84 (1.42)	4.46 (0.95)	0.004	4.50 (0.90)	4.88 (1.09)	0.380
EDV	148.10 (65.71)	147.50 (59.50)	0.657	149.50 (62.49)	146.00 (63.00)	0.590
ESV	103.85 (48.54)	93.59 (42.94)	0.016	86.73 (44.00)	86.58 (46.04)	0.178

Data presented as Median (IQR). Wilcoxon Signed Ranks Test. EF = ejection fraction, LVIDd = Left ventricular internal diameter end diastole, LVIDs = Left ventricular internal diameter end systole, EDV = End-diastolic volume, ESV = End-systolic volume.

There was a statistically significant difference in the proportion of NYHA classes pre- and post-ARNI treatment. Meanwhile, patients in non-ARNI group also showed a statistically significant difference in the proportion of NYHA classes between baseline and after at least 3 months of treatment with non ARNI (Table III).

Table III: Changes of NYHA classification at baseline and follow up (n=81)

Parameters		At Baseline	At Follow Up	P-value
ARNI (n=54)	NYHA Class 1 & 2	5	33	< 0.001*
	NYHA Class 3 & 4	49	21	
Non ARNI (n=27)	NYHA Class 1 & 2	4	6	0.031*
	NYHA Class 3 & 4	23	17	

McNemar Test, *P < 0.05. NYHA = New York Heart Association.

With regards to the rate of hospitalization during the next 90 days from initiation of ARNI or non ARNI, there was no statistically significant median difference of length of admission between patient treated with or without ARNI treatment. NT-proBNP mean value in ARNI group showed significant lower value as compared to non ARNI group. While in the quality of life assessment, patient with ARNI treatment recorded better quality of life based on higher KCCQ score as opposed to non ARNI group (Table IV).

Table IV: Median difference in heart failure hospitalization, cardiac biomarker and quality of life (n=81)

Parameters	ARNI (n=54)	Non ARNI (n=27)	U-Stat	P-value
Length of Admission Between Treatment	1.32 (0.00)	10.06 (0.00)	617.0	0.050
NT-proBNP	1459.50 (3353)	3148.00 (6363)	478.50	0.012*
KCCQ	82.81 (29.43)	54.17 (52.08)	351.50	< 0.001*

Data presented as Median (IQR). Mann-Whitney U Test, *P<0.05. NT-proBNP= N-terminal pro b-type natriuretic peptide, KCCQ=Kansas City Cardiomyopathy Questionnaire.

DISCUSSION

This study was conducted to assess the treatment outcome of HFrEF with or without ARNI in local setting for about 3 months' duration. A total of 81 patients from HUSM were prospectively enrolled with stratified sampling method: 2:1 ratio (ARNI group, non-ARNI group). The EF and other ECHO parameters was performed during the clinical visit using conventional ECHO. Then patients were compared based on their rate of hospitalization at 90 days while measuring their mean NT-proBNP and KCCQ score.

Our study shown that male patients were found to be dominant, 66 (81.5%) in both treatment group and control group which similar to the findings in other landmark studies including PARADIGM-HF 77-79%, EVALUATE-HF 74-79% and PROVE-HF 71.5%.[11,13,14]

Our study population had a median age of (SD) 62-64.5 years (9.95), which was comparable to other studies such as PARADIGM-HF 63.8 year (SD 11.4), EVALUATE-HF 67.3 year (SD 9.15), PROVE-HF 65.1 year (SD 12.4) and real-world data Italy 66.5 year (SD 11.5), Taiwan 67 year (SD 12.4).[11,13,14,17,18] The heart failure patients median age in this study supports local data which Raja Shariff *et al.* reported that heart failure median age of 63 years in Klang valley.[19]

Ischemic heart disease was the most common co-morbid which shared by both groups of patients (74.1% vs70.4%). The finding was similar to other studies which shown that IHD was the most common cause of HF as reported by other studies: PARADIGM-HF 60%, EVALUATE-HF 63%, PROVE-HF 53.7%, Italy 67% and Taiwan 54%.[11,13,14,17,18] This higher number of IHD may be explained by significantly higher prevalence of diabetes mellitus in this study population: 64.2% whereas only less than 35% in other clinical studies (except PROVE-HF with 45.5% diabetes mellitus subjects). Otherwise, all other co-morbidities were similar between two groups. All p-value in between groups was >0.05.

Our study also found that there was a lower usage of beta blocker in local study (75.3%) among HFrEF patients in

comparison to other studies which shown >85% of study patients were on beta blocker, such as EVALUATE-HF (86.5%).[13] This was likely due to recruitment of higher NYHA class (NYHA III-IV) subjects in our study (88.9%). These group of patients were more ill and tend to have lower BP at baseline and throughout the study period (mean BP 109/58). However, guideline directed medical therapy were well balanced in both study groups with all p-value in between groups were >0.05.

Our study shown that both improvement of EF and reduction in heart size were observed in ARNI group. Left ventricular reverse remodelling is clearly an outcome of interest in HFrEF patients as it associated with poorer outcome.[15] These structural changes were paralleled with lower NT-proBNP value and better KCCQ scores.[16,20] These data suggested that clinical benefits of ARNI compared with non ARNI in HFrEF patients.

Our study revealed that there were no statistically significant difference in terms of NYHA class changes and hospitalization of heart failure in the first 90 days of treatment in both treatment group. The finding may be explained by the shorter study length in comparison to PARADIGM-HF trial which demonstrated admission benefits from ARNI over non ARNI at 180 days onwards. The lesser number of hospitalization in ARNI treatment group with a p-value of 0.05 also suggested more benefits in ARNI group than in on-ARNI group. However, we believed that some patient may be admitted to other hospital without our notice.

This study was comparable with other study such as PROVE-HF trial, which had effect on reverse cardiac remodelling effect from ARNI usage.[14] This study results shown homogenous ECHO result compared to the trial on using advanced ECHO parameters. The mean reduction of LVEDV in our study was 6.21 whereas in PROVE-HF was 6.65. The mean reduction of LVESV in our study whereas was 5.6 whereas in PROVE-HF was 8.67. However, the less complex ECHO parameters were not found in landmark trials. Our study results were comparable to study by Liu *et al,* in which the mean reduction of LVIDd was 0.16 in our study, whereas in Liu *et al* was. 0.3. The mean reduction of LVISd in our study was 0.38 whereas in Liu et al. was 0.5.[18]

Point of care (POC) ECGO is a simplified echocardiography with easy portability while providing high-quality image resolution.[21] Limited ECHO by trained personnel may provide information such as EF, less complex LV parameters and even assessing Doppler flow velocities and gradients.[22] A previous study found negligible deviations on LV measurements by POC ECHO while compared to routine ECHO.[23] This may potentiate usage of POC assessment during routine clinic review or heart failure clinic assessment as routine ECHO appointment may require longer period of time.

LIMITATION

This study has many limitations, due to patients' characteristic, co-morbidities, concomitant medication drugs, baseline ECHO findings were already fixed. Hence we had to screen more patients in order to keep both group balance, which explained 2 patients were dropped out from our study. In view of higher numbers of poor LVEF patients with borderline BP in our study, dosage of ARNI were individualized. Moreover, a single center study may not represent the whole population in Malaysia as well. Finally, this study only looked at 2 ECHO readings. An repeated measure ANOVA analysis would be better tool to analyze between ARNI and non ARNI group outcome however it requires 3 sets of ECHO readings.

CONCLUSION

In summary, the use of ARNI within 3 months in this study has showed positive reverse cardiac remodelling with improvement of EF as compared to non ARNI usage. However, the lack of significant result on non ARNI group may be an isolated one due to the limitation of this study. We expect ACEi/ARB also to have possible reverse remodelling but not as strong as ARNI. There was lower value of NT-proBNP, higher KCCQ score which translated into better QOL as well as improvement of NYHA class when ARNI was used compared to non ARNI users. However, benefits of hospitalization were not significant at 90 days from initiation of drugs even though there was positive

trend towards ARNI users with lesser days of stay. In conclusion, this study suggest ARNI usage has more benefits compared to non ARNI for advance HF patients attending HF clinic. A large scale study will further validate these outcomes.

CONFLICT OF INTEREST

The authors declare no conflict of interests.

INSTITUTIONAL REVIEW BOARD (ETHIC COMMITTEE)

This study protocol was approved by the Human Research Ethics Committee Universiti Sains Malaysia, protocol code: USM/JEPeM/21020154. The study complied and conducted according to the Declaration of Helsinki. Permission from the HUSM management was also acquired before assessing the data and electronic health records. Signed informed consent form was obtained from the patients, prior their participation in this study.

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ORIGINAL ARTICLE

Parental Experience on Female Circumcision in East Coast of Malaysia

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ABSTRACT

INTRODUCTION: Female circumcision (FC) is a deep-rooted tradition in Malaysia, influenced by religious, cultural, and health beliefs. However, it has drawn criticism for its similarities to Female Genital Mutilation (FGM) in Africa, particularly during the 2018 Convention on the Elimination of All Forms of Discrimination against Women (CEDAW) by the United Nations Human Rights (UNHR). Amidst this backdrop, this study aimed to investigate parents' perspectives and experiences concerning female circumcision, as well as the factors driving its persistence. **MATERIALS AND METHODS:** This qualitative study used phenomenological approach and 20 in-depth interviews were conducted using a semi-structured questionnaire until data saturation were reached. We employed convenience and snowball sampling methods. The interviews were recorded, transcribed, and analyzed using NVivo 12 software. We used thematic analysis to identify key themes in the data. **RESULTS:** Three themes emerged from the interviews: (1) FC is a '*fitrah*', (2) maintaining good health is the main influence; and (3) FC causing no harm. The main influences on this practice are religious, cultural, and health factors. Parents described the procedure as simple and harmless for the baby and themselves, both physically and emotionally. **CONCLUSIONS:** This research shown that FC in Malaysia is not solely religious but deeply embedded in culture. The blend of cultural and religious aspects makes it a crucial practice for some.

Keywords
Female Circumcision, Religious Belief, Cultural Belief, Health Benefits, Harmless

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Received: 8th May 2024; Accepted: 16th July 2024

Doi: https://doi.org/10.31436/imjm.v23i04

INTRODUCTION

Female circumcision (FC) is a globally widespread practice and particularly common in regions such as Africa, the Middle East, and among Muslim communities in Southeast Asia.[1] Despite variations in methods and types across cultures, FC remains controversial. It is sometimes known as female *khitan*, derived from the Arabic term *"khitan,"* which signifies cutting and the subsequent healing process.[2]

There were several factors contributing to the persistence of the practice of FC with religion being a significant one, as in Malaysia. FC is deeply ingrained in the religious practice in those who follow the *Shafi'i* school of law in Malaysia, where it is considered obligatory to perform. However, it is not considered a sin for those who did not perform it.[3,4] There was also a hadith which emphasized that female circumcision in women should be done in modesty and just enough to make it look immaculate, not a whole removal of clitoris.

A Hadis narrated from Ummu 'Atiyyah al-Ansari stated that "*A woman in Madinah worked as a circumciser. Prophet p.b.u.h. said to her: Do not remove all. Truly, it will benefit the woman and make her loved more by the husband",* by Hasan: Dawud (5271).[5]

There were also other factors that were recognized as contributing factors for the persistent practice of FC which included controlling women's sexual pleasure and before they got married, traditions and cultural reasons, hygiene, pressure from the family and society, and the rite of passage to womanhood.[6,7]

The term FGM was introduced in 1990 by the Inter-African Committee on Traditional Practices Affecting the Health of Women and Children. In 1991, World Health Organization (WHO) recommended that the UN adopt this term.[8] By 2022, WHO defined FGM as procedures involving the partial or total removal of external female genitalia or other injuries for non-medical reasons. There are four main types: Type I, involves removing part or all of the clitoris. Type II, removes the clitoris, labia minora, and/or labia majora. Type III, narrows the vaginal opening and creates a sealed closure. Type IV includes other practices like pricking, piercing, or scraping.[9] Generally, in Malaysia, the procedures that have been practiced for years fall under WHO's type 1 or type 4.[10–12]

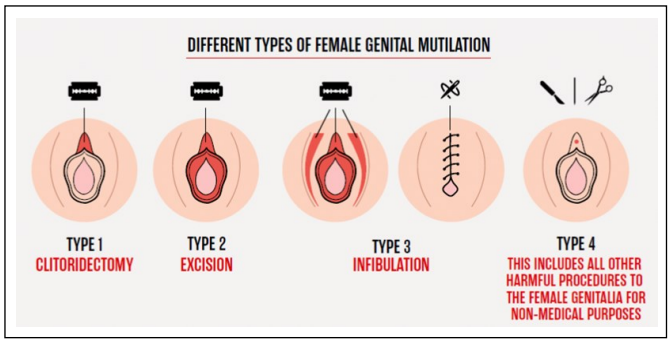

Figure 1. Different types of female genitalia mutilation.[13]

In Malaysia, there are divergent views on the practice of the FC. Some consider it unnecessary and advocate for its cessation,[6,14] while others support its continuation due to religious and cultural ties.[10,15] There is a dearth of research on the viewpoints of Malaysian parents who engage in female circumcision. Understanding their motivations and experiences is crucial given the cultural and religious significance attached to the practice. Majority of the existing studies on this topic concentrate on African countries, where the practice differs from that in Malaysia.

The aimed of this study was to explore parents' views on FC and the factors influencing their decisions. We utilized the Theory of Planned Behavior (TPB) to understand how people's likelihood of engaging in a behavior can be predicted based on their intention to do so, which will be explained further in our discussion. Ajzen explains that intentions regarding behaviors such as FC are shaped by attitudes, subjective norms, and perceived control.[16] Attitudes reflect personal feelings about the behavior, while subjective norms involve perceptions of others' opinions, such as family and community views. The theory helps to explain our findings.

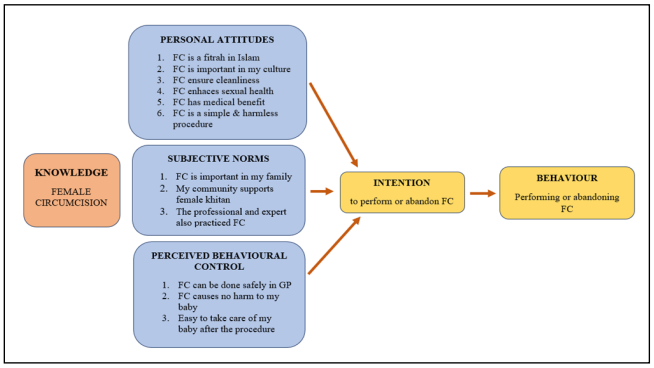

Figure 2. Factors that influence decision for performing a behaviour based on theory of planned behaviour (TPB)

MATERIALS AND METHODS

This qualitative study utilized the phenomenology approach to explore parents' perspectives on female circumcision. This study aimed to uncover how parents perceive and interpret the FC practice and how they react to it. The study was conducted in East Coast of Peninsular Malaysia and involved parents aged above 18 who had brought their daughters under 12 years old for circumcision and had prior experience with the practice. Purposive sampling including snowball sampling and referral from general practitioners' methods were employed, with sampling continuing until data saturation occurred. Saturation was reached after interviewing twenty participants.

WhatsApp Groups within the general practitioners (GPs) in the east coast of Peninsular Malaysia were utilized for study recruitment. Five GP clinics' agreed to participate as our key person, two from Kelantan, two clinics from Terengganu and one clinic from Pahang. After being briefed on the study, the GP had advertised the study posters' in their clinic and invited potential parents to participate. An interested parents signed consent forms to allow their contact numbers to be given to the main researcher. The main researcher then contacted participants, further explained the study, and scheduled face-to-face (F2F) interviews at agreed-upon locations. Prior to the

interviews, the study participants received a detailed explanation of the study, signed informed consent, and granted permission for audio recording.

A pilot study involving three parents was conducted in which the transcripts were reviewed by researcher with experienced in qualitative studies, to assess question clarity. The interview guide questionnaire was revised based on the feedback by the assigned researcher. The questionnaire had open-ended and probing questions to delve into participants' perspectives on FC and the factors influencing their decisions. Those who had undergone FC were questioned about any difficulties or complications they encountered post-procedure. Each parent interview lasted approximately 60 minutes.

All interviews were transcribed verbatim using NVivo 12 software. Thematic analysis was employed to identify key themes. Firstly, three researchers (N.H.S, R.Z, R.D.M) carefully read the first three transcripts to understand the parents' main ideas and perspectives. Secondly, the primary researcher (N.H.S) created an initial list of codes in NVivo and systematically coded the transcripts. Each of the transcripts was reviewed separately before being grouped into similar themes for an overall analysis and organized them into themes, subthemes, and connections. In order to ensure accuracy and consistency, all three researchers (R.Z, R.D.M, N.H.S) had reviewed all transcript codes, discussed on all disagreements, performed revision accordingly, and developed new preliminary themes as needed. Finally, the team reached consensus on the final codes, as well as the themes, subthemes, and their interconnections. In order to ensure confidentiality, all participants were given pseudo names.

As to maintain the study's rigor, all researchers discussed the findings in depth, including two experts in qualitative research (R.D.M and R.Z), to agree on the main themes. A master table of key themes was created to outline the themes' overall structure. The analysis aimed to find emerging themes and patterns from the participants' experiences, and contradictions, ambivalence, and paradoxes within and across different cases. In addition to that, the primary researcher (N.H.S) took field notes immediately after the interview throughout data collection and analysis to stay aware of her own perspective by observing the participants' verbal and non-verbal cues offered extra insights into the data gathered.

RESULTS

A total of twenty parents participated in the interview, in which eighteen were mothers and two were fathers. All study participants were aged between 20 to 50 years old. Nineteen of the participants had circumcised their baby and one participant had not circumcised her baby yet due to time constraint and planning to do it soon. Majority of the participants who had their babies circumcised by general practitioners in a clinic setting (n=13, 68.5%), whereas four had their babies circumcised by midwives and two had their babies' circumcised with both midwives and general practitioners (different babies). The Sociodemographic data are illustrated in Table I.

Table I: Sociodemographic Data

Variables	N (%)
Age	
20-30 years old	1 (5%)
30-40 years old	16 (80%)
40-50 years old	3 (15%)
Gender	
Male	2 (10%)
Female	18 (90%)
Localities	
Terengganu	9 (45%)
Kelantan	6 (30%)
Pahang	5 (25%)
Level of Education	
Primary school	0 (O%)
Secondary school	5 (25%)
Degree	11 (55%)
Master	4 (20%)
PHD	0 (0%)
Occupation	
Government	14 (70%)
Private sector	4 (20%)
Self employed	0 (0%)
Housewife	2 (10%)
Income	
< RM 1000	2 (10%)
RM 1000 - RM3500	1 (5%)
RM 3500 - RM5500	8 (40%)
> RM5500	9 (45%)
Are you daughter(s) circumcised?	
Yes	19 (95%)
No	1 (5%)
Where was your daughter(s) circumcised	
General practioner	13 (68.5%)
Midwives	4 (21%)
Both	2 (10.5%)

From the analysis, three primary themes were developed which include: (1) Female Circumcision (FC) is a Fitra, (2) Maintaining good health is the main influence; and (3) FC causing no harm.

Theme 1: Female circumcision (FC) is a Fitra

Almost all parents strongly believed in the strong relationship between FC and religious beliefs. 'Fitra' stems from the Arabic word, *fa ta ra,* and the verbal noun is '*fatrun'*.[17] Fitra refers to the natural disposition of human beings. In Islam, there is a belief that individuals are initially born with a fitra, which later influenced by the surrounding circumstances.[18]

There are several commands on circumcision stated in al-Quran and hadith as evidences. A hadith narrated by Abu Huraira stated that The Prophet Muhammad PBUH said, "Five practices are characteristics of the Fitra; circumcision, shaving the pubic region, clipping the nails and cutting the moustaches short", Sahih al-Bukhari (5439).[19] A hadith narrated by Abu Musa al-Asy'ari stated that The Prophet Muhammad PBUH said: "When a man is between four limbs of woman (having sex with woman) and the circumcised part meets the circumcised part, the bath is obligatory", Sahih Muslim (526).[20]

FC is a religious obligation

Parents hold the belief that FC is highly encourage in Islam and exemplifies religious perfection in Islam. *"Islam encourages this practice. So it is good for us and we need to do it. If we don't do it, it feels like something is missing and not complete in our fitrah", said Nurul, a 33-year-old, dental nurse.*

A participant with an Islamic education background mentioned specifically the knowledge that she obtained formally from her study on this matter. *"If we follow the Shafie' school of thought, female circumcision is considered mandatory (wajib). However, many people are unaware of this", said Ilani, a 40-year-old, Terengganu State Islamic Religious Affairs Department staff.*

Many parents strongly believe in the positive benefits of Islamic practices for the future, such as FC, even without clear scientific evidence. Faridah, a 35-year-old, housewife commented that *"It is in the Islamic teaching. So there is no argument, meaning it is good for us."*

FC is an assimilation of religious belief and culture

There were three parents who believed FC is influenced by religious and cultural factors. Ilani and Hamimah (36-years-old, medical officer) valued the interconnectedness of both these aspects and viewed the practice as a symbol of assimilation. Kamilah, 47-years-old, teacher shared her opinion and stated that *"For long time, khitan has been more to culture. It was only later that I learned it is encouraged in Islam as long as it is done in moderation and Rasulullah (Prophet) mentioned it too."*

There were also parents who believed FC is associated with obedience to parents, as good Malay demonstrate this in daily life. "Malay" refers to those practicing Islam, speaking Malay, and adhering to Malay customs, also known as *adat.*[17,20] *"When my mother asked me to do it, yes it was okay if I didn't do it but somehow I did it. I felt that if I didn't do it, it would be a sin for me. Yes, it is my responsibility for me as a daughter", commented Kamarina, a 37-year-old, master student.*

Theme 2: Maintaining good health is the main influence

Some parents mentioned cleanliness and sexual health as the main benefits of FC. Some parents have accepted FC to ensure their daughters' hygiene despite not clearly understanding the Islamic views of the practice, while others advocated for improvement of sexual health post-marriage.

Cleanliness and hygiene are integral components of Islamic teachings

The parents believed cleanliness are essential components of Islamic teachings, as FC symbolised purity and immaculacy as a Muslim. *"Especially about purity. There is a connection with cleanliness because Islam is pure right. So yes, there is a connection",* said Mastura, a 35-year-old, teacher.

Some parents believed that FC enhanced their children's confidence in worship. They saw it as a way to achieve purity, which improved their religious devotion and connection with the divine. *"Guys need to take care of their cleanliness, right? We have to do the same too to make sure*

we can pray properly, so both also need to be hygienic", commented Nani, a 33-year-old, housewife.

FC is good for sexual health

Many parents strongly believed in the cleanliness linked to FC improved marital intimacy, leading to a more satisfying sexual relationship. *"It is going to be clean... the relationship between husband and wife will be better", said* Hamimah, a 36-year-old, female doctor.

There was one male parent agreed with this opinion. *"If we don't do it, cleanliness will be affected, the quality of sexual intercourse will be bad, leading to unpleasant odours",* commented Nasrul, a 34-year- old, male nurse.

Many parents believed that FC promotes cleanliness, which they thought can prevent certain infections in the future. Liyana, a 42-years-old housewife, believed FC can preventsexual transmitted disease (STDs) later in life. Some participants argued that male and female circumcision are similar not just in terms of hygiene and health benefits but also from an Islamic legal standpoint (hukum). They believed both practices are justified by Islamic teachings, regardless of their differing outcomes. *"Just like boys, the obligation (for performing FC) is the same",* commented Faridah, a 35-year-old, housewife.

Theme 3: Female circumcision causes no harm

All parents shared that practicing FC is simple and has no negative effects on their babies or their own well-being. They felt assured and positive before, during, and after the procedure.

Female circumcision is a straightforward and harmless procedure

Some parents used midwives, while others chose a general practitioner clinic for FC. However, regardless of the setting, almost all parents found the procedure easy, short, and harmless. Nuha and Kamarina mentioned that their babies did not cry and had no noticeable changes.

Although some nervousness and anxiety were initially present, these feelings changed to calmness due to the simplicity of the procedure. *"I watched the procedure, it was so simple. My baby was okay. So my guilt had already disappeared",* said Wani, *35-year-old, dental office.*

Additionally, there was a sense of relief among the parents, as they believed they had fulfilled their responsibilities as parents and Muslims by carrying out this practice. "*Although this practice is only Sunna, I felt relieved because I had fulfilled my responsibilities as a mother and a Muslim",* said Zanariah, a 31-year-old, government officer.

A total of six parents shared their childhood circumcision experiences, describing them as smooth and trauma-free and influenced their decision to have their babies underwent the procedure. "During my time, *it was so simple. No bleeding and all. So I assume it is as simple as that",* commented Nafisah, a 34-year-old, businesswoman.

The babies were easy to take care of after the procedure

The parents managed their babies easily and did not notice any significant discomfort or complications. "*There was no problem for her to urinate or anything... everything was like normal",* said Anisah, a 39-year-old, female doctor.

The parents also reported no noticeable changes in circumcision location, stating that everything appeared normal and the procedure did not significantly alter their appearance or physical characteristics." *When I got home, I checked her first. Ha ha ha. Nothing has changed. I didn't even know where the procedure was done", commented Zanariah.*

DISCUSSION

This qualitative study delves into the meaning of female circumcision (FC) for parents, external factors that influence their beliefs regarding the procedure, and their experiences when carrying out FC on their babies. The findings yield several themes that elucidate their perspectives and comprehension of this practice. These themes are aligned with the TPB which proposes that behavior is predominantly influenced by the intention to engage in that behaviour and shaped by one's attitude toward the behavior, subjective norms, and perceived behavioral control.[22]

Attitude is a key factor in determining whether people choose to engage in certain behaviors or not. In this study, maintaining good health emerged as a major influence on parents' attitudes towards Fc. Parents with a positive attitude towards the practice are more likely to choose FC by believing that it promotes cleanliness and hygiene, which are important for good health. This viewpoint is reinforced by the findings of a previous study involving 605 participants, where hygiene emerged as the primary rationale for carrying out this practice, outweighing both health and religious considerations.[7]

Cleanliness is highly valued in the faith, allowing individuals to feel spiritually connected and prepared for acts of worship, known as ibadah. Muslims believe that maintaining hygiene is essential for prayers, known as solat, which is a fundamental part of Islam.[23,24] Furthermore, they attribute sexual health benefits to the practice, further solidifying their positive attitude toward it. Previous research has shown that FC can prevent the clitoris from becoming excessively enlarged during sexual intercourse, which can cause discomfort to the husband.[11] FC also helps to prevent accumulation of bacteria below the clitoral hood, which can cause a malodorous smell and poses risk of infection.[11,25]

Some parents also believe that FC offers similar health benefits to male circumcision. This assertion finds support in previous studies which explored the similarities in benefits between male and female circumcision, particularly related to improvements in sexual relations, cleanliness, and genital hygiene, concluding that circumcision is deemed necessary for both genders.[23,26] Parents' attitudes are shaped by their past experiences, whether personal or observed in others. Those who have had positive experiences with FC are more likely to feel confident and supportive of it especially among mothers and vice-versa.[27]

The perception of FC as religious obligation, or fitra, where the elements of attitude and subjective norms are overlapping is another theme explored in this study. Some parents expressed a strong conviction that FC is obligatory, citing the teachings of the Shafi'i school of law, which is widely followed by Muslims in Malaysia.[4] While the benefits of FC might not be as apparent as those of male circumcision, some parents believe that every practice endorsed in Islam has inherent benefits, even if they are not immediately obvious. This highlights their strong belief that FC reflects Islamic teachings, as supported by another study conducted in Malaysia.[28]

Most parents perceived FC as a blend of religious and Malay cultural practices. While some prioritize religion over culture, and vice versa, both factors were commonly cited together as influencing the decision to carry out this practice. This finding is similar to previous local study in which the findings suggested that the participants believed that culture and religious beliefs are an equal factor in performing FC.[28] Parents' opinions are crucial in the practice of FC, as they are considered important in Malay Muslim culture. Parents circumcised their babies based on their mother's instructions as they believed it would be sinful not to obey them. This practice is prevalent in Southeast Asia, where parents' encouragement is crucial for maintaining the tradition.[26]

In this study, some participants regarded FC as a ritual with a superstitious belief called *'pelepas'*, a Malay ritual to release bad omen from a person. This ritual is performed with a thought to ensure the baby's future of good health and behavior. The ritual practices in relation to FC in Malaysia varies from one state to another and the rituals are also differed between the midwives, also known as 'bidan'. Some parents present offerings like glutinous rice, betel leaves, and areca nuts, along with the fee on a plate.[12] However, some 'bidan' mentioned that there were no such rituals practiced.[29]

A perceived behavioral control, the third element in the TPB, refers to how easy or difficult it is for someone to carry out a behavior, influenced by both internal factors like personal ability and external factors like available resources. In our study, parents felt that FC didn't cause any physical or psychological harm. This finding is similar to another study where most female patients didn't see any complications with FC and would choose it for their daughters in the future.[3]

The parents also noted that their babies remained comfortable during and after the procedure. They feel confident in caring for their babies as usual, without any particular precautions or concerns about potential complications. This assertion is aligned with findings from a study conducted in South Sulawesi, where circumcised girls were able to resume regular activities the following day, suggesting that the procedure was minor in its impact.[10]

Some parents feel more comfortable continuing FC when it's done in a safe and controlled environment, like a clinic. They trust that medical professionals have the skills and tools needed for the procedure. Malaysia has become more urbanized, leading to better access to modern healthcare and education. As a result, more parents are choosing medical professionals to perform FC on their baby. This reflects a trend toward the medicalization of FC in Malaysia, as shown in studies conducted there.[7,28]

CONCLUSION

Female circumcision (FC) is beyond just a religious ritual. FC is deeply rooted in the culture and widely accepted by society, often seen as obligatory. The combination of cultural and religious aspects makes it a fundamental practice for many parents.

CONFLICT OF INTEREST

No conflict of interest declared.

INSTITUTIONAL REVIEW BOARD (ETHIC COMMITTEE)

This study is approved by ethical board committee of Universiti Sains Malaysia with the approval code of USM/JEPeM/22040268.

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ORIGINAL ARTICLE

The Effects of Psychological First Aid Training on Knowledge, Perceived Ability Using the Skill, and Quality of Life among Medical Students

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ABSTRACT

INTRODUCTION: The response to the crisis in Malaysia in recent years has led to a growing need for mental health services and a general dependence on mental health professionals to address the issue. It is widely acknowledged that providing psychological first aid (PFA) to survivors in the immediate aftermath of a crisis or disaster is an effective first step toward meeting their psychosocial needs. This study aims to determine the impact of PFA training on medical students' knowledge, perceived ability to apply skills, and quality of life. **MATERIALS AND METHODS:** 136 medical students were recruited for this non-randomized single group pre-post study, and trained using the World Health Organization (WHO) PFA guidebook. Measures include PFA knowledge, perceived ability to use PFA skills, WHO Quality of Life brief version (WHOQOL-BREF), and Helping Attitude Scale. Participants were required to fill in the questionnaire before and one month after the training. **RESULTS:** There were significant improvements in PFA knowledge (p<.001, Cohen's d = 0.60), perceived ability to use PFA skills (p<.001, Cohen's d = 0.80), psychological health (p<.001, Cohen's d = 1.52), and quality of life (p<.001, Cohen's d = 0.44). Brief training with various interactive teaching methods helps participants master the skills while paying attention to their emotional needs. **CONCLUSION:** Brief PFA training is as effective as a whole-day PFA workshop. However, regular training should be provided to increase the level of confidence of responders in dealing with crises.

Keywords
Psychological first-aid training, Medical students, Perceived ability, Quality of life

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Received: 15th January 2024; Accepted: 21st June 2024

Doi: https://doi.org/10.31436/imjm.v23i04

INTRODUCTION

A disaster can be defined as a significant interference with the normal functioning of a community or society as a result of hazardous events that interact with the conditions of exposure, susceptibility, and capacity.[1] This could lead to one or more of the following impacts: human, material, economic, and environmental losses. These impacts can be immediate and limited. However, the impacts are sometimes extensive and may persist longer than expected. The affected population may be unable to cope with their resources and, therefore, may need help from other external sources.[1] Although Malaysia is located in a geographically stable area and free of many natural disasters, it is vulnerable to several small-scale disasters such as haze, flooding, and landslides[2] and it is not uncommon for the most recent outbreak diseases such as the COVID-19 pandemic.

In Malaysia, disaster management has always been almost exclusively based on a centralised top-down government strategy and has adopted the 'loss reduction' model to mitigate natural disasters.[3] Typically, mental health professionals were referred to handle any psychological crisis. The fact is that mental health service providers are still lacking in Malaysia, with a population-to-psychologist ratio of 1.27 per 100,000 and approximately 400 clinical psychologists serving 33 million people.[4,5] More stakeholder participation is required to effectively mitigate the effects of a disaster. Enhancing their skills

and knowledge would enable prompt responses to disasters.[2] Given that the community is always the first responder to a disaster, empowering the communities through disaster risk reduction initiatives and promoting community resilience is essential.[3] Therefore, the public needs to be proactive in maintaining their mental health while equipping them with psychological first aid skills (PFA). This could help prevent overreliance on mental health professionals.[6]

American Psychiatric Association Committee on Civil Defense first adopted the term PFA during World War II in response to acute traumatised experiences in the year 1954.[7] The initial goal was to rapidly regain good function or, at the very least, stay comfortable until assistance could be provided. This concludes the PFA definition given by the National Child Traumatic Stress Network and National Center for PTSD as "*an evidence-informed modular approach for assisting people in the immediate aftermath of disaster and terrorism: to reduce initial distress and to foster short- and long-term adaptive functioning*".[8]

Over the years, despite many PFA models having been developed, the main principles generally emphasise the sense of safety, self and community efficacy, calmness, connectedness, and hope.[9] Among all, the World Health Organization outlined simple core actions of PFA: (1) Look – to observe those in urgent basic needs and emotional distress; (2) Listen–to focus on active listening, comforting and reassuring individuals, helping them express their feelings and concerns without the need to discuss the crisis event; (3) Link – to address the basic needs of individuals by identifying coping strategies, providing them with information and connecting them to relevant local social or professional services. (4) Know the limitations of PFA and recognise the need for professional care. It is important to understand that PFA is not a replacement for professional care and that those providing PFA need self-care too.[10,11]

Although PFA is widely recognized as a preferred post-disaster approach, there is limited evidence of its effectiveness.[12] Ethical concerns arise in studies involving vulnerable people who have just experienced a crisis.[13,14] Methodological limitations, long-term follow-up challenges, generalisability issues, and complex measurement of PFA, especially in mental health outcomes due to confounding factors and subjective assessments, limit the applicability of research results. Therefore, most studies that examined the effects of PFA training involved didactics, simulation, and role-playsand demonstrated improvements in participants' confidence and readiness to apply PFA as one of the mental health interventions after a crisis.[15-18] Most PFA training involved full-day training and helped improve providers' confidence in applying PFA.[19]

However, another pilot study observed by World Vision International after the Haiti earthquake in 2010 found that even two to three hours of briefer training was similarly beneficial to providers. They described the training as 'helpful, practical, and empowering', increasing their competence in handling survivors and motivating them to receive additional training.[20] Not only receivers, many believed that PFA training could help PFA providers become aware of their distress, thus reducing the burnout rate and practicing effective coping methods.[10,13] Knowing its effectiveness, Gispen & Wu[10] recommended that all healthcare workers should be able to practice PFA at their workplace. This approach ensures the provision of essential emotional support needed during mental health crises. Therefore, integrating PFA training into the co-curriculum activities of medical schools and nursing schools is imperative. Moreover, it should be accessible to lay people and non-mental health providers, ensuring widespread availability and knowledge dissemination.

In the present study, our objective was to examine the effects of PFA training by measuring the level of knowledge, perceived ability to apply PFA and quality of life among medical students. Ultimately, we hope to advise potential stakeholders such as universities, social welfare agencies, and non-governmental organizations to consider PFA training in their teaching modules for crisis response preparedness.

MATERIALS AND METHODS

Study Design and Participants

This is a non-randomized single-group pre-post interventional study that was conducted at a Malaysian public university, and participants were recruited via convenience sampling. An invitation mail was sent to 300 medical students randomly. One hundred and seventy-two students (response rate=57.3%) were agreed to participate in this study. However, only 136 university students (*mean age* =22.47, *SD*=1.19 years) were included in the final analysis (Table I) with an attrition rate of 20.9%.

Table I: Characteristics of the participants (N=136)

Variables	Frequency	%
Age	22.47 (1.19)*	
Male	24	25.0
Female	102	75.0
Malay	60	44.1
Chinese	28	20.6
Indian	5	3.7
Sarawak natives	37	27.2
Sabah natives	6	4.4
Islam	69	50.7
Christian	46	33.8
Buddhist	15	11.0
Hindu	4	2.9
Atheist	2	1.5

**Mean (SD)*

Measures

PFA knowledge

The PFA knowledge was assessed using 15 statements with yes or no answer options. The WHO working committee designed the questions that are readily available in Psychological First Aid: Facilitator's Manual for Orienting Field Workers.[21] The higher the score, the better the knowledge.

Perceived ability of PFA skill

A 12-item statement was used to measure the ability to support people who have experienced disasters or other extremely stressful events.[21] The respondents rated the items on a 5-point Likert Scale (1=Very Low, 5=Very High). The higher the score, the better perceived the ability to perform PFA skills. The internal consistency coefficient (Cronbach's α) was .89 for the pretest and .92 for the post-test.

Quality of life

The WHOQOL-BREF is a self-administered 26-item questionnaire that measures the general quality of life and general health (2 items) and other domains, including physical health (7 items), psychological (6 items), social relationships (3 items) and environment (8 items).[22] Each item was scored on a 5-point Likert scale to reflect the intensity, capacity, frequency, and evaluation of the past two weeks. Items 3, 4, and 26 require reverse scoring. The higher the score, the better the quality of life and the specific domain. The Cronbach's α were 0.92 and 0.94 for pretest and post-test, respectively.

Helping Attitude

A 20-item Helping Attitude Scale (HAS) was used to measure the beliefs, feelings, and willingness of the respondents to adopt prosocial attitudes that benefit others.[23] The respondent was expected to respond on a 5-point Likert scale ranging from 1 (strongly disagree) to 5 (strongly agree). A reverse score was done for items 1, 5, 8, 11, 18, and 19 before summing up the total score. A higher score reflects the higher levels of pro-social attitude. In the present study, Cronbach's alpha was .92 at pretest and .90 at post-test.

Procedures

The study was conducted between March 2021 and March 2023. The university students were invited to participate in the training via online registration. The participants were university students with no previous experience and voluntarily attended the PFA training. Participants were given a participant information sheet and were required to sign informed consent before the study. After an initial screening to review inclusion criteria, participants were asked to fill out questionnaires before training. All participants attended 4-hour PFA training provided by well-versed facilitators in WHO PFA modules. The modules were delivered as a didactic lecture by a psychiatrist, followed by role-plays in smaller groups guided by the facilitators, consisting of three counsellors and a clinical psychologist. After the role-play, the

facilitator provided constructive feedback to the participants. The participants were also asked to fill in the evaluation form at the end of the training. After a month, the participants were approached again to fill up the questionnaire (Figure 1). The participant repeated the questionnaire one month post-PFA training. Three consecutive emails were sent to remind the participants before considering the attrition rate (20.9%).

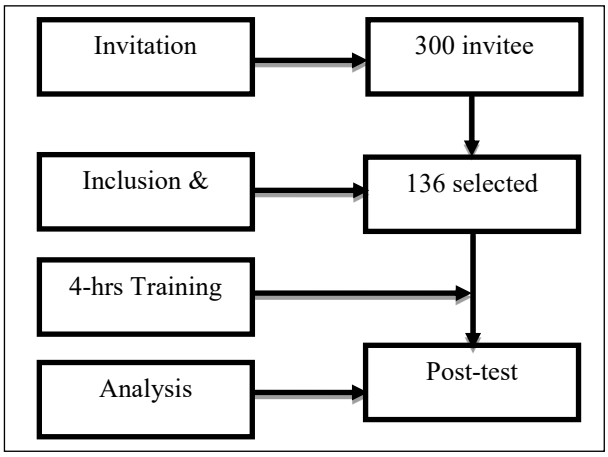

Figure 1. Study design and recruitment procedure

Data Analysis

IBM SPSS version 28 was used for data analysis.[24] A paired sample t-test was used to assess the impact of PFA training. This test compared the participants' knowledge, the perceived competency to apply the PFA skills, and the quality of life before and one month after training. Pearson's correlation method was used to determine the relationships between these variables. For a more comprehensive understanding of the results, practical significance was evaluated using Cohen's *d*, with established cut-off values to interpret the effect sizes.[25] The threshold for statistical significance was maintained at 5%.

RESULTS

Effect of the PFA training

A paired-sample *t*-test showed statistically significant improvements in knowledge, perceived ability using PFA skills, and psychological health domain of WHOQOL-BREF. The effect size was moderate for knowledge and small for overall quality of life, while it was large for perceived ability and psychological health (Table II).

Table II: Paired-sample test (Pre-Post) on each measure (N=136)

	Pretest Mean (SD)	Post-test Mean (SD)	*p-value*	*Cohen's d*
PFA knowledge	10.14 (1.69)	11.29 (2.03)	< .001**	.60
Perceived ability using PFA skill	27.26 (5.42)	32.17 (5.08)	< .001**	.80
WHOQOL- BREF				
Overall QOL	7.26 (1.56)	7.79 (1.21)	<.001**	.44
Physical Health	26.10 (4.09)	26.43 (4.10)	.263	.10
Psychological Health	17.12 (3.59)	20.97 (4.21)	<.001**	1.52
Social Health	7.42 (1.43)	7.39 (1.71)	.802	.02
Environmental Health	30.25 (4.21)	30.97 (4.50)	.022*	.20
HAS	82.38 (9.96)	82.72 (10.00)	.612	.04

Note. ** p<.05, **p<.001*
PFA=Psychological First Aid; WHOQOL-BREF= World Health Organization Quality of Life Brief Version; QOL= Quality of Life; HAS= Helping Attitude Scale, Cohen'd = Small (0.20), Moderate (0.50). Large (0.8).

The relationship between PFA knowledge, perceived ability to apply PFA skills and quality of life post PFA training

There was no significant relationship between PFA knowledge and other parameters in this study. The perceived ability to apply PFA skills has a positive correlation with all the domains of quality of life and helping attitude. All domains of quality of life were positively correlated with each other (Table III).

Table III: Correlation matrix of PFA Knowledge, Perceived Ability to Apply PFA Skills and Quality of Life

		1	2	3	4	5	6	7	8
1	**PFA Knowledge**	-							
2	**Perceived ability to apply PFA skills**	.086	-						
3	**Overall QOL**	-.120	.168	-					
4	**Physical health**	.054	.171*	.579**	-				
5	**Psychological health**	-.001	.248**	.713**	.749**	-			
6	**Social health**	.118	.241**	.463**	.609**	.689**	-		
7	**Environmental Health**	.155	.239**	.474**	.641**	.650**	.665**	-	
8	**HAS**	.104	.347**	.206*	.288**	.256**	.193*	.366**	-

Note. ** p<.05, **p<.001*
PFA=Psychological First Aid; WHOQOL-BREF= World Health Organization Quality of Life Brief Version; QOL= Quality of Life; HAS= Helping Attitude Scale

DISCUSSION

In general, the present training significantly improved the participants' PFA knowledge and the perceived ability to apply PFA skills. The results are consistent with other studies from several countries using different PFA training modules.[16,18] There was also a significant improvement in overall quality of life, especially psychological health. This is particularly useful, given that the training took place during the increased challenges of the post-COVID-19 mental health crisis. Most of the participants were able

to acquire the skills to effectively engage with those around them promptly using more appropriate strategies while simultaneously increasing self-efficacy in prioritizing self-care. Gispen and Wu[10] highlighted the tendency of healthcare workers to neglect their own emotional needs in their work lives. They believed that PFA training would facilitate self-awareness and be more sensitive to other colleagues' emotions, thus having an early intervention to reduce the rate of burnout. As such, integrating PFA training into the undergraduate curriculum could serve as a protective approach to address psychological distress, empowering them to actively support the emotional well-being of their peers effectively.

Participants' helping attitude remained consistently high following the PFA training despite no significant increase being observed. This dedication was believed to stem from their genuine desire to alleviate the suffering of others, motivating their voluntary participation in the current study. Studies indicate that PFA training has effectively enhanced PFA knowledge among non-mental health-trained personnel.[16,18] While many individuals may hesitate to offer support due to various obstacles, the participants in the present study were all medical students, inherently possessing strong pro-social tendencies. Instead of hindering them from responding to crises, awareness of the potential challenges while delivering PFA empowers them to persist in working with others' suffering. Their readiness to respond to crises was not hampered by their awareness of potential roadblocks when providing PFA; instead, it empowers them to continue working towards relieving others from suffering.

According to the previous study, the participants found it beneficial and were more likely to use PFA in responding to crises when equipped with the training. One key factor is that PFA prioritizes building genuine human connections, ensuring safety and accessibility rather than solely focusing on therapeutic outcomes in mental health. This is particularly useful for non-mental health professionals, relieving them of pressure to ensure positive psychological outcomes.[13,19] This allows them to focus on fostering meaningful connections and providing essential support in need. Furthermore, it is very time-consuming to train mental health professionals, while PFA responders can address various crises promptly and effectively on time.[6]

Most PFA training takes a full day or at least 6 hours. However, the present study applied the 4-hour training and found comparable effects in knowledge and self-efficacy in delivering PFA. Presumably, the effectiveness of current training may be affected by factors such as teaching methods, role-plays, constructive feedback, and a supportive environment. The easy-to-apply PFA concept facilitated the participants in acquiring knowledge and gaining confidence in applying it. This is consistent with previous qualitative studies that highlighted participants; preference for various teaching styles with shorter, regular training sessions over long ones.[26]

In light of the findings of the current study, we recommend increasing the accessibility of PFA training for people without mental health expertise and integrating it into the curriculum of medical and nursing schools. To provide high-quality PFA training in diverse populations, training more practitioners who can qualify as trainers is crucial. Ultimately, the researchers hope to establish a well-functioning community response structure capable of providing emergency crisis relief during disasters. Future research in this area could explore PFA training within communities or among people in remote areas.

To our understanding, the study represents the first study examining the effects of PFA training among university students in the region using WHO Psychological First Aid: Guide for Field Workers. Ethically, exploring the effects of PFA training on this demographic is essential, equipping university students with essential life skills to effectively respond to crises and fostering a campus community that is better prepared to respond to disasters and crises. Besides, the current training empowers them to support their peers emotionally and psychologically, promoting a healthier campus environment.

Limitations and Future Directions

This study has several limitations. Although the PFA concept is widely accepted, the WHO PFA model was not

rigorously researched on its module validity. Another limitation was the short follow-up period to examine the effects of training. It is recommended that participants attend revision courses regularly, especially in a setting with few disasters. Furthermore, there was no comparison of the control group in the study, which consisted of only one group intervention, which limited the depth of analysis. Conducting a comprehensive, randomized controlled trial with an extended follow-up period would provide a more in-depth understanding of the outcomes of PFA training.

CONCLUSION

Regardless of the severity of disasters, disasters can have an unpredictable impact on people's lives. Hence, it is important to prepare professionals and laypeople, enabling them to offer psychosocial support confidently without relying solely on mental health professionals. Regular brief revision training would help responders master the PFA skills, which would reduce the aftermath of a crisis. Furthermore, the use of these skills benefits the recipients and improves the psychological well-being of PFA providers, making them more aware of their own mental health. Therefore, it is worthwhile to encourage the university and other organisations to incorporate PFA into their regular training programmes.

FUNDING

The authors acknowledge Universiti Malaysia Sarawak [Small Grant Scheme, F05/SGS/2081/2021] for supporting this project.

INSTITUTIONAL REVIEW BOARD (ETHIC COMMITTEE)

The study was approved by the Medical Ethics of the Faculty of Medicine and Health Sciences, Univerisiti Malaysia Sarawak [FME/21/09].

ACKNOWLEDGEMENT

The authors express their gratitude to the World Health Organization (WHO) for the permission to reprint Psychological First Aid: Guide for Field Workers and use the WHOQOL-BREF questionnaire for research. We also thank Dr Nickell, who kindly permitted us to use the Helping Attitude Scale.

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ORIGINAL ARTICLE

Seroprevalence of Neonatal Herpes Simplex Virus Infection at A Tertiary Teaching Hospital in Malaysia

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ABSTRACT

INTRODUCTION: Neonatal herpes simplex virus (HSV) infection is generally infrequent, despite being a life-threatening illness. Knowledge of its prevalence is limited in Malaysia since most cases are asymptomatic infections and only limited routine neonatal HSV screening is conducted. This study therefore provides a comprehensive investigation of the seroprevalence of HSV-1 and HSV-2 in neonates. **MATERIALS AND METHODS:** Serological screening for HSV-1 IgG and HSV-2 IgG antibody tests using the Electrochemiluminescence assay was performed on serum samples of 215 neonates delivered from January until December 2022 at Hospital Universiti Sains Malaysia. **RESULTS:** Of the neonates, 54.4% were found to be HSV-1 positive, while 4.2% were HSV-2 positive. All the HSV-2 neonates were co-infected with HSV-1. Newborns aged 0–10 days were the most infected group by HSV-1 (92.3%) and HSV-2 (55.6%). The most reported clinical presentation was small gestational age (SGA) (60%). Microcephaly and macrocephaly were observed in one neonate each. The clinical presentations of reactive HSV-1 and HSV-2 cases revealed the presence of fever with rash in both cases. **CONCLUSION:** The high seroprevalence of HSV-1 is alarming. It is hoped that these data will support the advocacy of screening women for HSV before or during pregnancy as a precautionary approach to reducing the risk of vertical transmission.

Keywords
Neonatal HSV infection, HSV-1, HSV-2, Seroprevalence.

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Received: 18th January 2024; Accepted: 3rd July 2024

Doi: https://doi.org/10.31436/imjm.v23i04

INTRODUCTION

Herpes simplex virus (HSV) infection poses significant effects on the community. The virus has the capacity to induce a latent scenario within the sensory ganglia, persisting for the span of human longevity, and is capable of causing recurrent reactivation. In several instances, individuals infected with the disease may not display any noticeable symptoms, but those who do exhibit symptoms may present with painful blisters or sores in the afflicted region. HSV can be classified into two types: HSV-1 and HSV-2. These types differ in terms of their clinical characteristics, severity, genetic profile, primary mode of transmission, predominantly afflicted body site, seroprevalence, and reactivation rate.[1–5]

Both HSV-1 and HSV-2 demonstrate a high level of infectiousness in pregnant women, and it can be transmitted from the mother to the newborn through placenta or, more commonly, after vaginal delivery. This transmission leads to neonatal herpes, which causes higher rates of mortality.[6,7] Neonatal HSV infection is an infrequent occurrence but has serious effects for the neonate and is a potentially lethal illness.[6,8,9] The rate of neonatal HSV infection is determined by factors such as seroprevalence, birth rates, and infections during pregnancy. It is estimated that there are approximately 10 cases of neonatal HSV per 100,000 live births, with a yearly average of 14,000 cases.[10–12]

Neonatal HSV disease can show up in three different ways: i) as a localized infection affecting the mucous membranes, skin, or eyes; ii) as an infection mainly affecting the central nervous system; or iii) as a disseminated disease that may or may not involve the CNS.[3,13] The clinical presentations of this condition may include characteristic manifestations such as herpetic skin and mucosal lesions, as well as seizures. However, it is

important to note that symptoms can also be non-specific, encompassing fever, decreased fluid intake, lethargy, or even the absence of symptoms.[14,15]

Numerous diagnostic tests, including culture, molecular, and serological tests exist for detecting HSV infection, yet only a subset have been deemed useful and validated for use in newborns.[16] Serological diagnosis stands out as one of the most convenient and reliable tests available. At present, the direct fluorescent antibody assay (DFA) is a widely employed method in clinical virology laboratories for the direct detection of HSV in clinical specimens.[16] Moreover, serological tests are helpful given that antibodies specific to glycoproteins G-1 and G-2 can distinguish between HSV-1 and HSV-2, respectively.[3] It is worth noting, however, that negative serological testing in newborns does not entirely rule out HSV disease. Moreover, the presence of IgG in the neonate could be indicative of active or passive immunity from the mother.[16]

The available data on neonatal HSV infections in Malaysia is limited due to the fact that most cases are asymptomatic, and the absence of routine screening for HSV in neonates is not consistently implemented. Thus, the current study aims to fill this research gap by conducting a comprehensive investigation into the seroprevalence of HSV-1 and HSV-2 in suspected neonates. This study not only underlines the importance of conducting screenings for neonates with suspected HSV infection, but it also highlights the importance of screenings for pregnant women to minimise the risk of congenital transmission.

MATERIALS AND METHODS

Study Population

The present study consists of a total sample size of 215 neonates with clinically suspected cases of congenital HSV infections. The neonates were delivered at Hospital Universiti Sains Malaysia (HUSM) throughout the timeframe of January 2022 to December 2022. The neonates were divided into three groups; 0-10 days, 11-20 days and 21-28 days to see the frequency of seroprevalence and their correlation with clinical presentations.

Sampling Method and Laboratory Interpretation

Serum samples were obtained from the neonates and subjected to serological screening for HSV-1 IgG and HSV-2 IgG tests using an Electrochemiluminescence assay (ECLIA) (Roche, Germany) following the instructions given by the manufacturer. The outcomes of the samples were categorized as either 'reactive' or 'non-reactive' and were presented in the form of a cut-off index (signal sample/cutoff). Samples that have a cutoff index value of less than 0.6 in the Elecsys HSV-1 IgG and HSV-2 IgG assays are classified as 'non-reactive' and were interpreted as 'negative'. Samples that have a cutoff index falling within the range of greater than 0.6 and less than 1.0 are designated as 'Gray-zone'. In such cases, an additional sample is necessary to perform further testing. Samples exhibiting a cutoff index greater than 1.0 are classified as 'reactive'.

Selection Criteria

Newborns over 28 days of age were excluded. A total of 215 cases were analysed to investigate the serological profiles and then compared with the clinical data obtained from neonatal records.

Statistical Analysis

The data were gathered and examined using the statistical software SPSS, version 27.0. Descriptive statistics were used to analyse the data with the objective of determining the incidence of congenital HSV-1 and HSV-2 infections among newborns, as well as their relation to various clinical presentations, by using two types of statistical tests, namely, the Pearson chi-square test and Fisher's exact test. $p<0.05$ was considered significant.

RESULTS

Characteristics of the Specimen and Patients

In terms of the gender distribution, out of a total of 215 serum samples collected from neonates, 52% were female.

Regarding the age distribution, a significant proportion of individuals (89.3%) fell into the age range of 0–10 days.

Serological Detection of HSV-1 and HSV-2

The outcomes of the serological investigation revealed that 54.4% (n=117) of participants were reactive for HSV-1 antibodies, while 4.2% (n=9) for HSV-2 antibodies. All HSV-2 reactive samples were also found reactive to HSV-1, suggesting the presence of co-infection. In addition, it was observed that HSV-1 and HSV-2 reactivity were predominant among neonates aged 0–10 days, accounting for 92.3% (p=0.163) and 55.6% (p=0.002), respectively. Furthermore, the majority of newborns who were reactive for HSV-1 (59%, p=0.027) were females. Similarly, higher proportion of female neonates 55.6% (p=1.000) were reactive for HSV-2 (Table I).

Table I: Demographic data regarding reactive HSV-1 and HSV-2

Variables	Total (n)	HSV-1 reactive [n=117] n (%)	p value	HSV-2 reactive [n=9] n (%)	p value
Age (days)					
0-10	192	108 (92.3)	0.163[a]	5 (55.6)	0.002[b]
11-20	11	3 (2.6)		0 (0.0)	
21-28	12	6 (5.1)		4 (44.4)	
Gender			0.027[a]		1.000[b]
Female	112 (52%)	69 (59.0)		5 (55.6)	
Male	103 (48%)	48 (41.0)		4 (44.4)	

Note: [a] Pearson chi-square test, [b] Fisher's exact test

Clinical Presentations of Congenital HSV-1 and HSV-2 Neonatal Infections

The neonates suspected of having congenital HSV-1 and HSV-2 infections showed a predominance of certain clinical symptoms. The most frequently observed symptom was small for gestational age (SGA), which occurred in 60.0% (129/215) of cases. This was followed by neonatal jaundice (NNJ), which was present in 6.0% of cases. Additionally, sepsis and presumed sepsis were reported in 2.8% (6/215) of cases each (Table II). The clinical presentation of HSV-1 reactive neonates revealed that two neonates had fever with rash (2/2, p=0.502), whereas one presented with microcephaly, and another, with macrocephaly. Among neonates infected with HSV-1, a total of 72 out of 129 cases were found to have SGA, representing 55% of the sample. The results also indicated that two HSV-2 reactive neonates had fever with rash, with a statistically significant p-value of 0.002 (2 out of 2 cases). On the other hand, SGA was reported in 2.3% (3 out of 129 cases) of HSV-2 reactive neonates, with a p-value of 0.161, which was not statistically significant. These findings are summarized in Table II.

Table II: Clinical presentations of the neonates in relation to HSV status

Clinical symptoms n (%)	HSV-1 Non-reactive n (%)	Reactive n (%)	p-value	HSV-2 Non-reactive n (%)	Reactive n (%)	p-value
SGA 129 (60.0)	57(44.2)	72 (55.8)	0.615[a]	126 (97.7)	3 (2.3)	0.161[b]
Sepsis 6 (2.8)	5 (83.3)	1 (16.7)	0.095[b]	6 (100)	0 (0.0)	1.000[b]
Presumed Sepsis 6 (2.8)	4 (66.7)	2 (33.3)	0.415[b]	6 (100)	0 (0.0)	1.000[b]
NNJ 13 (6.0)	9 (69.2)	4 (30.8)	0.077[a]	13 (100)	0 (0.0)	1.000[b]
Premature 3 (1.4)	2 (66.7)	1 (33.3)	0.593[b]	3 (100)	0 (0.0)	1.000[b]
Fever with rash 2 (0.9)	0 (0.0)	2 (100)	0.502[b]	0 (0.0)	2 (100)	0.002[b]
IUGR 2 (0.9)	1 (50.0)	1 (50.0)	1.000[b]	2 (100)	0 (0.0)	1.000[b]
Microcephaly 1 (0.5)	0 (0.0)	1 (100	1.000[b]	1 (100)	0 (0.0)	1.000[b]
Macrocephaly 1 (0.5)	0 (0.0)	1 (100)	1.000[b]	1 (100)	0 (0.0)	1.000[b]

SGA: Small for gestational age, NNJ: Neonatal jaundice, IUGR: Intrauterine growth restriction
Note: [a] Pearson chi-square test, [b] Fisher's exact test

DISCUSSION

Neonatal herpes is an infrequent condition that impacts newborns, although it is classified as one of the most severe diseases acquired during pregnancy. Hence, it is imperative to include neonatal HSV infection in the list of potential diagnoses for every neonate presenting with an acute illness.[17,18] Performing seroprevalence studies on HSV-1 and HSV-2 is crucial to enhancing our understanding of neonatal herpes. This study aimed to analyse the seroprevalence of HSV-1 and HSV-2 in neonates, considering that there is limited knowledge on this issue, especially in the study area.

The present study revealed that 54% of neonates had HSV-1 IgG reactivity, whereas a mere 4.2% showed HSV-2 IgG reactivity and were co-infected with HSV-1. The significant variations in the seroprevalence rates of HSV-1 and HSV-2 have been based on factors such as age, gender, ethnicity, and geographical location. In several regions across the globe, the predominant cause of HSV infections is attributed to HSV-1.[19,20] The global prevalence of HSV-1 and HSV-2 infections is estimated at 67% and 11%, respectively.[21] According to a European study, the seroprevalence of HSV-1 in various countries was found to range between 52% and 84%. Additionally,

the seroprevalence of HSV-2 was between 4% and 24%.[22] In Asia, the seroprevalence of HSV-1 continues to be elevated, with a seropositivity rate of 50% among children and 75% among adults.[23] Meanwhile, the seroprevalence of HSV-2 is approximately 12% among the general population of this geographical area.[24] Various studies in Southeast Asia have shown the prevalence of HSV-1 in different populations. In Indonesia, the seroprevalence rate is 72.7%. In Thailand, it ranges from 61.1% to 92.9%, while in the Philippines, it is 82.5%. Rates ranging from 49% to 78% have also been reported in Singapore.[23] As for HSV-2, a combined seroprevalence of 21.2% was documented among different populations in Southeast Asia.[24]

The high prevalence of HSV-1 IgG indicates a high efficiency of transmission of HSV-1 from mother to infant, probably from primary infection or reactivation of genital HSV-1.[25,26] HSV-1 has emerged as the most prevalent viral agent associated with genital herpes, accounting for an important percentage (60%–80%) of genital herpetic infections in specific groups of young females [27,28]. It has the potential to cause severe newborn disease and can potentially result in an infection that is transmitted across the placenta.[29,30] The chance of newborn infection during delivery appears to be greater when HSV-1 is present in genital secretions than HSV-2.[25]

In this current study, a total of nine neonates (4.2%) who showed reactivity in HSV-2 IgG were also found to have reactivity in HSV-1 IgG. This finding suggests the presence of a co-infection involving both HSV-1 and HSV-2. The findings of our study are in line with Sauerbrei et al.'s investigation into the seroprevalence of HSV-1 and HSV-2 in Thuringia, Germany, between 1999 and 2006. Sauerbrei et al. observed that out of a total of 191 individuals who tested positive for HSV-2, 147 individuals (77.0%) were found to be co-infected with HSV-1.[1] Studies showed that the most common method used to differentiate between HSV-1 and HSV-2 infections in serological diagnostics is immunoassays or immunoblots that test for HSV type-specific IgG using type-specific glycoprotein G-1 (gG-1) from HSV-1 and gG-2 from HSV-2.[3] Recently, molecular techniques have become a feasible alternative to serological methods, providing several advantages, such as faster data collection, suitability for different clinical samples, and increased levels of sensitivity and specificity.[31]

Clinical presentation alone is not sufficient for a proper diagnosis due to the overlapping symptoms with various other illnesses.[32] In this current study, fever with rash was found in both reactive HSV-1 and HSV-2. Various viral and non-infectious disorders can lead to children presenting symptoms of fever with rash.[33] In the statistical analysis, none of the symptoms were related to HSV-1 or HSV-2 infection, except for fever with rash in HSV-2 infection. Therefore, it is recommended that future studies evaluate with a large sample size in order to further investigate the significance of the association.

Information regarding neonatal HSV in Malaysia is inadequate. A study was undertaken in 1976 by Tan et al.'s "TORCHES" program, which focused on congenital disorders, specifically in women of childbearing age. Using complement-fixing antibodies approach, up to 79% HSV infection was found in Malaysia.[34] Between 1961-1979, Tan and Stern conducted another serological study on the prevalence of CMV and HSV infections in Peninsular Malaysia. The study, conducted on a diverse group of people (aged 0-55 years) from different parts of Peninsular Malaysia, found that 954 of the 1554 people (61.4%) had HSV antibodies. These antibodies were also detected using complement-fixing antibodies.[35] Balasubramaniam et al. led a group of researchers in 1994. A total of 1688 infants, aged 0–4 months, presenting with congenital anomalies, underwent screening to detect the presence of congenital CMV infection and the rest of the TORCHES. The study, which also utilised the complement fixation test, indicated a 0% incidence of congenital HSV infection among infants in Malaysia.[36] In another study carried out by Hooi between January 1990 and December 1999, individuals presenting with a preliminary clinical diagnosis of mucocutaneous HSV infections in the oral and genital areas were examined. Out of the 504 specimens analysed, 18.0% showed positive results by direct immunofluorescence (IF) testing, while 55.0% tested positive through virus isolation.[37] However, the seroprevalence of HSV-1 and HSV-2 in newborns in Malaysia remains poorly understood due to

limited diagnostic virology facilities and a shortage of published studies. Consequently, the significance of these infections acquired during pregnancy, which can result in severe diseases in newborns, has not been adequately understood. Our study addresses this gap by reporting a substantial seroprevalence of HSV-1 and HSV-2 in newborns, highlighting the importance of screening pregnant women and suspected cases of neonates.

This study has certain limitations. Only serological method was used. The use of more advanced methodologies, such as polymerase chain reaction (PCR), in addition to serology, will help provide more robust result. Additionally, the duration of the data collection was limited. Also, a mother serum test was not conducted in order to define if the observed case of genital HSV was a result of primary infection or reactivation.

CONCLUSION

This study provides the first serological investigation that includes both HSV-1 IgG and HSV-2 IgG based approaches, as much as our current understanding allows. The ECLIA method revealed a high prevalence of HSV-1 IgG antibodies (54.4%) in neonates. Up to 4.2% of HSV-2 IgG antibodies were also observed in the neonates tested. It is suggested that future studies be conducted using techniques such as PCR to improve our understanding of active infections in neonates or passive immunity from the mother. Furthermore, the present study reveals the need for laboratory confirmation of HSV infection as clinical presentation alone is not sufficient for a proper diagnosis, especially due to the overlapping symptoms with other illnesses. It is hoped that these data will support the advocacy of screening of women before or during pregnancy as a preventive measure to reduce the risk of vertical transmission.

ACKNOWLEDGMENTS

We express our appreciation to the Department of Medical Microbiology and Parasitology and the School of Medical Sciences at Universiti Sains Malaysia for the provision of their facilities. Funding from Universiti Sains Malaysia under grant number 1001/PPSP/8012351 supported this research

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ORIGINAL ARTICLE

Prevalence of the Unmet Needs and its Predictors among Working Mothers of Children with Epilepsy Attending Specialist Hospitals in Kelantan

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ABSTRACT

INTRODUCTION: Unmet needs arise when everyday assistance is lacking. Epilepsy's unpredictability creates significant work-family conflict for working women, potentially leading to increased unmet needs. This study aimed to determine the prevalence and the factors related with the unmet needs of working mothers with children with epilepsy (CWE) in Kelantan. **MATERIALS AND METHODS:** A cross-sectional study using the validated Malay version of the Family Needs Survey questionnaire was conducted among 204 working mothers aged 18 to 60 of CWE attending specialist hospitals in Kelantan. Data were analysed using descriptive statistics and multiple linear regression. **RESULTS:** The highest unmet need was for community services (81.6%), followed by information (71.9%), social support (53.1%), financial (43.2%), explaining to others (28.9%), and family functioning (28.4%). Having follow-ups at tertiary hospitals reduced the unmet need score for information (B=-0.669, 95%CI=-1.276,0.081, p=0.021) and social support (B=-1.454, 95%CI=-2.225, -0.683, p=<0.001). Living in nuclear types of family reduced the unmet need score for community services (B=-0.669, 95%CI=-1.276,0.081, p=0.021) and information (B=-1.216, 95%CI=-1.965,-0.466, p=0.002). Working in the government sector increased the unmet need score for social support (B=0.437, 95% CI=0.062, 1.454, p=0.033). Full-time employment (B=-0.761, 95%CI=-1.244,-0.279, p=0.002) reduced the unmet need score for family functioning. Contract employment status (B=0.566, 95%CI=0.074,1.058, p=0.024) increased the unmet need score for family functioning. **CONCLUSION:** Mothers of CWE working in the government sector, under contract employment, and seeking follow-up care in secondary hospitals were related to higher unmet needs. The study findings may assist policymakers in introducing a child-friendly employment policy and flexible working arrangements among working parents of children with chronic illnesses such as epilepsy.

Keywords
Epilepsy, Unmet Needs, Working Mothers, Children.

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Received: 22nd January 2024; Accepted: 17th July 2024

Doi: https://doi.org/10.31436/imjm.v23i04

INTRODUCTION

Epilepsy is a neurological disorder characterized by recurrent, unprovoked seizures stemming from abnormal brain activity, with broad implications encompassing biological, psychological, and social dimensions.[1] It poses a significant global health challenge impacting millions worldwide, yet a considerable portion of these patients particularly in low- and middle-income countries, lacks access to effective treatment.[2,3] Children, notably during their first year, are susceptible to epilepsy, and while it profoundly affects them, research in this area remains limited.[4] Despite advances in understanding the disease and the availability of antiepileptic drugs, a substantial portion of patients, including children, remain untreated.[4,5]

The impact of epilepsy on children is significant; affecting their physical, cognitive and psychosocial development.[5] Children with epilepsy (CWE) often experience learning difficulties, social stigma and reduced quality of life.[6] The associated comorbidities and the long-term consequences of the condition further compound the global burden of epilepsy on children.[7]

In addition to the direct impact on children, epilepsy also places a significant burden on their caregivers. Families of CWE face numerous challenges, including financial strain, social isolation and emotional distress.[8–10] Caregivers often experience high levels of stress, anxiety, and depression, which can negatively impact their own health and well-being.[11] They have a range of unmet needs that can hinder their ability to provide optimal care for their children. These unmet needs are multifaceted, encompassing informational[12,13], emotional, financial [14], social [8–10], and family functioning aspects.[15]

Mothers, in particular, are often primarily responsible for caring for CWE. They are likelier to be the primary caregivers and face unique challenges in balancing their caregiving roles with other responsibilities, such as employment and household duties.[6,16,17] The demands of caring for a child with epilepsy can lead to increased stress, reduced work productivity, and strained family relationships.[18]

In Malaysia, despite improvements in epilepsy management and treatment, support for caregivers remains limited, with a lack of comprehensive support services such as counselling, respite care and educational resources. This gap in care is particularly challenging for working mothers. Various factors, such as lower socioeconomic status, lower educational levels, high seizure frequency, a high number of antiepileptic medications, having more sick children, being a single mother and employment characteristics, have been shown to impact caregivers' quality of life and contribute to unmet needs.[19–21]

In Malaysia, the increasing number of working mothers in recent years has further highlighted the challenges faced by those with caregiving responsibilities for CWE.[22] Working mothers often struggle to balance their professional and caregiving roles, leading to increased stress and burnout.[23,24] They may face difficulties finding appropriate childcare, managing work schedules, and accessing support services catering to their needs.[22,25] To our knowledge, although there were abundant research which focused on parents with chronic disease children, there is hardly any study among working mothers.

To address this issue, the first research study in Kelantan is being conducted to determine the prevalence and the factors associated with the unmet needs of working mothers with CWE. By understanding these mothers' unique challenges, the study aims to contribute actionable insights that may inform more inclusive and effective healthcare and social support, ultimately enhancing the quality of life for both CWE and their dedicated caregivers. While this study focuses on working mothers, it serves as a starting point for future research to explore the experiences of diverse caregivers and develop comprehensive support strategies that cater to the needs of all those involved in the care of CWE.

MATERIAL AND METHODS

Study Design and Participants

This cross-sectional study was conducted in Kelantan over four-month period from February until August 2023. The study participants were recruited from all government hospitals with specialists in Kelantan. The inclusion criteria were biological working mothers (18 to 60 years old) of CWE aged 18 years old and below registered at Pediatric Neurology Clinics in Kelantan. Working mothers who were diagnosed with chronic mental illnesses and non-Malaysians were excluded.

The sample size was calculated using a single proportion formula. The estimated sample needed was 219 participants using 5% type 1 error, 57.5% proportion, and an additional 10 % missing data.[13] There were 200 working mothers with CWE registered at the Pediatric Neurology Clinic in Kelantan. All eligible mothers were selected.

Research Tool and Data Collection

Data were collected using a self-administered proforma and the Malay version of the Family Needs Survey (FNS) questionnaire. The proforma consists of working mothers' sociodemographic characteristics, employment details, and children's characteristics. The FNS was initially developed by Donald B. Bailey and Rune J. Simeonsson to assess the needs of families with handicapped children.[46] Subsequently, the FNS was translated and validated in Malay version.[14] It consists of 35 items across

six subscales: information, social support, community services, explaining to others, financial support, and family functioning. Respondents who answered 0 (Not applicable), 1 (I definitely do not need help with this) or 2 (Not sure) were given a score '0' while respondents who answered 3 (I definitely need help with this) in the questionnaire were given score '1'. The minimum possible score is 0, the maximum possible score is 35. A higher score indicates higher unmet needs. Respondents rated each item from 0 to 3, indicating their need for help. Unmet needs were calculated by summing the items rated as '3'. Scores ranged from 0 to 35, with higher scores indicating more unmet needs.

Statistical Analysis

SPSS Statistics (IBM Corp. Released 2013. IBM SPSS Statistics for Windows, Version 24.0. Armonk, NY: IBM Corp) was used for data entry and analysis. The proportion of the unmet needs, the socio-demographic characteristics of working mothers and CWEs, and the employment characteristics were presented using mean, standard deviation (SD), frequency, and percentage. The predictors were analyzed using simple and multiple linear regression. Statistical significance was defined as a p-value <0.05.

RESULTS

Characteristics of Participants

Based on the study findings (Table I, Table II, Table III), most mothers were Malay (96.1%), married (97.5%), belonged to nuclear families (79.4%) and Muslims (96.1%). Most had attained higher education (93.2%) and had less than one child with chronic illness. Regarding employment characteristics, 76% of the mothers were engaged in full-time employment, and 63.7% held formal employment. Most worked at specific workplaces (71.6%) and had permanent job status (80.4%). About half of the mothers adhered to office hours (52.9%), and nearly half were employed in the government sector (48.5%). The CWE had a mean age of diagnosis of 3.05 (SD 2.84) years. Over a third of the children were registered with social welfare services (35.8%), and 15.7% had other comorbid conditions. On average, each child took 2.35 (SD 2.19) prescribed drugs and had a seizure frequency of 4.45(SD 15.86) times per month.

Operational Definition

The operational definition is as follows:

Working mother	Defined as a mother who engages in paid work, either full-time or part-time
Unmet needs	Needs or assistance from others to perform everyday activities is not provided or is inadequate. In this study, the unmet needs among working mothers of CWE were assessed using the Malay version of the FNS questionnaire and in-depth interviews. This questionnaire's scores range from 0 to 35; higher scores indicate higher unmet needs.
A child with epilepsy (CWE)	A child below the age of eighteen years diagnosed with epilepsy by a specialist
Full time	The total number of working hours is between 32 to 40 hours per week.[28]
Part-time	The total number of working hours is under 32 hours per
Formal worker	Formal working agreement between employers and employees. An employee usually: • Wear professional attire • Keep regular working hours • Work in the same place every day • Earn a regular salary or hourly wage
Informal worker	There is no formal working agreement between employers and employees. Employers may change their employees'
Permanent jobs	Persons whose main job is a work contract of unlimited duration or regular workers whose contract lasts 12 months
Contract jobs	Workers whose main job is a fixed-term contract lasting up to one year, occasional, casual, or seasonal work, or work
Government sector	All government-owned or government-affiliated organisations, including the federal government, states, and localities.
Private sector	They are owned and managed by private individuals or enterprises, taking risks to create jobs and generate a profit. They are competitive, and they have the incentive to be efficient.

Table I: Sociodemographic characteristics of working mothers of CWE, n= 204

Variables	Mean (SD)	Frequency (%)
Age (year)	38.68 (6.43)	
Distance hospital from house (km)	29.42 (42.05)	
Race		
Malay		196(96.1)
Non-Malay		8 (4.0)
Marital Status		
Married		199 (97.5)
Divorced		5 (2.5)
Family Type		
Mixed		42 (20.6)
Nuclear		162 (79.4)
Religion		
Islam		196 (96.1)
Other*		8 (4.0)
Number of children in house	2.9 (1.31)	
Education level		
Basic education		14(6.8)
Higher education		190 (93.2)
Chronic disease		
No		187 (91.7)
Yes		17 (8.3)
Own transport		
Yes		178 (87.3)
No		26 (12.7)
Number of children that has chronic disease (Excluding Patient)	0.15 (0.39)	

Table II: Employment characteristics of working mothers of CWE, n= 204

Variables	Mean (SD)	Frequency (%)
Types of employment		
Full time		155 (76.0)
Part-time		49 (24.0)
Work Formality		
Formal		130 (63.7)
Informal		74 (36.3)
Location of works		
Specific workplace		146 (71.6)
Work from home		58 (28.4)
Job status		
Permanent		164 (80.4)
Contract		40 (19.6)
Salary		
Monthly		145 (71.1)
Non-monthly		59 (28.9)
Employment Schedule		
Office Hour		108 (52.9)
Non-office hour		96 (47.4)
Work sector		
Government		99 (48.5)
Non-Government		105 (51.5)

Table III: Sociodemographic characteristics of CWE, n= 204

Variables	Mean (SD)	Frequency (%)
Age (year)	7.38 (4.34)	
Gender		
Male		117 (57.4)
Female		87 (42.6)
Age of diagnosis (year)	3.05 (2.84)	
Registered with JKM		
Yes		73 (35.8)
No		129 (63.2)
Other Comorbid		
Yes		32 (15.7)
No		172 (84.3)
Number of drugs	2.35 (2.19)	
Seizure frequency	4.45 (15.86)	
Hospital follow-up		
Tertiary centre		148 (72.5)
Secondary centre		56 (27.5)
Experienced adverse drug reaction		
No		172 (84.3)
Yes		33 (16.2)

The Prevalence of the Unmet Needs

The Family Needs Survey questionnaire comprised of six domains. The highest prevalence of unmet needs was observed in the Community Services domain (81.6%), followed by information domain (71.9%), social support domain (53.1%), financial domain (43.2%), and explaining to others domain (28.9%). The lowest unmet need was observed for the family functioning domain (28.4%). Result is shown in Figure 1.

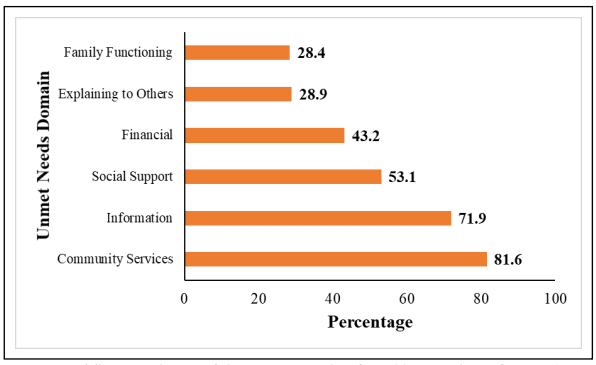

Figure 1. The prevalence of the unmet needs of working mother of CWE (n= 204)

Factors Related to the Unmet Needs among Working Mothers of CWE

Multiple regression analysis revealed several factors significantly related to the unmet needs scores across domains for working mothers of children with epilepsy (CWE). Mothers whose children received tertiary hospital follow-up and those from nuclear families had lower unmet information needs while having children with comorbidities and maternal illness increased information needs. Having follow-ups at tertiary hospitals reduces, while working in the government sector increases, the unmet social support needs. Nuclear family status lowered unmet community service needs. Having children with comorbidities increases unmet financial support needs. Full-time work reduces, while contract work and having children with more frequent seizures increase unmet family functioning needs. Greater distance from home to the hospital, having children with comorbidities, and having children with more frequent seizures increased unmet family functioning needs. Greater distance from home to the hospital, having children with comorbidities, and having male children increased unmet needs in explaining the condition to others.

DISCUSSION

The prevalence of Unmet Needs among Working Mothers of CWE

The Family Needs Survey (FNS) questionnaire provides valuable insights into the challenges faced by working mothers of children with epilepsy (CWE) highlighting diverse challenges across multiple domains. Addressing these unmet needs is a critical issue that requires attention. There is a scarcity of a global research specifically focused on this topic.

Our study showed the highest prevalence of unmet needs was on community services. The findings revealed that 86.8% of mothers struggled to obtain appropriate care for their CWE, while 83.3% encountered difficulties locating a suitable preschool. This indicates a shortage of specialized healthcare services or a lack of awareness about existing

Table IV: Factors related with the unmet needs among working mothers of CWE in Kelantan by simple and multiple linear regression (n = 204).

Variables	Crude b[a] (95% CI)	p-Value	Adjusted b[b] (95% CI)	*t*-Stat	p-Value	R^2
UNMET NEEDS FOR INFORMATION						
Types of hospital follow-up						
Secondary	1		1			
Tertiary	-1.405 (-2.120, 0.690)	<0.001	-0.883 (-1.599, -0.167)	-2.432	0.016	
Child's Comorbid						
No	1		1			
Yes	1.664 (0.785, 2.544)	<0.001	1.459 (0.609, 2.309)	3.386	0.001	0.211
Type of Family						
Mixed	1		1			
Nuclear	-1.036 (-1.841, 0.231)	0.012	-1.216 (-1.965, -0.466)	-3.197	0.002	
Mother's Illness						
No	1		1			
Yes	1.695 (0.522, 2.868)	0.005	1.901 (0.745, 3.057)	3.244	0.001	
UNMET NEEDS FOR SOCIAL SUPPORT						
Types of hospital follow-up						
Secondary	1		1			
Tertiary	-1.600 (-2.377, 0.823)	<0.001	-1.454 (-2.225, -0.683)	-3.723	<0.001	
Work's Institution						0.116
Non-Government	1		1			
Government	0.437 (-0.282, 1.156)	0.233	0.758 (0.062, 1.454)	2.148	0.033	
UNMET NEEDS FOR COMMUNITY SERVICES						
Types of family						
Mixed	1		1			
Nuclear	-0.669 (-1.257, 0.081)	0.026	-0.690 (-1.276, -0.104)	-2.323	0.021	0.038
UNMET NEEDS FOR FINANCIAL						
Child Comorbid						
No	1		1			
Yes	1.076 (0.323, 1.828)	0.005	1.073 (0.318, 1.828)	2.803	0.006	0.038
UNMET NEEDS OF FAMILY FUNCTIONING						
Types of employment						
Part-time	1		1			
Full time	0.208 (-0.272, 0.687)	0.394	-0.761 (-1.244, -0.279)	-3.111	0.002	
Job-status						0.106
Permanent	1		1		0.024	
Contract	0.545 (0.033, 1.056)	0.037	0.566 (0.074, 1.058)	2.269		
Seizure Frequency	0.018 (0.006, 0.031)	0.005	0.019 (0.007, 0.032)	3.114	0.002	
UNMET NEEDS EXPLAINING TO OTHERS						
Distance house to the hospital	0.008 (0.002, 0.014)	0.010	0.008 (0.002, 0.013)	2.607	0.010	
Child Comorbid						
No	1		1			0.102
Yes	0.766 (0.024, 1.509)	0.022	1.439 (0.562, 2.316)	3.234	0.001	
Gender						
Girl	1		1			
Boy	0.577 (0.070, 1.085)	0.026	0.614 (0.124, 1.104)	2.469	0.014	

[a]Simple linear regression
[b]Multiple linear regression
Stepwise/backward/forward multiple linear regression is applied; model assumptions are fulfilled; no interactions among independent variables are observed, and no multicollinearity is detected.

resources. This is particularly concerning, as CWE often requires ongoing medical management, including regular check-ups, medication adjustments, and monitoring for potential side effects or complications.[32] Without access to appropriate care, these children may be at risk of suboptimal seizure control, developmental delays and reduced quality of life.[33] However, our research highlights the importance of addressing these challenges and the need for supportive community services for working mothers with caregiving responsibilities, particularly those caring for CWE.[9] In Malaysia, the Facebook Malaysian Society of Epilepsy is a support group that helped to spread awareness about the illness and provide a platform for parents and children to share experiences and learn from each other.[34] In Malaysia however, no specific community services are available for working mothers. Previous research by Berger & Font (2015) demonstrated the positive impact of community services on the well-

being of families with children with chronic conditions, emphasizing the need for tailored interventions to bridge this gap.[35] Additionally, in the United States, community organizations such as the Epilepsy Foundation and the National Association of Epilepsy Centers offer various support programs and resources for individuals and families affected by epilepsy, which can be beneficial for addressing these needs.[36]

The prevalence of unmet information needs was 71.9%, with the most pressing issues being lack of information about their child's condition or disability (82.4%) and insufficient knowledge about the child's growth and development (80.9%) which reflected inadequate information. The information needs of parents of children with epilepsy is a critical aspect of caregiving. Several studies have shed light on the specific requirements and gaps in knowledge that parents experience. A systematic review study conducted by Nevin et al (2020) reported limited availability of detailed information and the need for clear realistic information.[37] Therefore, these caregiving parents had elevated stress levels, diminished psychosocial well-being, and decreased satisfaction with healthcare services. It was supported by the findings reported by Jain et al (2018) who emphasized the pivotal role of accessible information in empowering parents of CWE. Ali Maher Nashaat et al (2022) demonstrated the effectiveness of intervention programs in improving mothers' knowledge, attitude, and practice of epilepsy, directly addressing the unmet information needs and challenges faced by these mothers. This result emphasized the necessity of developing targeted informational resources to address the specific concerns of working mothers with CWE, aligning with recommendations from the *Epilepsy Foundation* (2015).

The prevalence of unmet needs of social support at 53.1% echoes the findings of a meta-analysis by Jones et al (2019), illustrating the pervasive challenges in establishing a supportive network for families affected by epilepsy.[41] The most pressing issues identified were the lack of time to communicate with the child's teacher or therapist (69.1%) and the absence of a family member to discuss problems with (63.2%). These results highlight the importance of addressing the social support needs of these mothers to promote better outcomes for both the child and the family. In Malaysia, the Malaysian Epilepsy Society works to improve the lives of individuals with epilepsy. They bring together medical professionals, caregivers and patients, including those with epilepsy, family members, doctors, nurses, healthcare workers, researchers and volunteers. Meetings provide a space to share experiences, learn about epilepsy and raise public awareness. The goal is to inspire everyone to collaborate for the betterment of people with epilepsy.[42] Additionally, the study revealed that family resilience and social support play mediating roles in the relationship between illness severity and depressive symptoms among primary caregivers of children with epilepsy, highlighting their significance in mitigating the negative impact of epilepsy severity on caregivers' mental health.[43]

The prevalence of unmet needs in financial support (43.2%) and explaining to others (28.9%) is consistent with the studies on the economic burden and societal misconceptions surrounding epilepsy.[47,48] In the financial domain, the primary areas of concern were paying for essential expenses such as food (59.8%) and accessing more counseling or assistance in securing employment (46.1%). A meta-synthesis of qualitative research studies highlighted the economic challenges caregivers face, including the need to be available to monitor the child, interrupting their career development and increased economic burden due to long-term care and medication needs.[44] Additionally, studies have highlighted the association between financial burden and maternal stress, anxiety and depressive symptoms.[45,46] Furthermore, the financial burden experienced by mothers of CWE has been linked to lower quality of life and increased parenting stress.[18] Currently, in Malaysia, a diagnosis of epilepsy alone does not guarantee eligibility for welfare assistance despite the significant financial and caregiving burden associated with the condition. This issue is not unique to epilepsy, as a large number of parents of children with various disabilities have reported similar challenges in obtaining necessary support.[47] This underscores the critical need for financial support to alleviate the economic strain on these mothers and improve their overall well-being.

Apart from financial assistance, CWE mothers also face difficulties articulating their child's condition to others. The decision to disclose a child's epilepsy diagnosis can be a source of stress and anxiety for parents, and they may face difficulties in navigating the disclosure decision-making process.[48] Furthermore, community perceptions of developmental and behavioral problems experienced by children with epilepsy can contribute to the stigma and challenges faced by mothers in explaining their child's condition to others.[49] This highlights the need for support and resources to help mothers effectively communicate and educate others about epilepsy, thereby reducing stigma and promoting understanding and acceptance.

The reported unmet need in family functioning is noteworthy, highlighting the broader impact on the family unit. This domain is a significant concern, as indicated by several studies. A study by Reilly et al (2018) found that mothers of children with epilepsy experience unmet expectations of the child, feel rejected by the child, or have not adequately bonded with the child, indicating specific areas of family functioning that are affected.[50] Other studies also emphasized the higher prevalence of disruption of family functioning among mothers of children with autism spectrum disorder (ASD), which is consistent with the challenges faced by mothers of children with epilepsy.[51] Additionally, stress levels are often high in caregivers of patients with chronic diseases such as epilepsy, leading to lower parent-child relationship quality and problems with family functioning.[51] Therefore, clinicians should be aware of the impact of epilepsy on maternal psychosocial outcomes and family life.[52] Family-centered care interventions should be considered in addressing these challenges and promoting resilience within the family structure.

Factors Related with the Unmet Needs among Working Mother of CWE

We found that working mothers who belonged to a nuclear type of family had lesser unmet needs as compared to those who belonged to an extended type of family. This finding showed the impact of extended family structure on communication patterns and decision-making dynamics. Cultural norms and stigma may hinder open discussion of the child's epilepsy within extended families. Mothers may face challenges accessing information due to larger family involvement and unclear role expectations.[34]

Our study also showed that having follow-ups at tertiary hospitals reduced the unmet need for information and social support. Specialized epilepsy clinics in tertiary hospitals provide comprehensive care from multidisciplinary teams, contributing to improved patient outcomes compared to secondary hospitals which have limited expertise. However, disparities exist globally regarding access to specialized care.[35] Communication gaps at secondary hospitals may contribute to unmet informational needs for parents.[36,37]

In our study, the presence of comorbidities in children with epilepsy was found to increase mothers' unmet need for information, explaining to others and financial costs. Managing comorbid health conditions in children with epilepsy places significant demands on parents. Factors such as treatment complexity, time constraints, financial costs, limited access to information and social support can heighten unmet needs for working mothers.[38,39]

Our study revealed that mothers working in government sector had higher unmet need for social support as compared to those working in non-government sector. Government work environments may have different policies, structures and levels of flexibility when compared to the private sectors. Public sector jobs may have more inflexible schedules and limited options for remote work, making it difficult for mothers to balance work and caregiving responsibilities.[40] This is further complicated by the unique considerations in diagnosing, working up, and managing pediatric epilepsy. Resource constraints in government sectors also limit family-friendly policies and caregiver support programs.[40] Research indicates that workplace flexibility, including working from home and part-time employment, can positively impact parent-child interactions, especially for mothers.[49]

Being a contract worker intensified the unmet need of the working mothers as reported in our study. This finding could be related to job insecurity. Job insecurity is

recognized as a critical factor influencing the well-being of employees, especially in the context of caregiving responsibilities. Contract employment may elevate anxiety about employment stability for these mothers, as highlighted by Picolli et al (2021) leading to increased stress and anxiety.[41] Anxiety due to lack of job security experienced by working mothers with caregiving responsibilities may have significant implications on their family life. Research has shown that mothers who were the primary caregivers experienced increased stress and anxiety about the stability of their employment, which can impact their overall well-being and family dynamics.[42] Overall, contract employment can contribute to job insecurity, limited benefits, work schedule inflexibility and family planning uncertainty for working mothers. This can exacerbate challenges in balancing caregiving and work demands.[43]

Our research found that mothers who had a male child diagnosed with epilepsy had higher unmet need for explaining to others as compared to those having a female child. There were societal stereotypes or misconceptions about epilepsy, and these was found to be more pronounced when the affected individual is a boy. Research has shown that societal stereotypes and misconceptions about epilepsy can lead to stigma and discrimination, impacting the well-being of individuals with epilepsy and their families.[45] Lack of understanding and misconceptions about epilepsy can contribute to social isolation and a sense of exclusion, particularly for families dealing with the condition. Therefore, addressing these stereotypes and misconceptions through increased awareness and education is essential to support families dealing with epilepsy and to promote understanding and empathy within the community.

CONCLUSION

In conclusion, our study findings underscore the pressing need for a holistic and multidimensional approach to support working mothers of CWE. Community services, targeted information dissemination, social support programs, financial interventions, and awareness campaigns emerge as key components of an effective strategy. Policymakers and healthcare providers may formulate comprehensive strategies to address the unique needs of this vulnerable population by integrating our study findings into evidence-based frameworks and interventions documented in existing literatures. Moreover, further research and initiatives are needed to better understand and meet the specific community service needs of this population.

INSTITUTIONAL REVIEW BOARD (ETHIC COMMITTEE)

Ethics approval was obtained from the Medical Review and Ethical Committee from National Institute of Health, Ministry of Health Malaysia [NMRR ID-23-00180-QCH (IIR)] and Research and Ethics Committee, Universiti Sains Malaysia [USM/JEPeM/ KK/23010067].

ACKNOWLEDGEMENT

We wish to extend our appreciation to the Tabung Insentif Pembangunan Pengajian Siswazah (TIPPS) for their generous research support. Additionally, we are grateful to the School of Medical Sciences, Universiti Sains Malaysia (USM), for covering the publication fees associated with this work. Without their support, this research would not have been possible.

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ORIGINAL ARTICLE

Role of Microstructural White Matter Changes of Somatosensory Cortex in Stress Among Non-Clinical Population: A Diffusion Tensor Imaging Study

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ABSTRACT

INTRODUCTION: Stress is a common response by people to stressors or potential threats, resulting in physical, affective, and cognitive changes. Emotions are associated with interpretations of physiological changes, and the processing of emotions is largely dependent on the somatosensory cortex which includes the postcentral gyrus. The objective of this study was to examine the correlation between stress and alterations in the microstructure of white matter in the somatosensory cortex among healthy non-clincal population. **MATERIALS AND METHODS:** A total of 30 participants were recruited. The participants were administered the Depression, Anxiety, and Stress Scale 21 (DASS-21) questionnaire. All subjects underwent Magnetic Resonance Imaging (MRI) brain scanning, with diffusion tensor imaging (DTI) used to assess white matter integrity. The association between stress scores in DASS-21 and DTI parameters was analyzed. **RESULTS:** A significant negative relationship was observed between stress scores and fractional anisotropy (FA) values in the left postcentral gyrus (r=-0.393, p=0.032), suggesting that stress has an early detrimental effect in this region, while no significant correlation was found in the right postcentral gyrus (r=-0.300, p=0.107). **CONCLUSION:** The findings of our study indicate that stress may lead to early impairments in the microstructural somatosensory cortex, particularly in the left postcentral gyrus. These alterations were observed using DTI technique. Hence, the alterations in the microstructure of white matter in the brain prior to the onset of the disorder may play a vital role and could serve as a new and promising biomarker for the early identification and treatment of the disease in the non-clinical population.

Keywords
Stress, DTI, Somatosensory, White Matter

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Received: 23rd April 2024; Accepted: 22nd June 2024

Doi: https://doi.org/10.31436/imjm.v23i04

INTRODUCTION

Stress is a negative psychological condition triggered by stressors or potential dangers in humans. It is a common response that involves changes to one's physical, emotional, and mental functions.[1] Stress is typically accompanied with physical symptoms. Indeed, nearly every people may encounter episodes of stress at some stage in their lives, and it has been indicated that sub-threshold stress and anxiety symptoms are commonly observed in the general population.[2] Prior research has predominantly examined the cognitive, emotional, and neurological dimensions of individuals experiencing anxiety and stress, neglecting the influence of their somatic brain networks.[3] It was suggested that emotional processing plays a crucial role in regulating behaviour, particularly decision-making.[4] All emotions are based on the body's homeostatic interpretations of changes in its condition.[5] It has been suggested that interoceptive memories and visceral emotional experiences serve as substrates for decision-making. The postcentral gyrus, which is part of the somatosensory cortex, plays a vital role in emotional processing and is often activated by physical sensations.[6]

The heightened neuronal activity in the postcentral gyrus is mostly associated with the impacts of social stress.[7] It has been demonstrated that the postcentral gyrus can be used to predict treatment responses during the analysis of pleasant facial expressions.[8] An enhanced connection between the postcentral gyrus and the amygdala indicates a heightened capacity to control emotions, while the association between the postcentral gyrus and the anterior cingulate cortex during rest is linked to negative emotional consequences.[9] Research indicates that the prefrontal cortex and limbic system have significant roles in chronic stress. However, it is suggested that more emphasis should be given to the participation of somatosensory brain regions, specifically the postcentral gyrus.[10] Therefore, it is crucial to determine how stress conditions underlie the post-central gyrus's effects.

Magnetic Resonance Imaging (MRI)- based diffusion tensor imaging (DTI) capability to assess the Fractional Anisotropy (FA) values of white matter tracts offers a promising avenue to explore how stress affects the brain's networks. FA values, indicative of the directional coherence of water diffusion in tissue, serve as a marker for white matter integrity. Reductions in FA are often interpreted as a sign of decreased white matter organization, potentially reflecting damage or alterations in neural pathways critical for emotional processing and cognitive function.[11,12,13] Increased activity was observed in the brain regions of the thalamus, para-hippocampal gyrus, middle frontal gyrus, and inferior temporal gyrus in the group with the mental disorder.[11] It was demonstrated notable alterations in the microstructure of cerebral cortical regions linked to mood disorders.[12] The rapid growth and subsequent reduction of dendrites and synaptic structures in certain groups of cortical neurons during specific sensory learning experiences may be responsible for the underlying microstructural alterations.[13] Despite the extensive research on the changes in brain function and structure associated with stress and stress-related disorders, there have been few studies that specifically focus on a healthy or non-clinical population. This group refers to individuals who do not have any known mental health conditions or diseases and are not currently seeking or receiving medical treatment. We hypothesized that the changes in brain function and structural integrity of the brain's white matter that precede the onset of a disorder may be crucial for the early identification and management of the illness in individuals who are not yet clinically diagnosed. The aim of this study was to investigate the relationship between early mental psychopathology and changes in the structural integrity of white matter in the brains of healthy non-clinical persons. Based on the functions of the postcentral gyrus and the somatic marker hypothesis, we hypothesised that changes in the microstructure of the white matter in the somatosensory cortex, notably the postcentral gyrus, would be linked to the level of stress.

MATERIALS AND METHODS

Participants

Thirty non-clinical healthy subjects were enlisted in total, consisting of 21 males and 9 females with a mean age of 40.83 years (range: 27–57 years). Participants were assessed for handedness, resulting in 25 right-handed and 5 left-handed individuals. Two qualified psychiatrists distributed the Depression, Anxiety, and Stress Scale 21 (DASS-21) questionnaire to the subjects. The methods were performed in compliance with approved protocols. Individuals who exhibited any movement during the scan, had neurological problems, severe psychiatric conditions, head injuries, or were pregnant or lactating were excluded from the study. Prior to the study, all participants provided written informed consent, which was approved by the Research Ethics Committee (ID -2022-047).

Instruments

The DASS-21 is a self-administered instrument designed primarily to evaluate the existence of negative emotional states, such as depression, anxiety, and stress. The tool consists of 21 items that measure levels of depression, anxiety, and stress using a Likert scale that ranges from 0 to 3. A score of 0 signifies that the state did not affect the individual in any way. A score of 1 suggests that it had some influence or occurred occasionally. A score of 2 indicates a significant impact or frequent occurrence. A score of 3 signifies a strong influence or a near constant presence. The DASS-21 is a simplified version of the DASS-42. The DASS-21 demonstrates high internal

reliability, as seen by Cronbach's alpha coefficients of 0.88 for the Depression scale, 0.82 for the Anxiety scale, 0.90 for the Stress scale, and 0.93 for the overall scale.[14] The stress level of the participants in this study was assessed by utilizing the 7 stress sub-items out of the total 21 sub-items in the DASS-21 questionnaire. The scores were calculated by summing the scores for each sub-item and then multiplying by two to ensure that they may be interpreted consistently with the lengthier form of the 42 items.

Data Acquisition

The Siemens 3-Tesla MR scanner was utilised for conducting MRI scans. Subjects were given instructions to maintain full motionless while inside the scanner. In order to reduce head movement, foam pads were placed on either side of the head, while earplugs were used to decrease the noise produced by the scanner. The diffusion tensor imaging (DTI) parameter sequence were set as follows: The repetition time: 7649 milliseconds, the echo duration: 72 milliseconds, the flip angle: 90 degrees, the field of view: 240 millimetres, the matrix size: 96 x 96, the section thickness: 2.5 millimetres, there is no section gap and the number of excitations: 1.0, with the acquisition time is 4 minutes and 28 seconds. The electrostatic repulsion model was used to apply diffusion-weighting gradients along 32 noncollinear directions. The imaging consisted of 2 images with b0=0 and 32 images with b1=1000 s/mm. In addition to the DTI scan, high-resolution anatomical T1, T2, and FLAIR weighted images were also obtained for each patient.

Image Processing

The diffusion imaging data was reconstructed using the DTI technique, in combination with the MRI Converter version 2.1.0 and DSI Studio software (http://dsi-studio.labsolver.org/). Initially, the DICOM data for each participant was imported using MRI Converter in order to convert the file format from DICOM (.dcm) to NIfTI (.nii). Subsequently, the converted files were opened in DSI Studio to generate the ".src" file. The ".src" file was subsequently reconstructed, yielding "fib" data, which was then used to obtain the FA value. The DSI Studio fibre tracking algorithm is a modified version of the deterministic tracking algorithm that utilises quantitative anisotropy as the termination criteria.[15] The deterministic technique was employed as the principal orientation of the tensor, aligning with the primary direction of the fibres and adhering to the most suitable pathway. The information provided reflects the predominant alignment of fibres inside each voxel. This approach seeks to illustrate the optimal balance between valid and incorrect connections.[16]

Region of Interest (ROI)s Localization

An expert radiologist assessed the postcentral gyrus on both the left and right sides of the brain, and manually designated a region of interest (ROI) on each side. Subsequently, using the program's auto-detection tool, the postcentral gyrus is automatically discovered on both sides of the brain (Figure 1) with the same ROIs. Minor adjustments were made to the automated selection of the ROIs for each unique case, taking into account the size and form of the postcentral gyrus. The FA values were obtained and analyzed after localising the ROIs.

Statistical Analysis

Data were analyzed using Statistical Package for the Social Sciences (SPSS) software. Quantitative data were described by calculating the means and standard deviations with p-values <0.05 considered as statistically significant. The Pearson correlation test was used to assess the relationship between the FA-value of post-central gyrus and stress score in DASS-21 sub-items.

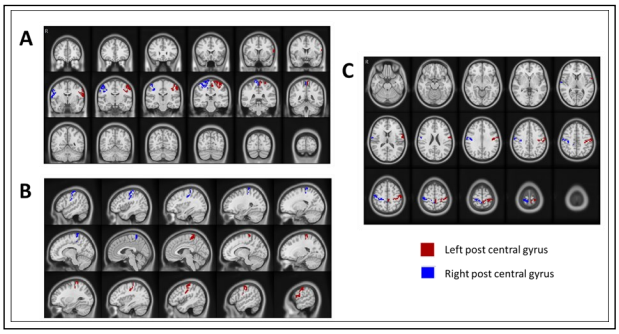

Figure 1. White matter tracts from the different cut sections; A) coronal view, B) sagittal view, C) axial view. FA values are extracted from the ROIs and analyzed in this work.

RESULTS

Clinical Variables

A total of 30 participants was enrolled in the study (21 males and 9 females; mean age=40.83, range=27–57 years). The results of the study showed that the average score ± standard deviation of the stress score of DASS-21 was 15.13 ± 7.34 (Figure 2).

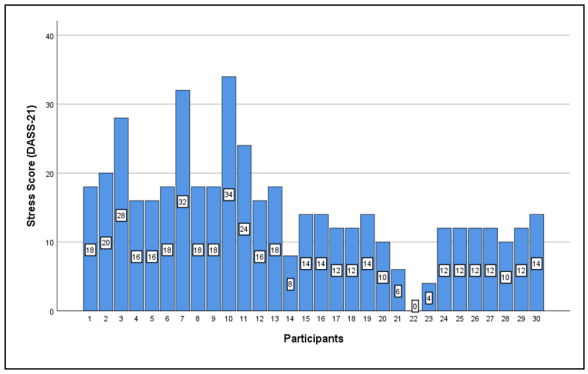

Figure 2. The distribution of the Stress Score in the healthy non-clinical participants.

Correlations Between FA-value of Postcentral Gyrus and Stress Scores

Our study has found that the MR-DTI FA value of the left post-central gyrus was negatively correlated with stress score (r=-0.393, p=0.032) (Figure 3), while no significant correlation was found between the MR-DTI FA value of the right postcentral gyrus and the stress score (r=-0.300, p=0.107) (Figure 4).

Table I: Brain regions with correlations between MR-DTI FA-value and Stress score

Brain Region	N	Mean FA value	r-value	p-value
Left post-central gyrus	30	0.208 ± 0.021	-.393	0.032*
Right post-central gyrus	30	0.179 ± 0.021	-.300	0.107

*Correlation is significant at the 0.05 level (2-tailed).

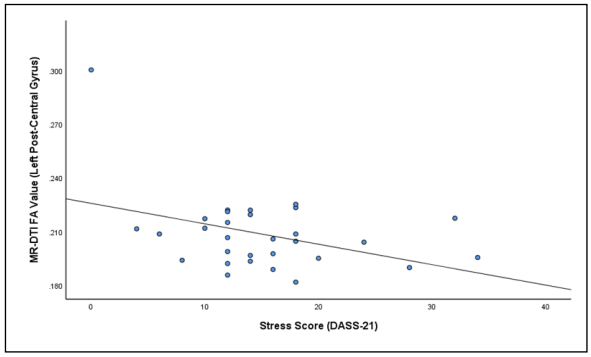

Figure 3. Correlation between MR-DTI FA value of left postcentral gyrus and stress score (r = -0.393, p = 0.032)

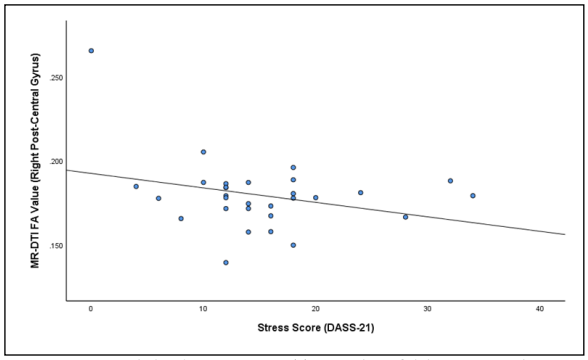

Figure 4. Correlation between MR-DTI FA value of right postcentral gyrus and stress score (r = -0.300, p = 0.107)

DISCUSSION

The aim of this study was to emphasize the significant involvement of the somatosensory cortex (specifically the postcentral gyrus) in response to stress among the groups of individuals who do not have any known mental health conditions or diseases and are not currently seeking or receiving medical treatment. This was achieved by examining the relationship between the structural alterations in the white matter of the postcentral gyrus and stress levels.

Consistent with our hypothesis, this study found that the somatosensory cortex, specifically the postcentral gyrus, which is part of the body-loop circuit, has a critical function in stress. Additionally, there is a notable inverse relationship between stress and the FA value of the left postcentral gyrus. Stress-related physical sensations can be linked to the postcentral gyrus in the somatosensory processing during stress exercises. This region is responsible for perceiving and analyzing the physiological alterations that are often induced in the body by stress.

Research has shown that the left somatosensory cortex has a greater influence on sensorimotor integration than the right somatosensory cortex.[17] The influence of handedness on brain structure and function has been well-documented with handedness can affect the lateralization of brain functions, including those related to sensory processing and stress responses.[17] In our study, the majority of participants were right-handed, which may have contributed to the observed lateralization effects.

Right-handed individuals often show stronger left-hemispheric dominance for motor and sensory functions, which could explain the significant association found between stress scores and the left postcentral gyrus.

Individuals experiencing high levels of stress tend to exhibit heightened sensitivity towards daily occurrences and stimuli, resulting in a state of perpetual vigilance and exaggerated reactions to stimuli. A plausible explanation for the association between the postcentral gyrus and stress is that individuals experiencing stress need to engage in increased brain activity in order to effectively manage stress and avoid developing pathological anxiety.[18] It was demonstrated that emotion perception is one of the functions of the somatosensory regions.[19] The postcentral gyrus, which forms connections with the frontal and parietal lobes, has been physically associated with cognitive functions such as control, memory, and attention.[20] The postcentral gyrus is a crucial component of the brain responsible for the recognition of basic emotions.[21] It is involved in the processing of somatosensory information, voluntary movement, and the regulation of emotions.[22] It was also explored that applying transcranial magnetic stimulation to the somatosensory cortex can influence the way people perceive emotions when performing social face recognition tasks.[23] Another study that looked at teens under emotional stress found that there was an alteration in the gray matter volume of the postcentral gyrus.[24]

The heightened neural activity observed in the postcentral gyrus during periods of stress, which has been associated with structural injury, could potentially indicate a rearrangement of its functional capabilities. Prior studies employing functional MRI (fMRI) in both people[25,26] and animals[27] have explored the functional reorganization of the central nervous system. According to these studies, the normal cortex undergoes substantial reconfiguration to compensate for the limitations of the affected area, resulting in behavioural adaptations. The greater extent of cortical activation, as indicated by the stronger fMRI response of the brain cortex, can be attributed to a bigger number of neurons and synapses involved in carrying out neurological function. These findings indicate that the extent of functional reconfiguration following stress, namely in the post-central gyrus region, may be influenced by the level of microstructural damage. Our study found a negative correlation between the FA value of the post-central gyrus and the subjects' higher stress levels, which might be explained by the increased neuronal activity to induce neuroplastic changes in the brain, such as axonal sprouting, dendritic growth, or synaptic remodeling. These processes may alter the organization and coherence of white matter tracts, leading to decreased FA values in DTI.

Clinical studies have found a correlation between a decrease in the volume of the somatosensory cortex and the presence of depression and other mental health disorders.[28,29,30] Glial cells, including astrocytes and oligodendrocytes, play crucial roles in supporting and maintaining the function of neurons. Preclinical research indicating significant glial atrophy in mental illnesses suggests that alterations in glial cell function or structure may be involved in the pathophysiology of these disorders.[31] Additionally, post-mortem investigations have found lower neuronal cell size and glial cell counts in individuals with mental disorders, further implicating cellular changes in the brain's structure.[32, 33] Reduced FA values are often associated with disruptions in white matter integrity, which can occur due to various factors, including changes in axonal structure, myelination, and fiber organization. The observed decrease in FA value in the postcentral gyrus (part of the somatosensory cortex) could be partially explained by reductions in cortical volume and supporting neuron cells. This implies that alterations in the microstructure of the somatosensory cortex, including changes in neuronal and glial cells, may contribute to disruptions in white matter integrity within this region, leading to decreased FA values.

Our findings revealed a significant negative correlation between stress scores and FA values in the left postcentral gyrus, whereas no significant correlation was observed in the right postcentral gyrus. This contrasts with previous studies that identified the right postcentral gyrus as being associated with stress scores in larger samples. For example, Li et al. (2019) found that state anxiety was linked to alterations in the right somatic brain network, including the postcentral gyrus.[34] Similarly, Kropf et al. (2019) reported that the right somatosensory cortex plays a crucial role in emotional regulation. [35] These

inconsistencies may be attributed to several factors such as differences in imaging techniques, data analysis methods, and the specific measures of stress used. For instance, our study used DTI to assess white matter integrity, while other studies might have employed different neuroimaging modalities or analytical approaches. Furthermore, lateralization of stress-related changes in the somatosensory cortex may vary across individuals. Factors such as genetic predispositions, environmental influences, and individual differences in stress perception and processing could lead to variability in which hemisphere shows stronger associations with stress.[36] The dominance of the left hemisphere in our predominantly right-handed sample could explain why we observed significant findings in the left postcentral gyrus.

Our study had certain limitations. The primary constraint was the limited population size, which impeded the ability to assess the variable degree of stress among healthy individuals. The findings from this study serve as preliminary evidence that can inform and justify the need for larger-scale studies. By demonstrating significant associations in a smaller sample, we provide a foundation for future research to build upon, potentially with larger and more diverse populations. Besides, this study only examined the somatosensory cortex. It is crucial to do additional research to examine other regions that are indirectly associated with stress. Furthermore, the study did not impose any age restrictions on the subjects, which means that the results may have encompassed brain deterioration associated with aging. In order to obtain precise prognostic forecasts for various age groups, it would be essential to have a bigger sample size. In addition, we did not assess the long-term impact of stress on the somatosensory cortex. Longitudinal studies would be necessary to investigate alterations in brain imaging and the relationship between stress and the somatosensory cortex. Lastly, the smaller subset of left-handed participants in our study did not provide sufficient data to examine potential differences based on handedness robustly. Future studies should include a more balanced representation of handedness to explore its impact more comprehensively.

CONCLUSION

In conclusion, we found that the stress eventually affected the integrity of the white matter in the postcentral gyrus. This may provide some insight into the mechanism of stress, as abnormal activity in the postcentral gyrus might affect decision-making and other activities. Individuals who experience stress exhibit emotional and cognitive processing biases that increase their susceptibility to bodily symptoms of stress, as compared to the normal population. Our research findings indicate that stress may lead to early damage in the microstructural somatosensory cortex, particularly in the postcentral gyrus, as observed using the DTI technique. Hence, the alterations in the microstructure of the brain's white matter prior to the onset of the ailment may play a vital role and could serve as a new and promising biomarker for the early identification and treatment of the disease in individuals who are not yet clinically diagnosed.

CONFLICT OF INTEREST

The authors declare that they have no competing interests.

ACKNOWLEDGMENTS

This work was supported by the Internal Universiti Malaysia Pahang Al-Sultan Abdullah Grant Scheme [UMP.05/26.10/03/RDU220306]. A special gratitude goes out to all authors for their expertise and assistance in completing the project and writing the manuscript.

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ORIGINAL ARTICLE

Outcomes of Negative Computed Tomographic Angiography in Management of Gastrointestinal Bleeding: A Cross-sectional Study

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ABSTRACT

INTRODUCTION: Acute gastrointestinal bleeding is a common gastrointestinal emergency. Only limited studies are available regarding the clinical outcomes after computed tomographic angiography (CTA) mesentery showed negative for active bleed. This study aims to determine the clinical outcome of negative mesentery CTA in patients with clinically active acute gastrointestinal (GI) bleeding. **MATERIAL AND METHODS:** A cross-sectional study with a universal sampling method was used. Patients who underwent CTA to detect gastrointestinal bleeding in the National University Hospital of Malaysia from December 2015 until March 2021 were retrospectively analysed. The outcome of each patient, risk of re-bleeding, and 30-days mortality rate were evaluated and assessed. **RESULTS:** In total, 280 CTAs were performed on 232 patients, with 186 of them showing negative results on their first initial CTA. 40.8% (76/186) of those with negative initial CTA had recurrent bleeding and 73.6% (56/76) of them required active intervention. We found that the risk of re-bleeding is lower in the upper gastrointestinal group compared to the lower gastrointestinal group (OR=1.5, 95% CI: 0.877- 2.852, p: 0.128). The overall 30 days mortality rate after the first negative CTA was 23.1% (43/186). Among those patients who experienced re-bleeding, 32.8% (25/76) died within 30 days, with 18.4% (14/76) succumbing to massive bleeding. **CONCLUSION:** From our analysis, it can be concluded **that a** clinically active GI bleeding with negative mesentery CTA has a 40.8% chance to re-bleed with 23.1% 30-day mortality rate. Close observation and follow-up of this population is recommended due to high rate of active intervention needed.

Keywords
Angiography, Gastrointestinal Haemorrhage, Mesentery, Computed Tomography Angiography, Cross-Sectional Studies

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Received: 2nd April 2024; Accepted: 18th July 2024

Doi: https://doi.org/10.31436/imjm.v23i04

INTRODUCTION

Acute gastrointestinal bleeding is a common gastrointestinal emergency with potentially critical outcomes and can be divided into upper gastrointestinal bleeding (UGIB) and lower gastrointestinal bleed (LGIB). In the United Kingdom, the incidence of UGIB per year is 84-172/100 000 and 25/100 000 for LGIB.[1] In Malaysia, the incidence of UGIB is approximately 72/100,000.[2] The average mortality rate is approximately 10% with no significant improvement over the past 50 years.[3] There is also an increase in the trend of hospitalization rate due to gastrointestinal bleeding in the United States of America which is approximately 375 per 100 000 patients.[4]

According to 2012 United Kingdom National Institute of Health and Care Excellence (NICE) guidelines, endoscopy should be performed urgently in severe acute gastrointestinal bleeding patients, and within 24 hours of admission in stable gastrointestinal bleeding cases. If there is an episode of re-bleeding clinically, a repeat endoscopy should be offered for endoscopic treatment. Prompt referral to interventional radiology or surgery should be made if recurrent bleeding despite endoscopic treatment occurs.[3] In more than 10% of patients, recurrence of bleeding occurs after the initial endoscopic treatment.[5] When endoscopy fails, it is mostly due to the

large pooling of blood in the bowels or poor bowel preparation. Hence computed tomographic angiography (CTA) of the mesentery is used to detect the source of bleeding.

CTA has high sensitivity and specificity of 97% and 100% respectively using arterial and portal venous phase images.[6] CTA can also provide a precise source of bleeding and its possible causes, which is very useful in facilitating further management.[7] If the CTA is positive, patients will usually undergo either interventional procedures, repeat endoscopy, endovascular embolization, or open surgery hence improving their clinical outcomes and rate of survival. However, limited studies are available on the clinical outcomes after a negative CTA of the mesentery for active bleeding.

In this retrospective study, our research aims to assess the clinical outcomes of patients who had active acute GI bleeding with negative initial CTA of the mesentery. Hopefully, through this study, clinicians will be able to predict the potential cases of re-bleed based on specific risk factors that are going to be discussed.

MATERIALS AND METHODS

Patient

The study was conducted in a tertiary centre National University Hospital of Malaysia. A universal sampling of all 232 cases of acute gastrointestinal bleeding that underwent mesentery CTA from December 2015 to May 2021 were retrospectively analysed. The data lists and CTA reports were retrieved from Radiology Information System (RIS) and Caring Hospital Enterprise system (C-HETs). The medical records were retrieved from the hospital RIS, C-HETs system, and manually from the record unit department. Inclusion criteria were patients who were referred for radiological investigation due to clinical symptoms of acute gastrointestinal bleeding by the primary team, either upper or lower gastrointestinal bleeding. Patients were excluded for the following reasons: variceal gastrointestinal bleeding, traumatic gastrointestinal bleeding, and detailed information were unavailable. Data collection included patient demographics, comorbidities, location and causes of bleeding, clinical outcome, radiological or surgical procedure, hemodynamic status during the CTA, and survival at 30 days within a single admission.

CTA of Mesentery Examination

CT examinations were performed with a 160-slice Toshiba Prime Aquillion or 640-slice Toshiba One Aquillion CT scanners using a multi-phase protocol:

1. Plain phase: baseline 1-mm acquisition was performed from diaphragm to symphysis pubis without IV contrast.
2. Arterial phase: 100 ml iodinated contrast medium (Ultravist-370) was administered by intravenous bolus injection, using a 21-G branula, at 4 ml/s using a bolus tracking technique and the region of interest (ROI) is centred at the celiac artery. Once the intraluminal contrast reaches the HU of 180, it automatically triggers the machine and scan is performed with an acquisition of 1 mm with a slice interval of 0.8mm.
3. Portovenous (delayed) phase: scan was done at 65 seconds post contrast administration with the acquisition of 1-mm with a slice interval of 0.8mm.
4. Further delayed phase: usually 5 minutes after post-contrast administration, to see further pooling of contrast.

A CTA mesentery was considered positive when contrast blush is seen in the arterial phase with further pooling in the portovenous and delayed phases. And a negative CTA mesentery was when there is no contrast blush seen arterial phase and no pooling of contrast in subsequent phases.

Terminology and Definitions

UGIB was when the origin of the bleed is proximal to the ligament of Treitz, while LGIB was when the origin is distal to the ligament of Treitz. Common symptoms of UGIB include hematemesis and melena; whilst in LGIB the symptom is mainly haematochezia. The re-bleeding case was when a patient had recurrent similar symptoms of gastrointestinal bleeding, or when they were referred for other secondary symptoms such as a drop in haemoglobin (Hb) or requiring blood transfusion after an episode of recovery. The hemodynamic status of the patient at the time of bleeding was considered stable or unstable based on

criteria from the Rockall score which is tachycardia (pulse rate>100/min) and hypotension (systolic blood pressure <100mmHg).

Data Interpretation and Analysis

Descriptive summaries were used to evaluate the clinical outcome after the initial negative CTA and to determine the frequencies, percentages, median, standard deviation, and as well as 30-day mortality rate. The rates of re-bleeding between upper and lower gastrointestinal bleeding were also compared using the Chi-squared test. The risk for re-bleeding between these two groups was also calculated. The–relationship between multiple possible related comorbidities and the CTA outcome was calculated using binary logistic regression analysis and the Chi-squared test. Data collected from the study were analysed using a software program, Statistical Package for the Social Sciences (SPSS) version 26. The p-value <0.05 was taken as a statistically significant difference.

RESULTS

A total of 232 patients underwent a total of 280 CTA mesentery for acute GI. 28 patients had 2 CTAs, 6 patients had 3 CTAs, 2 patients had 4 CTAs and 1 patient had 5 CTAs. Of the 232 patients 147 were males (63.4%) with a mean age of 65.4 and a standard deviation (SD) of 14.8. Out of 232 patients, 129 (55.6%) had UGIB, and 103 (44.4%) had LGIB. The aetiologies for the UGIB cases were peptic ulcer disease (PUD) (61.2%), tumour-related (10%), inflammation (7.7%), overwarfarinization (4.6%), and others (16.3%), while the aetiologies of the LGIB group were diverticulum (36%), ulcer (13.6%), colitis/inflammatory bowel disease (8.7%), tumour-related (6.8%), polyps (4.9%), angiodysplasia/arteriovenous malformation/pseudoaneurys (4.9%), overwarfarinization (1%) and others (24.3%).

Out of the 232 patients who underwent CTA mesentery, 186 (80%) of them had a negative first CTA. And 101 (59%) of these negative initial CTA had no episode of further bleeding, of which 92 patients (83.6%) were discharged well, and 18 (16.3%) passed away due to other unrelated causes and complications. However, 76 out of those 186 (40.8%) patients had recurrent bleeding, of which 29 patients (38.1%) ended up with surgical intervention, 24 (31.5%) had mesenteric angiography and embolization including prophylactic embolization, 8 (10.5%) had diagnostic mesenteric angiography, 3 (4%) underwent endoscopy treatment, and 12 (15.8%) had supportive therapy. Unfortunately 25 (32.8%) of those with recurrent bleeding after their negative initial CTA died, of which 14 (18.4%) died due to direct cause of bleeding, either after intervention or supportive therapy, and 11 (14.4%) died from other causes such as sepsis or multiorgan failure. The results summary is shown in Figure 1. The outline of the outcome according to the location of bleeding is shown in Figure 2.

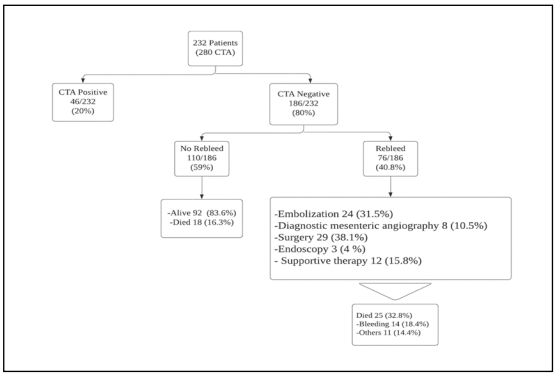

Figure 1. Flowchart of outcome summary of gastrointestinal bleeding after initial negative CTA.

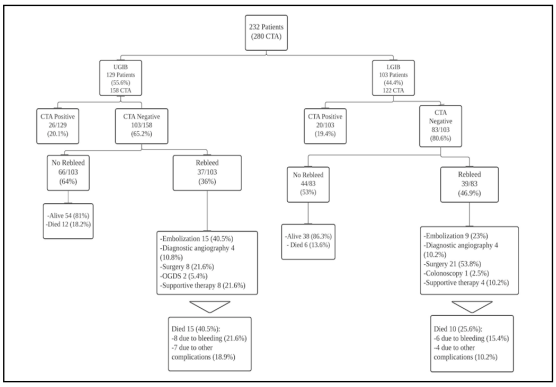

Figure 2. Flowchart of outcome summary of upper and lower gastrointestinal bleeding after initial negative CTA.

UGIB vs LGIB Re-bleeding Comparison

Within those without re-bleeding after the initial negative CTA, it was found that there was no significant difference between the upper and lower gastrointestinal groups; 66 out of 103 patients (64%) and 44 out of 83 patients in lower gastrointestinal bleeding (53%, p=0.127). The risk of re-bleeding after the first negative CTA is found to be lower in the upper gastrointestinal group as compared to the lower gastrointestinal group (OR=1.5, 95% CI: 0.877-2.852, *p*: 0.128).

In terms of the number of deaths directly related to bleeding following an episode of re-bleed, 8 patients (21.6%) were from the upper gastrointestinal group, and 6 (15.4%) were from the lower gastrointestinal group. They both showed rather similar mortality rate. So the decision whether or not to repeat CTA or directly go for intervention need to be weighed in equally in both cases.

30-Days Mortality Rate

The overall 30-day mortality rate for gastrointestinal bleeding after a negative initial CTA was 23.1% (43/186 patients). For those with re-bleeding after the negative first initial CTA, the mortality rate was 32.8% (25/76 patients), of which 18.4% (14/76) died directly due to bleeding; 5 after surgical intervention, 5 after mesenteric embolization, and 4 after supportive therapy. The remaining 11 (14.4%) deaths were due to other complications such as sepsis, myocardial infarction, and cancer-related. And of those who died directly due to bleeding episode following a negative initial CTA, there was not much difference either it was from UGIB or LGIB.

Most of the patients without re-bleed had a hospital stay of less than 7 days (82%). However, for those who had re-bleeding after the negative initial CTA, 39 out of the 51 patients who survived were admitted for more than 7 days (76.4%). The average total days of admission for this group was 13 days. Also important to note that most of these patients also had other health complications other than gastrointestinal bleeding per se such as sepsis and myocardial infarction.

Relation with Comorbidities

Binary logistic regression analysis assessing the association between CTA outcome and potential covariates or comorbidities is shown in Table I. All of these clinical predictors were analysed independently. While certain age group or patients with co-morbidities like end stage renal failure, coagulopathy and diabetes may have higher risk of gastrointestinal bleed, it is evident in our study that they are not significantly related to the result of their CT angiogram.

Table 1: Binary logistic regression analysis between covariates/comorbidities and CTA (n=232). *significant at p<0.05,

		CTA result of GI bleeding		Exp (B)	95% CI	*p* value
		Negative	Positive			
Age Category	15-30	8 (80%)	2 (20%)	0.375	0.051 - 2.772	0.337
	31-45	6 (60%)	4 (40%)			
	46-60	38 (80.9%)	9 (19.1%)			
	61-75	88 (85.4%)	15 (14.6%)			
	76-90	46 (74.2%)	16 (25.8%)			
Gender	Male	114 (77.6%)	33 (22.4%)	0.624	0.308 - 1.264	0.190
	Female	72 (84.7%)	13 (15.3%)			
Ethnicity	Malay	99 (81.1%)	23 (18.9%)			
	Chinese	76 (78.4%)	21 (21.6%)	1.189	0.613 - 5.602	0.608
	Indian	8 (80%)	2 (20%)			
	Others	3 (100%)	0 (0%)			
ESRF/CKD	No	144 (82.8%)	30 (17.2%)	0.547	0.272 - 1.098	0.09
	Yes	42 (72.4%)	16 (27.6%)			
Diabetes Mellitus	No	115 (81.0%)	27 (19.0%)	0.877	0.455 - 1.693	0.696
	Yes	71 (78.9%)	19 (21.1%)			
Hypertension	No	99 (80.5%)	24 (19.5%)	0.959	0.502 - 1.829	0.898
	Yes	87 (79.8%)	22 (20.2%)			
Coagulopathy	No	174 (81.3%)	40 (18.7%)	0.460	0.163 - 1.299	0.460
	Yes	12 (66.7%)	6 (33.3%)			
Haemodynamic	Stable	143 (80.8%)	34 (19.2%)	0.852	0.406 - 1.788	0.672
	Unstable	43 (78.2%)	12 (21.8%)			

DISCUSSION

Computed tomography angiography (CTA) of the mesentery is a widely used radiographic imaging to detect acute gastrointestinal bleeding mainly due to its availability in most tertiary hospitals, time-saving, and high accuracy to detect the bleeding point. The sensitivity and specificity of CTA were reported to be 97% and 100% respectively.[6,8] Our study focuses on the outcome of patients who presented with clinical symptoms of acute gastrointestinal bleeding but had a negative first initial CTA. In recent years, not many studies have been published regarding the clinical outcome after a negative CTA in gastrointestinal bleeding. Chan et al. in 2014 published their research regarding the prognostic indicator

of negative CTA and concluded that if no active gastrointestinal bleeding is detected in CTA, one can avoid unnecessary endovascular angiography intervention and supportive treatment might be sufficient.[1]

In this retrospective study, we found that the percentage of gastrointestinal re-bleeding cases in a single admission after the negative first CTA was 40.8%. This result was comparable to various studies from different other countries, with the re-bleeding rate after an initial negative study ranging from 27.4% to 51%.[1, 9-11] Some factors that may contribute to the re-bleeding episode in these patient include hemodynamic instability, cancer-related bleeding, the use of anticoagulants or antiplatelet medications and the performance status prior to admission.[1] All of these factors are associated with higher risk of bleeding to begin with hence would warrant a more vigilant monitoring. Almost three-quarters (73.6%) of the re-bleed group in our study population required further intervention, such as endovascular embolization, surgery, or an endoscopic treatment to stop the bleeding. A cross-sectional study in Korea also found quite a close number to our study, in which 60% of their study population required active intervention after a negative angiogram.[9] Chan et al. on the other hand showed that only a quarter (25%) needed further intervention to stop the bleeder.[1] In our setting, 31.5% had mesenteric angiography with embolization done to curb the recurrent bleeding in which mostly were prophylactic as localization of bleeding in angiography is even more challenging in a negative CTA cases. A few common factors making those with negative initial CTA needing active intervention include hemodynamic instability during the bleeding episode, overwarfarinization, and tumour-related bleeding.[1, 11] We can definitely relate with that as almost half of our patients (40%) needing active intervention after a re-bleeding episode was due to these factors; 27% were hemodynamically unstable, 9% had an underlying tumour, and 3.5% were overwarfarinized. However, these relationships require further in-depth research as it was not part of our study objectives.

Between the upper and lower gastrointestinal group, the rate of bleeding recurrence after a negative initial CTA was found to be higher in the LGIB group (46.9% vs 36%), with the risk of re-bleeding 1.5 times higher. The recently published data in 2020 by Fukuda et al also found a similar outcome[12] although some other studies observed a contradicted outcome in which bleeding recurrence was found to be higher in the upper gastrointestinal group.[1,13] The variable outcomes seen in these studies might be largely dependent on the expertise of the surgeons. On the other hand, the availability of medications like Proton-pump inhibitor (PPI) may contribute to the lesser bleeding recurrence in UGIB group.

The overall 30-day mortality rate of our study was 21.7% in the positive CTA group and 23.1% in the negative CTA group. In the negative CTA population, the 30-day mortality rate in the re-bleeding group was higher than in the non-rebleeding group (32.8% vs 16.3%). Mortality rate from this negative CTA group was reported to range from 8–48% in other centres.[1,7,9,12] Those who re-bleed and had UGIB after the first initial negative CTA had a higher mortality rate compared to the LGIB group (40.5% vs 25.6%). However, Chan et al., Fukuda et al. and Joo I et al. reported that the 30-day mortality rate was higher in the lower gastrointestinal group.[1,9,12] This conflicting result compared with our study was likely attributed to the more critically ill background of the upper gastrointestinal bleeding group in our population and faster surgical intervention in lower gastrointestinal bleeding. Although the 30-day mortality rate in the re-bleed group after their negative initial CTA was about one third of the studied population, only 18.4% of the death-related directly to massive bleeding, which is comparable to the previous study by Chan et al, Fukuda et al and Anthony et al.[1,7,12] The rest of the deaths were attributed to other complications and most of these patients had multiple comorbidities related to their mortality.

Several studies have shown that some specific comorbidities and factors could be the reasons for negative CTA in clinically positive gastrointestinal bleeding. Foley et al. concluded that in a hemodynamically stable patient, the result of an angiogram would likely to

be negative.[11] This is contrary to other studies that reported a significant association of age, haematocrit level and patients' heart rate (as manifestation of hemodynamic instability) with positive detection of bleeding on CTA.[14,15] And very recently, Sbeit et al. identified four parameters that were associated with positive bleeding on CTA which includes congestive heart failure, warfarin use, coagulopathy and low albumin level.[16] However, in our study, some of common comorbidities like hypertension, end stage renal failure, coagulopathy and hemodynamic stability were all shown not to be significantly related to the CTA result hence are not a suitable predictors of a positive CTA in those with clinical symptoms of gastrointestinal bleeding (Table I). Our result demonstrated that our patients had multiple variables which may interfere with the prognostication of the gastrointestinal bleeding outcome.

Finally, the variability in our study outcomes proved that managing gastrointestinal bleeding is ever challenging in general and our centre specifically. No fixed pathway or algorithm to find the cause of recurrent gastrointestinal bleeding is yet available, such as repeat CTA, tagged red blood cells (RBC) scan, and even diagnostic laparoscopy. However, a proposed diagnostic algorithm by Wortman JR et. al can be a useful guide. (Figure 3 & 4)

CONCLUSIONS

In our centre, a clinically positive gastrointestinal bleeding patient but with a radiologically negative CTA of mesentery has a 40.8% chance to re-bleed with a 30-day mortality rate of 23.1%. The lesson learnt from this study is that a negative initial CTA does not equate to no bleed. Hence, a close observation and follow-up in this group is highly recommended as the rate of these patients requiring active intervention is high with mortalities that may as well be prevented.

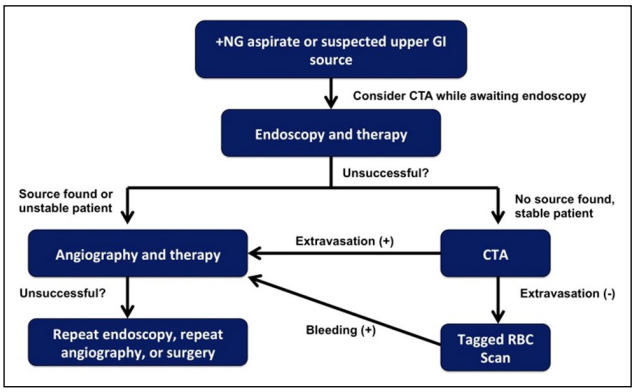

Figure 3. Proposed diagnostic algorithm for suspected upper GI bleeding cases[14]

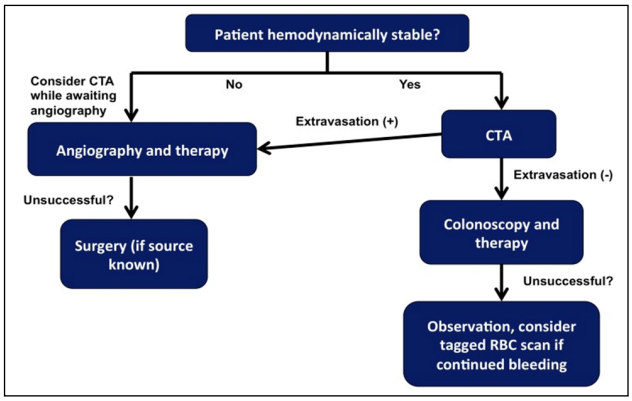

Figure 4. Proposed diagnostic algorithm for suspected lower GI bleeding cases[14]

LIMITATIONS

Since this study was a single centre retrospective study design, some of the data were difficult to obtain, as there was no direct contact with the primary physicians. Another limitation is that there was some variabilities in the clinical management of patients across different disciplines for example between surgical and medical patients.

RECOMMENDATIONS

A prospective and focused study of gastrointestinal bleeding with a larger sample size and standardized management algorithm should be conducted with collaborations from surgical and medical teams to evaluate the outcome.

CONFLICT OF INTEREST

No conflict of interest.

INSTITUTIONAL REVIEW BOARD (ETHIC COMMITTEE)

Ethical approval for this study was obtained from the institution's Research and Ethics Committee (Ethical approval code: FF-2021-037). This is a retrospective cross-sectional study, so informed consent was waived.

ACKNOWLEDGEMENT

We would like to express our gratitude to the healthcare professionals involved in the diagnosis, treatment, and care of the patients. Their dedication and expertise played a pivotal role in the successful management of these challenging cases.

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ORIGINAL ARTICLE

Effect of Tualang Honey-Mediated Silver Nanoparticles on TNF-α level, Caspase-3 Activity and Hippocampal Morphology in Kainic Acid-Induced Neurodegeneration in Male Rats

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ABSTRACT

INTRODUCTION: Despite being common disorder, the curative treatment for degenerative diseases are not yet available. Although Tualang honey (TH) has been reported to protect against neurodegeneration, but the effect of TH-mediated silver nanoparticles (THSN) on neurodegeneration is poorly understood. Thus, we conducted this study aimed to determine the effects of THSN on the levels of tumour necrosis factor alpha (TNF-α), caspase-3 activity, and hippocampal morphology in Kainic Acid (KA) induced neurodegeneration in rats. **MATERIALS AND METHODS:** A total of 72 Male Sprague Dawley rats were randomized into six groups which were the control, THSN 10mg, THSN 50 mg, KA only, KA+THSN 10 mg, and KA+THSN 50 mg groups. Each group was pre-treated orally with either distilled water or THSN (10 mg/kg or 50 mg/kg), according to their respective group. Following the last pre-treatment, each rat was injected with KA (15 mg/kg) or saline. After 24 h and 5 days of KA induction, all rats were sacrificed, and the hippocampus from each rat was harvested. Cresyl Violet and Fluoro Jade C staining were carried out to examine the number of viable cells and degenerating neurons. TNF-α level and caspase-3 activity in the hippocampus were measured using commercially available ELISA kits. **RESULTS:** Rats with KA-induced neurodegeneration demonstrated a significant increase ($p<0.05$) of TNF-α level and caspase-3 activity with a lower number of viable cells and increased number of degenerating neurons in the hippocampus. The pre-treatments of THSN groups improved these changes by lowering the TNF-α level and caspase-3 activity and decreasing the number of degenerating neurons. **CONCLUSION:** THSN could have potential neuroprotective effects in ameliorating TNF-α level, caspase-3 activity, and hippocampal damage in KA-induced male rats.

Keywords
Tualang Honey, Silver Nanoparticles, TNF-α, Caspase-3, Rats' Hippocampus

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Received: 29th August 2022; Accepted: 10th August 2024

Doi: https://doi.org/10.31436/imjm.v23i04

INTRODUCTION

Neurodegenerative diseases are associated with of neurodegenerative disorders involves excitotoxicity, in glutamatergic dysfunction[1] involving the production of which neuronal cells are injured and die as a result of free radicals, release of pro-inflammatory mediators and overstimulation of neurotransmitters, such as glutamate.[3] programmed cell death resulting in progressive The structural and functional changes in the brain affected neurodegeneration of the brain tissue.[2] The pathogenesis by neurodegenerative disorders are characterised by

cognitive and emotional dysregulation[4], and associated with reduced adult neurogenesis in the hippocampus.[5] The neurodegenerative process may be triggered by genetic factors related to intrinsic susceptibility, ageing, and environmental factors.[6] Previously, several studies used kainic acid (KA) as a chemical neurotoxicant in animal experiments to investigate the mechanisms involved in excitotoxicity and neurodegeneration by inducing pathological processes such as neuroinflammation and apoptosis.[7-9]

Previous findings suggested that TNF-α that derived from KA-activated microglia can increase the excitotoxicity of hippocampal neurons and induce neuronal apoptosis *in vitro* and *in vivo*.[10,11] Systemic administration of KA has been shown to cause neuronal degeneration primarily in the hippocampus hilus, CA1, and CA3 regions in animals.[12-14] The distribution and selective sensitivity of the KA receptors in the brain are related to KA-induced selective vulnerability in hippocampal neurons.[15] Therefore, this study used KA-induced neurodegeneration in rats as a model to explore the pathogenesis of excitotoxicity in neurodegenerative disorders.

Exogenous antioxidants are present in a variety of foods and natural products, including Tualang honey (TH). Studies have shown that TH contains many phytochemical components, such as flavonoids and phenolic acids, which display a variety of biological activities, including anti-apoptosis[6], anti-inflammatory[16], and neuroprotective effects.[17] The role of TH in neuroprotective activity against excitotoxicity in KA-induced rats' brain model has been reported in recent studies.[6,18] Moreover, TH has also been explored in nanotechnology fields and was found to be a good reducing and stabilising agent and, importantly, to function as a precursor in nanoparticles byproducts.[19-21] Nowadays, nanoparticles are one of the treatment options for several neurological disorders like Alzheimer's disease, Parkinson disease, and vascular dementia.[22] In addition, nanoparticles synthesized using antioxidants are becoming potential implications as a treatment and prevention of neurodegenerative illnesses.[23]

Various strategies have been explored to enhance honey's absorption, stability, and bioavailability, including the development of TH-mediated silver nanoparticles (THSN).[19-20] Nanotechnologies are extensively used to deliver neurotrophic agents to the brain, increasing the bioavailability of compounds and treatment for diseases affecting the brain tissue.[24,25] The nanoparticles increase the bioavailability and bioactivity of compounds by reducing the size of the particles[26], surface modification[27], attaching or entrapping the phytomedicine with different nanomaterials.[28] The rapid uptake of nanoparticles by cells is the main reason for their enhanced antioxidant capacity inside the brain cells.[29] THSN was shown to possess high antioxidant activity and ferric/reducing antioxidant power, with an average size of 22 nm, which most likely improves its bioavailability in the body.[20]

Our studies have recently reported that THSN was shown to ameliorate seizures, locomotor activity, memory function, and hippocampal oxidative damage in KA-induced rats.[30,31] However, the effects of THSN on neuroinflammation and apoptosis in an *in vivo* rat model of KA-induced neurodegeneration were poorly understood. Thus, the purpose of the current study was to investigate the neuroprotective effects of THSN in an *in vivo* rat model of KA-induced neurodegeneration, focusing on the levels of tumour necrosis factor alpha (TNF-α) and caspase-3 activity, as well as morphological changes in the hippocampus following KA administration.

MATERIALS AND METHODS

Animals

Male Sprague Dawley rats (200 to 250 g) were acquired from the Animal Research and Service Centre (ARASC) at the Universiti Sains Malaysia (USM) Health Campus. The animals were acclimatised at a temperature of 25±2° C with a light-dark cycle of 12:12 hours for seven days with free access to food and water. All procedures were carried out in accordance with the guidelines approved by USM Animal Ethics Committee [USM/IACUC/2018/(111)(904)].

Preparation of THSN

TH was purchased from the Federal Agricultural Marketing Authority (FAMA), Kelantan, Malaysia. THSN was prepared via the green synthesis method.[20] TH was diluted with distilled water before being heated in the oven (10-15 min, at 60°C). The honey solution was then adjusted to pH 8.0 before being added to the silver nitrate (0.1 M) solution. After that, the mixture was vigourously stirred. Lastly, the solution was dried overnight at 60°C. The nanoparticles were obtained in powder form and dissolved in 0.5 ml of distilled water before each use.

Experimental Groups

A total of 72 male rats were randomly divided into two major groups (24 h and 5 days post-KA induction), and each group contained six subgroups (n=6) as the following:

Group (1): Control	Group (4): KA only
Group (2): THSN 10 mg	Group (5): KA + THSN 10 mg
Group (3): THSN 50	Group (6): KA + THSN 50 mg

Each group was pre-treated orally with distilled water or THSN (10mg/kg or 50mg/kg), according to their respective group, five times at 12 h of intervals. The THSN dosages used in this study were based on previous research.[32,33] A recent study found that a daily dose of 10mg/kg silver nanoparticles (low dose) of *Azadirachta indica* extract may be safer for rats.[34] Therefore, the current study utilised THSN at 10mg/kg (10mg equivalent of TH/kg body weight) (low dose) and 50mg/kg (50mg equivalent of TH/kg body weight) (high dose) to compare their effects on KA induction in rats. After the last pre-treatments, the animals were injected subcutaneously with KA (15mg/kg) or saline.

In this experiment, two time points were selected, which were 24 h and 5 days. The rats were divided into two time periods of sacrifice as we expected that the different durations might have different effects on alteration of neuroinflammation, apoptosis and morphology in the hippocampus tissue. Following KA administration, an acute phase, lasting 24 to 48 h, corresponded to the damaging effect of neuronal apoptosis which occurred at early stage of excitotoxic insult.[7] Besides that, previous study showed a persistent hippocampal neuronal death in rodents at 1 to 5 days after KA treatment.[35] Therefore, the current study duration was extended to 5 days after KA injection to evaluate the potential protective effects at a later stage after KA injection.

Preparation of the Brain Homogenate

After 24 h and 5 days of KA induction, the animals were anesthetized with an overdose of sodium pentobarbital by intraperitoneal injection. The hippocampus from each animal was extracted. First, the isolated hippocampus was weighed and homogenized (10% w/v) in ice-cold 0.1 M phosphate-buffered saline (pH 7.4). Then, the homogenates were centrifuged (10,000 x g) for 10 min, and the supernatants were stored at -80°C until assayed.

Assay Procedures for Biochemical Analysis

TNF-α and caspase-3 activity were measured using commercially available kits (Qayee, Wuhan) according to the manufacturer's instructions. A double antibody enzyme-linked immunosorbent one-step process was used to determine the level of each parameter in the sample. The samples were analyzed at 450 nm using a microplate reader. The OD values were calculated according to the standard concentration using the standard curve linear regression equation.

Histological Analysis

Preparation of Brain Tissue

After all animals were anesthetised with an overdose of intraperitoneal injection of sodium pentobarbital, cardiac perfusion was performed. Then, the brain was removed and placed in the fixative solution, 4% PFA in 0.1 M sodium phosphate buffer (pH 7.4) at 4°C for overnight fixation. At post-fixation, the tissue samples were processed overnight in the automated tissue processing centre. The processed samples were embedded and blocked in paraffin for sectioning. The tissue blocks were serially sectioned (5 μm thickness) in the coronal plane using a rotary microtome. In each rat, three hippocampus sections were randomly taken from the anteroposterior Bregma -2.28 mm to Bregma -3.96 mm.

Cresyl Violet and Fluoro Jade C (FJC) Staining

Cresyl violet staining was performed to estimate the neuronal loss by identifying the positively bright purple, finely granular stained cells. Cresyl violet-positive cells were counted and defined as those with normal morphology and exhibiting round nuclei stained with granular-purple cresyl violet. Neuronal cells (pyknotic) that demonstrated shrunken nuclei or neuronal cells with no nucleoli or unclear bodies with surrounding empty spaces were excluded. Meanwhile, FJC staining was performed to further evaluate neuronal degeneration in the rats' hippocampus. FJC-positive cells were counted and defined as bright green fluorescence. In contrast, normal neurons that appeared darker than the background with lightly stained nucleoli or unstained nuclei were not counted. The sample sections were viewed and imaged using an Olympus BX41-32PO2 microscope (Olympus Corporation, Japan).

Six non-overlapping hippocampal areas were identified and photographed at 400X magnification for each slide. The number of cells was counted by taking the mean of six fields of CA1, CA2, and CA3 from each section. These regions were chosen as the function of CA1 pyramidal neurons provide a major output of the hippocampus proper.[36] Meanwhile, CA2 and CA3 pyramidal neurons will receive input from all the sources and this information will be segregated.[37] These regions are essential in the hippocampal function, like learning, long-term memory, spatial memory, and mood.[38] Therefore, these areas are important to consider in relation to neurodegenerative diseases.

Statistically Analysis

The IBM Statistical Package for Social Sciences (SPSS) software (Version 26, Chicago, USA) was used to analyze the results. Data with normal distribution and equal variance were analyzed using a parametric test; one-way analysis of variance (ANOVA) and followed by Tukey's post hoc test for multiple pairwise comparisons. The mean ± standard error of the mean (SEM) was used to express all values. The differences were considered statistically significant at $p<0.05$.

RESULTS

Level of TNF-α Level in Rats' Hippocampus

The level of hippocampal TNF-α was found to be significantly different ($p < 0.05$) between the groups at 24 h and 5 days post-KA induction (Figure 1). The post-hoc Tukey test demonstrated that the group pre-treated with 10mg/kg of THSN had significantly lower ($p<0.05$) TNF-α levels at both 24 h and 5 days post-KA induction when compared to KA only group. Meanwhile, pre-treatment with 50mg/kg of THSN reduced TNF-α level significantly ($p<0.05$) after 5 days but not at 24 h post-KA induction.

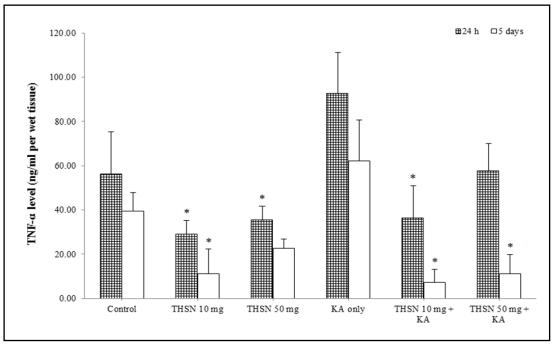

Figure 1. Level of TNF-α in the hippocampus of KA-induced rats. $p<0.05$ versus KA-only group. The results were expressed as mean ± SEM.

Caspase-3 Activity in Rats' Hippocampus

In the present study, the hippocampal caspase-3 level showed a significant difference ($p<0.01$) between groups at 24 h and 5 days post-KA induction (Figure 2). The post-hoc Tukey test revealed that, as compared to the KA-only group, pre-treatment with a low dose of THSN

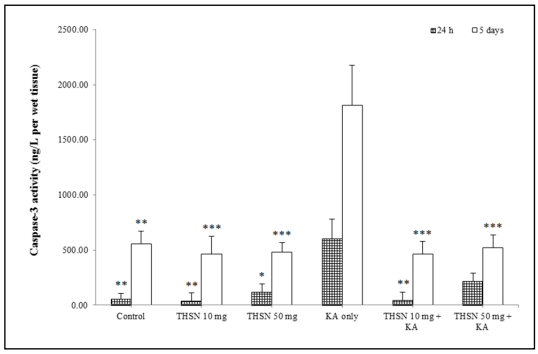

Figure 2. Level of caspase-3 in the hippocampus of KA-induced rats. *** $p < 0.001$ versus KA-only group; ** $p < 0.01$ versus KA-only group; * $p < 0.05$ versus KA-only group. The results were expressed as mean ± SEM.

(10mg/kg) remarkably decreased ($p<0.01$) caspase-3 activity in both time frames, while pre-treatment with THSN (50mg/kg) significantly reduced ($p<0.001$) the caspase-3 level after 5 days, but not at 24 h post-KA induction.

The Number of Cresyl Violet-Positive Cells in the Rats' Hippocampus

The number of cresyl violet-positive cells was shown to have significant differences ($p < 0.05$) between the groups in the CA1, CA2, and CA3 hippocampal regions, at 24 h post-KA induction. The post-hoc Tukey test demonstrated a significant decrease ($p<0.05$) in the KA-only group compared to other groups. Meanwhile, pre-treatment with THSN significantly improved ($p<0.05$) the number of cresyl violet-positive cells in the CA2 and CA3 regions at 24 h post-KA induction (Table I).

At 5 days of post-KA induction, the number of cresyl violet-positive cells in the CA1 and CA2 hippocampal regions revealed a significant difference ($p<0.05$) between the groups. The results revealed that as compared to the KA-only group, the pre-treatment with both doses of THSN significantly improved ($p<0.05$) the morphology of the CA2 region at both time frames. In contrast, in the CA3 region, the number of cresyl violet-positive cells was significantly increased ($p<0.05$) by the pre-treatment with THSN after 24 h, but not at 5 days of post-KA induction (Figure 3).

Table I: Number of cresyl violet-positive cells in rats' hippocampal regions at 24 h and five day of post-KA induction.

Groups	Number of cresyl violet-positive cells (0.01 mm²)					
	CA1		CA2		CA3	
	24 h	5 days	24 h	5 days	24 h	5 days
Control	64.65 ± 5.30	57.55 ± 3.283.28	74.71 ± 3.40[b]	41.39 ± 2.58[b]	41.39 ± 2.58[a]	41.59 ± 1.17
THSN 10 mg	69.45 ± 4.14[c]	69.95 ± 4.29[c]	79.74 ± 5.28[a]	43.67 ± 2.5[b]	43.67 ± 2.50[a]	43.72 ± 2.04
THSN 50 mg	62.69 ± 5.73	58.16 ± 3.22	74.91 ± 3.95[b]	38.77 ± 3.52[b]	38.77 ± 3.52[b]	39.36 ± 1.54
KA-only	49.02 ± 3.29	55.93 ± 2.99	47.89 ± 3.41	15.14 ± 2.66	15.14 ± 2.66	26.76 ± 5.50
KA + THSN 10 mg	65.55 ± 3.92	62.86 ± 2.73	72.69 ± 4.48[b]	38.98 ± 5.99[c]	38.98 ± 5.99[b]	28.14 ± 4.88
KA + THSN 50 mg	65.00 ± 4.25	66.68 ± 2.25	71.86 ± 5.89[b]	40.66 ± 3.51[c]	40.66 ± 3.51[c]	30.34 ± 6.66

The results were expressed as mean ± SEM. a $p<0.001$ versus KA-only group; b $p<0.01$ versus KA-only group; c $p<0.05$ versus KA-only group.

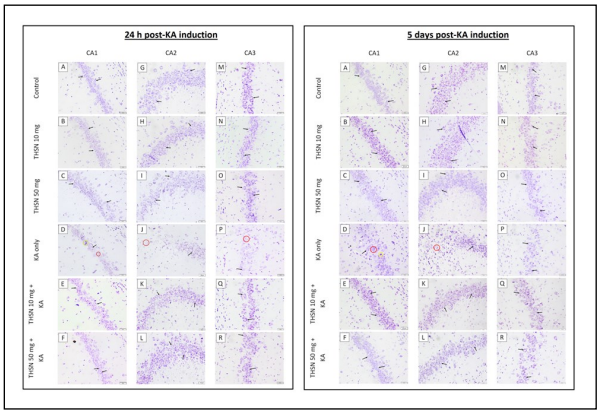

Figure 3. Morphology of CA1, CA2, and CA3 hippocampal regions among all groups after 24 h and 5 days of post-KA induction. (A – R) The arrows indicate the cells of interest; (D, J, and P) the neurons in the red circle show reduced staining intensity of the cresyl violet; (D) the appearance of pyknotic neuron in the yellow circle represents an injured neuron (Cresyl violet staining × 400, scale bar: 50 µm).

The Number of FJC-Positive Cells in the Rats' Hippocampus

In the present study, the post-hoc Tukey test demonstrated that the higher number of FJC-positive cells in the CA2 and CA3 hippocampal regions in KA-only group was significantly reduced ($p<0.05$) by both doses of THSN pre-treatments at 24 h post-KA induction (Table II). The hippocampal neuronal degeneration upon KA exposure was recognized by the appearance of shrunken or smaller-sized in their cell bodies compared to normal neurons (Figure 4).

Meanwhile, there was a significant difference ($p<0.05$) between the groups in the hippocampal CA2 and CA3 regions at 5 days of post-KA induction. The post-hoc Tukey test presented that, as compared to the KA only group, both doses of THSN pre-treatment significantly improved ($p<0.05$) the morphology of the CA2 region at both time frames, whereas the morphological alteration in the CA3 region could be seen at 24 h, but not at 5 days of post-KA induction. Therefore, THSN pre-treatments might exhibit protective effects on KA-induced neuronal degeneration in the hippocampus after 24 h.

Table II: Number of FJC-positive cells in rats' hippocampal regions at 24 h and five days of post-KA induction.

Groups	Number of FJC-positive cells (0.01 mm²)					
	CA1		CA2		CA3	
	24 h	5 days	24 h	5 days	24 h	5 days
Control	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00[a]	0.00 ± 0.00[a]	0.00 ± 0.00[a]	0.00 ± 0.00[c]
THSN 10 mg	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00[a]	0.00 ± 0.00[b]	0.00 ± 0.00[a]	0.00 ± 0.00[c]
THSN 50 mg	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00[a]	0.00 ± 0.00[b]	0.00 ± 0.00[a]	0.00 ± 0.00[c]
KA-only	4.06 ± 3.35	1.76 ± 1.75	14.67 ± 2.07	13.16 ± 5.74	23.20 ± 2.88	18.19 ± 4.75
KA + THSN 10 mg	0.24 ± 0.15	0.64 ± 0.39	1.81 ± 1.06[a]	1.46 ± 1.46[b]	6.31 ± 2.74[a]	12.01 ± 6.69
KA + THSN 50 mg	0.40 ± 0.35	0.11 ± 0.04	4.31 ± 2.78[a]	3.01 ± 1.37[c]	7.69 ± 3.62[a]	12.43 ± 6.18

The results were expressed as mean ± SEM. a $p<0.001$ versus KA-only group; b $p<0.01$ versus KA-only group; c $p<0.05$ versus KA-only group.

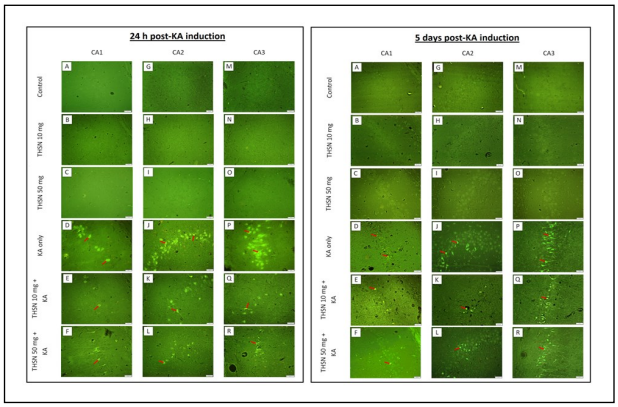

Figure 4. Morphology of CA1, CA2 and CA3 hippocampal regions among all of the groups after 24 h and 5 days of post-KA induction. The red arrows indicate the cell damages (FJC staining × 400, scale bar: 50 µm).

DISCUSSION

Our study found that hippocampal TNF-a level was remarkably increased in KA-only group at 24 h and 5 days of post-KA induction. The dysregulation of inflammatory factors like TNF-α, TLR-4, and NF-ϰb is thought to play a role in KA -induced excitotoxicity. Previous study reported that the high TNF-α level in rats' hippocampus can still be detected at 24 h, 3, and 21 days after KA induction.[39,40] Apart from that, previous finding suggests that the proinflammatory cytokine TNF-α derived from KA-activated microglia can promote neuronal apoptosis.[10] Apoptosis is the final stage of neurotoxicity and can be evaluated by measuring caspase- 3 activity. In the present study, a significant increase in caspase-3 activity was observed in KA- only group in both time frames, indicating a high rate of neuronal apoptosis in the hippocampus. These results closely resemble the previous findings that reported a significant increase in hippocampal caspase-3 level at 24 h after KA administration in rats.[41]

Our study demonstrated that the higher levels of TNF-α and caspase-3 were reduced by THSN pre-treatment after KA induction. A previous report found that silver nanoparticles can prevent protein denaturation, which is a common occurrence in inflammation and can interfere with the production of acute inflammatory mediators (such as histamines, serotonin, prostaglandins, and cyclooxygenase products) and counteract their action.[42] In a recent investigation, it was reported that the eco-friendly selenium nanoparticle produced using *M. oleifera* extract decreased melamine-induced nephrotoxicity in rats via reducing apoptosis.[43] Another finding also showed that green synthesis of silver nanoparticles was able to down-regulate the apoptotic pathways by reducing the caspase-3 level in rats' kidneys.[44] The reports cited above imply that green synthesized metallic nanoparticles can decrease inflammation more efficiently by inhibiting pro-inflammatory cytokines and preventing the apoptotic caspase-3 pathways.

Apart from that, the present study showed an increment in neuronal loss in hippocampus morphology, remarkably in CA1, CA2, and CA3 regions of KA-only group. However, the degenerated neuron in the CA1 region was detected at 24 h but not at day 5. This could be due to the cortical neuronal excitability that underwent an oscillating process after KA administration. Previous study showed that neuronal excitability increased rapidly in mice, decreased gradually at 8 h post-KA injection, and restored to a normal level one week later.[45] Concomitantly, kainate receptors were likewise found mostly in the CA2 hippocampal area with higher levels in low-excitability rats.[45] This observation can be explained by the existence of pyramidal neurons in different hippocampus regions, which demonstrated selective sensitivity to KA action, depending on the strain features of the animals' nervous system excitability. The present results showed that the morphology of the CA2 and CA3 regions were altered at both 24 h and 5 days after KA injection. This finding is consistent with previous reports demonstrating that CA2 and CA3 neurons were severely damaged due to KA-induced neurotoxicity.[46] Besides that, the CA3 region is the most sensitive and preferentially lesioned by KA since this region contains a high density of KA receptors.[47]

Our study result revealed that the morphological damage in the hippocampus after KA induction was attenuated by THSN. TH, a reducing agent used to synthesize the silver nanoparticles, contains various chemical compounds including acids, aldehydes, alcohol, ketones, terpenes, hydrocarbons, and furan derivatives as well as phytochemical compounds.[48] Meanwhile, THSN has been reported to contain alcohols, phenols, amides, carboxylate ions, and protein and exhibited excellent antioxidant activity.[20] The presence of antioxidant flavonoids and phenolic acids in TH could scavenge ROS and elevate enzymatic and non-enzymatic antioxidants

while decreases lipid peroxidation and inflammatory cytokine production; the combined effects contribute to the antioxidant and anti-inflammatory effects.[7,49] These compounds also reduce the degeneration of neurons and inhibit apoptosis via the downregulation of Bad, Bax, and cleaved caspase-3 expression levels and upregulate anti-apoptotic proteins such as Bcl-2 and Bcl-xL.[50] The presence of flavonoids and phenolic acids in TH and THSN, as well as the synergistic interaction between these bioactive compounds may have contributed to THSN's protective effects on neuroinflammation and apoptosis, which in turn contributed to the reduction of neuronal degeneration in the hippocampus of KA-induced rats.

Overall, our study findings suggested that THSN exhibited neuroprotective activity against KA-induced neurodegeneration. Establishing nanoparticles with natural antioxidants can increase stability and biocompatibility while diminishing toxicity, besides preserving the desirable properties of the natural compound in THSN as reported in previous study.[20] However, the chemical composition and profiling of THSN were not explored in detail in order to find out the possible mechanisms behind their protective effects against KA-induced neurodegeneration.

CONCLUSIONS

THSN may have a protective effect on neurodegeneration by reducing the level of TNF-α and caspase-3 activity as well as improving the morphological alteration in the rats' hippocampus after KA administration. Thus, a further research is required to investigate the molecular level of the mechanism to provide better elucidation of the protective effects of THSN against KA-induced neurodegeneration in rats.

CONFLICT OF INTEREST

The authors declare no conflict of interests

ACKNOWLDEGMENTS

This research was financially supported by the Universiti Sains Malaysia under Research University (Individual) (RUI Grant No: 1001/PPSP/8012249).

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ORIGINAL ARTICLE

Reliability and Validity of the Sexual Intention Questionnaire for Adolescent Boys (SIQ-AB) in Malaysian Context

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ABSTRACT

INTRODUCTION: The newly developed Sexual Intention Questionnaire for Adolescent Boys (SIQ-AB) is an adapted tool from the Youth Sexual Intention Questionnaire (YSI-Q) and Theory of Planned Behaviour (TPB), aimed to assess the influence of knowledge, permissive attitude, social norms, and self-efficacy toward the sexual intention. Thus, the aim of this study was to validate the newly developed SIQ-AB to determine Malaysian adolescent boys' sexual intention. **MATERIAL AND METHODS:** The SIQ-AB validity and reliability were assessed in this study. A total sample of 252 (Exploratory Factor Analysis, n=126 and Confirmatory Factor Analysis, n=126) among adolescent boys from Serian and Kota Samarahan, Sarawak were included in this study. The exploratory factor analysis (EFA) assessed three constructs of sexual intention which include attitude, self-efficacy, and social norms with a 4-point Likert scale with a total of 19 items. The confirmatory factor analysis (CFA) assessed the TPB constructs of sexual intention, attitude, self-efficacy, and social norms with a 4-point Likert scale with a total of 19 items adopted from YSI-Q. **RESULTS:** The four-factor structures were supported by the EFA which had 19 items which accounted for 65.79% of the variation overall. Four items were removed due to improper positioning or low factor loading (<0.50), even though EFA supported the four-factor structure. Using Cronbach's alpha, internal reliability varied between 0.77-0.89. Construct, convergent and composite validity of the SIQ-AB were further validated by the CFA with χ 2=199.0, df=129, p< 0.001, χ 2/df =1.54, CFI=0.98 and TLI=0.98 and RMSEA=0.06. **CONCLUSIONS:** The result proved that the SIQ-AB instrument is a valid and highly reliable tool to assess adolescent boys' sexual intention.

Keywords
Sexual intention questionnaire for adolescent boys (SIQ-AB), factor analysis, theory of planned behaviour

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Received: 18th January 2024; Accepted: 22th June 2024

Doi: https://doi.org/10.31436/imjm.v23i04

INTRODUCTION

Adolescents today are more prone to participate in early sexual activity, increasing the potential of multiple sexual partners through unsafe sexual.[1] The peak age for sexual initiation is between 15-19 years old, which affects about 30-50% percent young men and women from worldwide.[2] In Malaysia, about one-third of adolescents are engaged in sexual activities before the age of 14, which could negatively affect their health and development.[3] As a result, they are at higher risk of contracting sexually transmitted disease, unintended pregnancies, induced abortions, and psychological health consequences.[4] Some of most trending and social problems among adolescent in Malaysia include disciplinary problems, physical bullying, suicidal thoughts, free sex, tobacco consumption, alcohol abuse, drug abuse and media influence.[5]

The male adolescents sexual debut is at a higher prevalence rate than female adolescents.[2] One of the causes for increasing sexual debut is due to a strong drive to experiment sexual activities and a need for independence.[6] Adolescent boys may also face pressure to conform to societal expectations regarding masculinity and sexuality.[7] A research indicated that their first sexual intercourse happened because they 'had a crush' on the other person.[8] Furthermore, adolescent boys are more active in social networks than adolescent girls, hence exposing them to risky sexual behaviour.[9]

For this reason, the is an urgent need to measure sexual intention among adolescent boys in Malaysia. Sexual intention is the main driving force behind sexual activity after puberty. Currently, the tools used to measure sexual intention in adolescents are limited to ages older than 18 years old and for both genders. Youth sexual intention questionnaire (YSI-Q) was developed for unmarried youths aged 18-22 years old around Klang Valley, Malaysia with Cronbach alpha ranged between 0.89-0.94.[10] YSI-Q is also used to measure sexual intention among late adolescent (both genders) aged 18-19 years old in Kuantan government school.[11] In other Asian countries, an open-ended specific questionnaire was employed to identify the factor perceived to affect the intention of sexual behaviours among junior high school in both gender in Thailand.[12] In Indonesia, a Youth Sexual Intention Scale (YSIS) were used to measured sexual intention among students aged 15-18 in both genders from four high schools in Selayang district, Medan City.[13]

Another reason for the need of this questionnaire is due to Malaysia's diverse and various cultural and religious beliefs surrounding sexuality.[14] As such, sexual intention tool can be tailored to accommodate these perspectives, ensuring that the tool provided is culturally sensitive and relevant to the Malaysian context. Thus, the aim of this study was to validate a newly developed Sexual Intention Questionnaire for Adolescent Boys (SIQ-AB) to determine Malaysian adolescent boys' sexual intention.

MATERIALS AND METHODS

Study Design

This study applied three types of validation design namely i) content validity, ii) construct validity, and iii) internal validity, to ensure the validity and reliability of the Sexual Intention Questionnaire for Adolescent Boys (SIQ-AB).

Study Population

Boys between the ages of 13-17 years old who attended school made up the study sample. Participants who were unable to read or write were not allowed to participate in the study, nor were they allowed to do so without parental or guardian authorization.

Sample Size and Sampling Method

Sample size for exploratory factor analysis (EFA) and confirmatory factor analysis (CFA) were calculated based on sample-to-item ratio. The recommended ratio should not be less than 5:1.[15] Hence, this study with 20 items, required 100 respondents. This study used a purposive sampling.

Instrument

This study adopted the instrument YSI-Q instrument (20 items) to evaluate sexual intention, which is influenced by a permissive attitude, perception of social norms, and perceived self-efficacy in forecasting sexual intention.[7] The response was given on a 4-Likert scale, with 1 being "Strongly Disagree" and 4 being "Strongly Agree." On top of the 20 items from Muhammad et al., (2017), this study added 14 knowledge items specific for adolescent boy. Three out of 14 items were exclusively for adolescent boys. For example, *'Gonorrhoea among adolescent boy causes pus production form the urinary tract'. "Only gay, drug user and sex workers can be infected by HIV"* and *'the use of male condom can reduce the risk of HIV infection'.*

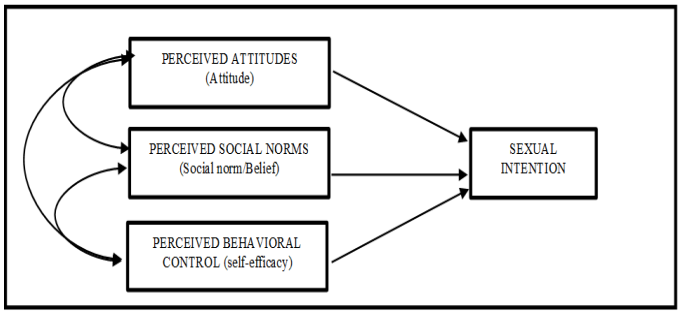

Figure 1: Theoretical framework for sexual activity based on Theory of Planned Behaviour

Data Collection Procedure

Schools under the Kuching Healthy City project were selected for this study. The researcher then obtained the necessary approvals from the Sarawak State Education Department and the principals of the selected schools. Research information and parental consent form were sent to parents via the students who were selected by respective school counsellors. Students with parental consent who took part in the study, were informed about study objectives and procedures. They were gathered in the school hall and answered the questionnaire with the presence of the researcher. Participants were encouraged to

ask any questions or verify any words they did not understand.

Content Validity

Content validity is the assessment on the validity of content for each item to identify whether the items represented domains of the scale in the instrument.[16] Five panel experts with the background of public health physician, health educationist, and public health nurse, were invited to determine the content validity of the 20 items of Youth Sexual Intention Questionnaire (YSI-Q). Each item was rated by each panel expert as 'essential', 'useful but not essential', and 'not necessary'. Once the items were rated, the content validity ratio (CVR) was calculated using Lawshe's formula.[17] Since the number of panel experts involved were five or less, only items with CVR of ≤0.99 or items received 'essential' rating from all experts were retained.[17] Any item that achieved less than 0.99 were removed. After performing content validity, the items that were subjected to a forward-backward translation technique starting with English to Malay and then back to English. It was conducted by two independent linguists who were fluent in Malay and English. Replacement of jargons and ambiguous words were done based on the comments from the translator.

Construct Validity

Construct validity was performed by using Exploratory Factor Analysis (EFA) and Confirmatory Factor Analysis (CFA) procedures.

Exploratory Factor Analysis (EFA)

To better understand the patterns of interactions between constructs, EFA analysed 19 items representing three domains of Theory of Planned Behaviour (TPB). The number of components in the model can be confirmed and verified using EFA.[18] Sample size and the degree of the link between the measurements were the two fundamental prerequisites for EFA. Even so, the investigator ought to consistently gather the greatest number of samples to represent each variable (for example, 5 samples per item).[19] This is to reduce the likelihood of over fitting the data. Thus, a sample size of 100 or greater in this study is desirable.[7] In this study, 126 adolescent boys were recruited EFA test.

The principal axis factoring methods were used to extrude factors from the correlation matrix, rotate the factors, and interpret the factor analysis results. The method of EFA includes i) generating factors whenever feasible combinations of the variables in a correlation matrix, ii) extruding factors from the correlation matrix, and iii) rotating the factors.[20] The process of rotating the factors or eigenvectors in an effort to attain simple structure is referred to as rotation.[21] In order to create the factor correlation matrix of values more than 0.50 for the purpose of this study, Varimax rotation was used. The Kaiser-Meyer-Olkin (KMO) procedure was used to assess the correlation matrix factorability.[22] Thus, a correlation matrix approach reveals if the correlation between a small group of variables is due to factors or random chance, >60 is a satisfactory value for KMO. [20]

Confirmatory Factor Analysis (CFA)

In order to establish the reliability of the SIQ-AB scale, the CFA was utilized to validate the factor structure suggested by the EFA technique.[22,23] Another set of 126 samples were subjected to the CFA. Goodness-of-fit was used to evaluate the model overall fit. It is believed that well-fitting models provide the data sufficiently and enable confidence to be applied to the entire population. Firstly, the chi-square (χ^2) that is not significant. The chi-square test however might result in an incorrect model fit if the sample size is excessive or if multivariate normality is violated.[24] Therefore, the normed chi-square test which is less than three (χ^2/df) is utilized to augment the chi-square test result.[25]

A good model fit is defined as a goodness-of-fit index (GFI) over 0.90.[24] Popular measures of fit include the Root Mean Square Error of Approximation (RMSEA), which does not need to be compared to the null model. An appropriate model fit is one with an RMSEA index value of less than 0.10 and up to 0.80.[26] Given their usefulness as measures of global model fit with a limited sample size, the CFA utilized the comparative fit index (CFI) and normed for index (NFI).[27] An index value of

more than 0.90 suggested a satisfactory fit.[25] In the modelling of linear mean and covariance structures, the Tucker-Lewis index (TLI) is well-liked in preventative research.[26]

Convergent Validity and Composite Reliability

The Average Variance Extracted (AVE) was calculated to evaluate the convergent validity of the questionnaire. The AVE value must meet a criterion of at least 0.50 in order for convergent validity to be accepted. If the Composite Reliability (CR) value is at least 0.60, composite reliability has been attained.

Discriminant Validity

By using the heterotrait-monotrait ratio (HTMT) correlations, discriminant validity is demonstrated between two reflective constructs if the HTMT value is less than 0.90.[28]

Internal Reliability

The internal reliability of this latent construct was assessed by determining the Cronbach Alpha value for each of its component elements or domains. Internal reliability for all domains and the complete latent construct was considered adequate with Cronbach Alpha values greater than 0.70.[29] Internal reliability tests were conducted twice for both EFA and CFA.

RESULTS

Socio-Demography of Participants

A total of 252 respondents ie 126 for EFA and 126 for CFA adolescent boys from Kota Samarahan and Serian areas in Sarawak participated in this study. The sociodemographic characteristics of respondents are shown in Table I.

Content Validity

In this study, 19 out of 20 items acquired an "essential" evaluation from five panel experts (CVR≤0.99). One item under the permissive attitude domain, '*I believe that having sex for the first time is the best'* scored only 0.4(below 0.99). Thus this item was dropped. The panel experts view this item unsuitable for adolescent boys as it may encourage

Table I: Sociodemographic characteristics of respondents

Pilot testing	**Exploratory Factor Analysis (EFA)**		**Confirmatory Factor Analysis (CFA)**	
Socio-demographic characteristics	Frequency (%) n=126	Mean (SD) n=126	Frequency (%) n=126	Mean (SD) n=126
Age				
13	126 (100.0)	13.0	25 (19.8)	14.7 (1.3)
14		(0.0)	35 (27.8)	
15			30 (23.8)	
16			19 (15.1)	
17			17 (13.5)	
Race				
Malay	51 (40.5)		41 (32.5)	
Chinese	2 (1.6)		2 (1.6)	
Dayak	68 (54.0)		83 (65.9)	
Others	5 (4.0)			
Form				
Form 1	126 (100.0)		25 (19.8)	
Form 2			35 (27.8)	
Form 3			30 (23.8)	
Form 4			19 (15.1)	
Form 5			17 (13.5)	
School location				
Kota	126 (100.0)		76 (60.3)	
Samarahan				
Serian			50 (39.5)	
SRH-communication 1: Discuss SRH with parent				
Never	86 (68.3)		104 (82.5)	
Seldom	28 (22.2)		18 (14.3)	
Sometime	5 (4.0)		1 (0.8)	
Often	7 (5.6)		3 (2.4)	
SRH-communication 2: Discuss SRH with siblings				
Never	93 (73.8)		101 (80.2)	
Seldom	24 (19.0)		14 (11.1)	
Sometime	7 (5.6)		6 (4.8)	
Often	2 (1.6)		5 (4.0)	
SRH-communication 3: Discuss with friends				
Never	39 (31.0)		50 (39.7)	
Seldom	55 (43.7)		34 (39.7)	
Sometime	11 (8.7)		10 (7.9)	
Often	21 (16.7)		32 (25.4)	
SRH-communication 4: Did SRH information be made available at schools?				
Yes	69 (54.8)		79 (62.9)	
No	57 (45.2)		47 (37.3)	
SRH-risky behaviour 1: Watching pornography				
Never	4 (3.2)		23 (18.3)	
Occasionally	94 (74.6)		83 (65.9)	
Always	28 (22.2)		20 (15.9)	
SRH-risky behaviour 2: Masturbation				
Never	55 (43.7)		41 (32.5)	
Occasionally	68 (54.0)		69 (54.8)	
Always	3 (2.4)		16 (12.7)	
Sexual intention				
Low	81 (64.3)		100 (79.4)	
High	45 (35.7		26 (20.6)	
Self-efficacy				
Low	47 (37.5)		64 (50.8)	
High	79 (62.7)		62 (49.2)	
Attitude				
Low	114 (90.5)		102 (81.0)	
High	12 (9.5)		24 (19.0)	
Social Norm				
Low	117 (92.9)		107 (84.9)	
High	9 (7.1)		19 (15.1)	
Knowledge				
Low	21 (16.7)		11 (8.7)	
Moderate	72 (57.1)		67 (53.2)	
High	33 (26.2)		48 (38.1)	

*SRH- Sexual and reproductive health

overall S-CVI for three domain (self-efficacy, social norm and permissive attitude) were 0.97. For example, words such as "old-fashion" changed to "not-up-to-date' and 'sexual intercourse' changed to 'having sex' for better understanding. Since the number of experts were fewer than five, only items with a CVR of at least 0.99 were retained. [17,30,31]

Construct Validity

Exploratory Factor Analysis (EFA)

Table II displays the results of the assessment of the 19 items related to sexual intention, self-efficacy, attitude and social norms. The subscales used a 4-point Likert scale. The Bartlett's test of sphericity χ^2 (171) =1388.74, $p<0.001$, along with the KMO of 0.855, Suggest that the correlations between the items were significant enough to support factor analysis. This study identified four components to be the most efficient for conceptual and statistical analysis; together, they accounted for 65.7% of the variation.

Table II: Factor loadings for EFA

Item	Factor				Uniqueness
	1	2	3	4	
C2	0.901				0.1555
C3	0.869				0.221
C1	0.823				0.273
C4	0.631				0.336
C5	0.559				0.324
F1		0.891			0.216
F2		0.765			0.342
F4		0.630			0.607
F3		0.606			0.549
E1			0.799		0.376
E4			0.504		0.428
E2			0.473		0.545
E6			0.432		0.526
D2*			0.431	0.402	0.512
E5				0.576	0.391
D1				0.539	0.544
D4				0.501	0.651
E3				0.443	0.641
D3				0.435	0.514

** Item deleted*

The Scree Plot in Figure 2 suggests that this latent construct has four dimensions or components. Which item corresponds to which components or domains was determined by the rotated component matrix. Items with factor loadings of at least 0.4 were kept for further analysis.[29] At this level, one item was removed. Thus, the 18 items were kept .

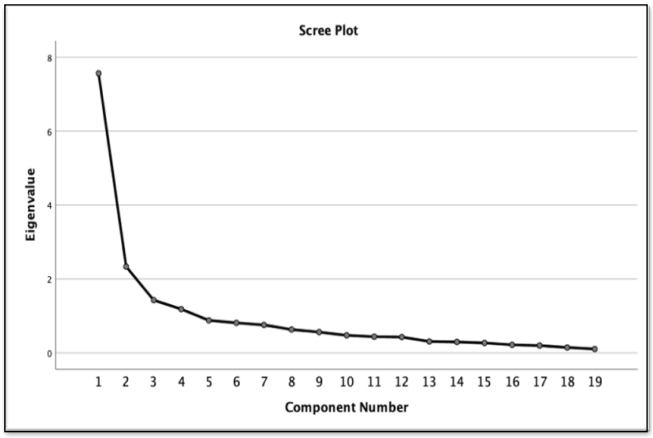

Figure 2: Scree Plot of Eigenvalues and component number

Which item corresponds to which components or domains was determined by the rotated component matrix. Further analysis was conducted on the items that had factor loading of at least 0.4.[29] At this level, one item was removed. The response to the attitude component's question, *'Youths should have sex before getting married to determine if they are compatible,'* has been removed. Hence the retained items were 18.

Confirmatory Factor Analysis (CFA)

CFA included four factors and 18 items to corroborate factor structure. The CFA procedures removed three items because of poor communality and low factor loadings after the factor loading characteristics of each item were analysed. Certain items were interpreted differently causing them to be splitting. The 4-factor/15-item scale that remains after three items are removed offers the minimal level of model fit. Absolute Fit category has met the thresholds of >0.05 and <0.08 respectively, with a p-value of 0.00 and the RMSEA of 0.06. The TLI was 0.98 and the CFI was 0.98, both of which met the >0.90 requirement for the incremental fit category. Meanwhile, the ratio of ChiSq/df for the Parsimonious Fit category was 1.54, which has achieved the threshold of <3.00. As a result, the questionnaire satisfies the criteria for the construct validity assessment. [32]

Convergent Validity and Composite Reliability

In order to evaluate the questionnaire's convergent validity, the Average Variance Extracted (AVE) was calculated. Based on the findings of AVE and CR for

every component or domain, the questionnaire satisfies all requirements for composite validity and convergent validity.[32]

Table III: AVE and CVR for each component in the model

Domain	Item	Factor loading	CR	AVE
Sexual intention	C1	0.86	0.93	0.77
	C3	0.89		
	C5	0.91		
	C4	0.85		
Self-efficacy	F1	0.93	0.88	0.64
	F2	0.93		
	F3	0.71		
	F4	0.58		
Attitude	E4	0.83	0.80	0.57
	E2	0.69		
	E6	0.75		
Social Norm	E5	0.83	0.82	0.54
	D1	0.65		
	D4	0.63		
	D3	0.81		

AVE indicates Average Variance Extracted, CR indicates Composite Reliability

Discriminant Validity

According to the results, the Heterotrait-Monotrait (HTMT) ratios of self-efficacy, social norm and attitude with sexual intention are acceptable at 0.53, 0.83 and 0.70. The ratio of attitude and social norm with self-efficacy also acceptable. Meanwhile, attitude toward social norm is acceptable under the cut-off point of 0.90.

Internal Reliability

Following dimension reduction, this latent construct internal reliability and each of its components or domains was assessed by determining the Cronbach alpha value which for the 15 items was 0.89.

The Final Questionnaire and Its Scoring Procedure

Four components were identified from the final set of questions in the Sexual Intention Questionnaire for Adolescent Boys (SIQ-AB), which consisted of 15 items. a rating derived from the answers on a 4-point Likert scale, where 1 represents strongly disagree and 4 represents strongly agree.The domains were; Domain 1: A higher intention to have sex is indicated by a higher total score on items 1-4 (siq1-siq4); Domain 2: A higher total score of items 5-8 indicates a higher perception of self-efficacy in performing sexual activity (siq5-siq8); Domain 3: A higher total score of items 9-11 indicates a higher perception of social norms on premarital sex (siq9-siq11); Domain 4: A higher score of items 12 to 15 indicates a higher permissive attitude towards premarital sex (siq12-siq15). Table IV tabulates the final items on the Sexual Intention Questionnaire for Adolescent Boys (SIQ-AB)

Table IV: Final items on the Sexual Intention Questionnaire for Adolescent Boys (SIQ-AB)

Item	Question
siq1	I expect to have sex with my partner.
siq2	I intend to have sex with my partner.
siq3	I would like to have sex now if I could find a partner who would do it with me.
siq4	I would like to have sex to see what it is like.
siq5	I know when I can have sex.
siq6	I know where I can have sex.
siq7	Whether to have sex or not is entirely up to me.
siq8	I can decide on my sexual activity.
siq9	Most of my friends think that men are allowed to have sex before marriage.
siq10	Most of my friends think it is mature to practice sex at my age.
siq11	Most of my friends think that youths who have never been involved in having sex before marriage are not-up-to-date.
siq12	Most of my friends think that you can have sex before marriage if you are in love.
siq13	I believe youths who have never been involved in having sex before marriage are not-up-to-date.
siq14	Youths can have sex if they are unable to control their sexual desire.
siq15	Youths can have sex provided they use methods to stop pregnancy.

DISCUSSION

The concerning trend is the decreasing age of adolescents who take risks of having sex when they are still young.[33] There are limited instruments targeted specifically to adolescent boys. The aim of this study was to assess the reliability and validity of the Sexual Intention Questionnaire for Adolescent Boys (SIQ-AB) to measure knowledge, permissive attitude, social norms, and self-efficacy toward sexual intention. The overall finding suggests four domains with 15 items to measure knowledge, permissive attitude, social norms, and self-efficacy toward sexual intention among Malaysian adolescent boys. In light of these findings, the SIQ-AB can be considered a reliable and practical method to measure Malaysian adolescent boys' sexual intentions.

Studies have shown that practitioners rarely ask about different aspects of adolescent sexuality since they do not know how to handle the situation and do not want to offend their young clients.[34,35] Conversely, adolescents' concerns and inquiries about their sexuality are growing.[36]

However, several studies have focused on how adolescents could respond to inquiries about their sexual orientation or what kinds of queries might be most appropriate. In order to comprehend how they react to sex better, this study validates a questionnaire originally developed to assess sexual intention.[10]

The current SIQ-AB solely focuses on adolescent boys. Muhamad's previous questionnaire was targeted at adolescents aged 18 years old and above.[10] Thus the researcher, hypothesized there are items that might not be suitable for adolescents below 18 years old. The current YSI-Q questionnaire used in Malaysian context, measured sexual intention among young people above 18 years old.[10] Since the peak age for sexual debut among adolescent worldwide is between 15-17 years old, and in Malaysia as low as 14 years old, there is a need to design questionnaire for this younger group. Hence, the availability of the SIQ-AB questionnaire, which targets adolescent boys between 13-17 years old, is suitable for a country like Malaysia. In the context of this study, early detection of sexual experience among younger age group can be used to plan first line prevention programmes.

In face validity, one item *'I believe that having sex for the first time is the best'* was excluded by the expert panel. The panel expert perceived that this item may encourage adolescents to have sex at an early age, which is culturally inappropriate. This was supported in a study which stated that in Asian countries, sex is prohibited at an early age and culturally inappropriate.[37] Premarital sex is forbidden in most major religions. In Islam, the person who commits premarital sex is committing 'zina' (adultery) which is considered a major sin.[38]

In this study, two items pertaining to the Social Norm; *'Most of my friends think that you can have sex before marriage if you are in love'* and *'Most of my friends think female youths do not have to maintain their virginity*', were loaded in Attitude factor. Theoretically, social norms and attitude are two different definitions, but in the context of Malaysian adolescent boys, it could be the same, in how you practice and follow it. When individuals are in groups, there are certain rules, norms, or beliefs about proper attitude or behaviour, and they may have certain behaviour regarding the reference group.[39] In adolescents, sexual behaviour is becoming more common. Thus, peer norms around sexual behaviour are likely more permissive than the norm established by parents.[40] In other words, peer norms are the individual's perceptions on whether close friends and family members believe he or she should engage in the behaviour. These perceptions are said to reflect the level of social pressures placed on a person to adopt a particular attitude or behaviour.[41]

Another two items originated from Attitude ie *'Teenagers need to have sex before marriage to ensure that the future partner is suitable'*, and Sexual Intention ie *'I want to have sex with my partner*' were removed. The removed sexual intention item is in accordance with Malaysian Child Act 211 (611) which stated that the consensual age for sex is 18 years old and above.[42] Hence, the item '*I want to have sex with my partner*' in this study is inappropriate for adolescents below 18 years old.

Developing a measurement tool using a behavioural theory such as TPB to assess sexual behaviour could help to design a tailored intervention targeted to adolescent boys. This is important as adolescent boys are at high risk for unhealthy sexual behaviour consequences.[7,33] While there may be some overlap with tools designed specific attention to both gender, boys may be pressured to conform with societal expectations of masculinity and traditional gender roles.[7] A new tools such as SIQ-AB could explore how these expectations influences boys' self-perception, relationships, emotional, expression and behaviours while also promoting healthy masculinity and challenging harmful stereotypes.

Besides some research suggest that adolescent boys are more likely than girls to engage in risk-taking behaviours, such as substance use and unsafe sexual practices. Tools for adolescent boys should provide strategies for assessing risks, making informed decisions, and resisting peer pressure to engage in risky behaviours.[5,43] In addition, the conduct of sex education for adolescent boy and girls should be separated to avoid embarrassment and discomfort.

Overall, the SIQ-AB instrument for adolescent boys had relatively good support for the four-factor model and 15 items which explained 65.7% of the variance. Internal consistency for the four-factor model (sexual intention, self-efficacy, social norm, and attitude) and 15 items were acceptable. According to the Theory of Planned Behaviour (TPB) introduced by Ajzen and Fishbein in 1988, predictions of behavioural performance are influenced by intentions, self-efficacy, social norms, attitude, and the perception of control over a conduct. Intentions are also hypothesized to be predicted by self-efficacy, attitudes, and subjective norms.[44] This showed that SIQ-AB instrument theory based-instrument supported by TPB construct. For example, study conducted in Tehran, Iran assessed the effect of TPB construct based educational intervention on attitude, social norms, parental control, behavioural control, and sexual intention among adolescent girls.[45] Furthermore, every TPB construct was essential in helping the students adopt better sexual and reproductive health practices.

CONCLUSION

The finalized Sexual Intention Questionnaire for Adolescent Boys (SIQ-AB) has 4 domains and 15 questions. Acceptable psychometric characteristics, strong internal consistency, and convergent and composite validity are all features of the sexual intention questionnaire for adolescent boys. It is intended that by making the questionnaire available, other academics may further validate it and possibly apply it in their studies.

FUNDING

This study was partially supported by the Ministry of Health and the Ministry of Education.

INSTITUTIONAL REVIEW BOARD (ETHIC COMMITTEE)

Ethical approvals were obtained from the National Medical Research Register (NMRR) [NMRR ID-22-00010 -DIT], and institutional Medical Research Ethics Committee (MREC) [UNIMAS/NC-21.02/03-02 Jld.3 (85)].

ACKNOWLEDGMENT

These authors would like to thank the University Malaysia Sarawak, the Ministry of Health, and the Ministry of Education. Thanks also to the respondents who participated in this study. Finally, not forgotten for all those who have been involved either directly or indirectly in the assistance and support given.

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ORIGINAL ARTICLE

Prior Knowledge, Acceptance, Adaptation, and Challenges Following Stoma Formation among Colorectal Cancer Patients in Northern Peninsular of Malaysia: A Qualitative Study

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ABSTRACT

INTRODUCTION: Stoma formation affects an individual in various ways, including physical, emotional, social, and cognitive functions. Diverse studies report ways of an individual lives with new stoma formation. However, the comprehensive understanding of the entire process by the patient, which includes knowledge before the surgical procedure, as well as the subsequent acceptance, adaptation, and challenges to living with a stoma is lacking. **MATERIALS AND METHODS:** In-depth interview session were conducted with 12 colorectal cancer patients who have undergone surgical procedures for intestinal stoma formation. The patterns and themes within the data were identified by thematic analysis, involving data familiarisation and coding followed by themes' generation and refinement of the themes. **RESULTS:** Four themes and 9 subthemes were identified, which revealed the sufficiency of stoma-related information and understanding prior to surgery as well as positive acceptance of self and family members reflected through their reactions and support. Nonetheless, the challenges were anticipated which highlights the complications of the stoma itself, obstacles surrounding social life, and financial burdens. **CONCLUSION:** This study provided valuable insights into the experiences of individuals living with a stoma following colorectal cancer surgery. The themes and subthemes highlight the need to address social stigma as well as financial issues to alleviate the burden of stoma-related expenses. Increasing public awareness and improving financial assistance could be a way to enhance the overall quality of life for patients living with stoma.

Keywords
Stoma, prior knowledge, acceptance, adaptation, challenges

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Received: 7th May 2024; Accepted: 26th July 2024

Doi: https://doi.org/10.31436/imjm.v23i04

INTRODUCTION

Living with a stoma among colorectal cancer (CRC) is a frequent condition seen in the communities in many parts of the world. Approximately one in 500 persons are living with a stoma, which contributes to an estimated 21,000 formation of stomas each year in the United Kingdom.[1] In Japan, it was reported that 29.6% of permanent stoma was done for male patients with poor healing of anastomotic leakage.[2] A study conducted in a single centre in Singapore reported about 93 colostomy were done in five years.[3] Intestinal stoma is created through colostomy or ileostomy surgical procedure, mostly done if gastrointestinal malignancy and other causes of intestinal obstruction, trauma, bowel ischemia, and inflammatory diseases, as part of the treatment that requires faeces diversio.[4-7] In stoma formation, the waste matter is collected in a bag that is attached to a small opening on the surface of the abdomen.[7-8] There are different types of stomas based on the location created along the colon, which can be temporary or permanent.[6,9] Temporary stomas are reversible, where reconnecting the remaining parts of the bowel by further surgery to allow the patient to defecate normally, while permanent stomas are irreversible and thus retained lifelong.[7,10,11] The formation of both types of stomas are to improve the situation of the patients by removing symptoms and parts of treatment.[10] Despite the benefits stomas provide, there were complications reported post construction of the stoma locally, causing skin irritation, re-construction issues, and loss of control

of bowel opening including loud flatulence and leakage.[4,12] The presence of stoma is also shown to have an impact the patients self-confidence concerning appearance and self-image.[4] In addition, previous studies demonstrated that the quality of life (QOL) among patients living with stoma also affected in general, regardless the type of stoma.[5,11,13] As a result, living with a stoma leads to an alteration in their physical activities and psychosocial responses.[14,15] Delays in adapting to living with a stoma and less social support from family members increase stress of these patients, leading to feeling of stigma and restricted.[13,16-18] This showed that living with a stoma influences the general aspect of their overall QOL[19] (ideas repeated). The data on stoma formation based on the perspectives patients is scarce including in Malaysia. Thus, this study was aimed to explore the perspectives on the acceptance, impact, and QOL CRC patients who must live with a stoma.

MATERIALS AND METHODS

Study Design and Participant

A phenomenological study design was adopted, using purposive sampling. An individual who was diagnosed with CRC in Hospital Sultanah Bahiyah (HSB), over the age of 18 years who had undergone stoma formation surgery (both elective and emergency surgery) within a month period and can communicate in Malay or English were recruited. In all 12 Patients were recruited. The reporting of this study complies to the information of the standard for reporting qualitative research (SRQR).[20]

Data Collection

The interview was conducted using a semi-structured guided, developed by the authors following a five-step process.[21] The process begun with important review by all authors in developing the interview questions to reflect the study objectives based on previous available knowledge.[4,13,14,22,23] To avoid biases and also ensure the appropriateness of the content, two other experts from the related field were also invited to revise, validate, and improve the interview questions. Later, the preliminary semi-structures interview guide was developed and tested for a pilot study with two participants who met the study criteria to refine the interview questions. Finally, a set of several interview questions was developed, covering the participants' knowledge regarding stomas prior to the procedure, their experiences with close friends or family members afterward, and also the difficulties they (as patients) and their caretakers encountered in adjusting to life with a stoma. The first author is a medical doctor, and the second author is a research officer both have experience in conducting qualitative research-and work in a clinical research department and are involved in multiple clinical trials surrounding the CRC area. The second author is a certified translator of Malay to the English language.

The third author is a nurse who- is a certified stoma care educator who works is a surgical department. Potential participants were approached during their admission in the wards in Hospital Sultanah Bahiyah and briefed them on the objectives and methods of the study. All queries and concerns were answered accordingly before consent from the patients were obtained. The interview sessions were arranged according to the availability of the patients. Some participants were willing to be interviewed immediately after the consent was obtained, but others for personal reasons wished to proceed with phone-call interviews. Phone-call interviews were arranged within a week of obtaining the consent. The interview sessions were done between 3rd July and 1st August 2022, conducted in the presence of all the authors. The duration for each interview session varied for approximately 34 to 57 minutes.

Data Analysis

The audiotaped interviews were transcribed verbatim and translated within a week of each interview session. To ensure accuracy, completeness, and consistency, the transcripts were reviewed carefully. We adopted the six-stage comprehensive thematic analysis (TA) approach by Braun and Clarke in conducting the TA, included reading the transcripts multiple times to immerse in the content, identifying the meaningful quotes and describing them into codes, and developing themes from the collated codes and subtheme to create a relationship with the theme.[24] To ensure data consistency, triangulation was conducted between all authors. The final themes that suited the

objectives are presented herein. The interview sessions were stopped when the data had reached saturation points.[25] No repeat interviews of patients were carried out.

RESULTS

Characteristics of Participants

Most of the 12 participants were females (~58%, n=7), of Malay ethnicity (~83%, n=10), with educational level up to secondary school (~75%, n=9), and married (~83%, n= 10) (Table 1).

Themes and Subthemes

Four themes and eleven subthemes were identified from the analysis.

Theme 1: Prior Knowledge

Subtheme 1: Information From the Attending Physician

The participants reported that they were informed by the attending physician about the importance of stoma formation, to relieve the symptoms and there will be some changes to their life routine.

"Right before the operation, the doctor had a brief chat with me and mentioned that because of the tumour, I wouldn't be able to pass stool as usual. He explained that getting a stoma could help solve this problem." (#01)

"Hmm, the doctor informed me that the tumour near my anus was causing severe constipation. He explained that after the surgery, it wouldn't be possible to reconnect the colon to the anus opening. That's why they recommended creating a stoma to help me defecate." (#03)

Subtheme 2: Patient's Understanding of Stoma Formation and Function

Following the explanation provided, the understanding of stoma formation and function among the participants is acceptable.

"The colon was connected to the abdominal wall like an opening for passing stool" (#02)

The participants understand that the stoma creation is part of the treatment strategy:

"Because I got tumour near the anus, I cannot pass faecal normally, so the doctor connecting my colon with a stoma bag, meanwhile, I need to undergo 12 cycles of chemotherapy. And if the healing process is well, the doctor might reconnect back." (#11)

These facts assisted them in appreciating the stoma formation even before the surgery happened:

"After the stoma formation, I feel better and comfortable, I can eat and pass stool well and it easier for me to go anywhere" (#04)

Theme 2: Acceptance

Subtheme 1: Individual Acceptance

In general, most of the participants felt unhappy the first time saw the stoma. Their most concerning issue was to manage the stoma on being discharged. Eventually, the participants reported that they could accept the stoma completely:

"Initially, I really cannot accept it. To manage the stoma and the bag is a bit complicated. But after, I get used to it." (#05)

"I felt worried in the beginning. I think it is difficult to wear and carry the stoma bag, I felt sad, but now…I'm okay" (#06)

Subtheme 2: Family Members' Acceptance

The participants claimed that their family members could accept the fact that they need to live with stoma but there is some concern regarding the development management and of later complications:

"My family was worried about me having to live with a stoma. Ultimately, they are fine and assisted me to manage it" (#07)

"My children supporting me and helped me to manage the stoma, I feel so grateful" (#01)

"Initially I felt that I'm a burden to my family. But they are fine and supporting me" (#09)

Theme 3: Adaptation

Subtheme 1: Self-Managing of Stoma

At initial phase, the participants reported that they were assisted by other family members in managing the stoma before they are on their own:

"Before this, my daughter helped me. But now I can do it myself, it is easy" (#06)

"At the beginning, it is difficult. But after a while, I get used to it. My children care and help me" (#05)

Subtheme 2: Support of Family Members

Participants reported that they get full support from their spouses, children, and their colleague. They have no problem doing routine activities like sleeping and eating together.

"All my family members are positive about the situation, and they advise me to accept this. There is no issue with eating and sleeping together with them" (#02)

"I'm grateful because my friends, and family members, they did understand and accepted my condition" (#09)

Theme 4: Challenges

Subtheme 1: Complication from The Stoma

Despite positive acceptance and adaptation, they claimed that wearing a stoma bag somehow makes them feel some discomfort and awkwardness in doing routine activities.

"I find it difficult to take care of this. It is dirty. I must change the bag every two to three days. My wife helped me. It is a bit challenging to go out and do activities. I did experience leaking and limitation in doing activities" (#08)

The complication is even harder for the participants with a visual disability and food allergies:

"My eyes can't see clearly where a hole is to stick the bag. So, I need help from my children. Sometimes I got some rashes surrounding the skin. If I ate certain food…I got itchy, but it resolved after applying cream" (#01)

Subtheme 2: Social Life

Almost all participants reported that their social life is very much affected, particularly when it comes to managing the stoma bag when they are out in public and surrounded by other people. One of the participants detailed:

"I'm unable to control the bowel open anymore. When I go visit some friends at their house, I'm afraid to eat because usually after eating the stools will come out...I felt embarrassed to manage this at someone's house. If I do it at a gas station, the equipment is not complete, and difficult for me. At the beginning, it was hard to adapt. Later, I get used to it and prepared everything in the car boot so that it will be easier for me to manage it everywhere I go" (#09)

A Muslim participant stressed the challenges when it comes to performing prayer:

"I went to the mosque for prayer, when the gas coming out from the stoma, occasionally it makes a loud sound and I feel embarrassed" (#10)

Meanwhile, participants clarified that the stoma formation indirectly affected their sex life.

"My genital...I think it is functional but not as good as before. I mean...sometimes it's not functioning at the right time but luckily my wife understands this situation. Sometimes this issue can cause marriage problems" (#09)

"I tried having sex with my wife. But it cannot last long, and I became tired instantly. My wife is not complaining as this is beyond my control" (#08)

Subtheme 4: Financial Burden

Most of the participants were unable to continue working after the surgery. They reported that living with the stoma increases their cost of living.

"For me, it is a bit of burden, I cannot work anymore. Need to buy the stoma bag and the price is quite expensive…depends on the quality" (#08)

"I need financial assistance to reduce my financial issues. In this situation, I regularly need to buy spray, cotton, and stoma bags. I did apply for financial assistance from the welfare department, but it was rejected since I got a business registered" (#09)

"For me, financially burden, I don't have enough money to buy all the stuff. It can cost up to RM10 per day or for 2 days depending on how frequently I changed the stoma bag. Estimated about RM300 needed to be spent per month, to buy the cotton, the sterile water, sometimes it will take up to RM400 per month." (#03)

Financial aid is an important element of support that is needed by the participants. At this stage of disease, they lose their employment due to physical incapability and treatment engagement. The participants claimed they got financial aid but not enough to cover the entire cost.

"I got financial aid but not enough, I still need to add from other sources to accommodate the cost…and I need to reapply for the financial aid every 6 months" (#03)

"I feel quite a burden… I got financial assistance from "*zakat*" every month… this helped me a lot of cause to buy the stoma bag is too expensive" (#07)

DISCUSSION

The study explored the individual perceptions of living with stoma among CRC patients. The information that is valuable to be brought into attention, especially to the health care provider and related agencies in achieving better outcomes to improve the quality of life among the patients and their caretakers. Majority of study patients had sufficient basic knowledge of stoma formation and its function, which they had acquired pre and post-operatively from the healthcare providers. This helped them psychologically in better accepting of the stoma. This is consistent with a previous study where understanding the stoma formation and its function is crucial in managing the stoma, and being mentally prepared in the aspect of stoma appliances, appropriate exercise, proper diet, and its complications.[26]

Furthermore, a comprehensive evaluation was conducted to determine whether education of patients with stoma formation improves their quality of life and is cost effective. This study demonstrated that structured patient education tailored to patients' psychosocial needs appears to have a positive effect on both quality of life and cost, and the procedure could be carried out before, during or following the admission.[27] Poor acceptance and adaptation among the stoma patients were anticipated. The participants felt insecure and concerned about managing the stoma at home immediately after getting discharged. However, they were fortunate to have great family support and empathy.

Realization and support from family members are critical in reducing the burden of the individual in coping with the situation.[22] Acceptance of bodily change following stoma formation is important to be achieved soon after operation to expedite the familiarity with the practical management skills of the stoma including its possible later complication.[28] The complications from stoma formation starting from the first year up to year five of the formation, are categorized into early and late complication, including skin excoriation and leakage from the appliance.[29-31] Early developed complications such as stomal ischemia, retraction, parastomal abscess, and mucocutaneous separation, and late complications included parastomal prolapse, hernia, retraction, and varices.[29,31] Participants clarified that they experienced similar to those commonly reported complications throughout the period. however, it is tolerable. Participants were assisted by their family members to manage the stoma in the early days but later, they were able handle it themselves. The stoma has been reported affected the individual relationships with their caretakers, in the aspects of emotional, health, and economic.[24,32,33]

However, this study found that the relationship between participants and their spouses, and the people surrounding them are significantly not affected in that the relationships were not changed.

Nevertheless, participants reported that their sexual relationship is affected indirectly by the stoma, as the root of the issue could be probably due to the complications of the cancer itself, which caused them to be easily tired on exertion and malfunction of the genital organ. Stoma formation can affect sexual ability in various ways, attributable to feelings of being unattractive, sexual performance anxiety, and issues related to stoma appliances like leakage and odour.[35] Moreover, participants reported that they needed a few months to resume back to the normal routine especially taking part in the heavy duties, due to fear of harming the stoma.[36] Social lives of the participants are affected in general, they felt embarrassed, stigmatized, and had low self-esteem while in public, especially for the Muslim community during performing prayer in congregation.

However, to overcome these issues, the National (Malaysian) Fatwa has announced the decision of the 79th National Fatwa Declaration concerning the Law on the Implementation of the prayer for patients with stoma bag which the patient with stoma bag is categorized as those who have an emergency problem and are not considered to be bearer of dirt. Therefore, he/she does not need to clean the stoma bag every time want to take *wudhu* and perform prayers. Notwithstanding, they should take *wudhu* every time before performs the prayer.[37] Moreover, previous studies have reported that stoma affected their physical, mental, and social well-being.[32,38] The participants clarified they were encountering financial constrain since living with the disease and stoma, as they are unable to continue working. Furthermore, it was reported that the individuals who were younger with income instability showed distress on their employment and financial status while individual who is retired and with continuous income reported to be more tolerant in accepting the stoma.[39] Most of the participants needed financial assistance in managing the stoma. Partly of them got financial aid from an authorized government agency, although it is not enough to cover everything, the financial support can ease parts of their financial burden. Thus, it is crucial for stoma educator (stoma nurse/physician) to provide correct education and management of stoma to improve the QOL of the patients, especially prior knowledge, so the patients are adequately prepared both physically and mentally before undergoing the procedure.

CONCLUSION

In this study, individuals with stoma formation were mentally prepared to start their journey with stoma alongside getting support from family members. So, they can cope with complication and social issues. However, sexual dysfunction is an issue mainly among male patients. An individual who is unable to earn for living faces a financial burden and is supported by other family members. This information could be a guide to relevant authorities in revising their priority of financial aid, or the public health sector in refining their health awareness campaign which ultimately could improve the QOL of patients.

RECOMMENDATION

A prospective study should be conducted in the future to address and understand the effect of stoma over a longer time. The combination of qualitative and quantitative research should be done to obtain richer information on this issue.

LIMITATION

This study did not include the perspective of the stoma educator, this information should be explored to determine any deficiencies in stoma education and could enhance patients' knowledge in the future.

Table 1: Summary of socio-demographic characteristics of the participants

Patient code	Age (years)	Gender	Ethnicity	Level of education	Occupation	Marital status
#01	71	Female	Malay	Secondary	Housewife	Married
#02	28	Female	Malay	Tertiary	Unemployed	Single
#03	53	Male	Malay	Tertiary	Unemployed	Married
#04	64	Female	Malay	Secondary	Housewife	Married
#05	69	Female	Malay	Secondary	Housewife	Married
#06	53	Female	Malay	Secondary	Housewife	Married
#07	22	Female	Malay	Secondary	Unemployed	Single
#08	56	Male	Chinese	Secondary	Retired	Married
#09	53	Male	Malay	Tertiary	Fisherman	Married
#10	56	Male	Malay	Secondary	Bank officer	Married
#11	63	Male	Chinese	Secondary	Retired	Married
#12	53	Female	Malay	Secondary	Housewife	Married

INSTITUTIONAL REVIEW BOARD (ETHIC COMMITTEE)

This study was approved by the Medical and Ethics Committee, Ministry of Health Malaysia (NMRR ID-22-00251-0XV).

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ORIGINAL ARTICLE

Evaluation of Tumour-Associated Macrophages and Colony-Stimulating Factor-1 Expression in Invasive Breast Carcinoma and Their Association with Prognostic Parameters

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ABSTRACT

INTRODUCTION: Recent breast cancer research has focused on tumour microenvironment (TME). Tumour-associated macrophages (TAMs) are the key players in TME as they provide pro-tumorigenic milieu for tumour progression and metastasis. These macrophages are primarily regulated by colony-stimulating factor-1 (CSF-1) secreted by breast cancer cells. This study investigated the association of localization of TAMs infiltration within breast carcinoma and CSF-1 expression by cancer cells with the pathological prognostic parameters. **MATERIALS AND METHODS:** TAMs were assessed in 128 cases of invasive breast carcinoma by CD163 immunohistochemical expression. The median TAM density in both the tumour nest and tumour stroma was utilized to classify TAMs into categories of low and high infiltration. The cancer cells were immunostained with anti-CSF-1 antibody and the staining intensity was evaluated as low or high expression. **RESULTS:** High nest and stromal TAMs were associated with higher tumour grades (p=0.005 and p=0.0001, respectively) whereas only high stromal TAMs showed significant association with negative oestrogen and progesterone receptors status (p=0.001 and 0.001, respectively); and triple-negative subtype (p=0.002). High CSF-1 expression was significantly associated with high stromal TAMs (p=0.031). High CSF-1 expression was associated with tumour grade and positive HER2 status (p=0.008 and 0.007, respectively). **CONCLUSION:** TAMs in tumour nest and stroma showed varying degrees of association with the clinicopathological parameters. High CSF-1 expression was associated with unfavourable prognostic parameters. Therefore, the evaluation of TAMs and CSF-1 expressions could potentially serve as prognostic markers and cellular targets for novel treatment modality in invasive breast cancers.

Keywords
breast cancer, colony-stimulating factor-1, tumour-associated macrophages, tumour microenvironment

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Received: 22th November 2023; Accepted: 11st July 2024

Doi: https://doi.org/10.31436/imjm.v23i04

INTRODUCTION

Breast cancer is the most frequently diagnosed cancer in females and is also a leading cause of death in most countries.[1] In Malaysia, breast cancer accounted for 34.1% of all cancers among women.[2] Breast cancer is a clinical and pathological heterogeneous disease. Vast evidence has suggested that breast cancers exhibit distinct behaviours and different treatment responses regardless of the histological subtypes.[3] Despite current recommendations regarding prognostic and predictive factors, breast cancer is difficult to treat, and additional parameters are required to further stratify patients for personalized and ideal treatment. Recent cancer research has partly shifted focus to tumour heterogeneity, particularly the molecular and cellular mechanisms of cancer cells as well as the tumour microenvironment (TME). The TME is the non-cancerous cells surrounding the tumour and encompasses heterogeneous populations of stromal cells and different types of immune cells.[4] Tumour cells recruit these supporting cells into the TME, which in turn promotes cancer cell growth and metastasis.[5] Macrophages are the major immune cells within the microenvironment, and in tumours, they are referred to as tumour-associated

macrophages (TAMs). Generally, TAMs can be classified into classically activated (M1) or alternatively activated (M2) subtypes depending on the specific provoking factors involved. In early-stage tumours, TAMs are predominantly of M1 phenotype which exerts pro-inflammatory effects. As the tumour advances, the macrophages polarized to M2 phenotype essential for tumour progression.[6]

CD68 and CD163 are glycoproteins expressed in monocytes and tissue macrophages and are widely used markers to detect TAMs in several cancer types. CD68 is relatively non-specific as it recognizes both M1 an M2 macrophages and is also expressed by a wide range of cells including fibroblasts, granulocytes, dendritic cells, endothelial cells, and some lymphoid subsets.[7] On the other hand, CD163 is a highly specific monocyte/ macrophage marker for M2-polarized macrophages.

High TAMs infiltration was associated with aggressive biological behaviours such as larger tumour size, higher tumour grade, lymphovascular invasion, and hormone receptor negative breast cancers.[7] However, there are conflicting data regarding association of TAMs and breast cancer prognosis. Some studies have found that there were no association between high TAMs infiltration with positive lymph node status, vascular invasion, and HER-2 expression.[8,9] These discrepancies may be due to the different methodologies used for histological assessment of TAMs, different cut-off values for definition of TAMs density, and different detection markers used.

Several studies have analysed association between total TAMs in tumour and poor prognosis without taking TAMs localization into account.[10,11] Meanwhile in other studies, they focused on the importance of TAMs localization in breast cancer tissue. One study found that increased CD163-positive TAMs in tumour stroma was correlated with unfavourable clinicopathological factors and overall survival (OS) of cancer patients; however, they did not find any statistical significance with TAMs in tumour nest.[12] On the contrary, another study showed that higher number of CD163-positive TAMs infiltration in tumour nest was correlated with unfavourable OS.[13] These conflicting findings warrants further investigations.

The crosstalk between tumour cells and the TME is initiated by various cytokines, chemokines and growth factors; and the main link between tumour cells and TAMs is colony-stimulating factor 1 (CSF-1). CSF-1, also known as macrophage colony-stimulating factor, is an important growth factor involved in cell differentiation, proliferation and activation via binding to the CSF-1 receptor (CSF-1R) expressed on macrophages.[11] CSF-1 is secreted by various types of cells such as monocytes, fibroblasts, endothelial cells and tumour cells. The paracrine signalling between breast cancer cells and TAMs is important for tumour progression and metastasis. Tumour cells secrete CSF-1, which is received by the CSF-1R on macrophages. In turn, TAMs upregulate the secretion of epidermal growth factor (EGF) and subsequently bind to the EGF receptor on the tumour cells.[14] EGF promotes the expression of CSF-1 by tumour cells, thereby generating a positive feedback loop. The EGF/CSF-1 positive feedback loop enhances the survival and proliferation of tumour cells and facilitates tumour cells to metastasize to secondary organs.

Studies have shown that breast cancer cells with high CSF-1 expression are associated with poor outcomes in both metastatic and non-metastatic breast cancers.[15,16] High CSF -1 expression is significantly correlated with poor clinicopathologic prognostic parameters such as larger tumour size, higher tumour grade, negative hormone receptor status, and HER2 positivity.[17] Hence, the detection of CSF-1 expression provides prognostic information in breast cancers. Moreover, new cancer treatments targeting CSF-1 and TAMs are emerging through reducing the number of TAMs in the TME and re-programming TAMs to anti-tumour phenotype.[18] LY3022855 is an example of monoclonal antibody directed against CSF-1R on macrophages by inhibiting the binding of CSF-1 on the receptor. Although a phase 1 study of LY3022855 in advanced refractory breast and prostate cancers showed limited clinical response of the subjects, there were evidence of immune modulation of TAMs in the tumour cells after therapy which warrants further evaluation.[19] In view of their prominent roles in breast cancer progression, more studies on TAMs and CSF-1 expression in breast cancer specimens as potential prognostic markers and their clinical application are required to stratify patients for targeted immunotherapies.

Therefore, this study aimed to evaluate the degree and histological localization of CD163-positive TAMs in invasive breast carcinoma, the proportion of CSF-1 expression and its association with the degree and histological localization of CD163-positive TAMs. We also aimed to investigate the association between degree of TAMs infiltration and CSF-1 expression with the pathological prognostic factors in invasive breast carcinoma at Sultan Ahmad Shah Medical Centre International Islamic University Malaysia (SASMEC@IIUM) and Hospital Tengku Ampuan Afzan (HTAA), Kuantan, Pahang, Malaysia.

MATERIALS AND METHODS

Sample Collection

This cross-sectional study involved 128 mastectomy specimens diagnosed as invasive breast carcinoma of no special type (NST) from January 2017 to December 2020 at SASMEC@IIUM and HTAA. Ethical approval from the IIUM Research Ethics Committee (IREC 2020-159) and National Medical Research Registry (NMRR-21-3749-38944 (IIR)) were obtained. The slides of these cases were reviewed by an experienced histopathologist to select the representative tumour tissue blocks containing the tumour nest and stroma. For each case, a representative formalin-fixed paraffin-embedded tumour tissue block including tumour stroma was retrieved. Clinicopathological data – patient age, gender, race, tumour size, histological grade, lymph node involvement, the status of oestrogen and progesterone receptors, and human epidermal growth factor receptor 2 (HER2) expression – were retrieved from pathology reports.

Immunohistochemical Staining Method

Tissue sections (3-µm thickness) were prepared on pre-coated slides and were heated in an oven for 20 min at 67° C. Two primary antibodies were used in this study: rabbit recombinant monoclonal CD163 antibody (Code EPR19518, Abcam, Cambridge, UK) and rabbit recombinant monoclonal CSF-1 antibody (Code SP211, Abcam, Cambridge, UK). Normal spleen and tonsil tissues were used as positive controls for CD163 and CSF-1, respectively. Immunohistochemical staining was performed using the VENTANA Immunohistochemistry Auto Stainer Benchmark ULTRA (Ventana Medical Systems, Inc., Oro Valley, AZ, USA). The tissue sections were baked for 16 min at 60°C and de-paraffinized in Ventana EZ Prep solution. Endogenous peroxidase blocking with ULTRA-View Universal DAB Inhibitor 3% was used for antigen retrieval and the slides were incubated in primary antibodies CD163 and CSF-1 at dilution 1:500 and 1:70, respectively for 60 min. Then, the slides were incubated in ULTRA-View HRP multimer, ULTRA-View Universal DAB H2O2, chromogen and copper. Finally, the slides were counterstained with haematoxylin 2 and bluing reagent.

Immunohistochemical Staining Analysis

Evaluation of TAMs

All CD163-stained slides were examined for quantification of TAMs. Positive cells expressed moderate to strong cytoplasmic staining. The areas with the highest density of CD163-positive macrophages (hot spots) were identified under low (100×) magnification. These areas included hot spots within the tumour nest and tumour stroma. Tumour nest TAMs is defined as macrophages in contact with tumour cells, whereas stromal TAMs are macrophages that reside at the tumour–stroma borders.[9] Large areas of necrosis were not included in the evaluation. Three hot spots for both tumour nest and tumour stroma were identified; and positive cells were manually counted using the plug-in cell counter in the ImageJ software in high-power fields (400× magnification). The mean of the three counts was calculated, and the median value of TAMs in both tumour nest and stroma was used as a cut-off point to categorize the patients into low- and high-TAM infiltration (Figure 1).

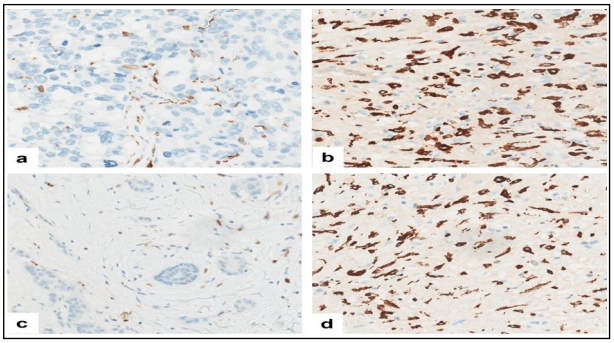

Figure 1: CD163-positive tumour-associated macrophages (TAMs) in tumour nest and tumour stroma. Examples of tissues with (a) low and (b) high TAMs infiltration in tumour nests; and (c) low and (d) high TAMs infiltration in tumour stroma (400× magnification).

Evaluation of CSF-1 Expression

The expression of CSF-1 by tumour cells was evaluated semi-quantitatively by the presence of diffuse brown cytoplasmic staining. The staining intensity of CSF-1 in tumour cells was scored as 0 (no staining), 1 (weak), 2 (moderate) or 3 (strong) as described in the study by Richardsen et al.[16] Score of 1 to 3 was considered positive staining. The CSF-1 staining intensity was further categorized into low expression (Score 0 and 1) and high expression (Score 2 and 3). The stained slides were assessed by two qualified histopathologists who were blinded to the clinicopathological data of the patients.

Statistical Analyses

Statistical analyses were performed using SPSS software version 25.0 (IBM Corp., Armonk, NY, USA). Continuous data were expressed as a mean with standard deviation. Categorical variables were presented as frequencies and percentages. The association between TAM infiltration and CSF-1 expression, and between TAMs infiltration and CSF-1 expression with clinicopathological parameters were calculated using Pearson's chi-square test. Fisher's exact test was used when appropriate. A p value of less than 0.05 was considered statistically significant.

RESULTS

Socio-Demographic and Clinicopathological Characteristics

The socio-demographic and clinicopathologic characteristics of the study subjects are illustrated in Table 1. The majority of the subjects were Malays (78.9%), while Chinese and Indians constituted 14.8% and 6.3% of the study participants, respectively. Eighty-three cases (64.8%) had tumour size between 2 – 5 cm, 32 cases (25%) had tumour size of more than 5 cm and only 13 cases (10.2%) had tumour size of less than 2 cm. Histologically, 73 cases (57%) were histological grade 2, 40 cases (31.3%) were grade 3 and 15 cases (11.7%) were grade 1. Positive lymph node metastasis was detected in 79 cases (61.7%). Cases with positive oestrogen receptor (ER), progesterone receptor (PR) and human epidermal growth factor receptor 2 (HER2) status were 82 (64.1%), 74 (57.8%), and 46 (35.9%) cases, respectively. Twenty-one cases (16.4%) were triple-negative breast cancers.

Table I: Socio-demographic and clinicopathological characteristics of the 128 invasive breast carcinoma cases.

Characteristic	Categories	Frequency	Percentage
Age (Mean ± SD)	55.29 ± 11.875		
Age group (years)	≤50 years	40	31.3
	>50 years	88	68.8
Gender	Female	128	100
Race	Malay	101	78.9
	Chinese	19	14.8
	Indian	8	6.3
Tumour size	≤2 cm	13	10.2
	2 – 5 cm	83	64.8
	>5 cm	32	25
Histological grade	Grade 1	15	11.7
	Grade 2	73	57
	Grade 3	40	31.3
Lymph node metastasis	Absent	49	38.3
	Present	79	61.7
ER status	Negative	46	35.9
	Positive	82	64.1
PR status	Negative	54	42.2
	Positive	74	57.8
HER2	Negative	82	64.1
	Positive	46	35.9
Triple-negative subtype	No	107	83.6
	Yes	21	16.4

SD, standard deviation; ER, oestrogen receptor; PR, progesterone receptor; HER2, human epidermal growth factor receptor 2.

Degree of TAMs Infiltration and Their Histological Localization within Breast Cancer Tissue

The degree of TAMs infiltration was variable. The median numbers of TAMs per high-power field was 30.5 (interquartile range: 21 - 42). High CD163-positive TAMs infiltration was more common in tumour stroma (55%) as compared to tumour nest (40%).

Association between Degree of TAMs Infiltration in Tumour Nest and Tumour Stroma with Pathological Prognostic Factors in Invasive Breast Carcinoma

High degree of TAMs infiltration was associated with poor prognostic parameters. High TAMs infiltration in both tumour nest and tumour stroma were significantly associated with higher histological grades. High nest TAMs was detected in 23 (57%) grade 3 tumour (p=0.006) whereas high stromal TAMs was detected in 33 (82.5%) grade 3 tumours (p=0.001). High TAMs infiltration in tumour stroma were also significantly associated with negative hormone receptor status. Thirty-seven (80.4%) ER-negative cases, 39 (72.2%) PR-negative cases, and 19 (90.5%) of triple-negative cases displayed high TAMs infiltration in tumour stroma (p=0.001, 0.001, and 0.001, respectively). The association between TAMs infiltration

Table II: Association between pathological prognostic factors and TAMs status in tumour nest and infiltration in stroma.

Factors			TAMs in Tumour Nest			TAMs in Tumour Stroma		
		N	Low n (%)	High n (%)	p	Low n (%)	High n (%)	p
Age	≤50	40	25 (62.5)	15 (37.5)	0.715	18 (45)	22 (55)	0.943
	>50	88	52 (59.1)	36 (40.9)		39 (44.3)	49 (55.7)	
Size	≤2 cm	13	9 (69.2)	4 (30.8)		7 (53.8)	6 (46.2)	
	2 – 5 cm	83	52 (62.7)	31 (37.3)	0.361	38 (45.8)	45 (54.2)	0.563
	>5 cm	32	16 (50)	16 (50)		12 (37.5)	20 (62.5)	
Grade	Grade 1	15	13 (86.7)	2 (13.3)		13 (86.7)	2 (13.3)	
	Grade 2	73	47 (64.4)	26 (35.6)	0.006	37 (50.7)	36 (49.3)	0.001
	Grade 3	40	17 (42.5)	23 (57.5)		7 (17.5)	33 (82.5)	
Lymph node metastasis	Negative	49	26 (53.1)	23 (46.9)	0.197	19 (38.8)	30 (61.2)	0.302
	Positive	79	51 (64.6)	28 (35.4)		38 (48.1)	41 (51.9)	
ER	Negative	46	25 (54.3)	21 (45.7)	0.315	9 (19.6)	37 (80.4)	0.001
	Positive	82	52 (63.4)	30 (36.6)		48 (58.5)	34 (41.5)	
PR	Negative	54	30 (55.6)	24 (44.4)	0.364	15 (27.8)	39 (72.2)	0.001
	Positive	74	47 (63.5)	27 (36.5)		42 (56.8)	32 (43.2)	
HER2	Negative	82	51 (62.2)	31 (37.8)	0.529	37 (45.1)	45 (54.9)	0.858
	Positive	46	26 (56.5)	20 (43.5)		20 (43.5)	26 (56.5)	
Triple-negative subtype	No	107	66 (61.7)	41 (38.3)	0.426	55 (51.4)	52 (48.6)	0.001
	Yes	21	11 (52.4)	10 (47.6)		2 (9.5)	19 (90.5)	

in tumour nest and tumour stroma with the clinicopathologic parameters are summarized in Table II.

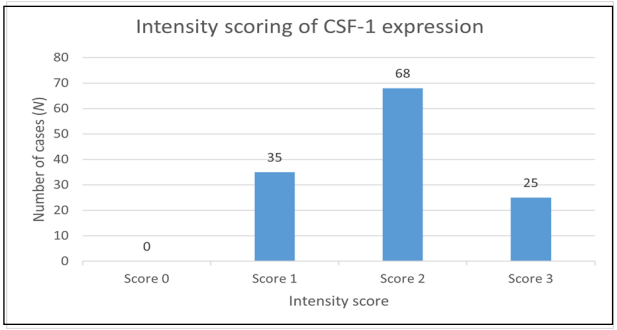

Figure 2: Intensity score of CSF-1 expression in breast cancer cells.

Association between CSF-1 Expression with The Degrees and Histological Localization of TAMs Infiltration in Breast Cancer Tissue

CSF-1 immunoreactive staining of tumour cells were observed in all cases with variable proportion and staining intensity (Figure 2). The CSF-1 expression scoring is illustrated in Figure 3. Cases that scored 0 and 1 were subcategorized into low CSF-1 expression whereas cases with scores 2 and 3 were considered as high expression. Ninety-three cases had high CSF-1 expression. Expression of CSF-1 by tumour cells was significantly associated with the degree and histological localization of TAM infiltration in breast cancer tissue (Table III). High CSF-1 expression was seen in 57 (61.3%) cases with high TAM infiltration in tumour stroma (p=0.031) as compared to only in 41 (44.1%) cases with high nest TAMs (p = 0.110).

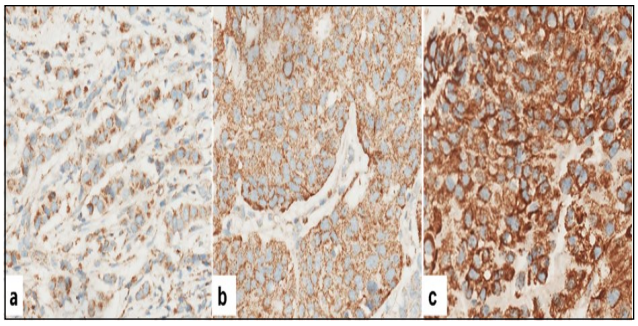

Figure 3: Scoring for CSF-1 expression in breast cancer cells. Tumour cells showed diffuse brown cytoplasmic staining with variable intensity scoring: (a) Score 1, (b) Score 2 and (c) Score 3 (400× magnification).

Table III: Association between CSF-1 expression with low and high TAM infiltration in tumour nest and tumour stroma.

CSF-1 Expression	TAMs in Tumour Nest			TAMs in Tumour Stroma		
	Low n (%)	High n (%)	p	Low n (%)	High n (%)	p
Low	25 (71.4)	10 (28.6)	0.110	21 (60.0)	14 (40.0)	0.031
High	52 (55.9)	41 (44.1)		36 (38.7)	57 (61.3)	

CSF-1, colony stimulating factor-1; TAMs, tumour-associated macrophages.

Association Between CSF-1 Expression with Pathological Prognostic Factors in Invasive Breast Carcinoma

CSF-1 expression was significantly associated with tumour grade and HER-2 cases, as illustrated in Table IV. High CSF-1 expression was significantly associated with higher histological grade (p=0.008). Furthermore, 40 HER-2-positive cases (87%) had a high expression of CSF-1 in breast cancer (p=0.007).

Table IV: Association between CSF-1 expression and pathological prognostic factors.

Factors		CSF-1 Expression		p
		Low n (%)	High n (%)	
Age	≤ 50 years	8 (20.0)	32 (80.0)	0.209
	> 50 years	27 (30.7)	61 (69.3)	
Tumour size	≤ 2 cm	3 (23.1)	10 (76.9)	0.856
	2–5 cm	24 (28.9)	59 (71.1)	
	> 5 cm	8 (25.0)	24 (75.0)	
Histological grade	Grade 1	9 (60.0)	6 (40.0)	0.008
	Grade 2	15 (20.5)	58 (79.5)	
	Grade 3	11 (27.5)	29 (72.5)	
Lymph node metastasis	Negative	9 (18.4)	40 (81.6)	0.073
	Positive	26 (32.9)	53 (67.1)	
ER status	Negative	8 (17.4)	38 (82.6)	0.058
	Positive	27 (32.9)	55 (67.1)	
PR status	Negative	12 (22.2)	42 (77.8)	0.267
	Positive	23 (31.1)	51 (68.9)	
HER2	Negative	29 (35.4)	53 (64.6)	0.007
	Positive	6 (13.0)	40 (87.0)	
Triple negative	Negative	30 (28.0)	77 (72.0)	0.691
	Positive	5 (23.8)	16 (76.2)	

CSF-1, colony stimulating factor-1; ER, oestrogen receptor; PR, progesterone receptor; HER2, human epidermal growth factor receptor 2.

DISCUSSION

Tumour-associated macrophages (TAMs), predominantly the M2 subtype are known to secrete various cytokines, chemokines, and proteolytic enzymes; which promote immunosuppressive activity, tumour proliferation, and tumour angiogenesis.[20] TAMs also play an important role in tumour metastasis by facilitating tumour cell invasion, migration, and tumour seedling to distant sites.[21,22] first part of this study, we investigated the degree of TAMs infiltration in invasive breast carcinoma and its association with the pathological prognostic factors of breast cancers.

In this study, we have demonstrated that high CD163-positive TAMs infiltration is more common in tumour stroma as compared to tumour nest. In breast tumours, TAMs are numerous within the stroma at the margins of breast cancer and becoming lesser towards the centre of the tumour.[14] These macrophages are also abundant at areas of tumour necrosis and preferentially associated with blood vessels. TAMs in various tumour locations had diverse phenotypes and functions. It was proposed that tumour stromal TAMs influence tubular architecture and, eventually, tumour grade.[23] During development, trophic macrophages are recruited to the growing breast ductal structures and play a role in tissue patterning and branching morphogenesis.[14] It is found that TAMs share similar properties to these trophic macrophages in tumour growth. Stromal TAMs also promote cell division by producing growth factors, cytokines and chemokines including transforming growth factor-β (TGF- β), basic fibroblast growth factor-2 (bFGF-2), platelet derived growth factor (PDGF), interleukin-10 (IL-10), and chemokine receptor type (CXC) ligand.[24] Stromal TAMs are associated with high expression epithelial-mesenchymal transition (EMT) markers contributes to rapid tumour progression and metastasis in these morphological variants.[25]

On the other hand, tumour nest TAMs is associated with hypoxia-induced angiogenesis and responses.[23] In poorly vascularised tumour especially intratumoural regions, nest TAMs upregulate hypoxia-inducible factor-1α (HIF-1α) and HIF-2 α which in turn stimulate the production of pro-angiogenic factors such as vascular endothelial growth factor (VEGF), bFGF, PDGF, and EGF to facilitate angiogenesis.[26] Hypoxia also augment nest TAMs with immunosuppressive features and further promote tumour growth and metastasis.

Our research findings showed there were significant associations between nest TAMs and stromal TAMs with pathological prognostic markers. We demonstrated a significant association between high TAMs infiltration in tumour nest and tumour stroma with higher tumour grades. Our study conforms with earlier research which demonstrated high numbers of CD163-positive TAMs in both tumour stroma and tumour nests were associated with higher histological grades.[13] In another study, high TAMs infiltration in tumour nest was significantly associated with high tumour grade but not with stromal TAMs.[9] It was postulated that increased density of CD163 -positive TAMs within high-grade tumours may be contributed by higher cytokines release by tumour cells to recruit TAMs such as CSF-1, IL-10, and TGF- β.[27] High stromal TAMs is recruited in tumours with solid architecture, hence, higher grade tumour as compared to tubular structure.[28] It was also suggested that TAMs in tumour stroma had more important roles that TAMs in the tumour nest in the aggressive behaviours of breast cancers.[23]

With regards to hormone receptor status, our study demonstrated a significant association between the high TAMs infiltration in tumour stroma and the ER and PR hormonal status. Most of the ER-negative cases (80.4%) and PR-negative cases (72.2%) had high density of TAMs infiltration in tumour stroma. These findings corroborated with earlier research findings that hormone receptor negativity was linked to increased expression of CD68 or CD163.[9,25] An *in vitro* model study demonstrated a novel mechanism of macrophage activation of kinase cascades in the cancer cells is responsible for loss of ERα expression in breast cancer cells.[29]

Our study also demonstrated a significant association between high TAM infiltration with triple-negative breast cancers (TNBC). Most of our TNBC cases (90.9%) is associated with high TAM infiltration within tumour stroma. Our finding is supported by previous study which showed that significant correlation between greater density of CD163-positive TAMs in tumour stroma and TNBC.[9] These findings are particularly important because presence of high TAMs infiltration affects the treatment response in TNBC; as the common chemotherapeutic drugs given to TNBC patients can activate TAMs and induce chemotherapy tolerance and inhibit immune killing of tumour cells by CD8+ T cells.[30]

In the second part of this study, we investigated the association between TAMs and CSF-1 expression in invasive breast carcinoma. CSF-1 stimulates TAMs to polarize from the M1 type to the M2 type; promotes TAM differentiation, proliferation and survival; and attracts monocyte-macrophage lineages to extravasate from peripheral circulation into the tumour tissues.[14] The positive feedback loop between tumour cells and TAMs through CSF-1 and EGF enhances tumour growth and metastasis. In our study, we demonstrated a significant association between the degree of TAM infiltration and CSF-1 expression. We also found that high CSF-1 expression was associated with high TAM infiltration in tumour stroma. This finding supports the crucial role of the surrounding microenvironment in breast cancer progression. CSF-1 and CSF-1R expressions are correlated with poor prognostic parameters in breast cancers. An experimental study demonstrated that breast cancers behaved more aggressively through CSF-1 secretion as more macrophages were recruited into the TME, thus creating a pro-tumorigenic milieu.[31] In our study, we demonstrated that CSF-1 expression was significantly associated with histological grade.

Our findings are in agreement with previous research that revealed significant correlation between high CSF-1 expression levels with higher pathological grade and worse prognosis in breast cancer.[32] We also found that there was a significant association between high CSF-1 expression and positive HER2 status. HER2-positive breast cancer is aggressive and has a poor prognosis if untreated; but due to the effectiveness of HER2-targeted therapies, its prognosis has improved. Studies showed that CSF-1 enhances invasiveness of cancer cells by signalling the macrophages to secrete EGF which causes the alteration of cellular morphology into elongated protrusions, which in turn promote tumour invasion.[33]

In this present study, we observed some discrepant results. An experimental study of hormone-independent breast cancers demonstrated a more aggressive behaviour than hormone-dependent breast cancer through CSF-1 secretion and TAMs recruitment.[28] However, our study failed to find association between CSF-1 expression and negative hormone receptor status. Another study has found significant correlations between CSF-1 expression in breast cancer with lymph node metastasis; however, our finding was nonsignificant.[14] The conflicting data produced may be due to different inclusion and exclusion and different methodologies to study the expression of CSF-1 in breast cancers. There are various methods that can be used to investigate CSF-1 level and expression such as fluorescence in situ hybridization (FISH), polymerase chain reaction (PCR), Western blot, ELISA and immunohistochemistry that can affect and produce variable results.[14,28,34] Immunohistochemistry method is reliable, specific, cost effective and feasible in most diagnostic laboratories. These differing results can also be attributed to the fact that the effect of CSF-1 in breast cancer is influenced by not only the genotype and phenotype of the carcinoma cells but also other cells in the TME. [35] Therefore, more data and further studies are required to better understand the correlation of CSF-1

with clinicopathological parameters and breast cancer prognosis.

CONCLUSION

In conclusion, we demonstrated that different TAM localization within invasive breast carcinoma NST have different degree of association with the clinicopathological parameters. High CD163-positive TAMs infiltration is more prevalent in tumour stroma as compared to tumour nest. High stromal TAMs were significantly associated with poor prognostic parameters. In this study, we also demonstrated that CSF-1 expression has varying degrees of association with TAM infiltration in the tumour nest and tumour stroma. High TAM infiltration in the tumour stroma was strongly associated with high CSF-1 expression. High CSF-1 expression was associated with adverse prognostic factors in breast cancers. Therefore, evaluation of TAMs infiltration and CSF-1 expression in breast cancer while taking into account the histologic localization is important. It has potential to serve as valuable biomarkers for patient prognosis and as targets for personalized cancer treatment strategies.

CONFLICT OF INTEREST

There are no potential conflicts of interest for any of the authors.

ACKNOWLEDGEMENT

This research was funded by Sultan Ahmad Shah Medical Centre International Islamic University Malaysia (SASMEC@IIUM) under the SASMEC Research Grant (SRG-21-036-0036). We also would like to extend our sincere gratitude to the staff of the Department of Pathology Hospital Tengku Ampuan Afzan for the support received.

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ORIGINAL ARTICLE

Upregulation Mechanism of *CCND1* in Apoptosis on MCF-7 Cell Line upon Treatment with Quranic Verses

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ABSTRACT

INTRODUCTION: Ruqyah Shar'iyyah, one of the complementary and alternative medicine (CAM) has shown therapeutic benefits in breast cancer by utilizing Quranic verses in reducing symptoms and enhancing their quality of life as a result of invasive standard therapies. However, scientific evidence is required to demonstrate Ruqyah Shar'iyyah's effectiveness on gene expression and molecular pathways in carcinogenesis. Therefore, this study was conducted to evaluate the effectiveness of Ruqyah Shar'iyyah on breast cancer cell line on apoptosis and *CCND1* expression in related signaling pathways. **MATERIALS AND METHODS**: MCF-7 cell lines were treated with direct recitation of several Quranic verses for 12 hours and 24 hours. Cell viability was observed using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. The expression of *CCND1* was determined using RT-qPCR technique. The molecular mechanism and signalling pathways were evaluated using Reactome database for *in silico* analysis. **RESULTS:** Cell viability data showed 95.69% and 93.54% exhibiting slight reduction for 12-hour treatment in untreated and treated MCF-7, respectively. Meanwhile more reduction was observed in 24-hour treatment with 95.11% in untreated cell line as compared to 92.34% in treated cell line. The cell morphology also exhibited apoptotic activity in the treated group for both two time points. Gene expression analysis of *CCNDI* also demonstrated upregulation with 1.81-fold change. The data was supported by the *in-silico* analysis in which 25 relevant significant signalling pathways related to *CCND1* highlighting the role of the gene in breast cancer development. **CONCLUSION:** *CCND1* may have a function in signalling pathways that control the proliferation of mammary epithelial cells.

Keywords
apoptosis, breast cancer, Quranic verses, CCND1, mechanism

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Received: 19th February 2024; Accepted: 19th August 2024

Doi: https://doi.org/10.31436/imjm.v23i04

INTRODUCTION

In 2020, Malaysia recorded 8,418 new cases of breast cancer, highlighting it as a significant health concern requiring attention and effective treatment. The precise cause of breast cancer remains unknown and is attributed to various potential risk factors. Some key factors such as gender, are uncontrollable, with women being 100 times more susceptible than men.[1] Moreover, advancing age is also linked to an increased incidence of breast cancer, with most diagnoses occurring in women aged 55 and above.[1] Notably, a study in 2016 revealed that American women over 40 and 60 contribute to 99.3% and 71.2% of breast cancer-related deaths, respectively.[2] To address this, it is recommended that women over 40 undergo regular mammography screenings for early detection of breast cancer.

Moreover, the likelihood of developing breast cancer is higher in women with a family history of the disease. A United Kingdom cohort study indicates that women with first-degree relatives (mother, sister, or daughter) diagnosed with breast cancer face a 1.75-fold increased risk compared to those with unaffected relatives. The risk further escalates to 2.5-fold higher for women with two or more first-degree relatives affected by breast cancer.[3] This inherited susceptibility is associated with genetic mutations in the BRCA1 and/or BRCA2 genes.[4]

Presently, the primary treatment modes available for breast cancer include surgical procedures combined with adjuvant therapies like radiation therapy, chemotherapy, targeted therapy, hormonal therapy, and immunotherapy.[4,5] However, these treatments may result in undesired side effects, prompting some patients to consider complementary and alternative medicine (CAM) in conjunction with conventional approaches. The National Cancer Institute defines CAM as medical products and practices outside the realm of standard medical care. It encompasses five main categories: alternative medical systems, exemplified by traditional medicine and homeopathy; mind-body interventions, including practices like yoga and cupping; biologically based therapies, such as dietary supplements; manipulative and body-based methods, like reflexology and massage; and energy therapies, encompassing practices like Qigong and Tai Chi.[6,7]

One of the practices within CAM involves Islamic spiritual healing. This is manifested through the recitation of Quranic verses known as Ruqyah Shar'iyyah. This approach is commonly embraced by Muslim patients, underscoring the profound influence of religion, particularly when confronted with life-threatening illnesses. Islamic spiritual healing employs recitation of Quranic verses or the traditions of Prophet Muhammad (peace be upon Him), known as sunnah, for healing purposes. This is practiced globally and even among non-Muslim regions. There are two main approaches to Islamic spiritual healing: direct recitation of Quran to patients and the use of herbs or water (healing water) infused with pre-recited Quranic verses.[8] Muslims attribute healing powers to Quranic recitation, citing verses such as Surah Al-Isra' verse 82, which states, "We send down (stage by stage) in the Quran that which is a healing and a mercy to those who believe..." Additionally, Surah Fussilat verse 44 emphasizes the Quran as a guide and healing for believers, and Surah Ash-Shu'ara verse 80 mentions, "And when I am ill, it is He (Allah) who cures me." Previous studies have indicated that Islamic spiritual healing significantly contributes to enhancing the mood, coping with symptoms and side effects, and alleviating anxiety in breast cancer patients, facilitating spiritual and psychological healing. It is important to note that complementary medicine is intended to mitigate the side effects of breast cancer or enhance quality of life and should not replace conventional medical treatments.

Numerous articles assert that Islamic spiritual healing positively influences cancer patients in terms of mood, spirits, and psyche. However, only a limited number of articles delve into the impact of Ruqyah Shar'iyyah on cell cultures and the associated genetic mechanisms.[9] As mentioned earlier, genetic factors significantly contribute to breast cancer development. Multiple genes play a role in breast cancer pathogenesis across various signalling pathways, necessitating an exploration of their functions. Among these genes is Cyclin D1 (*CCND1*), a candidate gene involved in cell cycle regulation. *CCND1* encodes a cyclin protein crucial for CDK kinase regulation, forming a complex with CDK4 or CDK6 to facilitate G1/S transition during the cell cycle. Alterations to *CCND1*, such as missense mutations, in-frame deletions, insertions, amplifications, and overexpression, have been observed in various carcinomas, including breast carcinoma, impacting cell cycle progression. A previous study reported *CCND1* mutations seen in 16.28% of breast carcinomas. *CCND1*'s role in breast cancer can influence tumour growth, cell cycle, and cell migration, as evidenced by cyclin protein expression in breast cancer tissue samples. Consequently, *CCND1* has been associated with the prognosis of breast cancer, with higher expression levels correlating with poor metastasis-free or recurrence-free survival.[9]

Given that the prevalent practice of Islamic spiritual healing is favoured in Malaysia, particularly among Muslim cancer patients, this study aimed to evaluate the efficacy of Ruqyah Shar'iyyah in regulating *CCND1* by inhibiting cell proliferation of breast cancer cells. Our investigation focused on assessing the expression level of the *CCND1* gene and its impact on the cell proliferation and apoptosis of the MCF-7 breast cancer cell line after treatment with Ruqyah Sha'iyyah.

MATERIALS AND METHODS

Selection of Quranic verses

General and specific Quranic verses were selected in this study. Table 1 shows a list of Quranic verses in Rukyah Shar'iyyah that commonly used in breast cancer treatment.[11,12] The selection of Qur'anic verses was based on the several well-established endorsed by Islamic healers who were used in breast cancer treatment. Six Islamic healers in Malaysia were chosen for the Quranic verses selection in which they use similar verses in their breast cancer treatment

Table 1: Series of Qur'anic verses used in Ruqyah Shar'iyyah

Category	Surah and Verses
General	al-Fatihah:1-7, al-Baqarah: 255, al-Hasyr:21-24, al-Ikhlas: 1-4, al-Falaq:1-5, al-Ikhlas:1-6
Specific	al-Baqarah:1-5, al-Baqarah:163-164, al-Baqarah:284-286, Ali `Imran:18, al-A`raf:54-56, al-Mu'minun:116-118, al-Jin:3, al-Saffat:1-10, Ali `Imran: 4, al-'An`am:133-135, Yunus:69-70, Yunus:73,Yunus:88, al-A`raf:90-93, Ibrahim:13-17, Ibrahim:22, Ghafir:70, Ghafir:75-77, al-Kahfi:29, al-Kahfi:59, al-Kahfi:52-52, al-Kahfi:102, al-Kahfi:105-106, al-Nahl:45-46, al-Nahl:85, al-Nahl:88, al-Anbiya':39-41, al-Anbiya':76, al-Anbiya':98, al-Hajj:44, al-Hajj:48, al-Hajj:57, al-Hajj:72, al-Nur:57, al-Zumar:47, al-zumar:51, Fussilat:41, Fussilat:50, al-Saffat:66, al-Saffat:111, al-Furqan:12, al-Furqan:19, al-ahzab:64, al-Isra':97, Taha:97, Taha:100, Hud:39, Hud:58, Hud:66, al-Qasas:81, l-Zukhruf:51, Maryam:37, al-Fil:1-5.

MCF-7 cell culture

The human breast cancer cell line (MCF-7) ATCC HTB-22tm) (Human breast adenocarcinoma) was cultured in 25 cm2 cell culture flask in Roswell Park Memorial Institute (RPMI) 1640 medium and supplemented with 10% (v/v) foetal bovine serum (FBS; TICO Europe, Netherlands) and 1% (v/v) penicillin-streptomycin (Gibco, Thermo Fisher Scientific, USA). The cells were cultivated in the incubator containing humidified atmosphere of 5% CO_2 at 37°C.

Treatment Procedure

The treatment sessions were carried out in a carbon dioxide (CO_2) incubator to ensure controlled in-vitro environment for optimum cell culture growth throughout the procedure. MCF-7 cells were divided into two groups: untreated and treated. Cells in the untreated group served as a control and were not exposed to Qur'anic recitation. The treated group was exposed to Qur'anic recitation for 12 hours and 24 hours using a sound level meter at 50-60 decibel played in the closed chamber. The plates containing the cells were placed inside a sterile chamber with four sterile speakers at each corner to avoid any other interfering factors. During the treatment sessions, a compilation of several Qur'anic verses from the Qur'an was played by the speakers. The Qur'anic verses used are as shown as in Table I according to the category of either general Qur'anic verses or specific Qur'anic verses. General verses were first introduced in the treatment, followed by the specific verses for the breast cancer treatment.

Cell Proliferation Assay

The effect of Islamic spiritual healing on the proliferation of MCF-7 cell line was assessed using Trypan blue exclusion assay. Cells at a density of 3x104 cells /ml (100 ul/well) were seeded in 6-well plate and incubated overnight under 5% CO_2 at 37 °C, followed by exposure to Quranic verses recitation using speaker for 12 hours and 24 hours incubation. The cells without the treatment of Islamic spiritual healing served as the control group. After treatment, MCF-7 cells were trypsinised and incubated in Trypan blue dye (0.2%) for 5 minutes at room temperature. A 20uL aliquot was removed and placed on a Neubauer haemocytometer. The number of viable and non-viable cells were counted under a microscope. The percentage of cell viability was calculated using the following equation in which the non-treated cells were set as 100%.

$$\text{Cell viability (\%)} = \frac{\text{Treated cell OD} - \text{black OD} \times 100}{\text{Non treated cell OD} - \text{blank OD}}$$

Morphological Evaluation using Phase-Contrast Microscopy

Morphological evaluation following the exposure to Islamic spiritual healing on MCF-7 cells was performed using a phase-contrast microscopy. Changes in cell morphology of treated cells were imaged at 100X magnification using an inverted phase contrast microscope (Nikon Instruments, Tokyo, Japan) and were compared to the control group.

Gene expression level

CCNDI gene was determined for the expression level upon Rukyah Shar'iyyah treatment to the MCF-7 in triplicates. Based on the percentage of cell viability, the reduction of cell growth was prominent after 24-hour treatment. Thus, this time point was selected for gene expression analysis. After 24-hour treatment session, MCF-7 cells were harvested, and total RNA was extracted using the innuPREP RNA Mini Kit (Analytik Jena AG, Jena, Germany) according to the manufacturer's instructions. RNA quality was measured, quantified, and analyzed using NanoDrop (Thermo Scientific).

50 µL of RNA was eluted from each sample by using Rnase-free water provided with the kit. Only 1 µL of RNA was dropped into the well of QuickDrop™ Micro-Volume Absorbance Spectrophotometer (SpectraMax, USA) to obtain a ratio of 260/280 and 260/230 reading to determine the purity of RNA sample. RNA samples were diluted and standardized to 100 ng/µL prior to cDNA conversion process.

cDNA synthesis

Reverse transcription was performed using the Viva cDNA Synthesis Kit (Vivantis Technologies Sdn Bhd, Malaysia) following the manufacturer's recommended protocol. The following mixture was prepared to give a 10 ul reverse transcriptase (RT) solution: 1 ug/ul of total RNA was added to 1 ul of random hexamers, 11 ul of 10 mM dNTPs mix and top up to 10 ul with nuclease-free water. After proper mixing, the mixture was incubated at 65℃ for 5 min and chill on ice for 2 minutes. Then, a mixture of 2 ul of 10x buffer M-MuLV, 100 unit of M-MuLV reverse Transcriptase with a final volume of 10 ul with nuclease-free water was added to the previous mixture and was mixed gently prior to incubation at 42℃ for 60 min. Later, reverse transcription was inactivated at 85℃ for 5 min and later the mixture was stored at -20℃.

Quantitative real time PCR (RT-qPCR)

Quantitative Real time PCR reactions were performed by TaqMan Universal PCR Master Mix and TaqMan Gene Expression Assays using a StepOne™Plus Real-Time PCR Systems (Applied Biosystems, USA). The assay identification number for targeted gene used in this study was *CCND1*: Hs00765553_m1. In a 20 ul reaction solution. 2 ul of cDNA template, 1 ul of TaqMan Gene Expression Assay, 10 ul of TaqMan Universal PCR Master Mix was added to 7 ul of RNAse-free water. The reaction was completed with the following steps: 10 min at 95°C, then followed by 40 cycles of 15 sec at 95°C, 1 min at 60° C. The relative expression of target gene was normalized with b-actin as the endogenous control and calculated using the 2ΔΔCt method. All samples were in measured independently in triplicates.

Statistical Analysis

PRISM 5 Software (GraphPad, La Jolla, CA, USA) was used to perform statistical analysis. The statistical significance of data was obtained by performing one-way ANOVA for multiple-group comparison. p values less than 0.05 were considered to indicate statistical significance.

In silico analysis

Reactome database from Cytoscape software version 3.7.1 was used to perform the in-silico analysis for functional interaction and gene enrichment. The most relevant signaling was based on false discovery rate (FDR) value after rejecting the type 1 error.

RESULTS

Cell viability

The inhibitory effects of Quranic verses used in this study on the cell proliferation is shown in Table 2 and Figure 1. The study demonstrated that the inhibitory effect on MCF-7 cell line gradually increased with the duration of exposure time. After 12-hour treatment with Quranic verses, the non-treated cell lines yielded 95.69% of mean cell viability as compared to the treated group with 93.54%. In a longer duration of treatment with 24 hours, the reduction on cell proliferation was higher with 95.11% for non-treated as compared to 92.34% for treated MCF-7 cell lines.

Table 2: Summary for mean cell viability (%) for 12-hour and 24-hour treatment

Time	Group	Experiment	Cell viability (%)			Mean cell viability (%)	Std. deviation
			Replicate 1	Replicate 2	Replicate 3		
12h	Control	1	96.25	94.74	97.40	95.69	1.535
		2	97.73	95.65	93.83		
		3	96.34	93.15	96.15		
	Treated	1	94.59	94.44	91.55	93.54	1.857
		2	90.70	94.05	92.13		
		3	96.51	95.00	92.86		
24h	Control	1	97.12	95.50	95.15	95.11	1.466
		2	93.58	97.48	94.69		
		3	93.39	93.75	95.33		
	Treated	1	93.20	92.00	92.52	92.34	0.916
		2	91.07	93.16	92.17		
		3	92.31	90.99	93.64		

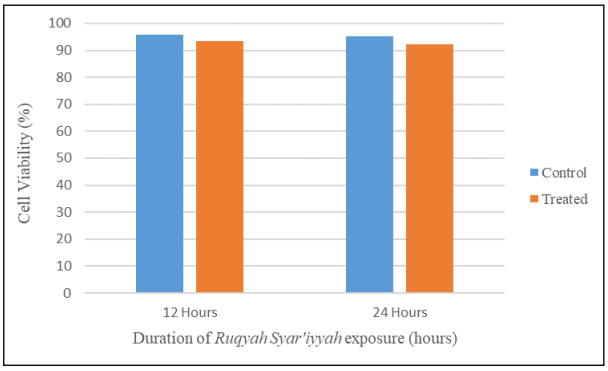

Figure 1: Cell viability (%) of MCF-7 cells after being treated with Ruqyah Shar'iyyah recitation for 12 and 24 hours. The data represents the mean of three independent experiments done in triplicates (n=12) using one-way ANOVA.

Apoptosis

The cell proliferation was consistent with cellular changes for these two time points upon treatment with Quranic verses. The observation using phase-contract microscopy demonstrated the evidence of apoptosis activity with membrane blebbing, cell shrinkage, karyorrhexis and pyknotic bodies. The cellular changes due to apoptosis was more observed in 24-hour treatment as compared to 12-hour treatment. Meanwhile the cellular features such as halo and shade-off contrast patters were also present as shown in Figure 2.

Upregulation of CCND1

The expression level of CCND1 was upregulated upon treatment with Rukyah Sya'iyyah for 24 hours as exhibited in Table 3 with 1.81-fold change based on threshold cycle.

Table 3: CCND1 expression level

Sample	Ct (mean)	Ct of ACTB (mean)	ΔCt	ΔΔCT (ΔCt Treated - ΔCt Non Treated (mean))	RQ
Non-Treated	29.292	36.728	-7.436	-0.858	1.813
Treated	27.454	35.748	-8.294		

Abbreviation: Ct=Threshold cycle, ΔCt=delta threshold cycle, ΔΔCT=delta delta threshold cycle, RQ=relative quantification

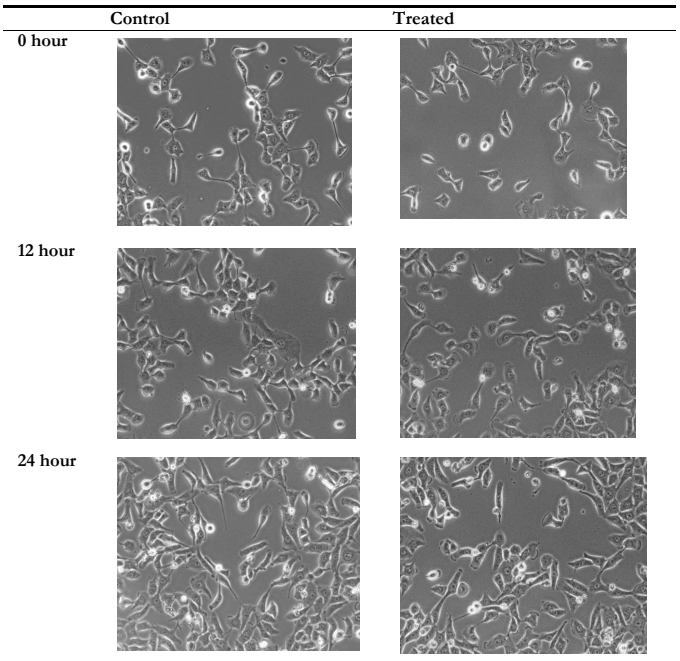

Figure 2: Morphology analysis by phase-contrast microscopy of MCF-7 cells treated with Ruqyah Shar'iyyah recitation for 12- and 24 hours. All images were obtained at a magnification of x100.

Mechanism of CCND1

According to the pathway analysis generated by Reactome database, there were 25 most relevant signalling pathways sorted by p-value for *CCND1*. The top five pathways were *RUNX3*-regulates *WNT* signalling (FDR: 2.05e-05), Oestrogen-dependent nuclear events downstream of *ESR*-membrane signalling (FDR: 8.44e-05) and Transcriptional regulation by *VENTX* (FDR: 1.51e-04), Extra-nuclear oestrogen signalling (FDR: 5.47e-04), Transcriptional regulation by *RUNX3* (FDR: 6.07e-04). Figure 3 and Table 4 represents the top 25 most significant pathways related to *CCND1*.

Figure 3: Genome-wide overview of the results of pathway analysis for *CCND1* generated using Reactome

DISCUSSION

Breast cancer has emerged as a silent threat and killer affecting women across the global population. Complementary and alternative medicine (CAM) has gained significant traction among breast cancer patients

who are Muslims worldwide, serving to alleviate signs and symptoms associated with both pre- and post-operative conventional invasive therapies. It is advisable to incorporate CAM as a supportive measure alongside conventional medicine to address treatment-related side effects.[13] In the present study, the impact of *CCND1* in upregulating multiple signalling pathways in breast cancer was demonstrated, showcasing a decrease in the numbers of cancer cell proliferation and apoptotic activity following treatment with Quranic verses. Notably, there was a slight marked decline in cell viability percentages observed after 12-hour and 24-hour treatments.

Table 4: The 25 most relevant pathways related to CCND1 sorted by p-value.

Pathway Name	Entities	
	p-value	FDR*
RUNX3 regulates *WNT* signaling	4.36e-07	2.05e-05
Estrogen-dependent nuclear events downstream of *ESR*-membrane signaling	3.67e-06	8.44e-05
Transcriptional Regulation by *VENTX*	1.00e-05	1.51e-04
Extra-nuclear estrogen signaling	5.37e-05	5.47e-04
Transcriptional regulation by *RUNX3*	6.07e-05	5.47e-04
Estrogen-dependent gene expression	1.03e-04	7.24e-04
Interleukin-4 and Interleukin-13 signaling	1.94e-04	0.001
ESR-mediated signaling	2.88e-04	0.001
Signaling by Nuclear Receptors	6.53e-04	0.003
Drug-mediated inhibition of CDK4/CDK6 activity	7.92e-04	0.003
PTK6 Regulates Cell Cycle	0.001	0.004
Signaling by interleukins	0.002	0.006
RUNX3 regulates p14-ARF	0.002	0.006
Defective binding of *RB1* mutants to E2F1, E2F2, E2F3	0.002	0.007
Aberrant regulation of mitotic G1/S transition in cancer due to RB1 defects	0.002	0.007
Regulation of RUNX1 expression and activity	0.004	0.008
Aberrant regulation of mitotic cell cycle due to RB1 defects	0.005	0.008
Cytokine signaling in immune system	0.005	0.008
Cyclin D associated events in G1	0.007	0.008
G1 phase	0.007	0.008
Ubiquitin-dependent degradation of cyclin D	0.007	0.008
RMTs methylated histone arginine	0.007	0.008

Since this study only covered up to exposure of 24 hours to Qur'anic recitation, the results were not significant enough to draw any definitive conclusion. Therefore, it is hypothesized that longer treatment durations would may produce more meaningful and significant outcomes, as suggested by Syed Bidin et al. (2020).[14] Therefore, future research should extend the exposure period to 72 or 96 hours to better understand the effects of Ruqyah Shar'iyyah on cancer cells, as indicated by several studies.[14] Additionally, it is worthwhile to further investigate using water as a medium, which might offer better effects. The vibrations from the sound wavelengths can penetrate and alter the water molecules, potentially enhancing their effectiveness in killing cancer cells.[15, 16]

Significantly, alterations in cellular membranes were evident, characterized by cell shrinkage, blebbing of the cellular membrane, and the formation of pyknotic bodies, indicative of the apoptosis mechanism. Despite the minimal difference observed between the 12-hour and 24-hour treatments, a more pronounced effect may potentially be achieved with prolonged exposure and repetitive treatments, as recommended by Quranic healers for their patients. Previous reports have highlighted the therapeutic properties of Quranic verses, demonstrating their ability to impede cell proliferation in normal chondrocytes during the process of wound healing.[17]

The direct recitation of Quranic verses through a speaker emitting 50-60 decibels within an enclosed chamber aims to facilitate wave transmission into cells, potentially inducing alterations in the cell membrane and nucleic acid within the nucleus.[15] Furthermore, the utilization of an anechoic chamber in this research ensures the exclusion of environmental interference, including noise contamination and sound inconsistencies.[15]

Regarding cellular changes, this study delved into the molecular level by examining gene expression. Numerous genes have been implicated in the development of breast cancer, serving as either negative or positive regulators in signalling pathways. Genetic aberrations can alter the normal functioning of these expressed genes. To substantiate the involvement of genes, the study focused on evaluating CCND1 gene expression in the MCF-7 cell line treated with Quranic verses compared to the untreated MCF-7 cell line, revealing a 1.81-fold change in its threshold cycle. *CCND1* was overexpressed in 50% of breast cancers, and its gene amplification is linked to breast cancer recurrence and decreased chemosensitivity.[18,19,20] Cyclin D1 (*CCND1*) encodes crucial oncoproteins involved in cancer cell proliferation across various cancers, including breast carcinoma. Located on chromosome 11q13, its upregulation has been observed in tamoxifen-resistant breast cancer cells. Suppression of *CCND1* leads to a reduction in Cyclin D1

protein, thereby inhibiting the proliferation of cancer cells in tamoxifen-resistant breast cancer cell lines.[21]

Given the evidence of cellular changes, cell proliferation, and *CCND1* upregulation, the Reactome database furnished information on 25 associated signalling pathways. Among these, the top five most significant pathways predominantly participate in regulating cellular proliferation and cell survival by targeting the oestrogen receptor. As shown in Table 4, the pathway most closely linked to *CCND1* is the *WNT* signalling pathway, regulated by *RUNX3*. The binding interaction between *RUNX3*, beta-catenin (*CTNNB1)*, and *TCF/LEF* prevents the *CCND1* and MYC gene promoters, thereby interfering with the *WNT* signalling-mediated activation of *CCND1* and *MYC1* transcription. Consequently, this regulation inhibits *WNT*-induced cellular proliferation.[22] Additional pathways associated with *CCND1* include *MAPK* and *PI3K/AKT*, whose activation can influence oestrogen levels and contribute to cell proliferation through gene dysregulation.[23,24] These pathways, specifically *MAPK* and *PI3K/AKT*, play a role in stimulating the secretion of MMP9, a proteolytic enzyme that degrades the extracellular matrix (ECM), thereby promoting cell invasion and metastasis.[25] It is noteworthy that oestrogen, recognized as a mitogenic agent, disrupts the cell cycle by shortening the G1 phase and advancing it to the S phase, with aberrant expression of *CCND1*.[26] This mechanism has been implicated in over 50% of mammary tumour development.

Regarding apoptosis activity, *VENTX* plays a role in inducing cell cycle arrest by promoting the transcription of cell cycle inhibitors *TP53* and *p16INK4A*. This activation subsequently triggers tumour suppressor pathways regulated by *TP53* and *p16INK4A*, leading to cell differentiation.[27] Additionally, *VENTX* inhibits the transcription of *CCND1*. This mechanism has been observed in the development of various cancers, including colon and breast cancer.[28]

CCND1 has been shown to participate in oestrogen-dependent transcription, where beta oestradiol (E2) binds to receptors on the plasma membrane, serving a non-genomic role in extra-nuclear signaling.[29,30] This extra-nuclear signalling induced by E2 operates independently of the transcriptional activity of oestrogen receptors. The ongoing mechanism activates various signalling pathways, including the *RAF/MAK* kinase cascade and *PI3K/AKT* signaling cascade, governing processes such as apoptosis, cellular proliferation, and metastasis.[24] Furthermore, extra -nuclear signalling engages in cross-talk with nuclear oestrogen receptor signalling and is essential for controlling ER protein stability, thereby contributing to the development of breast cancer.[31]

RUNX3 functions as a transcription factor, playing a crucial role in DNA binding through heterodimerization with *CBFB* (CBF-beta), thereby regulating gene expression in various essential developmental pathways.[32] Its participation in neurogenesis and its identification as a tumour suppressor gene in carcinogenesis have been demonstrated. Previous studies have revealed the inactivation of *RUNX3* in human breast cancer cell lines, evident through hypermethylation of the RUNX3 promoter, hemizygous deletion of the RUNX3 gene, and cytoplasmic sequestration of the RUNX3 protein.[28] Consequently, the silencing of *RUNX3* via promoter hypermethylation in breast cancer predisposes Runx3+/- individuals to the development of breast cancer by targeting the oestrogen receptor alpha (ER+SR1) protein.[33] RUNX3 inactivation typically occurs as an early event in breast cancer progression, characterized by downregulation.[34]

CONCLUSION

Our results indicate that Ruqyah Shar'iyyah as a complementary medicine is a promising non-invasive treatment for breast cancer patients to use alongside conventional treatments such as surgery, radiotherapy, and chemotherapy. The treated breast cancer cells exhibited cell death due to apoptotic activity. Although the differences between the control and treatment groups were minimal and insignificant, the treatment groups did show positive effects, particularly over extended exposure times. It can be concluded that longer treatment durations may yield better results. Therefore, it is recommended to practice Qur'anic recitation consistently as a supplementary therapy along

with conventional medicine to improve the condition of breast cancer patients.

This suggestive evidence reveals the reduction on cell viability of MCF-7 breast cancer cell line was due to the upregulation of *CCND1*. The identification of associated signalling pathways with *CCND1* elucidates potential biomarker implicated in the pathogenesis of breast cancer upon treatment with selected Quranic verses in Ruqyah Shar'iyyah.

CONFLICT OF INTEREST

The authors agree that this research was conducted in the absence of any self-benefits, commercial or financial conflicts and declare absence of conflicting interests with the funders.

ACKNOWLEDGEMENTS

This research has been funded by Universiti Sultan Zainal Abidin under LabMat grant (UniSZA/ LABMAT/2018/07).

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EXPERT OPINION

Vaping as a Harm Reduction Strategy: Lessons from the Past

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INTRODUCTION

The rise of electronic cigarettes (e-cigarettes), or vaping devices, has led to debates on their role in harm reduction for smokers. While proponents argue that vaping reduces exposure to harmful chemicals from tobacco, it is important to recognise the potential risks and lack of comprehensive evidence regarding its long-term effects. Malaysia is facing a public health challenge, with an increase in vaping, especially among the youth, as the *Control of Smoking Products for Public Health Act 2023 [Act 852]* remains unenforced.

The Unknown Safety Profile of Vaping

One of the most pressing concerns with adopting vaping as a harm reduction tool is the lack of long-term data on its safety profile. E-cigarettes are relatively new, having entered the global market in the early 2000s. This short timeframe is insufficient to assess the long-term health risks posed by regular e-cigarette use. Proponents claim that vaping is less harmful than traditional smoking, but "less harmful" does not mean "safe." Emerging research highlights that vaping still poses serious health risks.

Aerosols produced by e-cigarettes contain harmful substances such as heavy metals, volatile organic compounds, and known carcinogens such as formaldehyde and acrolein.[1] Furthermore, the impact of inhaling flavouring agents, approved for ingestion but not for inhalation, remains largely unstudied.[2] In the United States, cases of e-cigarette or vaping-associated lung injury (EVALI) underscore the dangers of these products, especially when they contain unregulated additives.[3]

Historical Parallels with Cigarette Smoking

History provides a cautionary tale about the dangers of adopting products before fully understanding their health risks. In the early 20th century, cigarettes were widely consumed, and their harms were not recognised until the 1950s, when researchers began linking smoking to lung cancer, heart disease, and other illnesses.

Pioneering studies by Ernst L. Wynder and Evarts A. Graham in the United States and Sir Richard Doll and Sir Austin Bradford Hill in the United Kingdom provided the first epidemiological evidence of smoking's lethal effects. In 1950, Wynder and Graham linked cigarette smoking to lung cancer,[4] while Doll and Hill conducted similar research in the UK.[5] Later, the British Doctors Study followed 40,000 doctors and confirmed the severe health impacts of smoking.[6] However, by the time these harms were widely acknowledged, millions were already addicted, resulting in a global tobacco-related health crisis.

This historical oversight, where cigarettes were normalised before their risks were fully understood, serves as a stark reminder of the dangers of prematurely endorsing new products like e-cigarettes.

The Situation in Malaysia: Unregulated Access and Youth Vaping

Malaysia's *Control of Smoking Products for Public Health Act 2023 [Act 852]* is designed to regulate the sale and marketing of tobacco and vaping products. Still, the delay in enforcement is allowing the vaping industry to grow, particularly among adolescents. In July 2024, the Ministry of Health Malaysia (Kementerian Kesihatan Malaysia, or KKM) raised concerns about the unregulated sale of vaping products through vending machines, which is prohibited under Act 852.[7] The lack of enforcement allows young people to access vaping products without sufficient oversight.

The rise in youth vaping is especially alarming. Research shows that nicotine exposure during adolescence can disrupt brain development and lead to long-term cognitive and behavioural impairments.[8] Additionally, vaping often acts as a "gateway" to smoking traditional cigarettes, a trend observed in several studies.[9] This normalisation of vaping risks undoing decades of progress in reducing smoking rates, particularly among vulnerable youth populations.

The appeal of vaping products to young people is exacerbated by the widespread availability of sweet, fruity flavours, such as mango and bubble gum, which are highly attractive to adolescents. Studies have shown that flavoured e-liquids increase experimentation and regular use among youth.[10] Without enforcing Act 852, Malaysia's youth remain vulnerable to nicotine addiction and the harmful effects of vaping.

The Case Against Vaping as a Harm Reduction Tool

The argument that vaping can serve as a harm-reduction tool rests on the assumption that it is safer than traditional cigarettes. While vaping may pose fewer immediate risks, it is not without serious health concerns. Evidence suggests that vaping can lead to respiratory and cardiovascular issues.[9]

One of the main problems with promoting vaping as a harm reduction tool is the phenomenon of "dual use," in which individuals use both e-cigarettes and traditional cigarettes. This behaviour fails to reduce exposure to harmful chemicals and may even exacerbate health risks by combining the dangers of both products.[11] Rather than reducing harm, dual-use prolongs exposure to toxins and impedes the transition to complete cessation.[11]

Moreover, promoting vaping as a harm-reduction tool could undermine efforts to discourage smoking initiation and encourage cessation. Presenting vaping as a "safe" alternative weakens the social unacceptability of nicotine use, especially among younger populations. Normalising vaping threatens the significant progress made in tobacco control efforts.

RECOMMENDATIONS

The uncertainty surrounding the long-term safety of vaping, combined with the increasing trend of youth vaping, underscores the need for caution. The *Control of Smoking Products for Public Health Act 2023 [Act 852]* is expected to come into force on 1st October 2024. This is a significant step forward for public health in Malaysia, but its effectiveness will depend on stringent enforcement and comprehensive regulation. The Act needs to be enforced effectively to curb the rising vaping epidemic, particularly among youth, and to ensure that the sale and marketing of smoking products, including e-cigarettes, are controlled to protect public health.

Strong regulation is necessary to prevent the widespread use of vaping products, particularly among the youth, who are vulnerable to the harmful effects of nicotine.

History teaches us that delays in responding to emerging public health risks can lead to devastating consequences. The parallels between vaping and the early days of cigarette smoking are too striking to ignore. We must act now to enforce Act 852 effectively, regulate the vaping industry, and protect future generations from the long-term dangers of nicotine addiction and vaping-related harm. Immediate action is essential to safeguard the health and well-being of Malaysians.

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