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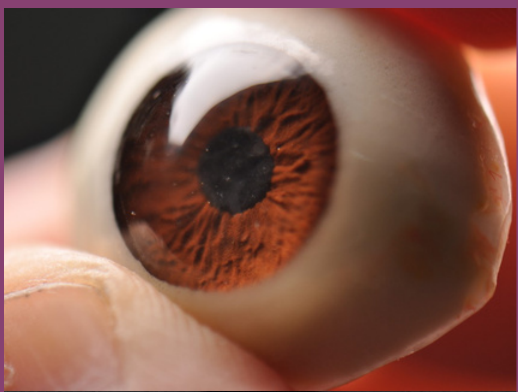

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AIMS AND SCOPE

IIUM Journal of Orofacial and Health Sciences (IJOHS) is a peer reviewed biannual international journal dedicated to publishing high quality of scientific research in the field of orofacial sciences, health sciences and interdisciplinary fields, including basic, applied and clinical research. The journal welcomes review articles, original research, case reports and letters to the editor. Areas that are covered include but are not limited to dental sciences, oral microbiology and immunology, oral maxillofacial and craniofacial surgery and imaging, dental stem cells and regenerative medicine, dental biomaterial, oral maxillofacial genetic and craniofacial deformities, dental public health and health sciences.

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EDITORIAL Open Access

Application of 2017 new classification of periodontal diseases and conditions: A commentary

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Introduction

Periodontal diseases such as gingivitis and periodontitis are among the common burden of oral health diseases. Gingivitis, by definition, is an inflammatory disease that happened due to bacterial dysbiosis in the gingival area (Trombelli *et al.*, 2018). It is characterized by bleeding on probing, erythematous gingival as well as gingival swelling. The lesion is limited to the gingival area only and does not involve other periodontal structures such as cementum, alveolar bone process, periodontal ligaments, and connective tissue fibers. Meanwhile, periodontitis is a more severe form of gum disease characterized by clinical attachment loss, bone loss, gingiva recession, and tooth mobility (Caton *et al.*, 2018).

In Malaysia, the prevalence of periodontal disease can be considered as high. Based on the information from the National Oral Health Survey of Adults (NOHSA), in the year 2010 (Ministry of Health, 2011), 94% of the adult population has gum problems whether it is gingivitis or periodontitis. Gingivitis is a well-known diagnosis at the early stage of periodontal disease. Based on the new classification of periodontal disease (Trombelli *et al.*, 2018), the patient is considered as having gingivitis when the bleeding on probing (BOP) score is more than 10%. It is further classified into localized and generalized, with 30% of the BOP score as the cut point. If BOP is more than 30%, the patient is then diagnosed with generalized gingivitis, and less than 30% is considered localized gingivitis. However, this classification of gingivitis may raise some issues as it is disregarding the factors such as the presence of calculus and smoking habit. It seems that even if the patient has a lot of calculus deposition, due to the BOP score of less than 10%, they are still considered healthy, whereas calculus is a well-known risk factor for the initiation of periodontal disease. Regarding calculus, the previous NOHSA survey revealed that 42.2% of the adult population have calculus deposition inside the oral cavity. This report indirectly showed that calculus is not a healthy deposit inside the mouth, and it can cause harm to the periodontium if it is not removed properly. Similarly with smoking habit. This habit is only mentioned in periodontitis classification, but not in gingivitis. Patients with a smoking habit may have a small percentage of BOP due to the constriction of blood vessels at the gingival area. However, the patient is still considered healthy even though smoking provides a negative impact on oral health.

Meanwhile, periodontitis has multiple factors for its initiation and progression.

Untreated gingivitis, together with the patient's susceptibility and environmental factors may lead to periodontitis (Caton *et al.*, 2018). Once a patient is diagnosed with periodontitis, the patient is considered a periodontitis case for the rest of their life as this disease is not reversible and the destruction of the periodontium is not going to be regenerated in total (Chapple *et al.*, 2018).

Recently, periodontitis has been classified into various stages and grades (Tonetti *et al.*, 2018). The stages and grades will give information regarding the disease's severity, extension, and progression. Stages consisted of three main components which are severity, complexity, and extension. The severity component consisted of interdental clinical attachment loss (CAL), radiographic bone loss, and tooth loss due to periodontitis. Meanwhile, complexity refers to the additional clinical findings such as the pattern of bone loss, presence of deep probing depth, tooth mobility, and furcation involvement that indicate more complex treatments are required. The stages of periodontitis range from stage I to stage IV. The treatment for stage I is considered simple while treatment for stage IV is considered complex and may require multidisciplinary efforts (Tonetti *et al.*, 2018).

Grades in periodontitis consisted of two components which are primary criteria and grade modifiers. The primary criteria are looking at the amount of bone loss based on radiographic findings, while grade modifier consists of two main risk factors of periodontitis which are smoking and diabetes mellitus (Tonetti *et al.*, 2018).

In Malaysia, 18.2% of the population has severe gum disease which is defined as having a probing pocket depth measurement of more than 6mm. If we transfer the information of this cohort into the new classification system, it means that around 20% of our population is having Stage III or Stage IV periodontitis.

On the other hand, the recent diagnosis classification of periodontitis and gingivitis may give an easier understanding to classify the problems and prepare a proper treatment plan. Oral hygiene instruction and mechanical debridement are mandatory to prevent and treat the problems. It is undeniable that more advanced treatment modalities are available, but those are only adjunct to the regular scaling and root surface debridement. European Federation of Periodontology (EFP) provided a guideline of treatment according to the stages and grades, but it is still focusing on prevention, removal of soft and hard deposits, and controlling systemic factors such as smoking and diabetes mellitus.

Nevertheless, early prevention, accurate diagnosis, and proper planning will provide better periodontal care for the community. The current diagnosis system can help clinicians to understand better the diseases, hence providing accurate treatment for any degree of periodontal problems. It is hope that we can implement the new classification on next NOHSA survey, and perhaps we can see a different status of periodontal diseases in Malaysia.

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ORIGINAL ARTICLE Open Access

Demographic of orthognathic cases seen in Kulliyyah of Dentistry IIUM

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Abstract

Orthognathic treatment is a combination of orthodontics and surgery, aimed to restore functional occlusion and the facial aesthetics. KOD orthognathic team has been established since 2018. There was no published demographic profile of the orthognathic patients referred. This study aimed to enumerate the demographic data and correlations for the orthognathic cases seen in KOD, IIUM from 2018 until December 2020. A total of 28 patients were referred for consultation. One patient was excluded as no referral letter was attached. Data were obtained from patients' case notes, photos and study models. Data collected were then categorized into patients' demographic profiles, clinical features, patients' psychological status and treatment. The demographic pattern and correlations were analysed using SPSS version 25.0. Out of 27 patients, most patients were females (66.7%), Malay (55.6%) and within the age group of 21 to 30 years old (44.4%) and originated from Pahang (88%). Majority of patients presented with Class III skeletal base discrepancy (66.7%), facial asymmetry (76.0%), increased vertical dimension (51.9%), shifted chin point (83.3%), Class III incisal relationship (70.4%), reversed overjet (70.4%), reduced overbite (56.0%) and crossbite (77.7%). Significant relationships were found between skeletal base discrepancy with incisal relationship, overjet, crossbite, canine relationships and right molar relationship. 57.1% agreed to orthognathic surgery, 32.1% opted for orthodontic treatment alone whilst 10.7% refused any treatment. It is hoped that this baseline demographic data may aid in the management and resource planning for this team in the future.

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Introduction

Orthognathic surgery has been an approach in correcting skeletal defects. Usually, in cases with mild to moderate skeletal discrepancy, orthodontic treatment is sufficient to achieve a good aesthetics and function within a balanced facial harmony. However, unlike orthodontic treatment alone, orthognathic surgery assists in correcting the underlying skeletal discrepancy directly (Raposo *et al.*, 2018).

Orthognathic treatment is effective for moderate to severe facial discrepancy, be it in the antero-posterior, vertical or transverse plane. The combination of orthognathic surgery and orthodontic treatment aids in correcting both skeletal imbalance and malocclusion with the ultimate aim of achieving acceptable facial harmony with a good functional bite (Zamboni *et al.*, 2019).

Multidisciplinary collaboration in the field of orthodontics, oral maxillofacial surgery and psychology is essential for orthognathic treatment. For the initial consultation, the psychological health of the patient must be understood (Reyneke, 2011). A clinical psychologist evaluates (Littlewood & Mitchell, 2019):

- Patients' expectation and the ability to cope with the whole life-changing treatment process.
- Patients' underlying motives or reasons in seeking for orthognathic treatment.
- Patient who are suffering from any psychological or even psychiatric disorder who needs to be managed professionally (i.e. Body Dysmorphic Disorder).

Upon psychological evaluation, a thorough joint assessment by the orthodontist and oral maxillofacial surgeon is conducted to obtain consensus of the best possible treatment options available for patient to achieve good functional occlusion with harmonious facial esthetic (Khechoyan, 2013). Amongst the records that are taken to help achieve diagnosis and treatment planning are lateral cephalogram, panoramic radiograph, posteroanterior skull radiograph, orthodontic study models with centric bite relation of the patient.

Orthognathic surgery had started way back in the early 19th century. Over the years, numerous orthognathic surgeries had been conducted with various surgical method introduced. These include vertical ramus osteotomy, distraction osteogenesis, genioplasty and many more. Today, Le Fort 1 for maxillary procedure and bilateral sagittal split osteotomy for mandibular procedure (Bagheri *et al.*, 2011) are identified to be most widely performed osteotomies. Despite various choices of surgical procedures to choose from, the implementation of pre-surgical orthodontics and post-surgical orthodontics play a great part in the success of orthognathic surgery (Graber *et al.*, 2017). Currently, orthognathic treatment has gained its reputation and more centers have been set up globally to offer orthognathic treatment.

There are only a few studies that indulge in the epidemiological aspect of orthognathic treatment. Studying the orthognathic treatment patients' demography could aid the health care system and for clinicians to establish optimum care for patients.

IIUM orthognathic team has been established since 2018. This establishment is to systematically cater to numerous orthognathic patients from the east coast of Malaysia, especially patients from Pahang. This joint clinic is achieved with the synchronized coordination between orthodontist, oral maxillofacial surgeon, and psychologist in IIUM. To date, there was no demographic study conducted regarding all the orthognathic cases presented in this clinic. The establishment of demographic data will further facilitate the management of this joint clinic. Therefore, this study aimed to establish the demographics and correlations for orthognathic treatment cases in Kulliyyah of Dentistry (KOD), IIUM since the establishment of the joint clinic from 2018 until December 2020.

Materials and Methods

This retrospective study investigated all the orthognathic treatment cases referred, consulted, and treated by the specialists at IIUM Orthognathic Joint Clinic of Kulliyyah of Dentistry, IIUM Kuantan. Ethical approval was obtained from IIUM Research Ethics Committee (Reference number: IIUM/504/14/11/2/ IREC 2021-026). A total of 28 patients were referred and listed for the orthognathic joint clinic from 2018 up until December 2020. Patients who were referred to the joint clinic were included in this study.

These data were obtained and examined from patients' case notes, photos, and study models (Table 1).

Table 1. Details of data.

Patients' Case Notes	• Patients' details were extracted from case notes such as age, gender, locality, the progress of treatment for each patient, treatment options etc.
Patients' Photos (Extra-Oral & Intra-Oral)	• Extra-oral photos were used to assess patients' facial deformity in terms of skeletal base relationship and soft tissue features. • Intra-oral photos were used to assess patients' dentoalveolar features which were integrated with the findings from patient's study models.
Patients' Study Models	• Study models were used to measure and assess the patients' dentoalveolar features such as overjet, overbite, crowding and others.

A calibration session between researcher who extracted the data with an orthodontic specialist was carried out, to achieve a standardized agreement on data collection. After good agreement was achieved, full data collection was done. These data were categorized into few parts. The first part recorded the demographic profile of orthognathic treatment cases seen in terms of gender, age, locality, race, and patients' motivation in seeking out treatment. The next part recorded the clinical features of orthognathic patients in relation to skeletal base relationship, soft tissue feature and dentoalveolar feature. Lastly, patients' treatment and management were noted.

The data measured and collected were analyzed using the IBM SPSS system version 25.0. Descriptive analysis was used to describe and categorize the data. Crosstabulation was also used to summarize the relationship between different variables of the categorical data. At the same time, statistical analysis was conducted using Phi Correlation Coefficient to assess the significant correlation between skeletal base discrepancies and other variables.

Results

Demographic profile of orthognathic patients

A total of 28 cases included in this research were referred and presented to IIUM orthognathic joint clinic from 2018 until December 2020. One patient was excluded as the patient refused treatment and did not handover the referral letter to the team. Therefore, the detailed data of 27 orthognathic patients were collected and presented here. Table 2 demonstrated the demographic profile of these patients. Patients' age varies from as early as 15 years old up until 42 years old. The majority of the patients were within the age group of 21 to 30 years old (44.4%). In terms of gender, 66.7% were females while the rest were male patients. Malay patients were slightly higher (55.6%) compared to Chinese patients (44.4%). There were no Indian or other ethnic groups referred to IIUM orthognathic joint clinic. With regards to the locality, most patients were from the state of Pahang except for 6 of them from Terengganu (22.0%).

Table 2. Demographic profile of orthognathic cases seen in KOD IIUM.

		Number of Respondents (n)	Percentage (%)
Age Group	**0 – 20 years old**	9	33.3
	21 – 30 years old	12	44.4
	31 – 40 years old	5	18.5
	40 years old & above	1	3.7
Gender	**Female**	18	66.7
	Male	9	33.3
Race	**Malay**	15	55.6
	Chinese	12	44.4
Locality	**Pahang**	21	77.8
	Non-Pahang	6	22.2

Clinical features of orthognathic patients

All patients were assessed in view of skeletal features, soft tissue features and dento-alveolar features. Table 3 showed that most orthognathic patients presented with Class III skeletal pattern (66.7%), increased vertical dimension (51.9%) and asymmetrical transverse dimension (76.0%). As for the soft tissue features, most of the patients had competent lips (86.4%) along with an average nasolabial angle (52.2%). 83.3% of patients had chin point deviation, of which 62.5% of them deviated to the left while 20.8% deviated to the right.

Concerning dentoalveolar features, most patients that sought or were referred for orthognathic consultation had a Class III incisal relationship (70.4%), reversed overjet (70.4%), reduced overbite (56.0%). Other than that, 77.7% of patients presented with crossbite, be it anterior crossbite, unilateral right or left posterior crossbite, bilateral posterior crossbite or a generalized crossbite. Most of the patients had mild crowding on the upper arch (57.7%) and lower arch (65.5%). Most patients had proclined upper incisors (45.5%) and retroclined lower incisors (40.0%) indicating dental compensation towards Class III skeletal base.

Table 3. Clinical features of orthognathic patients.

Clinical Features		Number of Respondents (n)	Percentage (%)
SKELETAL FEATURES	**Antero-Posterior Dimension**		
	• Class I	3	11.1
	• Class II	6	22.2
	• Class III	18	66.7
	Vertical Dimension		
	• Average	9	33.3
	• Increased	14	51.9
	• Reduced	4	14.8
	Transverse Dimension		
	• Symmetry	6	24.0
	• Asymmetry	19	76.0
SOFT TISSUES FEATURES	**Lips Competency**		
	• Competent	19	86.4
	• Incompetent	3	13.6
	Nasolabial Angle		

	• Average	12	52.2
	• Acute	5	21.7
	• Obtuse	6	26.1
	Chin Midpoint		
	• Coincide with facial midline	4	16.7
	• Shifted to left from facial midline	15	62.5
	• Shifted to right from facial midline	5	20.8
DENTOALVEOLAR FEATURES	**Incisal Relationship**		
	• Class I	1	3.7
	• Class II	7	25.9
	• Class III	19	70.4
	Overjet		
	• Average overjet	5	18.5
	• Reduced overjet	0	0.0
	• Increased overjet	3	11.1
	• Reversed overjet	19	70.4
	Overbite		
	• Average	7	28.0
	• Increased	4	16.0
	• Reduced	14	56.0
	Centreline		
	• Coincide with upper and lower facial midline	5	19.2
	• Not-coincide with either upper or lower facial midline	21	80.8
	Crossbite		
	• Absent	6	22.2
	• Anterior crossbite	7	25.9
	• Unilateral posterior crossbite	3	11.1
	• Bilateral posterior crossbite	2	7.4
	• Generalized crossbite	9	33.3
	Upper Alignment		
	• Well-aligned	1	3.8
	• Spacing	4	15.4
	• Mild crowding	15	57.7
	• Moderate crowding	3	11.5
	• Severe crowding	3	11.5
	Upper Arch Incisors Inclination		
	• Average inclination	9	40.9
	• Proclined	10	45.5
	• Retroclined	3	13.6
	Lower Arch Alignment		
	• Well-aligned	2	7.7
	• Spacing	5	19.2
	• Mild crowding	16	61.5
	• Moderate crowding	1	3.8
	• Severe crowding	2	7.7
	Lower Arch Incisors Inclination		
	• Average inclination	6	30.0
	• Proclined	6	30.0
	• Retroclined	8	40.0

Crosstabulation between skeletal base discrepancies with other variables

Table 4 showed that most patients presented with Class III skeletal base discrepancy despite being different in age group, gender, and locality. However, in terms of race, most Malays (26.7%) presented with Class II skeletal base discrepancy.

With regards to skeletal features, most patients with various vertical and transverse dimensions also exhibited Class III skeletal base discrepancy. Similar pattern can be seen with parameters of soft tissue features.

For dentoalveolar features, the crosstabulation showed an apparent number of patients with Class III skeletal base discrepancy presented with Class III incisor relationship (94.7%), reversed overjet (94.7%) and anterior crossbite (85.7%).

Table 4. Crosstabulation table between skeletal base discrepancies with other variables.

Variable	Skeletal Base Relationship			Total
	CLASS I	CLASS II	CLASS III	
DEMOGRAPHIC				
Age Group				
0 – 20 years old	11.1%	22.2%	66.7%	100.0%
21 – 30 years old	16.6%	16.7%	66.7%	100.0%
31 – 40 years old	0%	20.0%	80.0%	100.0%
41 years old & above	0%	100.0%	0%	100.0%
Gender				
Female	11.1%	22.2%	66.7%	100.0%
Male	11.1%	22.2%	66.7%	100.0%
Race				
Malay	6.6%	**26.7%**	16.7%	100.0%
Chinese	16.6%	16.7%	16.7%	100.0%
Locality				
Pahang	9.6%	19.0%	71.4%	100.0%
Non-Pahang	16.7%	33.3%	50%	100.0%
SKELETAL PATTERN				
Vertical Dimension				
Average	11.2%	44.4%	44.4%	100.0%
Increased	14.3%	7.1%	78.6%	100.0%
Reduced	0%	25.0%	75.0%	100.0%
Transverse Dimension				
Symmetry	16.7%	33.3%	50.0%	100.0%
Asymmetry	10.5%	21.1%	68.4%	100.0%
SOFT TISSUE FEATURE				
Lips Competency				
Competent	10.5%	21.1%	68.4%	100.0%
Incompetent	33.3%	33.3%	33.3%	100.0%
Nasolabial Angle				
Normal	8.3%	16.7%	75.0%	100.0%
Acute	20.0%	20.0%	60.0%	100.0%
Obtuse	16.7%	33.3%	50.0%	100.0%
Chin Midpoint				
Coincides	25.0%	25.0%	50.0%	100.0%

Shifted Left	13.3%	20.0%	66.7%	100.0%
Shifted Right	0%	40.0%	60.0%	100.0%
DENTO-ALVEOLAR FEATURES				
Incisal Relationship				
Class I	0%	100.0%	0%	100.0%
Class II	42.9%	57.1%	0%	100.0%
Class III	0%	5.3%	**94.7%**	100.0%
Overjet				
Average	40.0%	60.0%	0%	100.0%
Reduced	0%	0%	0%	100.0%
Increased	33.3%	66.7%	0%	100.0%
Reversed	0%	5.3%	**94.7%**	100.0%
Overbite				
Average	14.3%	0%	**85.7%**	100.0%
Increased	0%	50.0%	50.0%	100.0%
Reduced	14.3%	21.4%	64.3%	100.0%
Centreline				
Coincide	20.0%	20.0%	60.0%	100.0%
Not-Coincide	9.5%	23.8%	66.7%	100.0%
Crossbite				
Nil	33.3%	66.7%	0.0%	100.0%
Anterior	0.0%	14.3%	**85.7%**	100.0%
Unilateral Post	33.3%	33.3%	33.3%	100.0%
Bilateral Post	0.0%	0.0%	100.0%	100.0%
Generalized	0.0%	0.0%	100.0%	100.0%
Upper Arch Alignment				
Normal	0.0%	100.0%	0.0%	100.0%
Spacing	0.0%	50.0%	50.0%	100.0%
Mild	20.0%	20.0%	60.0%	100.0%
Moderate	0.0%	0.0%	100.0%	100.0%
Severe	0.0%	0.0%	100.0%	100.0%
Upper Arch Inclination				
Average	0.0%	22.2%	77.8%	100.0%
Proclined	20.0%	10.0%	70.0%	100.0%
Retroclined	0.0%	66.7%	33.3%	100.0%
Lower Arch Alignment				
Normal	0.0%	0.0%	100.0%	100.0%
Spacing	0.0%	20.0%	80.0%	100.0%
Mild	18.7%	31.3%	50.0%	100.0%
Moderate	0.0%	0.0%	100.0%	100.0%
Severe	0.0%	0.0%	100.0%	100.0%
Lower Arch Inclination				
Average	0.0%	33.3%	66.7%	100.0%
Proclined	16.7%	33.3%	50.0%	100.0%
Retroclined	0.0%	0.0%	100.0%	100.0%

Correlation between skeletal base discrepancies with other variables

Based on Table 5, there was no significant association illustrated between skeletal base discrepancies with other variables except with incisal relationship, overjet and right canine relationship, molar relationship and crossbite. For these variables, the p-values were less than 0.05 indicating significant correlation with skeletal base discrepancies. Significant correlation coefficient demonstrated between skeletal base discrepancies and incisal relationship (0.966), overjet (0.925), right canine relationship (0.777), left canine relationship (0.701), right molar relationship (0.715) and crossbite (0.879). Very strong correlations (Phi Correlation Coefficient: 0.80-1.00) were found between skeletal base discrepancies with incisal relationship, overjet and crossbite. Meanwhile, strong correlations (Phi Correlation Coefficient: 0.60-0.79) found between canine relationships and right molar relationships.

Table 5. Correlation between skeletal base discrepancies and other variables.

Variable	Skeletal Pattern	
Age Group	Sig. (2-tailed)	0.583
	Phi Correlation	0.417
Gender	Sig. (2-tailed)	1.000
	Phi Correlation	0.000
Race	Sig. (2-tailed)	0.638
	Phi Correlation	0.183
Locality	Sig. (2-tailed)	0.617
	Phi Correlation	0.189
Vertical Dimension	Sig. (2-tailed)	0.285
	Phi Correlation	0.431
Transverse Dimension	Sig. (2-tailed)	0.715
	Phi Correlation	0.164
Lips Competency	Sig. (2-tailed)	0.436
	Phi Correlation	0.275
Nasolabial Angle	Sig. (2-tailed)	0.848
	Phi Correlation	0.245
Chin Midpoint	Sig. (2-tailed)	0.758
	Phi Correlation	0.280
Incisal Relationship	Sig. (2-tailed)	**0.000***
	Phi Correlation	0.966
Overjet	Sig. (2-tailed)	**0.000***
	Phi Correlation	0.925
Overbite	Sig. (2-tailed)	0.363
	Phi Correlation	0.416
Centreline	Sig. (2-tailed)	0.804
	Phi Correlation	0.130
Right Canine Relationship	Sig. (2-tailed)	**0.003***
	Phi Correlation	0.777

Left Canine Relationship	Sig. (2-tailed)	**0.047***
	Phi Correlation	0.701
Right Molar Relationship	Sig. (2-tailed)	**0.014***
	Phi Correlation	0.715
Left Molar Relationship	Sig. (2-tailed)	0.066
	Phi Correlation	0.674
Crossbite	Sig. (2-tailed)	**0.008***
	Phi Correlation	0.879
Upper Arch Alignment	Sig. (2-tailed)	0.307
	Phi Correlation	0.602
Upper Arch Inclination	Sig. (2-tailed)	0.176
	Phi Correlation	0.536
Lower Arch Alignment	Sig. (2-tailed)	0.739
	Phi Correlation	0.446
Lower Arch Inclination	Sig. (2-tailed)	0.183
	Phi Correlation	0.558

**Phi-Correlation Test, p < 0.05 (two-tailed); Correlation is significant. Phi-Correlation Coefficient: 0 to 0.19 indicate very weak correlation; 0.20 to 0.39 indicate weak correlation; 0.40 to 0.59 indicate moderate correlation; 0.60 to 0.79 indicate strong correlation; 0.80 to 1.00 indicate very strong correlation (Campbell & Swinscow, 2009)*

Patients' status on orthognathic treatment

Figure 1 illustrated that out of 28 patients listed for orthognathic consultation, 10.7% (3) patients had either failed to attend their first appointment or patient came but was suggested on treatment option other than the orthodontic and surgical intervention. Out of 25 patients that attended the consultation session, 32.1% (9) had either declined surgery and chosen to proceed with orthodontic treatment only or were advised against surgical intervention as management due to case unsuitability, motivational or financial issues.

On the other hand, 52.1% (16) of patients that attended, had agreed to proceed with orthognathic management suggested by the team of specialists. From this group of patients, only two of the patients had completed the surgical treatment. While other 9 patients were either in pre-surgical orthodontics (fixed appliance) or in the growth stabilization monitoring process. Two patients were still contemplating the orthognathic surgery option. Unfortunately, three out of sixteen patients (18.7%) withdrew from orthognathic surgery treatment after initially agreed to it, due to a few reasons such as medical, dental, and patient factors.

Discussion

A large number of patients in this study were within the age group of twenty-one years old to thirty years old. This tallies with an orthognathic treatment study conducted in one of the dental schools in Thailand which stated that most orthognathic treatment patients were at the mean age of 22.8 years old (Aschaitrakool & Udomrat, 2014). Also similar to the United States, most of their patients were at an average age of 27.6 years old (Venugoplan *et al.*, 2012).

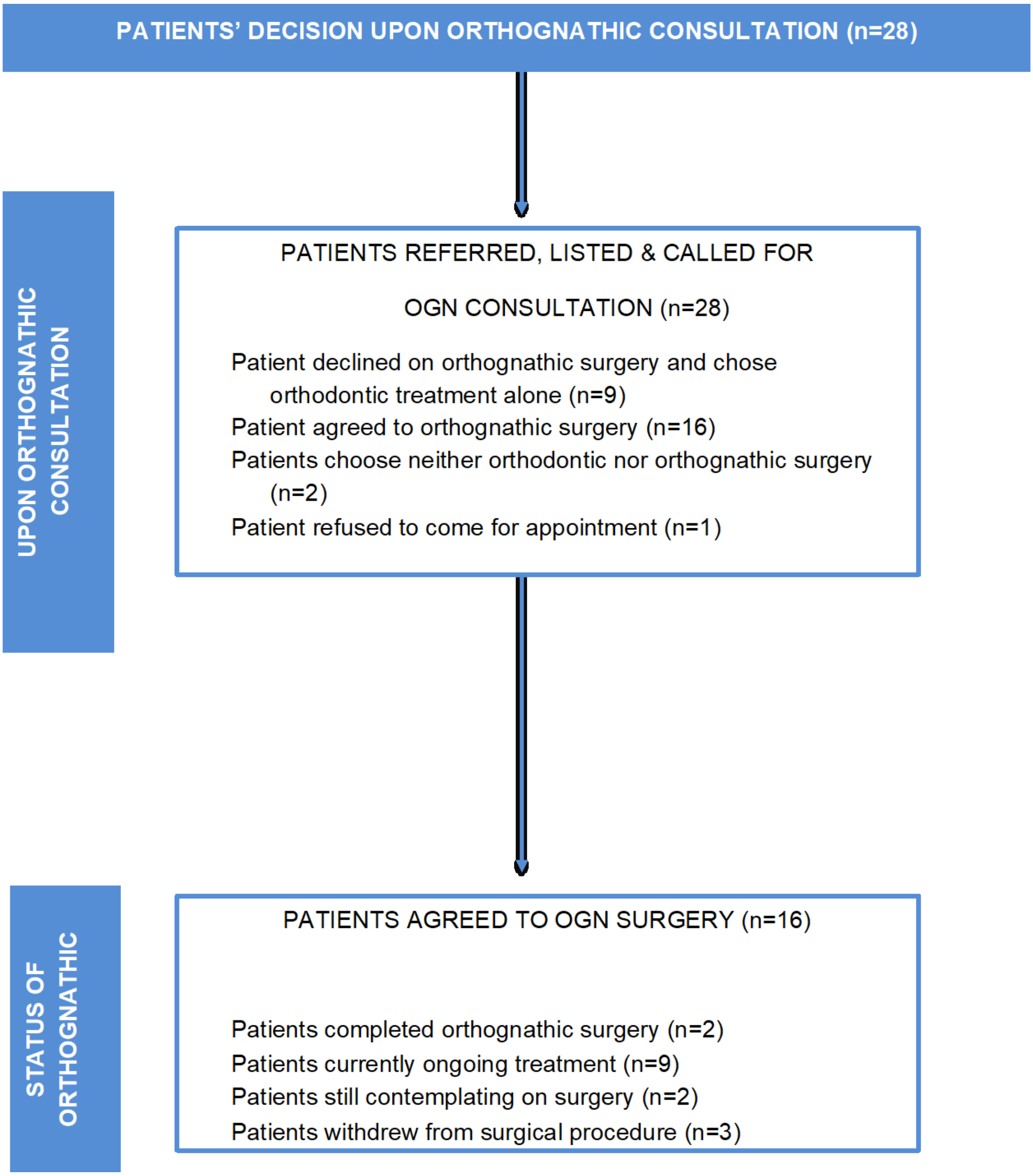

Figure 1. Patients' decision upon orthognathic consultation

In the present study, 66.7% which was more than half of the orthognathic treatment patients were females. This may be because females were more motivated and determined to do surgery than males for the sake of improving their aesthetic appearance (Ong, 2004). Females are very particular with regards to their appearances. Most Asians felt that females appear unpleasant in a community whenever they have profile of prognathic mandible (Ming, 2006). Another study also found that despite gender, both males and females had rated protrusive mandible as the least attractive profile while normal profile of maxilla and mandible as most attractive (Soh *et al.*, 2005). One is considered to have an attractive appearance when they presented with acceptable facial harmony. This is assessed by the rule of thirds, rule of fifth and smile parameters (Elsalanty *et al.*, 2007). Although beauty lies in the eyes of the beholder, studies have shown that attractiveness is more in straight profile (Lew *et al.*, 1992), thus is more acceptable by the community.

This study also recorded slightly higher Malay patients (55.6%) compared to Chinese patients (44.4%). The predominant number of Malay patients reflects the higher Malay population living in the state of Pahang followed by Chinese population. Only a small portion 4.7% are Indians and other ethnicities found in Pahang population (Department of Statistic Malaysia, 2020). Out of 27 patients that were referred to the joint clinic, 6 patients originated from out of Pahang state. As an orthognathic treatment team on the east coast of Malaysia, KOD can play a major role in consulting and treating orthognathic treatment patients both from Pahang and other states along the east coast of Malaysia.

In terms of skeletal features, demographic findings from current research correspond with findings from the literatures. Most orthognathic patients that were referred to or came to IIUM KOD orthognathic joint clinic presented with Class III anteroposterior dimension (66.7%), increased vertical dimension (51.9%) and asymmetrical transverse dimension (76.0%). Similar features were evident in an orthognathic treatment study conducted on orthognathic treatment patients from fifty Oral Maxillofacial Surgery clinics in Sweden. Most patients presented with the discrepancy in sagittal dimension (46.9%), vertical dimension (13.0%), transversal (9.9%) or combination (30.2%) of all dimensions (Andrup *et al.*, 2015). This correlates with another study which reported that more than 10% of orthognathic treatment patients in one of the dental schools in Japan presented with facial asymmetry (Inoue *et al.*, 2019). In addition, Class III skeletal discrepancies was reported to be more dominant in Southeast Asian population. Especially for Mongoloid population which has been reported to exhibit a Class III skeletal discrepancy for more than 20% of the population (Ruslin *et al.*, 2015).

In this study, more than half of the patients had Class III skeletal discrepancy in addition to deformity of dentoalveolar features such as Class III incisal relationship, reversed overjet and crossbites. Based on Table 4, significant strong correlations were found between skeletal base relationship (Class I, Class II and Class III) and incisal relationship (Class I, Class II and Class III), overjet (average, increased and reversed), crossbite (absent, anterior, unilateral posterior, bilateral posterior and generalized crossbite), right molar relationship (Class I, Class II and Class III), right and left canine relationship (Class I, Class II and Class III). Generally, patients with Class III skeletal patterns had glenoid fossa displaced anteriorly causing the head of condylar to be positioned more anteriorly, thus leading to mandibular prognathism. This causes patients to be presented with anterior crossbites which are when one or more than one lower incisor is positioned more labially or even worse, patients might have reversed overjet when all lower incisors are positioned labially to upper incisors.

Most patients in this study had a low incidence of soft tissue deformity. The majority of patients presented with competent lips and average nasolabial angle. A small portion of patients reported with incompetent lips (13.6%). There was also no significant association was found between skeletal discrepancy and soft tissue parameters in this study.

Orthognathic treatment has shown rapid growth throughout the world including Southeast Asia. However, the suggestion for surgical intervention may come as an extreme option to some of the patients. This study showed 12 out of 28 patients refused surgery and some of them opted for more conservative intervention instead. In addition, the remaining five that initially agreed to proceed with surgery were either still contemplating (n=2) or already withdrawn from surgical intervention (n=3). Hence, clinicians are recommended to provide some space and time for patients to make their own decisions after delivering the explanation of risks, the procedure involved, advantages, disadvantages or complications that may come with this life-changing surgical option (Reyneke, 2011).

One study conducted in 2019 had questioned some Malaysians with and without dental background to answer a questionnaire regarding the need for orthognathic surgery in the community. Less than half of respondents showed acceptance in correcting severe facial deformities with orthognathic surgery. This low in acceptance may be due to some Malaysians believing that surgery that enhances facial appearance is against their moral and religious views. This also may be due to financial problems and lack of support from close family members (Abdul Halim Chong *et al.*, 2019).

Hence, the future goal for IIUM orthognathic team should include the promotion of orthognathic consultation in providing treatment options for patients experiencing severe malocclusion with facial deformity especially for people within the location of east coast of Malaysia. Furthermore, this first organized demographic data of orthognathic patients in KOD IIUM might aid in management of the joint clinic along with improving health care access in terms of budget and resources allocations for orthognathic patients.

Limitation

The limitation of current study includes the small cumulative sample size of the orthognathic treatment patients. Hence, significant findings of this tiny sample size might not truly represent the whole population. There was also a drop of total number of cases between the end of 2019 until the end of 2020 during the hit of Covid-19 pandemic, as the Specialist Dental Clinic in KOD was forced to close during this period which eventually affect the number of orthognathic treatment cases referred or seen during this time frame.

Conclusion

To sum up, a total of 27 cases were referred, consulted, and treated in KOD IIUM orthognathic joint clinic. Majority were Malay female patients in age group between 21 to 30 years old, originated from Pahang. Most of these patients were presented with Class III skeletal discrepancy. A significant relationship was found between skeletal base discrepancy with several dentoalveolar deformities such as incisal relationship, overjet, crossbite, canine relationship and right molar relationship. 57.14% of orthognathic patients agreed to orthognathic treatment, 32.14% of them opted for orthodontic treatment alone whilst 10.7% refused any treatment. From the 57.14% (n=16), patients who initially agreed to orthognathic surgery, 10.7% (n=3) of them eventually pulled out, 7.14% (n=2) still undecided on surgery, 7.14% (n=2) completed surgery and whilst another 32.14% (n=9) ongoing treatment. This demographic hoped to give an initial description for the joint clinic for administration and future planning.

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ORIGINAL ARTICLE Open Access

The effect of probiotic *Lactobacillus rhamnosus* GG on *Candida albicans* and *Candida tropicalis* biofilm formation: A preliminary study

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Abstract

Lactobacillus rhamnosus is Gram-positive and lactic acid-producing bacterium. Meanwhile, *Candida albicans* and *Candida tropicalis* are opportunistic fungi that cause oral candidiasis. This study aimed to determine the effect of *L. rhamnosus* GG (LGG) on the biofilm formation of *C. tropicalis* and *C. albicans* with the hypothesis that LGG inhibits the biofilm of the yeasts. C. albicans ATCC MYA-4901 and C. tropicalis ATCC 13803 were standardised to 1x106 cells to form a mono-species biofilm. LGG was standardised to $1x10^7$ cells, equivalent to absorbance 0.5 at OD620nm. The microorganisms were cultivated in nutrient broth in a 96-well plate and incubated at 37°C for 24 h and 48 h. Co-culture biofilm was developed by combining *Candida* spp. with LGG in the same well at a similar concentration as the mono-culture. Crystal violet assay was conducted to assess the biofilm biomass with absorbance measured at OD_{620nm} wavelength. After 24 hours, polymicrobial biofilms of *C. albicans* with LGG decreased by 37.1 ± 9.2%. At 48 hours, it further decreased to 44.7 ± 5.9%. For *C. tropicalis*, co-culture biofilms with LGG decreased by 16.3 ± 5.9% and 35.7 ± 7.6% after 24 h and 48 h incubation, respectively. LGG significantly reduced *C. albicans* biofilm compared to *C. tropicalis* (P<0.05). In conclusion, LGG has antibiofilm activity against *C. albicans* and *C. tropicalis.* However, further study is needed to conclude the effect against other species strains.

Keywords: *biofilm, Candida albicans, Candida tropicalis, Lactobacillus rhamnosus GG, probiotic*

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Introduction

Candida species are common human opportunistic pathogens and capable of colonising the oral cavity, despite being the normal oral microbiome. The fungi commonly infect immunocompromised persons, causing severe mucosal and systemic infections such as oral candidiasis (Hernday *et al*., 2010; Geng *et al.*, 2018). Candidiasis is the most common disease associated with *Candida* spp. infection. The disease results from the overgrowth of fungi and is frequently observed in patients with HIV and those under steroid (Martins *et al.*, 2014; Erdogan *et al.*, 2015).

Although *C. albicans* is the most common aetiology for oral candidiasis, other *Candida* spp. such as *Candida tropicalis*, contribute to the infection (Hu *et al*., 2019). In oral infection, *C. tropicalis* is commonly found in a mixed culture with *C. albicans, C. glabrata* and *C. parapsilosis* (Miranda-Cadena *et al.*, 2018). *C. tropicalis* has been identified as the leading cause of candidemia in Algeria, followed by *C. albicans* (Megri *et al.*, 2020). Furthermore, the formation of true hyphae is also observed in *C. tropicalis,* increasing the virulence of the species (Zuza-Alves *et al.*, 2017).

Biofilm formation is a virulent factor of *Candida* spp. It begins with the adhesion of a single yeast cell to the substrate base, forming a groundwork of the basal yeast layer. The initiation step begins as proliferation and filamentation of the yeast cells occur, as they spread across the surface and form elongated projections, arising to filamentous hyphal form. Then, the accumulation of the extracellular polysaccharide matrix follows, indicating the maturation of the biofilm. Finally, non-adherent yeast cells are dispersed to colonise other mucosal surfaces. It is clinically significant, broadening the infection by forming new biofilm or disseminating it into the host tissue (Andes *et al*., 2004; Uppuluri *et al*., 2010; Tournu *et al*., 2012; Tsui *et al*., 2016).

Probiotics can benefit human health through three mechanisms of action which are 1) inhibition or exclusion of pathogen through direct inhibitory/bactericidal/fungicidal action or by influencing the commensal microbiome; 2) improvement of the epithelial barrier through modulation of signalling pathways; and 3) modulation of host's immune response by exercising strain-specific local and systemic immune response (Mack *et al.*, 2003; Lebeer *et al.,* 2008; Corr *et al.*, 2009; Segers *et al.*, 2014). Furthermore, the biofilm formed by probiotics can also act as a protective barrier against oral pathogens from colonising the cavity (Alok *et al.*, 2017).

Lactobacillus rhamnosus GG (LGG) is a gram-positive, non-spore-forming and lactic acid-producing bacterium classified as a beneficial probiotic to the gut (Doron *et al.*, 2005). For example, the pre-treatment of LGG to septic mice decreased inflammatory cytokines, reversed colonic proliferation, and recuperated gut microbiota diversity (Chen *et al.,* 2019).

Although probiotic LGG is commonly associated with gastrointestinal health, the effect on the colonisation of *Candida* spp. in the oral cavity remains unclear. Thus, this study aimed to determine the effect of LGG on the biofilm formation of *C. albicans* and *C. tropicalis* with the hypothesis that LGG inhibits the biofilm of the yeasts.

Materials and Methods

Growth of microorganisms

C. albicans ATCC MYA-4901*, C. tropicalis* ATCC 13803 and *Lactobacillus* GG (LGG) were used in the study. The inocula were standardised using a spectrophotometer (UviLine 9400, Secomam, Ales) in nutrient broth to give a final cell density of 10^6 cells/ml for *C. albicans* and *C. tropicalis*, while 10^7 cells/ml for LGG, which equivalent to an absorbance of 0.5 at 620 nm wavelength (OD_{620nm}).

Static biofilm formation

Biofilm formation in a static environment was conducted according to the protocol outlined by Alnuaimi *et al.* (2013). To develop mono-species biofilm, 60 µl of *C. albicans*, *C. tropicalis* or LGG standard suspension was pipetted into different wells of a 96-well plate. Following that, 120 µl of nutrient broth was added to the wells. To develop a dual-culture biofilm, 60 µl of *C. albicans* or *C. tropicalis* and 60 µl of LGG were pipetted into the same well. Then, 60 µl of nutrient broth was added to give a final volume of 180 µl. The plates were incubated for 24 and 48 hours at 37 °C.

Crystal violet assay

Crystal violet (CV) assay was conducted according to the protocol outlined by Badri *et al.* (2022). Briefly, non-adherent cells were washed with sterile phosphate buffer saline (PBS) (Amresco, Ohio). Fixation of biofilm was done by pipetting 200 µl of ethanol into each well, followed by incubation at room temperature for 15 minutes. The supernatant was removed, and the 96-well plate was air-dried for 45 minutes. Next, 200 µl of CV solution was added to the wells and was incubated for another 20 minutes at room temperature. The plate was washed with distilled water twice, and 200 µl of 33% acetic acid was pipetted into each well, followed by five minutes of incubation. Finally, 100 µl of the solution from each well was transferred into a new sterile 96-well plate, and the absorbance was measured at 620 nm using a microplate reader (Infinite 200 PRO, Tecan, Switzerland).

The inhibitory effect of LGG on *Candida* biofilm was assessed by calculating the percentage of biofilm reduction at 24 and 48 hours. The calculation was performed according to Subramenium *et al.* (2018) using the formula below:

$$\textbf{Reduction (\%)} = [(OD_{620nm}X - OD_{620nm}Y) / OD_{620nm}X] \times 100\%$$

Where X is the expected biofilm, and Y is the obtained biofilm. The expected value is the sum of the mono-culture biofilm biomass of *Candida* spp. and LGG, while the obtained value is the observed biofilm biomass of *Candida* spp. when co-cultured with LGG.

Statistical analysis

All experiments were conducted in three biological and three technical replicates (N=9). Statistical analysis was performed using SPSS software version 27.0. An independent t-test was used to compare biofilm biomass between the mono- and co-culture biofilm and between incubation times. Data with a value of $P<0.05$ were considered statistically significant.

Result and Discussion

C. tropicalis had more biofilm biomass than *C. albicans* after 24 h and 48 h incubations in mono-culture and co-culture. However, no significant differences were observed ($P>0.05$; Table 1). In addition, mono-culture *C. albicans* and *C. tropicalis* had significantly decreased biofilm biomass at 48 h incubation compared to 24 h ($P<0.05$). Meanwhile, only *C. albicans* exhibited significantly higher biofilm biomass at 24 h incubation than at 48 h in dual-culture biofilm ($P<0.05$).

The biofilm of both *C. albicans* and *C. tropicalis* was observed to develop as early as 24 h. This is attributed to the secretion of adhesin by both *C. albicans* and *C. tropicalis* that aid in the initial adherence to the host during the infection period (Yang, 2003; Martin et al., 2020).

Furthermore, a previous study reported that the biofilm formation by *C. albicans* and *C. tropicalis* was at the intermediate phase during the first 24 h (Cavalheiro *et al.*, 2018). At this phase, the extracellular polymeric substances (EPS) increased, and the bilayer formation of biofilm, commonly composed of yeast, germ tubes, and/without young hyphae, was formed. This phase lasts for 12 to 30 h. The maturation phase proceeds with a dense network of hyphae and yeast, forming a thicker EPS, which will take approximately 38 to 72 h (Cavalheiro *et al.*, 2018). It is suggested that the decreased biofilm biomass at 48 h in this study was due to the competition for nutrients and microhabitats by microorganisms in the dense biofilm consortium at the maturation phase.

C. albicans biofilm biomass was reduced by 37.1 ± 9.2% at 24 h when co-cultured with LGG (Figure 1). The biofilm decreased by 44.7 ± 5.9% after 48 h. As for *C. tropicalis*, the biofilm biomass of the yeast was reduced by 16.3 ± 5.9% when co-cultured with LGG after 24 h. The biofilm was further decreased by 35.7 ± 7.6% after 48 h incubation with LGG. These results are suggested due to the antagonistic interaction of LGG with *C. albicans* and *C. tropicalis* that compete for the binding site on the oral surface to initiate colonisation (Jiang *et al.*, 2016; *Jørgensen et al.*, 2017; Meurman *et al.*, 2018).

Furthermore, metabolites such as bacteriocin-like peptides that LGG produces have also been reported to contribute to the biofilm reduction (Dimitrijević *et al.,* 2009; Zhang *et al.,* 2018). In addition, these secondary metabolites also act antagonistically against biofilm formation by interfering with the adhesion of *Candida* spp. to the surfaces and by reducing the sturdiness of the formed biofilm (Barzegari *et al.,* 2020).

Table 1. Biofilm biomass produced by *C. albicans*, *C. tropicalis* and LGG in mono-culture and dual-culture biofilms.

Inocula	Incubation period	
	24 h	48 h
C. albicans	0.157 ± 0.046*	0.067 ± 0.006
C. tropicalis	0.217 ± 0.082*	0.109 ± 0.042
LGG	0.077 ± 0.006	0.073 ± 0.009
C. albicans + LGG	0.147 ± 0.033*	0.077 ± 0.006
C. tropicalis + LGG	0.215 ± 0.087	0.120 ± 0.043

The data were CV assay and expressed as mean value ± standard deviation from nine replicates, N = 9. *Significant differences were observed between 24 h and 48 h. Data were considered statistically significant when $P<0.05$.

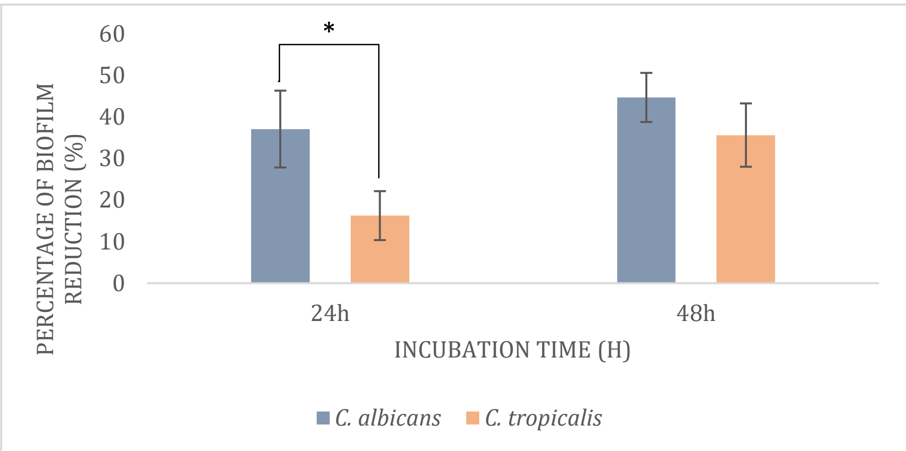

Figure 1. Percentage of biofilm reduction in nutrient broth (NB) at the 24 hours and 48 h incubation, N = 9. *Significant difference was observed between *C. albicans* and *C. tropicalis* ($P<0.05$).

Conclusion

This preliminary *in vitro* study demonstrated that LGG inhibits the biofilm formation of *C. albicans* and *C. tropicalis* at 24 h and 48 h incubation. However, more *Candida* strains are suggested to elucidate the mechanism of inhibition by LGG to the oral pathogen.

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ORIGINAL ARTICLE Open Access

Prevalence and orthodontic management of missing permanent maxillary lateral incisor at a referral centre in Sabah

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Abstract

This was a cross-sectional study of the prevalence and orthodontic management of developmental missing permanent maxillary lateral incisor of patients referred for orthodontic treatment from year 2010 to 2020. The dental records of written case notes, radiographs, and study models that fulfilled the inclusion criteria were selected using systematic sampling and were assessed retrospectively. Sample were taken from every five dental records. Dental records that did not fulfil the inclusion criteria were substituted. Data taken were the demographic data, side of the missing maxillary lateral incisor, and the orthodontic management. All variables were analysed descriptively. The differences between orthodontic open space and orthodontic close space with respect of unilateral and bilateral missing permanent maxillary lateral incisor were tested using Fisher's exact test. From the total of 291 samples, there were 11 (3.78%) patients presented with missing permanent maxillary lateral incisor. The mean age of the patients was 17.46 ± 1.52 years. All 11 patients were females. There were six (54.55%) patients presented with bilateral missing permanent maxillary lateral incisor, while five (45.45%) were unilateral. From the unilateral group, there were three (27.27%) patients presented with right side missing permanent maxillary lateral incisor. Six (54.55%) patients were treated with orthodontic open space, while five (45.45%) patients were treated with orthodontic close space. In conclusion, the prevalence of missing permanent maxillary lateral incisor was 3.78%. All patients were females. More patients presented with bilateral missing permanent maxillary lateral incisor. The treatments provided were orthodontic open space and orthodontic close space.

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Introduction

The permanent maxillary lateral incisor is the most common missing tooth (not including the third molars) (Fauzi *et al.*, 2019; Mani *et al.*, 2014; Nik-Hussein, 1989; Shakirah Said *et al.*, 2017) and can contribute to malocclusion (Caterini *et al.*, 2017). Two-thirds of the missing maxillary lateral incisor were unilateral, while the remaining one-third were bilateral (Arandi & Mustafa, 2018). A study on the Malaysian population found that more missing teeth observed at the maxilla and on the right side (Fauzi *et al.*, 2019), although there was another study found that missing permanent maxillary lateral incisor were more commonly occurred on the left side (Arandi & Mustafa, 2018).

The prevalence of missing permanent maxillary lateral incisor varied among different regions and populations. The prevalence of missing permanent maxillary lateral incisor among orthodontic patients was 3.77% (Sahoo *et al*., 2019; Swarnalatha *et al*., 2020). Meanwhile, the prevalence of missing permanent maxillary lateral incisor among hypodontia orthodontic patients were 26.5% (Acev & Gjorgova, 2014), 19.8% (Kim, 2011), and 17.8% (Gracco *et al*., 2017; Zakaria *et al*., 2021). However, the prevalence of missing permanent maxillary lateral incisor was found to be lower among non-orthodontic populations; 1.91% (Arandi & Mustafa, 2018), and 2.56% (Musaed *et al*., 2019).

The treatment of missing permanent maxillary lateral incisor is challenging and complex, requiring very careful treatment planning, good communication with the patient, and coordinated interdisciplinary efforts of the orthodontist, periodontist, prosthodontist, and restorative dentist. The two orthodontic treatment options are open space with prosthetic replacement and close space with canine camouflage. Careful diagnosis and treatment plan are important because the spacing is available on the anterior part of the upper arch that affects the dental function and aesthetic. The decision whether to open space or to close space could be controversial (Gupta & Rauniyar, 2021). Both orthodontic close space and orthodontic open space with implant replacement produced similar well-accepted aesthetic results (Jamilian *et al*., 2015). Other simpler treatment option is the maintenance of the retained primary teeth with composite build-up.

At the present, the two most recommended procedures for prosthetic replacement are the single-tooth implant and the fibre-reinforced resin-bonded bridge with a ceramic overlay (Dudney, 2008). Each available treatment has its own advantages, disadvantages, indications, and limitations. The treatment plan should not be influenced by the clinician's bias, but the patient's realistic expectation should be taken into consideration (Kavadia *et al*., 2011). Other prosthetic option is the removable partial denture if patients preferred less complicated and less expensive treatment.

Patients with spaces closed by substituting permanent canines had significant healthier periodontal tissues than patients with prosthetic replacement (Jamilian *et al*., 2015; Nordquist & McNeill, 1975). Moreover, orthodontic space closure appeared to be reasonably stable and better accepted by the patients than prosthetic replacements. There was no difference in the prevalence of dysfunction and impaired temporomandibular joint function, but there was greater tendency of plaque accumulation and gingivitis development in subjects with prosthesis replacement (Robertsson & Mohlin, 2000). Infraocclusion greater than 1 mm was found in implant patients (Jamilian *et al*., 2015).

Missing permanent maxillary lateral incisor can have a major impact on the dental and facial aesthetics from a very young age, which may affect the self-esteem and social well-being of the patient. This condition was often complicated by other dental anomalies associated with hypodontia, such as impacted teeth, microdontia, hypodontia of posterior teeth, delayed eruption, and taurodontism (Ministry of Health Malaysia, 2012).

The demand for orthodontic treatment is high due to its impact on both dental function and facial aesthetics. Knowledge and updates on the prevalence and orthodontic management of missing permanent maxillary lateral incisor are important to assist clinicians in early diagnosis and timely referral for interceptive treatment to prevent developing malocclusion. Therefore, the aim of this study was to assess the prevalence and orthodontic management of developmental missing permanent maxillary lateral incisor at a government orthodontic clinic in Kota Kinabalu, Sabah. The findings from this study may help us to know more of the needs of the community, to plan treatment, and also to serve as a baseline reference for future multicentre study for missing permanent maxillary lateral incisor.

Materials and Methods

This was a cross-sectional study of the available dental records of patients referred for orthodontic treatment from year 2010 to

2020 at a government orthodontic clinic in Kota Kinabalu, Sabah. Ethical approval for this study was obtained from the Medical Research and Ethics Committee (MREC), Ministry of Health Malaysia and registered with the National Medical Research Register (NMRR-20-2316-56893).

The dental records consisted of written case notes, relevant radiographs (dental panoramic radiograph, periapical radiograph and upper standard occlusal radiograph), and study models that fulfilled the inclusion criteria, were recruited and assessed retrospectively by a researcher (LJH). The sample size for this study was estimated to be 156, with 0.03 (3%) precision based on 3.8% prevalence with at least one missing permanent maxillary lateral incisor in an orthodontic population by Sahoo *et al*. (2019).

The inclusion criteria were patients who have no previous orthodontic treatment before and presented with developmentally missing permanent maxillary lateral incisor. The exclusion criteria were patients who presented with congenital syndromes, genetic disorders and craniofacial deformities e.g. cleft lip and palate, the missing permanent maxillary lateral incisor was due to dental extraction or trauma, and inadequate evidence from the dental records, due to poor quality radiograph or study model to diagnose or to confirm the missing maxillary lateral incisor and previous treatment.

The patients' dental records were selected using systematic sampling. Sample were taken from every five dental records in the storage. Dental records that did not fulfil the inclusion criteria were substituted. Data taken were the demographic data (age, gender, and ethnic group), side of the missing maxillary lateral incisor, and the orthodontic management of the missing maxillary lateral incisor.

The data was entered into a standardised data collection form. All variables were analysed descriptively using Stata 15. The differences between orthodontic open space and orthodontic close space with respect of unilateral and bilateral missing permanent maxillary lateral incisor were tested using Fisher's exact test. The level of significance was set at 5% ($p < 0.05$).

Results

A total of 291 samples were taken from the dental records and assessed. There were 11 (3.78%) patients presented with missing permanent maxillary lateral incisor (Figure 1). The mean age of the patients with missing permanent maxillary lateral incisor at the time of referral, was 17.46 ± 1.52 years. All of the patients presented with missing permanent maxillary lateral incisors were females (n=11, 100.00%).

The ethnic groups with missing permanent maxillary lateral incisor were Kadazan Dusun (n=2, 18.18%), other Bumiputera Sabah (Bajau and Brunei) (n=3, 27.27%), Chinese (n=4, 36.36%), Malay (n=1, 9.10%), and other ethnicity (n=1, 9.10%) (Table 1). The prevalence of missing permanent maxillary lateral incisor was highest in other Bumiputera Sabah (n=3, 5.46%), followed by Malay (n=1, 4.76%), Chinese (n=4, 4.30%), and other ethnicity (n=1, 3.70%). Kadazan Dusun had the lowest prevalence of missing permanent maxillary lateral incisor (n=2, 2.11%) (Table 2).

There were six (n=6, 54.55%) patients presented with bilateral missing permanent maxillary lateral incisor, while the remaining five (n=5, 45.45%) were unilateral missing permanent maxillary lateral incisor. From the unilateral group, there were three (n=3, 27.27%) patients presented with right side missing permanent maxillary lateral incisor (Figure 2).

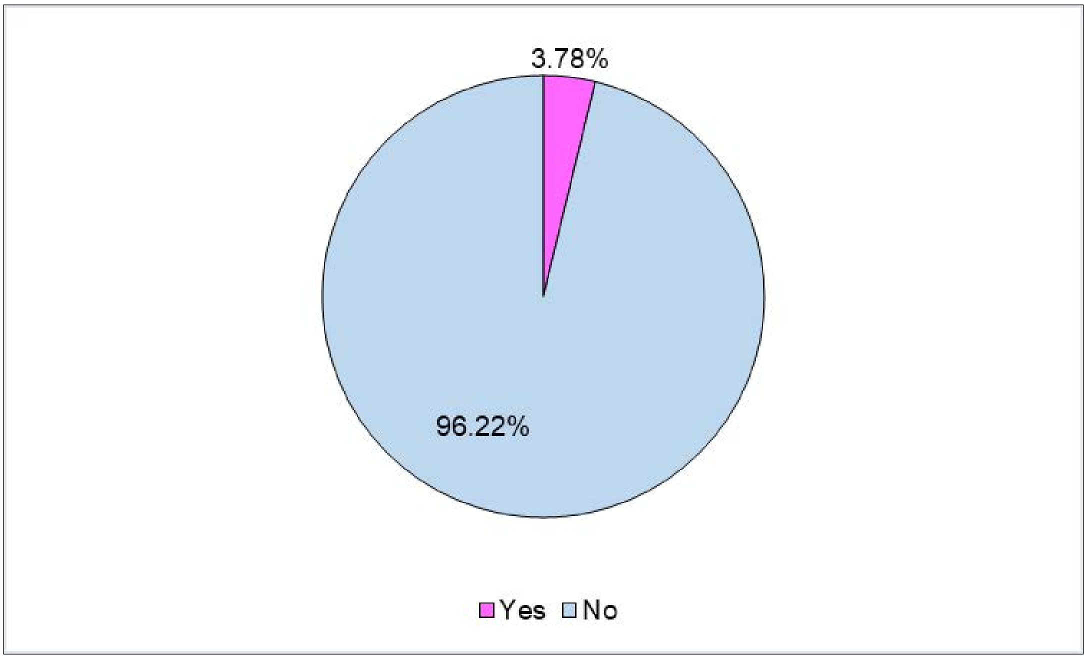

Figure 1. Prevalence of missing permanent maxillary lateral incisor.

Table 1. Demographic profile of the patients with missing permanent maxillary lateral incisor.

Variables		n (%)	Mean ± SE
Age		-	17.46 ± 1.52
Gender	Female	11 (100.00)	
	Male	0 (0.00)	
Ethnic groups	Kadazan Dusun	2 (18.18)	
	Bumiputera Sabah	3 (27.27)	
	Chinese	4 (36.36)	
	Malay	1 (9.10)	
	Other ethnicity	1 (9.10)	

Table 2. Prevalence of missing permanent maxillary lateral incisor among ethnic groups.

	Missing	Total sample	Prevalence (%)
Kadazan Dusun	2	95	2.11
Other Bumiputera Sabah	3	55	5.46
Chinese	4	93	4.30
Malay	1	21	4.76
Other ethnicity	1	27	3.70
Overall	**11**	**291**	**3.78**

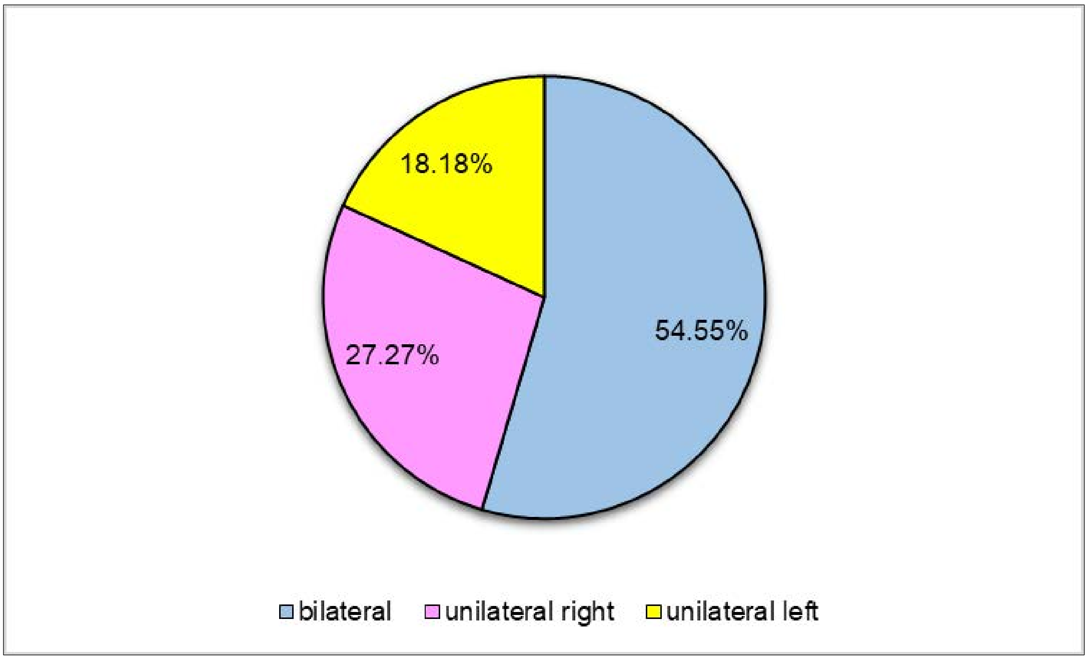

Figure 2. Pattern of missing permanent maxillary lateral incisor.

From the total of 11 patients who had missing permanent maxillary lateral incisor, six (n=6, 54.55%) patients were treated with orthodontic open space, while the remaining five (n=5, 45.45%) patients were treated with orthodontic close space. Among the patients with unilateral missing permanent maxillary lateral incisor, more than half of them (n=3, 60%) were treated with orthodontic close space. In contrast, among the patients with bilateral missing permanent maxillary lateral incisor, more than half of them (n=4, 66.67%) were treated with orthodontic open space. These findings were not statistically significant, $p > 0.05$ (Table 3).

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Table 3. Type of orthodontic management based on unilateral or bilateral missing permanent maxillary lateral incisor.

Type of orthodontic management	Unilateral missing (%)	Bilateral missing (%)	Total (%)
Open space	2 (33.33)	4 (66.67)	6 (100.00)
Close space	3 (60.00)	2 (40.00)	5 (100.00)

Fisher's exact = 0.740

Discussion

In Malaysia, the three major ethnic groups are Malay (51.0%), Chinese (24.2%), and Indian (7.2%) (Nagaraj *et al*., 2015). Meanwhile in Sabah, the largest ethnic group is Kadazan Dusun, which is about one-third of the total population. Chinese is the largest non-Bumiputera ethnic group in Sabah

(Kerajaan Negeri Sabah, 2023). In this study, the prevalence of missing permanent maxillary lateral incisor was higher in Malay compared to Chinese, similar to a hypodontia study conducted in Peninsular Malaysia (Zakaria *et al*., 2021). Other Bumiputera Sabah had the highest prevalence, whilst Kadazan Dusun had the lowest prevalence of missing permanent maxillary lateral incisor. However, there is no similar study done before on Sabah population. Therefore, these findings might serve as a baseline data for future hypodontia studies.

The prevalence of missing permanent maxillary lateral incisor in this study was same with the finding by Sahoo *et al*. (2019) and Swarnalatha *et al*. (2020). However, this finding was not comparable to other studies by Acev & Gjorgova, Kim, and Gracco *et al*. as their prevalences were determined from orthodontic patients with other missing teeth or hypodontia (Acev & Gjorgova, 2014; Gracco *et al*., 2017; Kim, 2011).

All of the patients in this study who were presented with missing permanent maxillary lateral incisor were females. It seemed that congenitally missing teeth usually occurred more commonly in females than males (Alhaddad *et al*., 2019; Kafantaris *et al*., 2020; Rakhshan, 2015; Swarnalatha *et al*., 2020; Zakir *et al*., 2015).

There were more patients presented with bilateral missing permanent maxillary lateral incisor in comparison to unilateral missing permanent maxillary lateral incisor. From the unilateral group, more were presented with right side missing permanent maxillary lateral incisor. These findings were similar to other studies (Sahoo *et al*., 2019; Swarnalatha *et al*., 2020). It is important for the clinician to diagnose the missing lateral incisor, either bilateral or unilateral before planning for the treatment due to the differences in the spaces available to be managed.

Among the patients with unilateral missing permanent maxillary lateral incisor, more were treated with orthodontic close space. In contrast, among the patients with bilateral missing permanent maxillary lateral incisor, more were treated with orthodontic open space. There were more spaces to be managed in bilateral missing permanent maxillary lateral incisor. Therefore, orthodontic open space might be a more suitable treatment compared to orthodontic close space. However, besides localised spaces due to the missing permanent maxillary lateral incisor, other factors that might influence the treatment choice were the malocclusion and skeletal pattern (Sahoo *et al*., 2019). There was no statistically significant association between orthodontic close space and orthodontic open space in this study.

Treatment for missing permanent maxillary lateral incisor must be interdisciplinary to get the most predictable outcome (Gupta, 2022). It involved the orthodontics, prosthodontics, restorative, periodontics, and implantology. Interdisciplinary approach ensured good occlusion, natural smile and stable treatment outcome for the patients. From a prosthodontic and restorative study, the factors affecting the decision-making for missing permanent maxillary lateral incisor were patient's age at treatment commencement, individual characteristics of each clinical situation, and the clinician's preference (Kafantaris *et al*., 2020). Nevertheless, the patient's preferences such as complexity and cost of treatment should be taken into consideration too.

This study provided knowledge of the prevalence, pattern, and orthodontic management of missing permanent maxillary lateral incisor, which are important for treatment planning. An interdisciplinary treatment, if done properly and timely, could prevent the patients from aesthetic and functional discrepancies that might reduce their self-esteem and social well-being and also to prevent developing malocclusion. The limitation for this study is the data taken from one government orthodontic referral centre in Sabah which lacks generalisability and the stability of the treatment outcomes were not assessed. For future studies, the author would like to suggest involvement of all government orthodontic referral centres in Sabah, for prospective cohort studies to follow up and to compare the stability of the treatment outcomes of orthodontic open space and orthodontic close space. The true prevalence among the population might be lower than this prevalence in orthodontic referrals.

Conclusion

The prevalence of missing permanent maxillary lateral incisor was low, at 3.78%. All of the patients with missing permanent maxillary lateral incisor were females. More patients presented with bilateral missing permanent maxillary lateral incisor. The treatments provided at this centre were orthodontic open space and orthodontic close space.

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Conflict of interest

The author declared no conflict of interests in this research.

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ORIGINAL ARTICLE Open Access

Molecular docking study of hyaluronic acid against interleukin-6 (7DC8 protein) in COVID-19 patients with periodontitis

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Abstract

Coronavirus disease 2019 (COVID-19) is a new disease caused by a *coronavirus*, namely SARS-CoV-2. This virus was entered inside the host by angiotensin-converting enzyme receptors (ACE2). Recent evidence suggests that sulcus fluid in the periodontal pockets of patients with periodontitis may be a source of SARS-CoV-2 and a potential reservoir for increasing oral viral load in patients with confirmed COVID-19. ACE-2 is expressed in stratified squamous epithelium mainly on the dorsal tongue and gingiva. The gingival sulcular epithelium is the entry point for SARS-CoV-2 into the periodontal pocket epithelium through the *gingival crevicular fluid* (GCF). Hyaluronic acid (HA) is a high molecule of heavy polysaccharide (*glycosaminoglycan*) which has several functions, such as anti-inflammatory and accelerated wound healing. It could decrease the levels of several cytokines. This study aims to analyse the interaction of HA against the IL-6 coronavirus receptor in periodontitis through a molecular docking study using MOE 2015.10 software with IL-6 receptor (7DC8) as the protein model to predict the binding of HA with 10 poses. The 7DC8 protein was prepared by adding charge and the validation method was performed with RMSD <2Å which indicates this method is valid. The results of this study showed that there are interaction between HA and the IL-6 receptor via amino acid residue interaction at the Leucine 98 (bond energy -0.7 kcal/mol), Serine 52 (bond energy -1.7 kcal/mol), Glycine 53 (bond energy -1.5 kcal/mol), and Glycine 299 (bond energy -1.6 kcal/mol). HA has an interaction with coronavirus at the IL-6 receptor of periodontitis based on *molecular docking* study and can potentially be used as a therapeutic option in COVID-19 with periodontitis. In conclusion, hyaluronic acid has the potential as an anti-inflammatory drug of choice in COVID-19 patients with periodontitis.

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Introduction

Coronavirus disease 2019 (COVID-19) has been declared a worldwide pandemic (Indonesian Lung Doctors Association, 2020). This disease causes progressive respiratory failure leading to death (Zhou *et al.*, 2020). ACE-2 is the main receptor for the entry of SARS-CoV-2 into human cells located in the lungs, nasopharyngeal mucosa, salivary cells, and oral epithelial cells (Badran *et al.*, 2020). In oral epithelial cells, ACE-2 is expressed in the stratified squamous epithelium of the dorsal tongue and gingiva. The gingival sulcular epithelium is the entry point for SARS-CoV-2 into the periodontal pocket epithelium through the gingival crevicular fluid (GCF) (Sakaguchi *et al.,* 2020).

Periodontitis is a chronic inflammation that attacks the supporting tissues of the teeth and has a high prevalence in the adult population with the main clinical manifestations of periodontal pockets. Sulcus fluid in the periodontal pocket of patients with periodontitis can be a source of SARS-CoV-2 and a potential reservoir for increasing the viral load in the oral cavity of patients with confirmed COVID-19 (Bertolini *et al.,* 2020).

In periodontitis patients there is an increase in the level of interleukin-6 (IL-6) which is a mediator in the process of periodontal destruction (Molayem & Pontes, 2020). Gingival fibroblasts are capable of producing increased levels of IL-6 when exposed to polysaccharides (LPS) or IL-1. IL-6 is involved in osteoclastogenesis and is used as a potential marker to predict the progression of COVID-19 patients (Molayem & Pontes, 2020.; Wang, 2020). High levels of IL-6 have been associated with a higher risk of pulmonary complications.

Non-surgical periodontal treatment to reduce cytokine levels is considered to be able to reduce lower IL-6 levels and inflammation due to periodontal treatment has the potential to protect COVID-19 patients from life-threatening respiratory complications (Molayem & Pontes, 2020). Hellman *et al.* (2020) has conducted a study that in patients with COVID-19 hyaluronic acid was seen in the alveolar walls and pulmonary perivascular tissue.

Hyaluronic acid (HA) has bacteriostatic and anti-inflammatory effects that play a role in the wound healing process. HA works by weakening the bonds of tissue cells that are chronically inflamed so that they are easily released and replaced by the regeneration of new healthy tissue cells (Wijayanto *et al.,* 2014). HA significantly suppresses the secretion of inflammatory cytokines IL-6 and IL-8 (Rooney *et al.,* 2015). HA reduces the proliferation of epithelial cells such as fibroblasts and lymphocytes which play an active role in chronic inflammatory conditions thereby accelerating the regeneration of new cells (Mesa *et al.,* 2004). There have been no scientific studies related to the interaction of hyaluronic acid with IL-6 in COVID-19 patients with periodontitis using molecular docking study. Molecular docking is a genetic-based method that can be used to find the most appropriate and involving interaction patterns between two molecules, namely receptors and ligands. The test was carried out to see the docking score and the interaction between hyaluronic acid and the IL-6 receptor (GDP: 7DC8) using Molecular Operating Environment (MOE) 2015.10 software.

Materials and Methods

Software and program

Molecular docking test using Molecular Operating Environment (MOE) 2015.10 software.

Preparation of protein structures for Interleukin-6 (7DC8)

Interleukin-6 receptor protein obtained from protein data bank (PDB), with ATP agonist ligand (PDB: 7DC8) via https://www.rcsb.org/structure/7DC8. The protein was chosen based on the element of origin of this protein which is human (Homo sapiens), with an unmutated protein, and has an active single ligand. The docking method used is the optimal method that has the best RMSD value. The receptors were prepared using MOE 2015.10 software by removing unnecessary molecules, such as water. Redocking of antagonist ligands is carried out on the ligand receptor to obtain the active site of the receptor.

Preparation of hyaluronic acid ligand

Hyaluronic acid SMILES code obtained from PubChem at https://pubchem.ncbi.nlm.nih.gov/compound/Hyaluronic-acid-sodium-salt. The HA structure was prepared directly using Molecular Operating Environment (MOE) software 2015.10 and saved in .mdb format.

Molecular docking

The test was carried out to see the docking score and the interaction between hyaluronic acid and the IL-6 receptor (GDP:

7DC8) using Molecular Operating Environment (MOE) 2015.10 software. First, the redocking process was carried out to see the position of the complete protein with its native ligands before and after docking. The redocking process will look at the value of the Root-mean-square-deviation (RMSD) as a method validation parameter with an ideal value of <2 Å. The docking positions tested were 10 best positions of hyaluronic acid (HA) against the IL-6 receptor. Manually selected a ligand position that has the best docking score. The docking score of the test ligand was compared with that of the agonist ligand to compare the binding affinity of the two to the receptor.

Result and Discussion

The molecular docking was carried out using the IL-6 target protein obtained from PDB, namely 7DC8. Redocking of IL-6 (GDP: 7DC8) with ATP agonist ligand is shown in Figure 1; and obtained the value of RMSD = 0.9807. Since RMSD < 2Å (Jain and Nicholls, 2008), the PDB:7DC8 method and receptor are valid for use in molecular docking assay ligands.

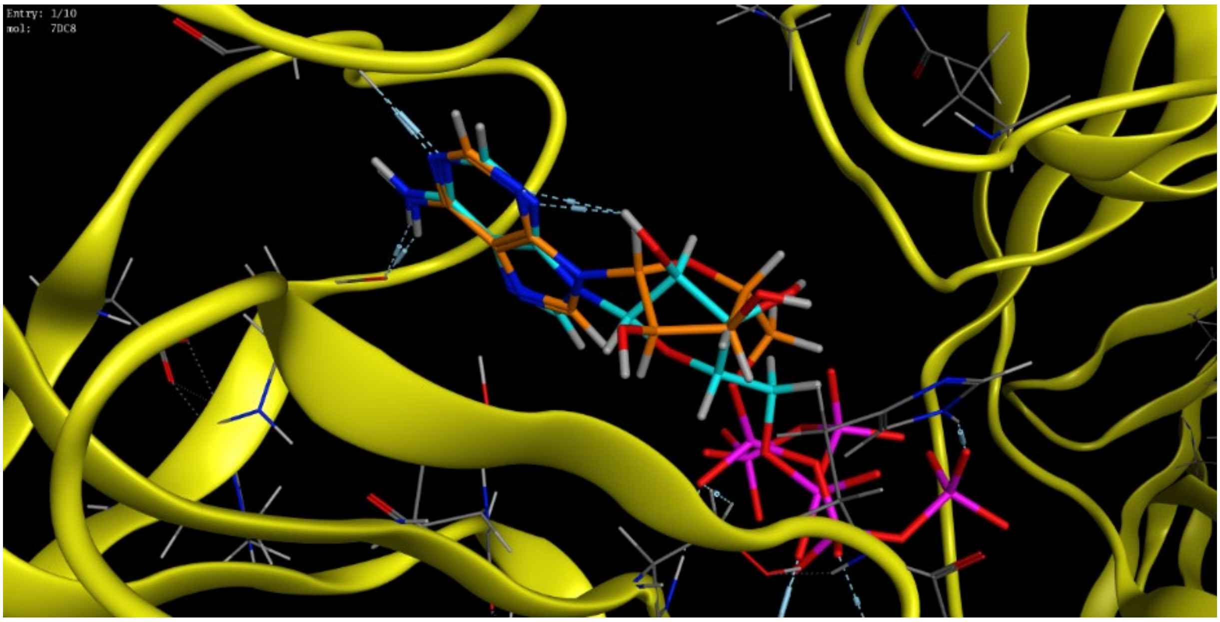

Figure 1. Redocking of IL-6 (PDB-7DC8) (light blue) with ATP agonist ligand (orange).

The molecular docking agonist ligands ATP on the amino acids from IL-6 are *Phenylalanine* (Phe) 298, *Glycine* (Gly) 96, *Leucine* (Leu) 100, Leu 98, *Tyrosine* (Tyr) 95, *Serine* (Ser) 52, and *Glutamine* Gln 53 (Table 1). The hyaluronic acid test ligand has a bond with the amino acids Gly 299, Leu 98, Ser 52, and Gln 53, so that hyaluronic acid has the potential to inhibit IL-6. Molecular docking of IL-6 with agonist ligand (ATP) and test ligand (hyaluronic acid) is shown in Table 1. While the overlay of molecular interactions of IL-6 with agonist ligand and test ligand (hyaluronic acid) is presented in Figure 2.

Table 1. Molecular interaction of IL-6 (GDP: 7DC8) with hyaluronic acid test ligand

Ligand	Docking Score	Amino Acids	Interaction	Distance(Å)	E (kcal/mol)
Agonis (ATP)	-5.4722	PHE 298	H-donor	3.26	-0.7
		GLY 96	H-donor	2.87	-2.2
		LEU 100	H-donor	3.07	-1.9
		LEU 98	Pi-H	3.68	-1.0
		TYR 95	Pi-pi	3.96	-0.0
		SER 52	H-acceptor	2.50	0.9
		GLN 53	H-acceptor	2.76	-5.4
Test (Hyaluronic acid)	-7.3179	GLY 299	H-donor	2.99	-1.6
		LEU 98	H-acceptor	3.05	-0.7
		SER 52	H-acceptor	2.95	-1.7
		GLN 53	H-acceptor	2.86	-1.5

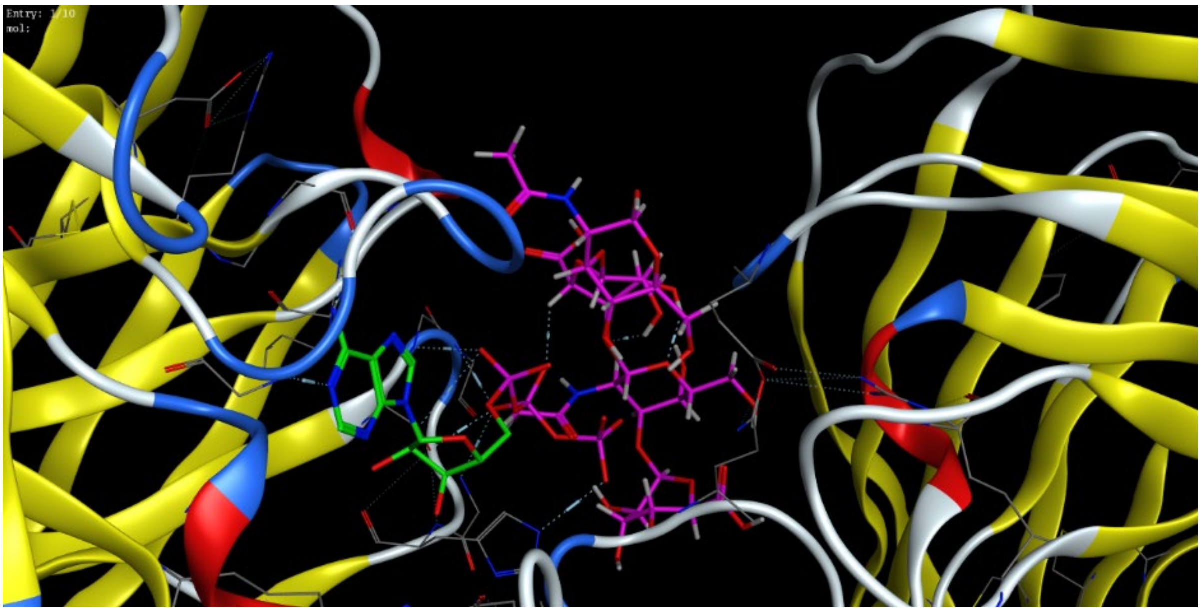

Figure 2. Overlay of IL-6 molecular interaction with agonist ligands (magenta)

Hyaluronic acid inhibits ATP in increasing inflammation through increase IL-6 receptors on the amino acids Leu 98, Ser 52, and Gln 53. This indicates that hyaluronic acid has potential as an anti-inflammatory in COVID-19 patients with periodontitis. The molecular interactions of the test ligands that have the potential as inhibitors are shown in Figure 3.

Docking Score is the scoring function used to predict the binding affinity of both ligand and target once it is docked. Docking score the ATP agonist ligand was -5.4722 and the test ligand (hyaluronic acid) was -7.3179. The lower the bond energy value between. the ligand and the target protein, the more stable the complex formed. This indicates that hyaluronic acid has potential as an anti-inflammatory in COVID-19 patients with periodontitis through inhibition of IL-6 receptors.

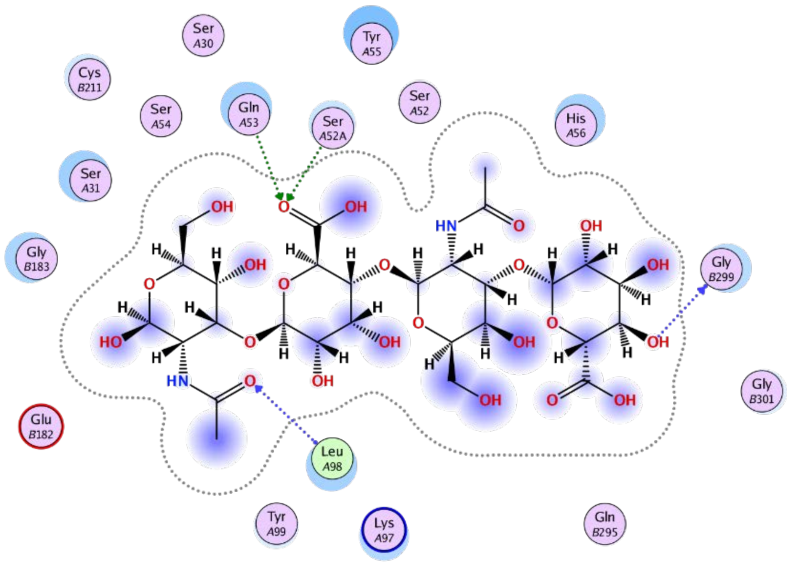

Figure 3. Molecular interaction of test ligands on IL-6

Conclusion

Hyaluronic acid (HA) can interact with IL-6 receptors in COVID-19 patients with periodontitis using molecular docking. Molecular interaction of hyaluronic acid to inhibition of IL-6 (GDP: 7DC8) with a docking score of -7.3179 was able to inhibit the attachment of agonist ligands by -5.4722 to the amino acids Leu 98, Ser 52, and Gln 53. So, hyaluronic acid has the potential as an anti-inflammatory drug of choice in COVID-19 patients with periodontitis.

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Anti-MRSA activity of *Stereospermum fimbriatum's* stem bark extracted using subcritical and supercritical carbon dioxide

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Abstract

Antibiotic resistance is a major challenge in healthcare, and this is further worsened by the presence of the dreadful Methicillin-resistant *Staphylococcus aureus* (MRSA) infection. This has urged scientists to find new effective antimicrobial drugs. Earth is enriched with natural resources such as plants that have been used traditionally to cure diseases. *Stereospermum fimbriatum* or "Chicha" had been used traditionally to treat several illnesses such as stomachache, earache, itchy skin, and postpartum illness. Thus, this study was designed to investigate the antibacterial potential of *S. fimbriatum's* stem bark against MRSA. Subcritical (Sub-CO_2) and supercritical carbon dioxide (Sup-CO_2) extractions were used to extract the stem bark, with and without the addition of co-solvent (ethanol). The antimicrobial assay was carried out using disc diffusion (200, 400 and 600 μg/disc), minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) tests. There was no anti-MRSA activity observed on both the Sub-CO_2 and Sup-CO_2 extracts without 10% ethanol. The most potent anti-MRSA was observed by the Sup-CO_2 extract with 10% ethanol with the MIC value of 500 μg/mL. Therefore, the use of ethanol (10 %) in the extraction increased the antibacterial activity of the *S. fimbriatum*'s extract against MRSA. These research findings could potentially facilitate future investigations into the utilization of green extraction methods to uncover promising antibacterial agents that can effectively target MRSA, a formidable pathogen. Future studies on the other parts of *S. fimbriatum*, its potential toxicity, and the possible mechanisms of action are needed to investigate its promising therapeutic values on MRSA infections.

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Introduction

The use of solvent extraction in herbal or medicine production has become a global concern regarding its effect on human health and the environment as well (Gil-Ch´avez *et al.*, 2012; Opuni *et al.*, 2021). Besides, the search for new effective medicines to treat infectious diseases has also increased in demand as microorganisms are getting more resistant to the available antibiotics. The mortality rates caused by MRSA infection are considerably higher in comparison to infections caused by Methicillin-sensitive *S. aureus* (MSSA). This disparity is exacerbated by the emergence of antibiotic resistance (Garoy *et al.*, 2019). Plant-derived drugs in the market such as topotecan, vincristine, and Taxol (anti-cancer agent) are well-known for their effectiveness (Cragg and Pezzuto, 2016). Within the period of between 1981 and 2019, 1,394 small molecules were approved of which 33.6 % can be traced back to natural products (Newman and Cragg, 2019).

S. fimbriatum, commonly known as 'Chicha' or 'lempoyan' locally, earns the nickname 'snake tree' due to its elongated, coiled fruits. This species primarily thrives in lowland and hill forests and widely grows across Peninsular Malaysia, specifically in Kelantan, Terengganu, Kedah, Malacca, Langkawi, and Tioman Island. Apart from that, it is also distributed in Sumatra, Myanmar, and Laos. According to local Malaysians, the dried flowers of this plant were incorporated into desserts to impart flavour (Awang *et al*., 2016). This plant was traditionally used for medicinal purposes, with the roots and shoots being boiled to relieve postpartum ailments and stomachaches, respectively. Additionally, the leaves were crushed to extract their juice, which was applied to treat earaches, and when combined with lime, it served as a remedy for itchy skin (Awang *et al*., 2016). Despite being a rare plant species, it is crucial not to overlook its valuable traditional attributes, but rather delve into its unexplored therapeutic potential such as its antimicrobial properties.

Modern approaches using green technology extraction methods had been applied in drug discovery from natural resources such as subcritical and supercritical fluid extraction. Green technology extraction methods refer to environmentally friendly and sustainable techniques that minimize the use of hazardous chemicals and energy consumption while maximizing efficiency and yield. These methods align with the principles of green chemistry and are considered more eco-friendly alternatives to traditional extraction techniques. Subcritical and supercritical fluid extraction are two specific methods that have shown promise in drug discovery (Carpentieri *et al*., 2021). Previous studies on *S. fimbriatum* had been reported using supercritical carbon dioxide and Soxhlet extractions which analysed its bioactive compounds and enriched the isolated compounds (Fadhlina *et al*., 2020; Fadhlina *et al*., 2021; Izyani Awang *et al*., 2020). In view of the scanty information on *S. fimbriatum*, the present study aims at evaluating the antibacterial activity of *S. fimbriatum* Sup-CO_2 and Sub-CO_2 extracts against *Methicillin-resistant Staphylococcus aureus* (MRSA).

Materials and Methods

Sample collection

The plant material (stem bark) was collected at Kampung Chicha Tinggi in Kelantan, Malaysia. It was verified by a botanist (Dr. Shamsul Khamis, Universiti Putra Malaysia) and a voucher specimen (PIIUM 0249) was deposited at herbarium, Kulliyyah of Pharmacy, International Islamic University Malaysia. The collected plant part was further cleaned and dried in a drying room for a week. After the drying process, the plant was ground into powder for extraction purposes.

Subcritical carbon dioxide (Sub-CO_2) extraction

About 200 g of dried and ground stem bark was placed in a 1 L extraction vessel of the Sub-CO_2 system (Figure 1A). The preliminary Sub-CO_2 extraction with and without ethanol (10 %) as co-solvent was performed at 28°C under the pressure of 70 bar for four hours. The extracts were collected after cycles of extraction completed with every cycle spanned for 3 to 5 minutes (Alam *et al*., 2017).

Supercritical carbon dioxide (Sup-CO_2) extraction

Waters SFE 1000 was utilized as the Sup-CO_2 extraction system with a maximum working pressure of 1000 bar and a temperature range of up to 80°C, featuring a 1 L extraction chamber (Figure 1B). The extraction process was conducted according to Fadhlina *et al*. (2020), with some modifications such as, the pressure was set at 200 bar, the addition of 10% ethanol as a co-solvent ethanol, and the temperature was set at 60°C. About 20 g of powdered stem bark was loaded into the extraction chamber. The CO_2 was supplied from a gas cylinder and compressed using a diaphragm compressor to the desired pressure, which was then regulated by the pressure controller and heated to the desired temperature via a heat exchanger to achieve the supercritical state. The CO_2, along with the extracted material, was depressurized at the extractor exit to separate the material. The flow rate was kept constant at 2g/min and the average yield of three experiments was recorded.

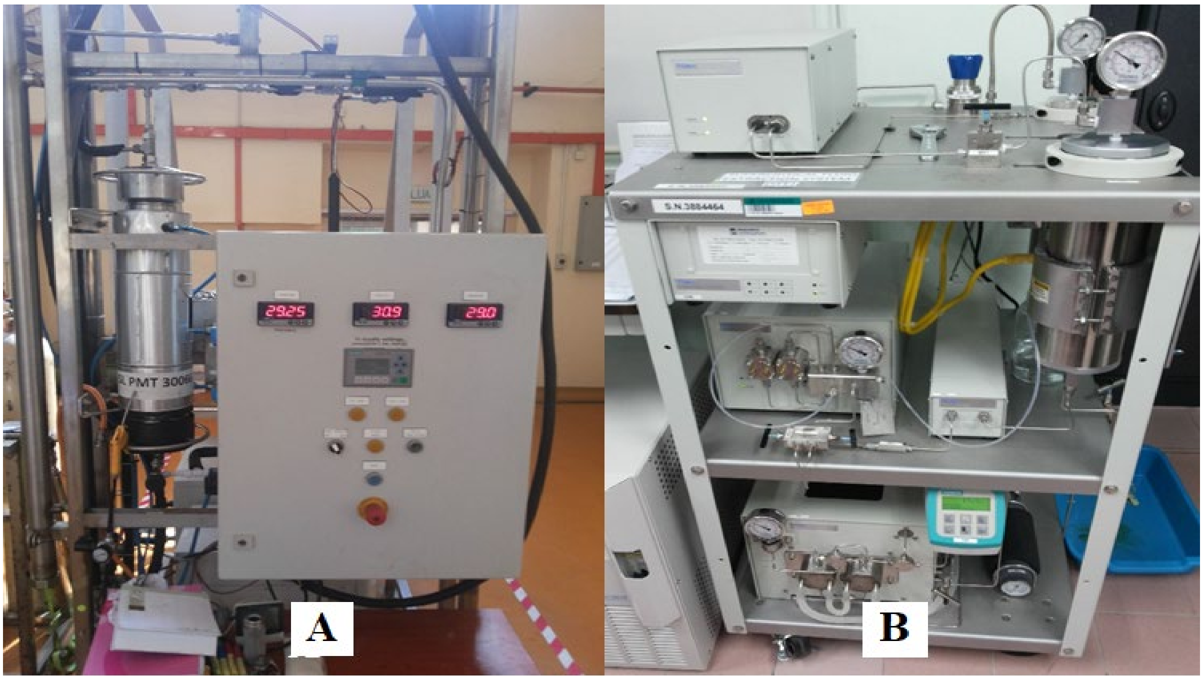

Figure 1. Subcritical (A) and Supercritical (B) CO_2 extractor.

Sample preparation

The sample was prepared by dissolving 10 mg of the sample in 1 mL of dimethyl sulfoxide (DMSO). This stock sample was stored in the chiller (4°C) until further use.

Microorganisms

A clinically isolated Methicillin-resistant *S. aureus* (MRSA) was used in the antimicrobial assay. The inoculum preparation was done using 0.5 McFarland standard which provided the standard optical density (OD) of MRSA. Single colonies growth on agar culture was transferred into growth medium, Mueller-Hinton Broth (MHB). Incubation took place at 37 °C for 24 hours. A spectrophotometer was used to check the OD of the incubated inoculum at 600 nm (Fadhlina *et al.*, 2021).

Antimicrobial assay

Disc diffusion method

A disc diffusion assay was carried out to screen for the anti-MRSA activity of each sample. All extracts were tested in three different dosages which were 200, 400, and 600 µg/disc. For disc preparation, filter paper number three was punched and sterilized in the autoclave. Using sterile forceps, each of the extracts was loaded into the discs and dried for 30 minutes. The inoculum adjusted to 0.5 McFarland standard was swabbed (100 µL) on the Mueller-Hinton agar plate evenly. The loaded discs were laid on the inoculated agar and incubated. The positive control used for this test was vancomycin (30 µg/disc), while for the negative control, the solvent carrier, DMSO was employed. All tests were done in triplicate and the mean from triplicate measurements of the inhibition zone (diameter) with standard deviation was recorded (Fadhlina *et al*, 2021).

Micro-dilution method

The MIC of extracts was determined by the broth micro-dilution test according to the previous method (Fadhlina *et al.*, 2021) with some modifications. Adjusted inoculum using 0.5 McFarland standard was loaded into 96-well plates and 3 µL of the sample stock (10 mg/mL) was transferred into the inoculated well (197 µL). Then, different concentration of the sample was prepared (1000-7.8 µg/mL) with the final volume of 100 µL each well. The tested plate was incubated at 37 °C for 24 hours. The positive control (antibiotic) was tested in the same manner as the stated steps (100-0.2 µg/mL). After the incubation period, an indicator which was 0.01% (wt/v) resazurin sodium salt (30 µL) was added to each well and incubated for two hours. MIC was considered as the lowest concentration that preserved the blue or purple colour of resazurin. Treated cultures with MIC value and above were swabbed onto the agar plate. MBC was considered as the concentration that give no growth upon completion of the incubation period.

Statistical analysis

IBM SPSS Statistics 20 was used for the statistical analysis. The results were presented as the means of three readings ± the standard deviation (SD). Statistical significance was determined using one-way ANOVA analysis, followed by post-hoc test, with a significance value set at $p<0.05$.

Result and Discussion

Supercritical carbon dioxide (Sup-CO2) and subcritical carbon dioxide (Sub-CO2) extraction are two commonly used methods for extracting plant extracts. Both extractions are an eco-friendly process that does not require the use of harmful solvents and have their advantages and disadvantages in terms of extraction efficiency as well as biological activities (Awang *et al.,* 2016). Sup-CO_2 extraction involves using CO_2 at temperatures and pressures above its critical point, which results in a highly efficient extraction process. This method is preferred for extracting non-polar and semi-polar compounds, such as essential oils, fatty acids, and some alkaloids. The extraction efficiency of Sup-CO_2 is generally higher than that of Sub-CO_2, due to its ability to extract a wider range of compounds. On the other hand, subcritical carbon dioxide (Sub-CO_2) extraction involves using carbon dioxide at temperatures and pressures below its critical point, which results in a less efficient extraction process compared to Sup-CO_2. This method is preferred for extracting polar compounds, such as flavonoids, glycosides, and some alkaloids. While Sub-CO_2 extraction is less efficient, it has some advantages over Sup-CO_2 extraction. For example, Sub-CO_2 extraction can preserve the bioactivity of heat-sensitive compounds and can also extract a broader range of polar compounds (Gallego *et al.*, 2019).

The present study was the first work to report on the Sub-CO_2 extraction of *S. fimbriatum* using an eco-friendly co-solvent, ethanol. The percentage of yields (Table 1) for extract with the use of 10 % ethanol as co-solvent (6.32 %) was more than the percentage of yields obtained without the use of ethanol (4.19 %). The anti-MRSA activity (Table 2) of Sub-CO_2 extracts were only observed on the stem bark extract with the addition of 10% ethanol (Figure 2). A similar observation was recorded for Sup-CO_2 extract with 10% ethanol. However, there was no extract yielded by Sup-CO_2 extraction without the addition of 10% ethanol. All the anti-MRSA activity observed was in a dose-dependent manner (8 -10 mm). Extracts that exhibit an inhibition zone of 13 mm or greater are classified as potent extracts (Fadhlina *et al.*, 2021), and achieving this level of potency may be possible by increasing the dosage of the extract since the concentrations employed in this study were relatively low. Based on the micro-dilution assay of the active extracts (Table 3), Sup-CO_2 extract exhibited the lowest MIC value of 500 ug/mL, while its MBC value was 1000 ug/mL, against MRSA.

Table 1. The yield of Sub-CO_2 and Sup-CO_2 extraction with and without 10% ethanol.

Stem bark (200 g)	Condition	Yield (%)
Sub: CO_2	70 bar at 28°C	4.19
Sub: CO_2 + 10 % ethanol	70 bar at 28°C	6.32
Sup: CO_2	200 bar at 60°C	0.00
Sup: CO_2 + 10 % ethanol	200 bar at 60°C	4.15

Table 2. Disc diffusion assay of extracts against MRSA.

Extracts	CO_2 (mm)			CO_2 + 10 % Ethanol (mm)			Antibiotics (mm)
	200 µg	400 µg	600 µg	200 µg	400 µg	600 µg	V30
Subcritical	NI	NI	NI	7.67*±0.58^{c}	9.33±0.58^{b}	10.67±0.58^{b}	19.33±0.58^{a}
Supercritical	NI	NI	NI	8.33±0.58^{c}	9.67±0.58^{b}	10±0.00^{b}	

*=Partial inhibition; NI=No inhibition; V30=Vancomycin 30 µg/disc; $^{a, b, c}$: Small superscript alphabets represent post-hoc analysis tested at p<0.05.

Table 3. Micro-dilution assays of extracts against MRSA.

Extracts (ug/mL)	MRSA	
	MIC	MBC
Sub-CO_2 + 10 % Ethanol	>1000	-
Sup-CO_2 + 10 % Ethanol	500	1000
Vancomycin	0.78	1.56

Sub-CO_2 and Sup-CO_2 offer a valuable option for green technology extraction as it uses a non-toxic solvent of carbon dioxide and less solvent consumption (Awang *et al.,* 2016) with only 10 % co-solvent so that it increased the polarity and allowed the tuning of compound's solubility in the plant extraction. The addition of 10 % ethanol at a constant temperature and pressure in both of the extraction methods might increase the solubility of antimicrobial compounds in the stem bark of *S. fimbriatum*. A similar observation was reported in a previous study on the extraction of bilberry using Sub-CO_2 extraction whereby it was found that the addition of 10 % ethanol as a co-solvent had increased the efficiency of anthocyanin recovery as well as the bioactivity (Babova *et al*., 2016). Meanwhile, a previous study on *S. fimbriatum* (Fadhlina *et al.*, 2020) using Sup-CO2 extract (addition of 6% ethanol) showed lower MIC value against MRSA, operated at 40°C and 300 Bar. A novel anthraquinone compound had been isolated from the stem bark of *S. fimbriatum* which was semi-polar (Izyani Awang *et al.*, 2020). Thus, further optimization on the extraction conditions (pressure, temperature, % of co-solvent) may be conducted to enrich its bioactive compounds and improve its antimicrobial activity against a broad range of microorganisms.

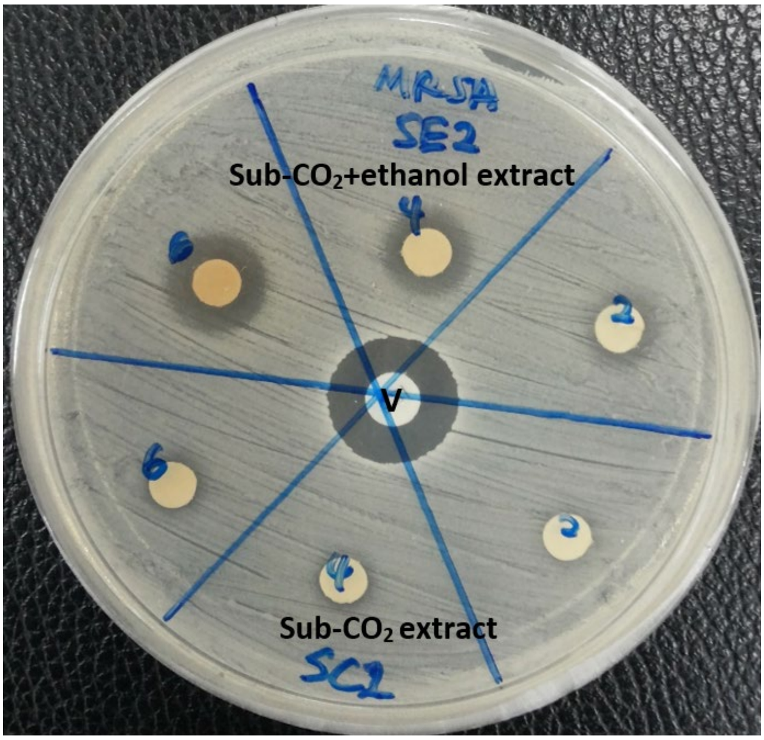

Figure 2. Inhibition zones of Sub-CO_2 extracts against MRSA in disc diffusion assay.
2: 200 µg/disc; 4: 400 µg/disc; 6: 600 µg/disc; V: Vancomycin 30 µg/disc.

Conclusion

The findings of this study highlight the potential of Sup-CO_2 in discovering valuable antibacterial agents for targeting MRSA. Sup-CO_2 extraction of *S. fimbriatum's* stem bark operated at 60°C, 200 Bar, and the addition of 10% ethanol showed higher antibacterial activity against the dreadful bacteria, MRSA, compared to Sub-CO_2 extraction. The use of 10% ethanol in both extractions is crucial to improve the yield and anti-MRSA activity of *S. fimbriatum* extracts. Further studies on optimizing the extraction of antibacterial extracts are needed to obtain an optimum condition against a broader range of microorganisms. The present study focused mainly on the stem bark of *S. fimbriatum*, highlighting the need for future research to explore other plant parts and assess their potential toxicity or side effects. Moreover, it is essential to further investigate the possible mechanisms responsible for the demonstrated anti-MRSA activity.

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TECHNICAL REPORT Open Access

Pressurised putty technique: A technical report

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Abstract

The surface detail reproducibility of conventional putty impressions is hindered by high viscosity and low flowability. In the plastic industry, injection moulding application utilizes an influx of pressure to achieve better flowability. Leveraging this concept, this technical report investigated the impact of pressure on surface detail reproducibility and structural homogeneity, featuring the pressurised putty technique. Surface detail reproducibility and structural homogeneity of three techniques (non-pressurised, putty & light body wash and pressurised) were visually assessed and differences in surface detail reproducibility were observed among all techniques. Whilst a pressurised clear-based putty index presented a more uniform glassy finish. The outlined technique suggests a simplistic and cost-effective way of improving a putty index, which may benefit many clinicians in terms of prosthodontic success.

Keywords: *pressurised putty, surface detail reproducibility, structural homogeneity*

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Introduction

The ability to register good dental impressions is an important skillset to be developed by all clinicians, as it accounts for a wide array of uses in everyday dentistry. Regardless of its many applications, the ultimate purpose of all dental impressions is to create an accurate, void-free negative imprint, so that a true positive reproduction of surface details can be correctly transferred to dentitions or study models, ultimately improving chances of treatment success. In the field of prosthodontics, diagnostic wax-ups are particularly useful in the evaluation of treatment options, dental aesthetics, and fabrication of short/long termed provisional restorations.

For the last-mentioned, indexes are used and they are commonly made up of a silicone impression material in a putty consistency as it is rigid and dimensionally stable. However, one major disadvantage of a putty silicone impression material is its poor ability to record fine surface detail. This is attributed to its high filler content, which leads to high viscosity and low flowability, thus hindering its ability to capture fine details (Ghahremanloo *et al.*, 2017). Although this can be overcome by the application of a wash technique with a light body silicone impression, either in a one-step or two-step technique, this is seemingly technique sensitive and not cost effective. In most situations, light-bodied silicone impression has a tendency of being displaced and torn when the putty index is loaded and removed, which in turn will lead to a compromise of impression accuracy (O'Brien, 2002). Therefore, the fabrication of conventional putty indexes warrants further improvements.

Based on the fundamental principle of differential pressure flow, particles flow from high to low pressures within a system. The plastic industry incorporates this concept in injection moulding applications, where the influx of pressure forces polymers into a mould so that proper uptake of shape is ensured (Kirk & Patrick, 2011). Leveraging on the aforementioned concept, this report aimed to examine the impact of pressure on surface detail reproducibility and structural homogeneity of putty impressions.

Report

The materials included a maxillary dental model (Frasaco AG3, VDDI Dental Solutions, Germany) to act as a framework for the fabrication of putty index, a putty consistency silicone impression (Aquasil Soft Putty, Dentsply Sirona, United States), a light body silicone impression (Aquasil Ultra+, Dentsply Sirona, United States), a clear-based silicone impression (Exaclear, GC Corporation, Japan), and a polymerising pressure pot (R-030420, Mestra, Spain).

Three techniques, specifically non-pressurised, putty & light body wash and pressurised were employed, and a full-arch putty silicone impression was made for each. The first two techniques were allowed to polymerise under ambient pressure, while the pressurised putty index was polymerised under 1.5 atmosphere of pressure (atm). Based on the manufacturer's recommendation, 1.5-2.0 atm is desirable to achieve optimum polymerisation and prevention of damage to the pressure pot (R-030420, Mestra, Spain). Putty & light body wash was carried out in a two-step technique, in which the intaglio surfaces of polymerised putty index were scrapped to accommodate light body impression material. For fabrication of clear based putty indexes, Exaclear was dispensed onto the Frasaco model and seated with a pre-loaded non-perforated stock tray. They were set under pressurised and non-pressurised manner.

Full arch putty indexes were divided into three sections for inspection of surface detail reproducibility, while the clear-based putty indexes were assessed for structural homogeneity from a labial view. Figure 1 outlines all three techniques according to their polymerisation conditions, while figure 2-4 illustrate the comparison of three techniques from different viewpoints. Figure 5 depicts the comparison of non-pressurised and pressurised clear-based putty indexes.

In figures 2 and 3, non-pressurised putty and putty & light body wash presented with crude voids and improper registration from maxillary lateral incisor to lateral incisor, and left first premolar up to third molar (pinpointed by yellow arrows),

In the maxillary anterior region, both surface detail reproducibility of putty & light body wash and pressurised were seemingly comparable. However, due to the inherent properties of low-filled light body impression, some parts of the putty & light body wash succumbed to tears. In figure 4, all three depictions were almost identical with no conspicuous voids from the non-pressurised putty. As for figure 5, multiple minute voids were seen in the non-pressurised putty, while the pressurised counterpart has a more uniform glassy finish.

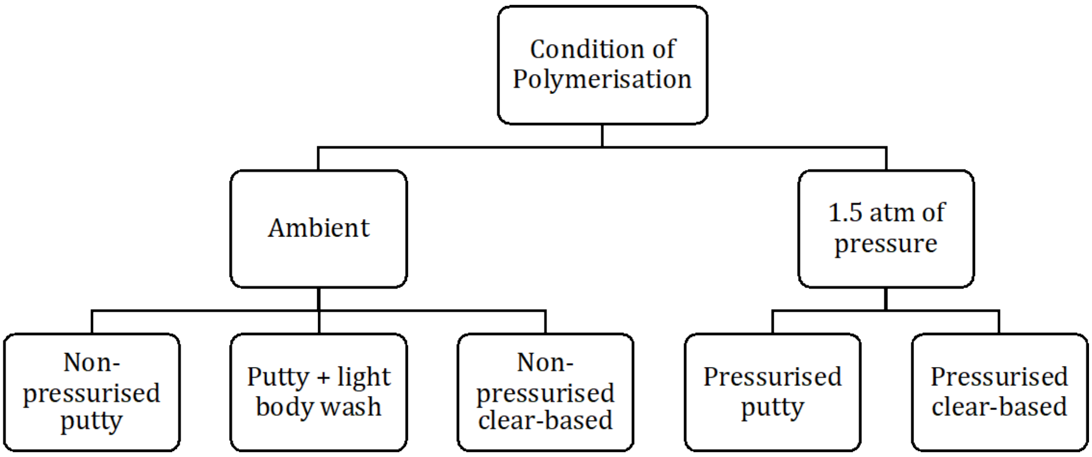

Figure 1. Flowchart of techniques according to their polymerisation conditions.

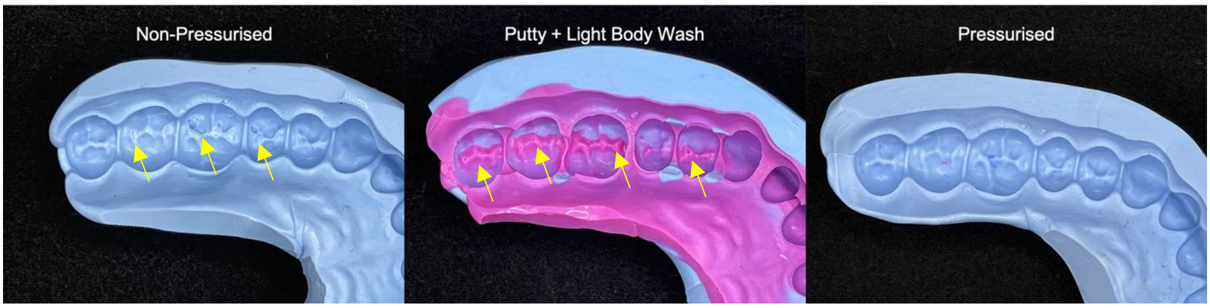

Figure 2. Comparison of surface detail reproducibility of maxillary left region.

Figure 3. Comparison of surface detail reproducibility of maxillary anterior region.

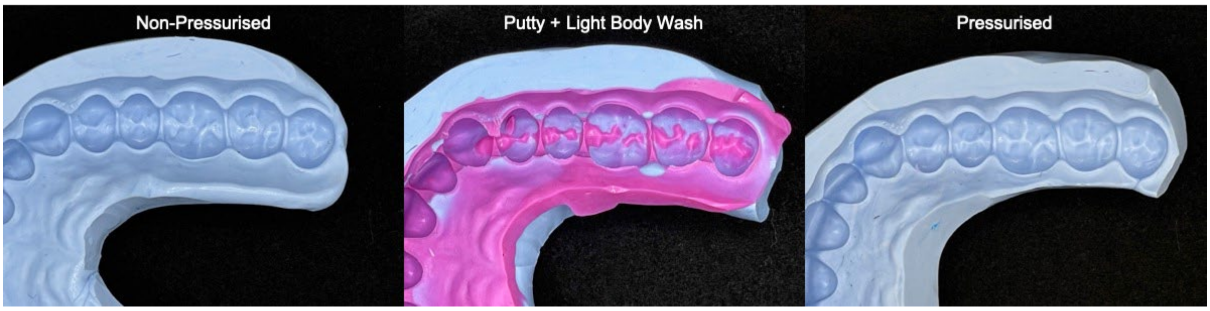

Figure 4. Comparison of surface detail reproducibility of maxillary right region.

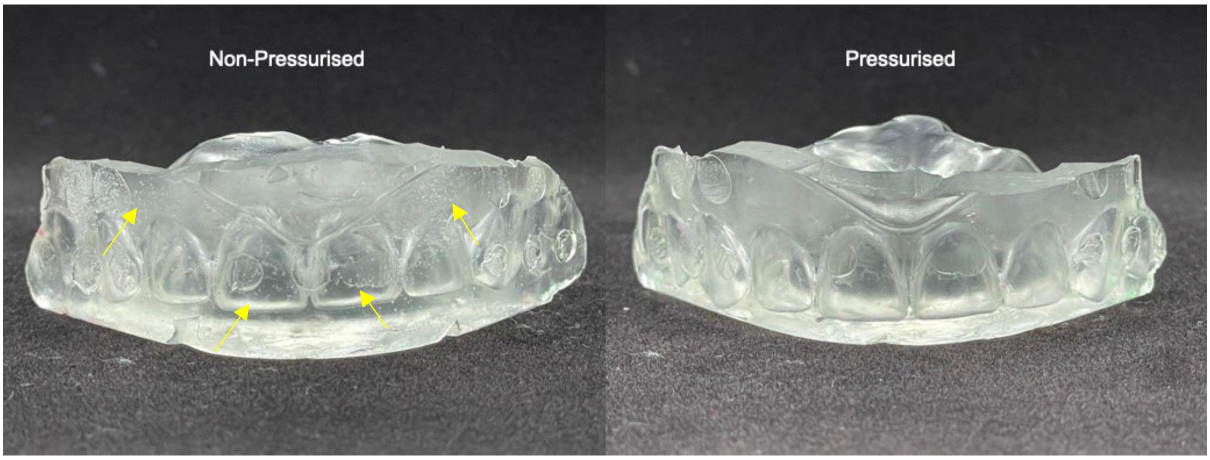

Figure 5. Comparison of structural homogeneity of clear-based putty indexes.

Discussion

This report has demonstrated how pressure has affected the surface detail reproducibility and structural homogeneity of putty indexes. Based on a study (Nishigawa *et al.*, 2013), they have demonstrated an apparent pressure-induced flowability using a theoretical model, in which a Newtonian viscoelastic material (*e.g.,* a silicone-based dental impression) was able to travel through a channel when pressure is applied. This is seemingly congruent with the author's speculation. Hence, on the latter basis and the outcomes of this report, the initial hypothesis is duly accepted.

Pressure polymerisation is not a whole new endeavour, in fact, such approach was first described about a century ago, where isoprene was subjected to polymerization under 0.9-1.2 GPa of pressure (Conant & Tongberg, 1930). Over time, this method was adopted and experimented in other polymeric materials as well. Reportedly, the introduction of pressure had improved the mechanical and physical properties of polymethyl methacrylate (PMMA) and composite resin blocks via a high degree of resin cross-linkages, reduced intermolecular distances and free volume, ultimately an increase in bulk density and fracture toughness. Moreover, the occurrence of defects and voids can be effectively mitigated due to the isotropic compaction of the polymeric structures (Kojima *et al.*, 2002; Nguyen *et al.*, 2012; Schettino *et al.*, 2008). Pressurised putty indexes, especially clear-based ones will be greatly beneficial to clinicians when injection moulding technique with flowable composite resin is used as it helps in achieving proper contouring and better operational visualization. In addition, the stiff nature of pressurised putty indexes may reduce the incidence of flexing when seated intraorally. On the whole, pressurised putty index seems desirable as it enhances prosthodontic efficiency and success.

This report has its setbacks and can be improved in several ways. Firstly, the magnitude of improvements in surface detail reproduction and structural homogeneity was not quantified but relied on the author's perspective. Secondly, the material chosen in this study does not represent the spectrum of all silicone impressions available in the market. With all things considered, the evidence mentioned should not be hastily concluded, but as mere adjunctive evidence to clinical practice and a pathway for more impactful and evidence-based studies to be conducted in future. For future recommendations, microscopic in-vitro studies should be carried out to measure the amount of surface detail reproduction and structural homogeneity improvement on a more precise scale, with the inclusion of more silicone impressions. Lastly, the effects of higher pressure should also be investigated as the pressure used in this report was limited by the manufacturer's instructions.

Conclusion

Within the limitation of this report, differences in surface reproducibility were seen between non-pressurised, light body wash and pressurised techniques. A uniform glassy finish of a pressurised clear-based putty index was observed. The outlined technique suggests a simplistic, sensible and cost-effective alternative for an improved putty index. These admirable traits may benefit many clinicians, given that they all need to use a putty index at some point in their careers. In hindsight, the uses of dental pressure pots are not limited to the conventional denture reline and repair, but also can be used for the application of the pressurised putty technique. In the author's opinion, the investment of an inexpensive dental pressure pot would be more than repaid in time saved for later procedures, adjustments, and remakes.

Conflict of Interest

None declared

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CASE REPORT Open Access

Full mouth rehabilitation of young adults with oligodontia: Two case reports emphasizing implantology, prosthodontics, and restorative intervention

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Abstract

Oligodontia, a severe form of hypodontia characterized by the absence of multiple permanent teeth, presents considerable challenges in dental treatment. Successful management of this condition requires early detection and a comprehensive, multidisciplinary approach. While the complete restoration of missing teeth is typically postponed until all permanent teeth have erupted or after orthodontic therapy, it is crucial to consider additional factors such as the patient's oral hygiene status, socioeconomic circumstances, and long-term maintenance therapy for achieving optimal treatment outcomes. This case reports showcase the effective treatment of oligodontia in two young male patients: a 17-year-old Chinese individual and a 19-year-old Caucasian individual. These cases serve to emphasize the significance of early diagnosis, multidisciplinary collaboration, and personalized treatment planning in addressing the challenges posed by oligodontia. Although orthodontic intervention was not pursued in the presented cases, it is important to recognize its integral role in the overall treatment of oligodontia. The restoration of missing dentition has a profound positive impact on aesthetics, function, and the overall quality of life. The presented cases underscore the importance of early intervention and sustained motivation throughout the treatment process. By emphasizing the value of early detection, comprehensive teamwork, and individualized treatment approaches, clinicians can strive to achieve optimal outcomes for patients with oligodontia.

Keywords: *full mouth rehabilitation, oligodontia, prosthodontics, restorative intervention, severe hypodontia*

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Introduction

Oligodontia is a rare congenital dental anomaly characterised by the absence of more than six permanent teeth excluding the third molar (Chandwani & Suvarna, 2011; Tavajohi-Kermani *et al.*, 2002) The aetiology of oligodontia is multifactorial, involving both genetic and environmental factors. Several genes, such as PAX9, MSX1, and AXIN2, have been associated with the development of permanent tooth agenesis (Der Weide *et al.*, 1994; Mostowska *et al.*, 2005; Zhou *et al.*, 2021). Environmental factors, such as maternal smoking, prenatal exposure to teratogens, and certain medical conditions, may also contribute to the development of oligodontia. The prevalence of oligodontia varies among populations, ranging from 0.08% to 0.33%, with a higher incidence reported in females (Jepson *et al.*, 2003; Tangade & Batra, 2012). Congenitally absent maxillary lateral incisors, maxillary second premolars, and mandibular central incisors are most often seen in oligodontia cases (Bural *et al.*, 2012; Polder *et al.*, 2004), while the agenesis of maxillary central incisors, maxillary or mandibular canines, or first permanent molars is rare (Dhanrajani & Al Abdulkarim, 2002).

Despite numerous studies indicating the prevalence and distribution of hypodontia throughout the globe, very few studies have reported the prevalence and distribution of this dental anomaly in the Malaysian population. A recent retrospective and cross-sectional study on the prevalence of hypodontia and supernumerary teeth among dental patients was conducted at the Dental Clinic, Advanced Medical and Dental Institute (AMDI), Universiti Sains Malaysia, the tertiary referral centre for Northern Malaysia. The prevalence of hypodontia was reported to be 15.9% and was more prevalent in females (Bahoudela *et al.*, 2022). In addition, they reported that the incidence of hypodontia increased with the subjects' age. The result contradicts the prevalence reported by another study conducted in the central region of Malaysia in 1989, which found that only 2.8% of the Malaysian population had hypodontia (Nik-Hussein, 1989). Despite the fact that both studies only included subjects from specific regions, the results may not reflect the actual prevalence of hypodontia in the entire Malaysian population. The prevalence of hypodontia in Malaysia appears to be high, and dentists are likely to encounter this anomaly in their patients.

The clinical manifestations of oligodontia extend beyond the absence of teeth, often leading to significant functional impairments. The remaining teeth may exhibit space closure, rotation, or supraeruption, resulting in malocclusion and compromised masticatory efficiency. In addition, the absence of multiple teeth can lead to difficulties in chewing, speech articulation, and overall oral function (Bural *et al.*, 2012; Der Weide *et al.*, 1994; Dhanrajani & Al Abdulkarim, 2002; Jepson *et al.*, 2003; Kotsiomiti *et al.*, 2007; Polder *et al.*, 2004; Tangade & Batra, 2012). Furthermore, the compromised aesthetics resulting from tooth loss can significantly impact self-esteem and social interactions, leading to psychological distress and decreased quality of life. Therefore, early detection and intervention are crucial to mitigate the negative consequences associated with oligodontia (do Valle *et al.*, 2011).

The dental management of oligodontia requires a multidisciplinary approach involving orthodontists, prosthodontists, oral surgeons, and paediatric dentists. Treatment options include orthodontic interventions to optimize occlusion and align the remaining teeth, prosthetic solutions such as removable or fixed partial dentures, and surgical interventions like dental implants or autogenous grafts for tooth replacement. It must be emphasised that oligodontia generally necessitates lengthy and complex treatments, ranging from multiple restorations to surgical intervention and complex prosthodontic rehabilitation, as well as lifelong maintenance (Demes *et al.,* 2023). Each treatment plan must be tailored to the individual's specific needs, considering their age, skeletal growth, dental development, and functional requirements (Aronovich *et al.*, 2022; Bural *et al.*, 2012; Kotsiomiti *et al.*, 2007). Furthermore, when planning the appropriate treatment, the financial implication of the patient must also be considered, as well as providing a long-term oral care to which the patient can comply to (Dhanrajani & Al Abdulkarim, 2002).

This article presents two case reports focusing on the full-mouth rehabilitation of young male adolescents diagnosed with oligodontia, emphasising periodontics, prosthodontics, and implantology interventions. Although the treatment plans for both cases were not the most ideal and unpopular decisions had to be made due to the patients' choice of treatments, the outcome for both cases, considering that they were in a compromised situation, showed a good result and promising long-term stability.

Case 1: Combined periodontics-prosthodontics-oral surgery intervention.

A 17-year-old Chinese young man with severe hypodontia was referred for restorative management. His medical history was not significant, and no family history of any oral or dental anomalies was reported. He was not a regular dental attendee and had no history of dental extractions. Clinical examination revealed a reduced lower facial height, a convex facial profile, a Class II skeletal base with a Class II division 1 malocclusion, a deep overbite, an asymmetrical anterior gingival zenith, and a low smile line. Furthermore, the occlusal plane was slanted in relation to the interpupillary line, and a slight canted and deviated midline was also noted (Figure 1). Oral hygiene and gingival status were good, and no caries were found.

An orthopantomogram (OPG) confirmed that nineteen teeth were congenitally missing: 18, 14, 15, 23, 24, 25, 26, 38, 35, 34, 33, 32, 31, 41, 42, 43, 44, 45, and 48. Six retained deciduous teeth were present: 53, 63, 65, 81, 82, and 83, and significant external root resorption was found in 63, 81, 82, and 83 (Figure 2).

Several treatment alternatives were discussed and evaluated, but the patient declined to undergo orthodontic treatment due to a possible longer treatment duration and additional cost, and as a result, a combination of surgical and restorative therapy was planned. His wishes were to at least have a set of fixed prostheses to function normally while having a set of "teeth" to boost his self-confidence.

A diagnostic wax-up was prepared, and an intraoral mock-up was performed to assess the viability of the proposed treatment plan and get feedback from the patient and his parents prior to the start of the treatment. Due to the poor long-term prognosis of 53, 63, 81, 82, and 83, they were extracted. The remaining retained maxillary left deciduous molar (65) was sound and was used as an abutment for a future bridge.

Two 4-unit provisional fixed-fixed bridges were constructed on abutments 13, 12, 22, and 65. This was followed by surgical crown lengthening to improve the position of the gingival zenith and gingival aesthetic. Before the surgical procedure, a cone beam computed tomography (CBCT) was taken, and the dimension of the labial crestal bone to the cementoenamel junction (CEJ) was analysed to determine the amount of crestal bone to remove during ostectomy (Figure 3). In the mandibular arch, six Nobel Active® (Nobel Biocare, Switzerland) implants were placed between the first molars, followed by a temporary removable partial denture. After a healing period of 6 months, the mandibular implants were restored with three segmented cement-retained full zirconia bridges on customised titanium abutments (Figure 4). Although the authors prefer screw-retained prostheses, due to the limitation of the bone and thus the non-ideal positioning of implant fixtures during placement, cement-retained prostheses on custom-milled titanium abutments were more favourable for aesthetic outcomes. The authors also took the hygiene and motivation of the patient into consideration before making the decision.

The final prostheses construction for the maxillary arch was initiated after a one-month review of the mandibular prostheses. This was to make sure that the occlusion was stable and the patient was able to function well with the existing provisional bridges. The impression of the provisional prostheses on the maxillary arch was sent to the dental technician as a template to copy for the final prostheses. Two 4-unit porcelain-fused-zirconia (PFZ) bridges for the posterior quadrants and two PFZ crowns for maxillary central incisors were constructed and cemented with dual-cured resin cement (NX3 Nexus™, Kerr Restoratives) (Figure 5). Modified ovate pontics were digitally designed for the prostheses with the gingival convex portion positioned more facially on the residual ridge rather than on the crest. This design provides a better aesthetic appearance while allowing easy hygienic care for long-term maintenance.

The patient was reviewed regularly at 1-week, 1-month, 3-month, and 1-year intervals, followed by annual reviews. The most recent review was a 5-year review in which the peri-implant mucosa and prostheses were stable and the patient expressed positive feedback that he could function very well and was delighted with the outcome (Figure 6).

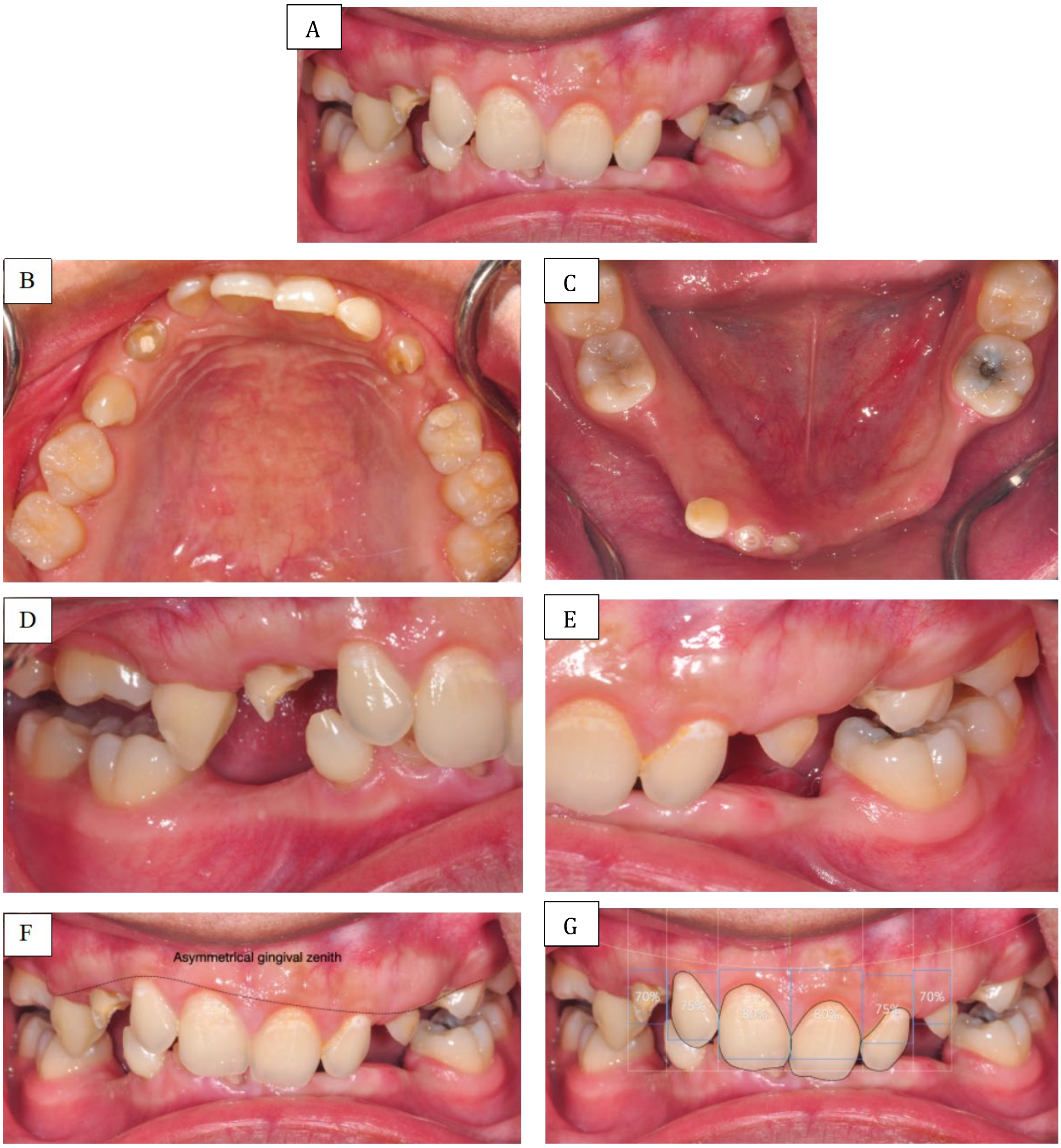

Figure 1. Pre-operative intraoral photographs: (A-E) Pre-operative intraoral view in maximum intercuspation; (F) Asymmetrical gingival zenith; (G) Digital smile analysis to preliminarily assess the amount of tooth reduction and crown lengthening needed.

Figure 2. Orthopantomogram (OPG) to assess the pre-operative oral condition.

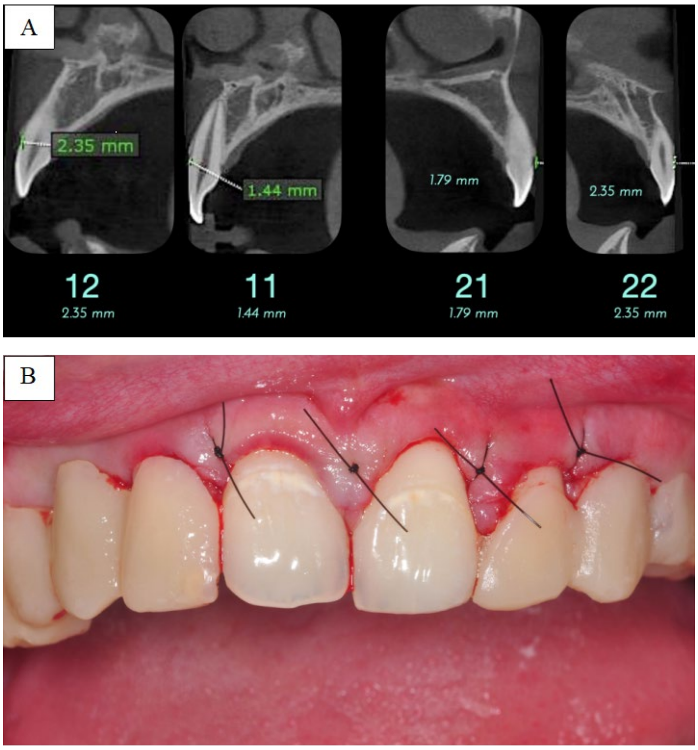

Figure 3. (A) cone beam computed tomography (CBCT) analysis of the dimension of the labial crestal bone to the cementoenamel junction (CEJ); (B) Immediately after surgical crown lengthening.

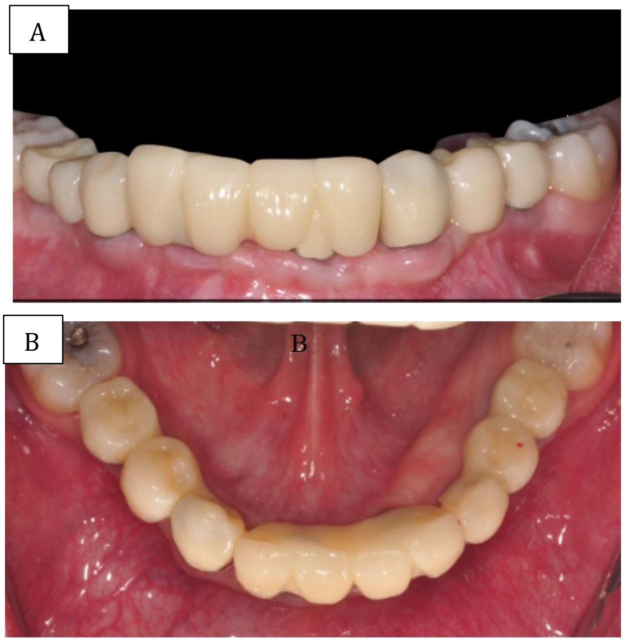

Figure 4. (A, B) Three segmented, cement-retained implant-supported bridges in situ.

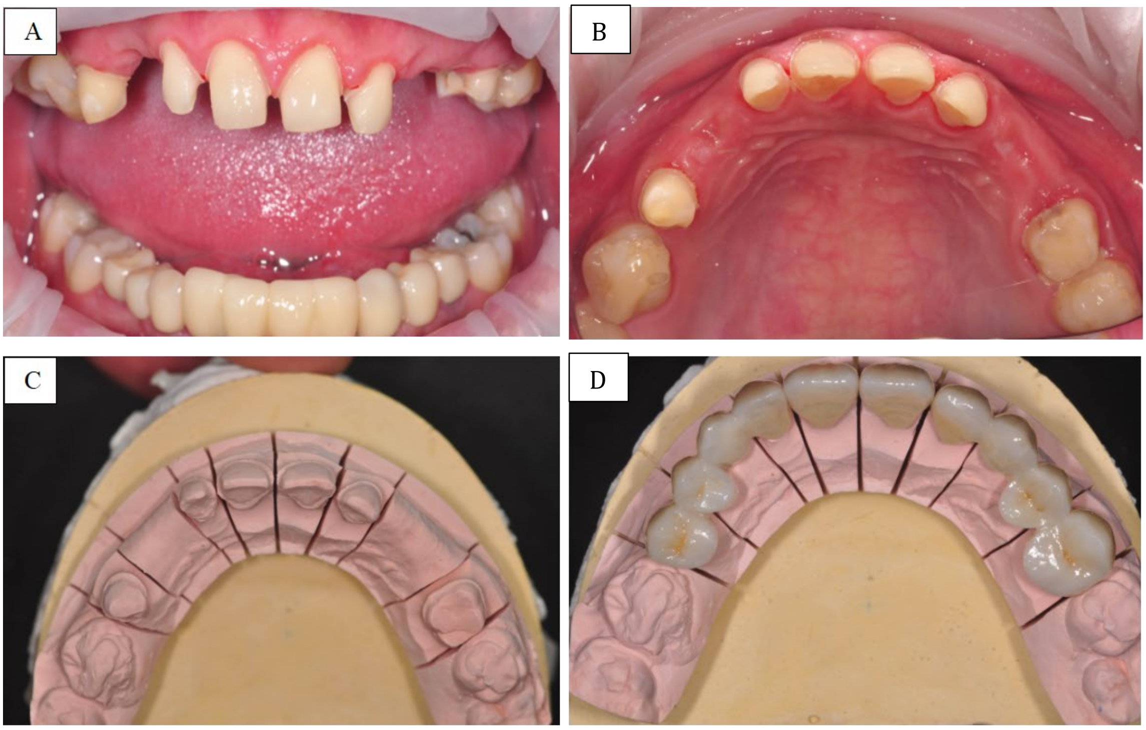

Figure 5.(A, B) Intraoral view of minimal tooth reduction on the maxillary abutment teeth; (C, D) laboratory-milled full zirconia crowns and bridges on master cast; (E-H) Post cementation.

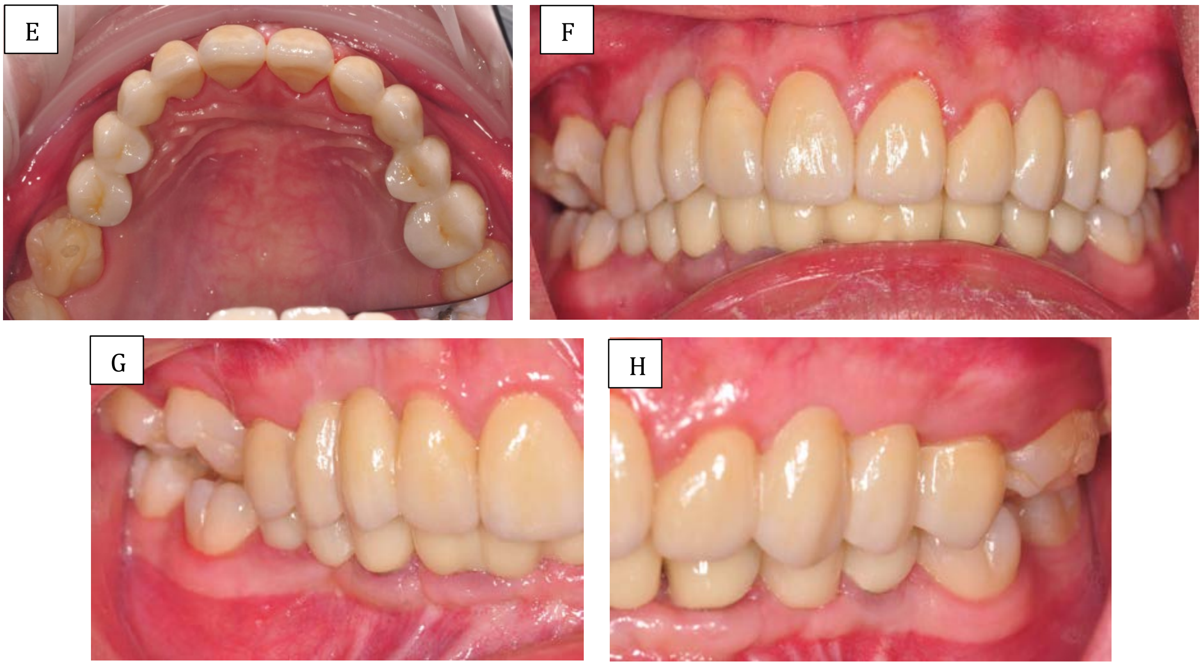

Figure 5. *(continued)*

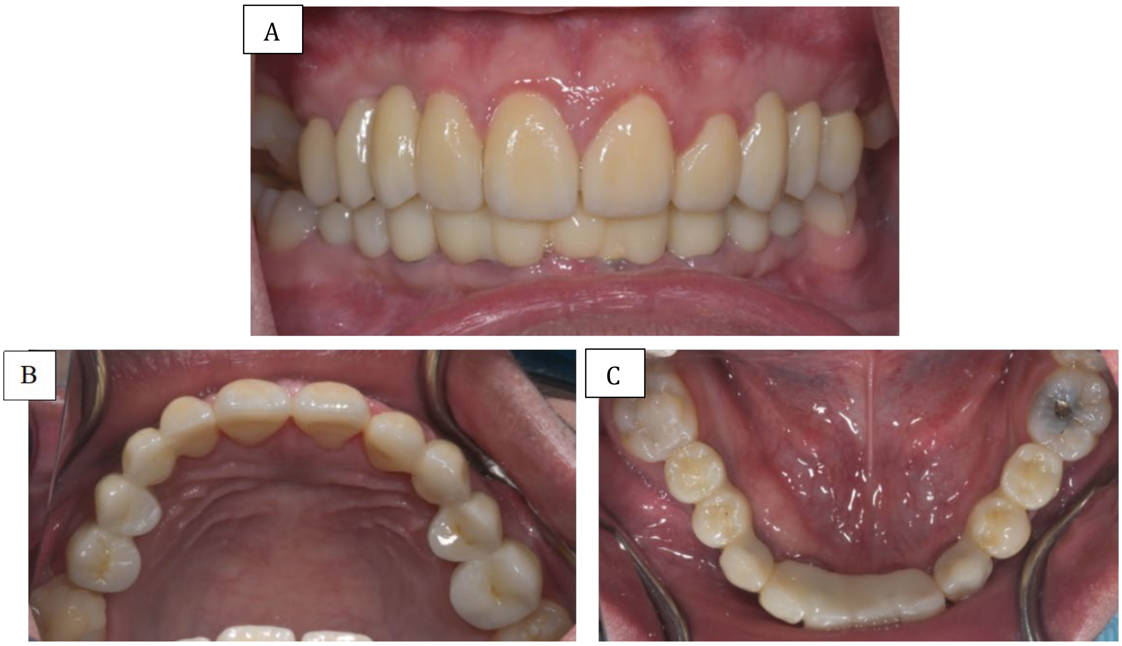

Figure 6. Five-year review. Apart from minimal gingival inflammation around the maxillary crowns and bridges, the peri-implant mucosa and the prostheses were stable.

Case 2: Prosthodontic intervention in a compromised treatment planning

A 19-year-old Caucasian young man with severe hypodontia was referred for the restoration of multiple implant fixtures. The patient had previously undergone unsuccessful removable denture therapy and had rejected orthodontic therapy, in which eight implants were subsequently placed by his oral surgeon. Due to a severe lack of opposing dentition, the patient could not masticate normally and was suffering from social anxiety due to the appearance of his teeth. Since the patient was planning to leave the country soon, he requested to only have the implant restored to help with his mastication and to improve his appearance without prolonging the treatment duration.
Clinically, only nine teeth were present, and eight implant healing abutments were noted (Figure 7). The patient also presented with reduced lower facial height and a deep overbite. Moreover, the interach space for restoration was limited, which was also complicated by constricted maxillary and mandibular arches. The upper midline was shifted about 3mm to the left, and there was a large median diastema with a low smile line. An orthopantomogram (OPG) showed satisfactory implant fixtures in relation to the crestal bone, and no abnormality could be detected on the remaining dentition (Figure 8).

A diagnostic wax-up and intraoral mock-up were performed (Figure 9) to gauge the patient's acceptance of and feedback on the proposed outcome. In the subsequent visit, all the healing abutments, except the one on fixture 44, were replaced with multiunit abutments that were torqued to 35 Ncm in accordance with the manufacturer's recommendation (Figure 9). These multiunit abutments will serve as the final abutments to support the superstructure. Following that, abutment-level impressions and fixture-level impression (implant on 44 site) were taken with polyether (Impregum™, 3M ESPE).

Acrylic jigs were constructed on the master cast, which were later used to verify the passive fit of the definitive abutments intraorally (Figures 10). Once verified, wax rims were used to register the patient's jaw relationship at increased OVD, which was pre-determined at the mock-up stage.

Customised titanium frameworks (AtlantisTM, Dentsply Sirona) were constructed and tried intra-orally to further verify their passive fit, and jaw registration was registered again with the frameworks in situ before the frameworks and bite registration record were sent back to the technician for porcelain layering. (Figure 11) The final prostheses were inserted and torqued according to the manufacturer's recommendation (Figure 12). Modified ovate pontics were constructed for the prostheses with reduced buccolingual width to reduce the size of occlusal table and thus reduce occlusal stresses during function. The convex gingival portion of the ovate pontics were positioned more facially on the residual ridge rather than on the crest to create aesthetically-pleasing appearance as well as better hygienic control for long-term maintenance. A minor occlusal adjustment was performed during the review appointment, but overall, the patient expressed great satisfaction with his appearance, and the outcome definitely boosted his self-confidence and positively improved his social anxiety.

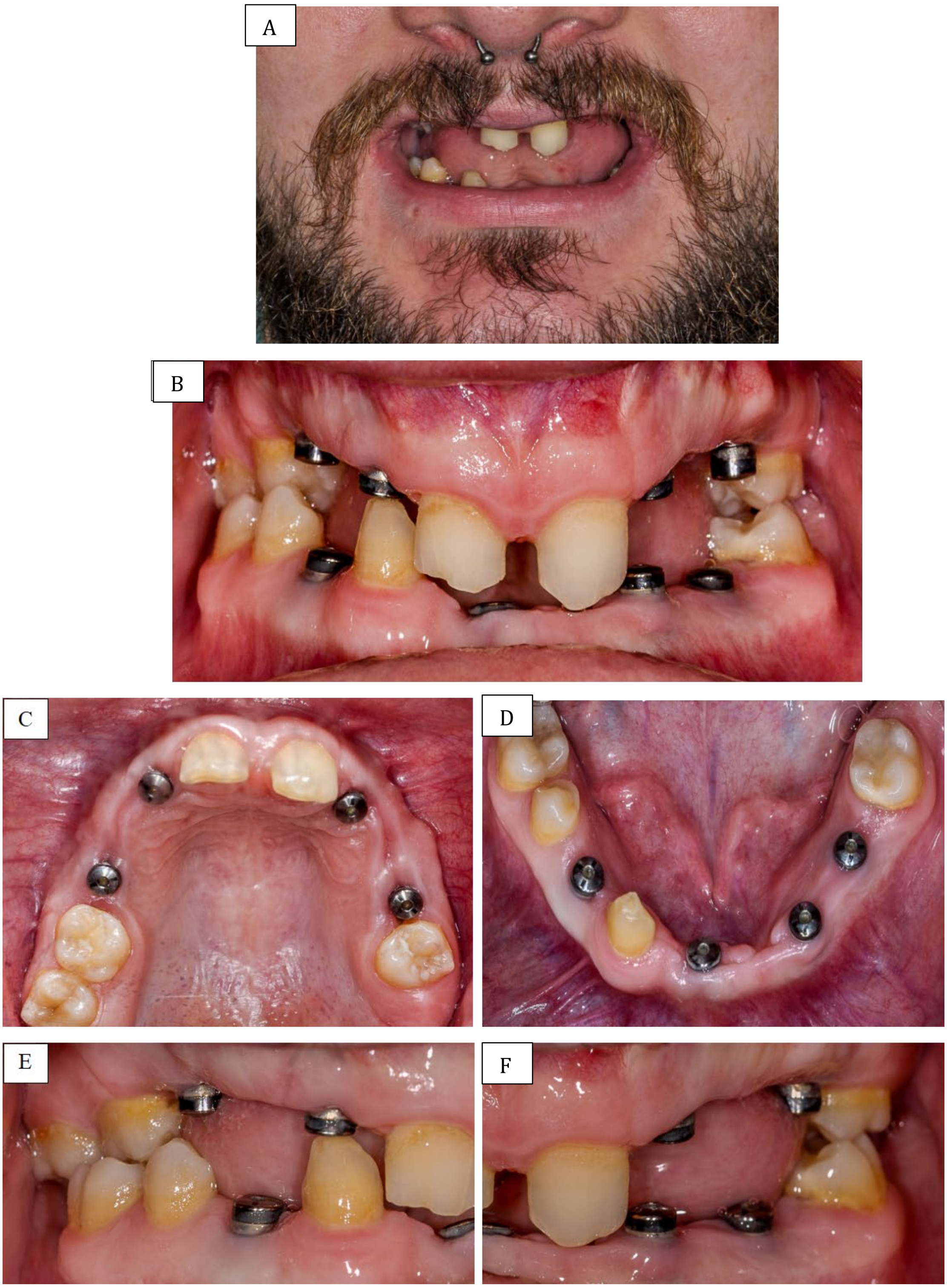

Figure 7. (A-F) Pre-operative extraoral and intraoral view. All implants and healing abutments had been placed by his oral surgeon.

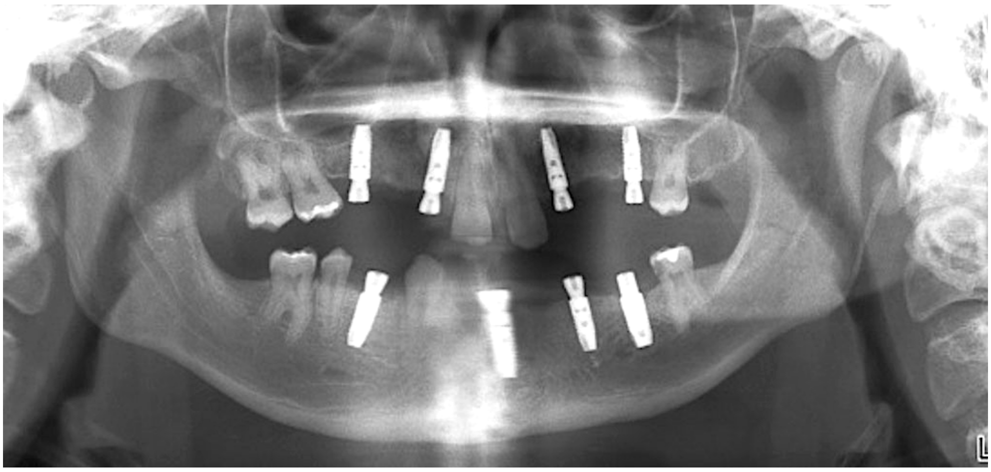

Figure 8. Pre-operative orthopantomogram (OPG) to assess the remaining dentition and the relation of the implant fixtures to the alveolar bone height.

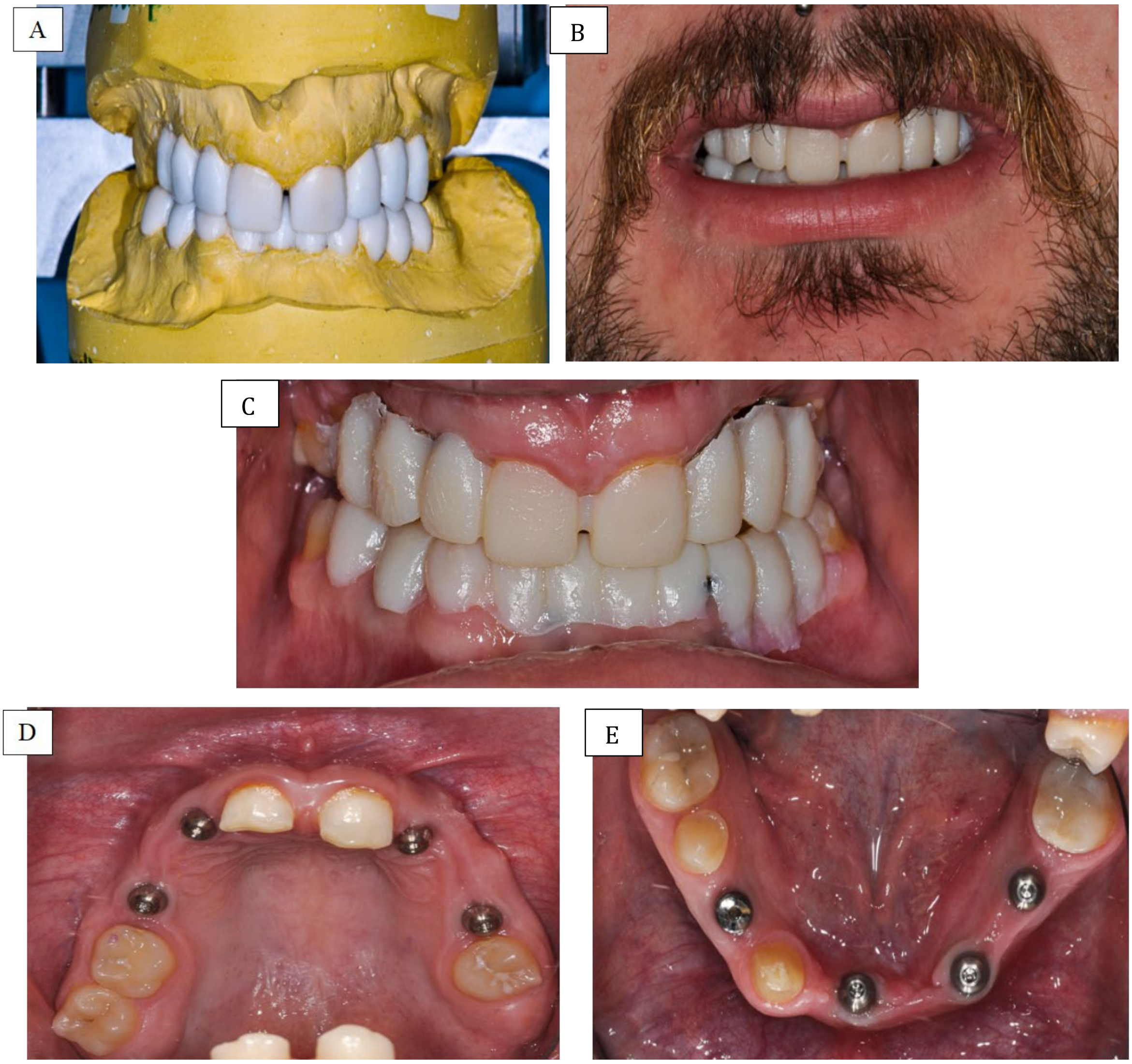

Figure 9. (A) Diagnostic wax up on articulated casts; (B-C) intraoral mock-ups; (D-E) Multiunit abutments were torqued into all fixtures except the fixture on 44.

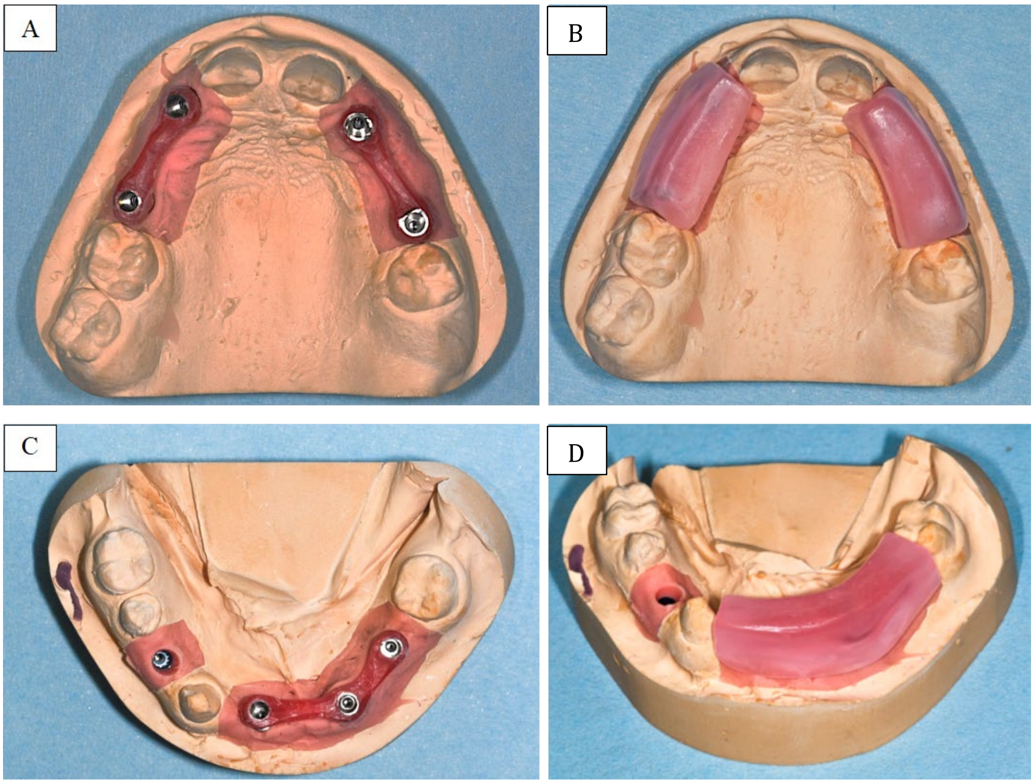

Figure 10. (A-D) Acrylic jigs were constructed on the master casts to allow for assessment of the passive fit of the definitive abutments, followed by wax rims above them to record the jaw relationship.

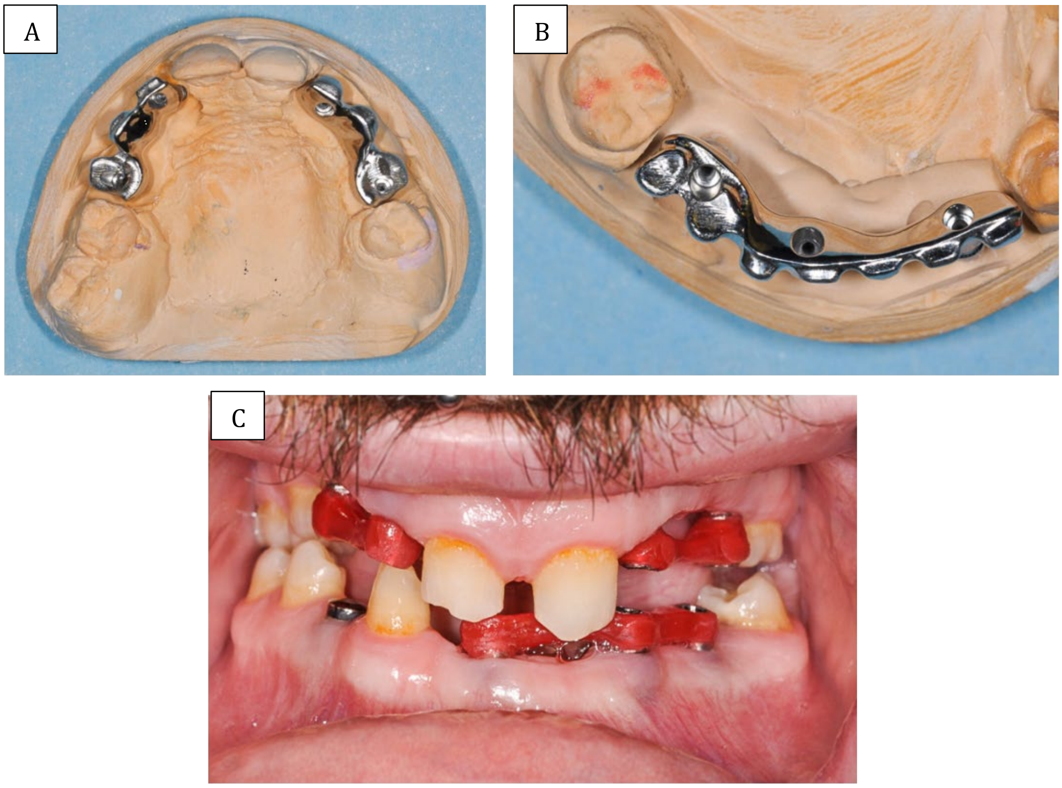

Figure 11. (A-C) Customised titanium frameworks were constructed and tried in intra-orally to further verify their passive fit before jaw registration.

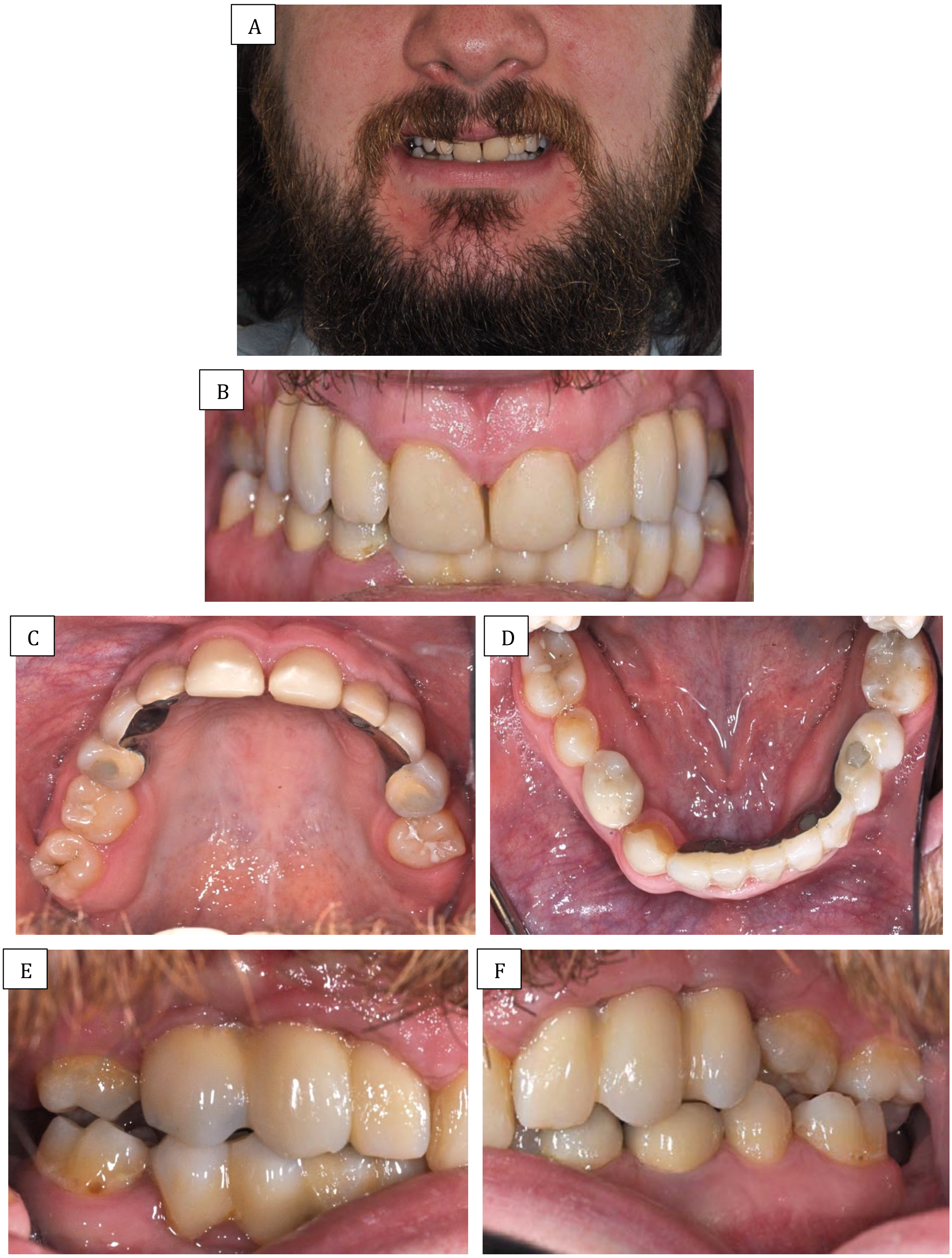

Figure 12. (A-F) Post-operative intraoral view in maximum intercuspation and extraoral view.

Discussion

Both cases highlighted the complexities encountered in managing severe hypodontia or oligodontia, particularly in the absence of orthodontic interventions. Orthodontic tooth movements could have improved the treatment outcomes, such as reducing overbite and closing the midline diastema. The restoration of the occlusal vertical dimension was a significant challenge, and provisional prostheses played a crucial role in monitoring and adapting before the final restorations. The psychological aspect of treatment acceptance and maintenance was also emphasised.

Although there are some similarities to the processes involved in providing fixed prosthodontic treatment, the cases presented the complexities encountered in managing severe hypodontia in adolescents that required highly specific techniques. Not only did the presented cases focus on the clinical and technical aspects of management, but also the inherent psychological aspect that was critical to the patient's acceptance of the prostheses and adherent to their regular maintenance.

Both cases demonstrated the lack of orthodontic intervention, which hampers the more optimal final treatment outcome that can be achieved. Orthodontic tooth movements, especially of the maxillary incisors, were essential to retrude the supra-erupted incisors and reduce the overbite in Case 1 and to close the midline diastema in Case 2. Both cases would have benefited from a more favourable space distribution for subsequent prosthesis construction. Furthermore, loss of occlusal vertical dimension (OVD) is a common manifestation among patients with severe hypodontia, and re-establishing the ideal maxilla-mandibular jaw relationship by means of fixed or removable prostheses is one of the most challenging clinical procedures. Hence, provisional prostheses restored to the planned occlusal vertical dimension are crucial, as this step allows for initial monitoring and adaptation before committing to the final restoration (Marin *et al.*, 2015; Tariq *et al.*, 2021).

The long-term success of oral implants in partly edentulous patients has led other doctors to use implants in younger patients who are missing teeth because of agenesis or trauma. Although removable prostheses have always been the treatment choice for younger patients who are partially edentulous, they are often rejected by the patients. They may also lead to an increased caries rate and residual ridge resorption, as well as periodontal complications (Cronin & Oesterle, 1998; Mankani *et al.*, 2014). Young patients and their parents frequently insist on reducing the treatment duration and opting for implant therapy as soon as feasible due to discomfort from removable prostheses. In the absence of maxillary teeth, the alveolar ridges will not develop, and the maxilla will remain underdeveloped both sagitally and vertically. In contrast, mandibular growth is not dependent on the presence of teeth (Epker, 1998; Shah *et al*., 2013). Therefore, in the presence of hypodontia, the relationship between two jaws will tend to be disproportionate. The pressure to begin implant therapy as soon as possible is further increased by physiological and psychological factors. Additionally, it has been demonstrated that paediatric implants can promote the growth of alveolar bone (Mankani *et al.*, 2014; Shah *et al.*, 2013).

Implant placement in adolescents has long been controversial, with some academics and physicians strongly supporting its use in this patient population while others are totally against it. According to Brugnolo *et al.* (1996), infraocclussion was observed in implants installed in patients aged 13 to 14.5 years due to the vertical growth of the subjects, resulting in the need to re-construct new prostheses. Other studies warned of the possibility of implant loss in the anterior maxilla due to resorption in the infradental fossa and nasal floor (Oesterle *et al.,* 1994; Oesterle, 2000), as well as submergence of implants within the mandibular alveolar process due to a rotational growth pattern (Cronin & Oesterle, 1998). In contrast, Smith *et al.*

(1993) reported that implant use in children with ectodermal dysplasia is a treatment of choice since its placement in the mandibular anterior region of a 5-year-old patient did not affect the adjacent tooth buds; however, they admitted that the prosthesis of their subject needed remodelling due to implant submergence. Similarly, Guckes (1997) found that dental implants placed in the maxilla and mandible had not moved despite growth in a 3-year-old patient with ectodermal dysplasia. Furthermore, a case report by Kearns *et al.* (1999) could not prove the presence of restrictions to transverse and sagittal growth due to implant use in children with ectodermal dysplasia. However, re-construction of new prostheses was necessary in some patients secondary to implant submergence.

In cases of severe anodontia or oligodontia in the mandible, it is possible to place implants before the pubertal growth spurt because few growth changes occur in the anterior region after the age of 5–6 years, owing to the absence of teeth. In contrast, implant placement in the maxilla is advised to be delayed until after growth spurt (Koch, 1996; Graber, 1997). During the 1995 Scandinavian Consensus Conference meeting in Sankoping, Sweden, it was determined that implant placement, particularly in partially edentulous cases, should be delayed until the end of craniofacial and skeletal growth (Koch, 1996).

The anterior mandible holds the greatest potential for early use of dental implant prostheses when compared to the posterior mandible and the maxilla (Fouda, 2020; Op Heij *et al.*, 2003; Shah *et al.*, 2013). Although implants are not routinely recommended for adolescents due to concerns about jaw development, not all adolescents with missing teeth must wait until growth is complete prior to implant placement. This decision should be based not only on growth but also on the number and location of the missing teeth (Sharma & Vargervik, 2006).

The advantages of implant use in adolescents are as significant as the risks associated with their premature use, but they can be beneficial to the growing adult if a meticulous diagnosis and treatment plan are followed. The authors strongly suggest avoiding placing implants in the growing maxilla until early adulthood. This is due to the resorptive aspects of maxillary growth at the nasal floor and the anterior surface of the maxilla, which may cause unpredictable implant dislocations in the vertical and anteroposterior directions. Moreover, the maxilla has a thinner cortical plate and lower bone density than the mandible (Fouda, 2020; Kim & Lee, 2010). Therefore, osseointegrated implants in the maxilla of growing patients must be undertaken with a great deal of caution since anterior maxillary implants were 2.8 times more likely to fail than those placed in the anterior mandible (Mankani *et al.*, 2014). In the mandible, transversal skeletal or alveolodental alterations are less pronounced when compared to the maxilla (Mankani *et al.*, 2014; Oesterle *et al.,* 1994; Skieller *et al.,* 1984). The major portion of the mandible's transverse development occurs early in childhood, while the anteroposterior growth occurs mainly at the posterior mandible. High failure rates in the mandibular posterior region have been determined to be caused by overheating of the bone (Fouda, 2020; Park *et al.,* 2010). However, in children with severe hypodontia, the anterior mandibular growth seems relatively small; therefore, many case reports have shown favourable outcomes and concluded that the anterior mandible represents the most suitable site of implant placement in these subjects (Mankani *et al.,* 2014).

Finding the optimal timing for implant treatment in children or adolescents can be very challenging, as numerous factors must be considered in determining the optimal individual treatment strategy. In view of the evidence from the literature, it is highly recommended to delay implant therapy until the completion of dental and skeletal growth. However, should the need arise and the treatment planning favour implant use before skeletal maturation, the operator should consider the implant location and gender of the patient, and most importantly, the patient and their parents need to be informed about the benefits, risks, and possible complications of the treatment. In addition, careful attention must be given to the prosthesis design and regular reviews.

Conclusion

The management of oligodontia requires a comprehensive approach that includes early diagnosis, multidisciplinary collaboration, and individualised treatment planning. These elements are crucial in addressing the challenges associated with this condition effectively. When considering implant therapy for patients with severe hypodontia, several factors should be taken into account, such as the implant location, the patient's gender, and skeletal maturation level. These factors play a significant role in determining the optimal timing for implant placement, ensuring favourable outcomes for the patient. The cases presented in this study highlight the significance of various dental disciplines, including implantology, prosthodontics, and restorative intervention. These disciplines work together to achieve both functional and aesthetic results for patients with severe hypodontia. The positive impact of dental restoration on these aspects underscores the importance of early intervention and sustained motivation throughout the treatment process. While orthodontic intervention may be necessary in some cases, the restoration of missing teeth significantly improves aesthetics, function, and overall quality of life. These cases emphasise the importance of timely intervention and ongoing patient motivation in achieving optimal outcomes for individuals with severe hypodontia or oligodontia.

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Conflict of Interest

The authors have no conflicts of interest to declare. We certify that the submission is original work and is not under review at any other publication**.**

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CASE SERIES Open Access

Digital and clinical approach to quantifying periodontal tissue changes after crown lengthening surgery: A case series pilot study

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Abstract

Crown lengthening surgery is a common periodontal procedure carried out to increase the amount of tooth exposure for aesthetic or restorative purposes. It is crucial for clinicians to understand the effect of crown lengthening surgery on the periodontal tissues to prevent relapse or over treatment. Even though various clinical parameters have been used by researchers in the past to quantify the amount of suprastructure exposed, the use of digital technology has not been widely adopted. The aim of this case series was to evaluate the changes in periodontal tissue after crown lengthening surgery with both clinical and digital approaches. This case series included five patients who underwent surgery at the Postgraduate Periodontics Clinic at the University of Otago over the period of six months. Clinical parameters such as probing depth, gingival recession, keratinised tissue height, plaque accumulation, gingival inflammation, crown height, gingival phenotype, and bone height were measured with a customised probing stent at baseline and 1-, 3-, and 6-month post-surgery. Digital impressions were also taken along with the clinical parameters to measure the volumetric changes. Most significant changes were observed in crown exposure, gingival recession and bone levels, followed by probing depth reduction for treated sites. Minimal changes were seen for the width of keratinised tissues, plaque levels and gingival scores. Volumetric changes were only significantly reduced after 6 months of healing. This case series found that crown lengthening resulted in an increase in tooth exposure and a reduction in tissue volume. Volumetric changes measured through sequential digital impressions were also comparable to clinical findings.

Keywords: *crown lengthening, periodontium, surgical flaps, volumetric analysis*

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Introduction

Surgical crown lengthening is a procedure designed to increase the amount of supragingival tooth structure for restorative and aesthetical indications (Gupta *et al.*, 2015). It can be carried out by clinicians to improve access and manage subgingival caries, tooth fractures, root resorptions or endodontic therapy perforations (Bennani *et al.*, 2017; Brägger *et al.*, 1992; Jepsen *et al.*, 2018). On the other hand, patients with excessive gingival display (delayed passive eruption) can benefit aesthetically by removing the excess periodontal tissue.

In order to have a predictable result after crown lengthening surgery, several factors need to be considered. The morphology and dimension of the dentogingival unit (DGU), which is the soft tissue compartment that is located at the cervical area of the tooth coronal to the alveolar crest has to be evaluated carefully prior to crown lengthening surgery. It comprises of the junctional epithelium and the supracrestal connective tissue attachment of the gingivae. It has been reported that the average measurement for the supracrestal tissue attachment, also known as the "biological width", is on average 0.97 mm for the junctional epithelium and 1.07 mm for the supracrestal connective tissue attachment (Gargiulo *et al.*, 1961). Feasibility of performing this procedure is also highly influenced by the amount of keratinised tissue and supporting alveolar bone (Gupta *et al.*, 2015; Nobre *et al.*, 2017).

An adequate periodontal-restorative interface determines tissue health. Accurate location of the prospective restoration margin prevents tissue inflammation associated with pathological probing depths and loss of periodontal supporting tissue. Likewise, it facilitates optimal access for oral home care procedures (Brägger *et al.*, 1992; Carvalho *et al.*, 2020). Earlier studies measure periodontal tissue changes after crown lengthening surgery through various clinical parameters such as the distance between a custom stent fitted on the teeth to the free gingival margin and base of the probable pocket (Brägger *et al.*, 1992). Even though this is a highly accurate method of tracking changes, other methods based on current technology are now emerging.

Intraoral scanners have increased in popularity in dentistry to create digital impressions instead of traditional impressions and stone casts (Richert *et al.*, 2017). In the last 5 years, multiple studies concluded that intraoral scanners are as accurate as physical stone casts (Mennito *et al.*, 2019, Güth *et al.*, 2016). However, only a few studies have used intraoral scanners to evaluate volumetric alterations. A study by Zhang and colleagues in 2021, concluded that intra-oral scanners can be recommended to evaluate morphological changes of the gingiva after initial periodontal therapy. Similar digital techniques have been successfully used to evaluate volumetric changes in periodontal plastic surgery to measure mucosal thickness a year after grafting with acellular dermal matrix (Papi *et al.*, 2021). Furthermore, three-dimensional quantitative measurements have been carried out to assess buccal augmented tissue after modified coronally advanced tunnel technique combined with subepithelial connective tissue graft *(Fei et al.*, 2021). The results of this study concluded that digital measurement by intraoral scanning is a non-invasive and reliable method to monitor volumetric changes after periodontal plastic surgery.

As of now, no guidelines nor recommendations exist for clinical studies regarding the use of a series of digital impressions to prospectively evaluate soft tissue volume changes after surgery (Tavelli *et al.*, 2021). Currently, there is limited research evaluating volumetric periodontal tissue alterations after crown lengthening surgery (CLS).

Aim

The aim of this pilot study is to assess the volumetric and clinical changes in periodontal tissues after crown lengthening surgery at 1-, 3- and 6-month healing time.

Materials and Methods

Inclusion criteria

- Participants of ≥ 18 years of age who are being treated for crown lengthening surgery at the Postgraduate Periodontics Clinic at the Faculty of Dentistry, University of Otago
- Not undergoing active orthodontic therapy
- Absence of periodontitis
- Absence of pathologic tooth mobility or furcation involvement.

Exclusion Criteria

- Participants with systemic conditions contraindicated for periodontal surgery
- Pregnant and lactating females
- Smokers/vapers

Ethical approval was obtained from the University of Otago Human Ethics Committee (Health) (H21/167) and Research Consultation with Māori.

Parameters

As a part of the periodontal screening appointment, patients referred to the clinic for crown lengthening surgery over a recruitment period of six months underwent a standardised periodontal evaluation. Five patients, with one site requiring crown lengthening surgery each, met the criteria above and were included in this case series. Four of the sites were in the posterior sextant while one was in the anterior region Only one site was of a thin gingival phenotype. Intraoral scans were taken for both arches and bite registration to fabricate a customised probing stent as seen in Figure 1. Clinical photographs and radiographs were taken at different time points as shown in Figure 2a-2h.

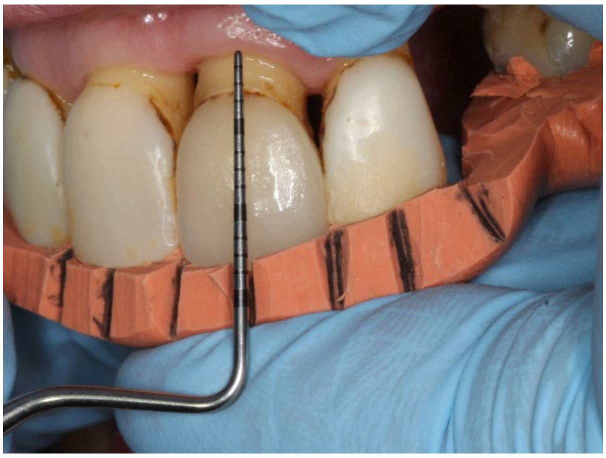


Figure 1. Buccal measurement with customised stent and UNC-15 probe.

(a)

(b)

Figure 2. Clinical and radiographic images before and after crown lengthening surgery of 36. (a) Pre-operative buccal view, b) Pre-operative lingual view, c) Intra-operative buccal view, d) Intra-operative occlusal view, e) Pre-operative periapical radiograph, f) Post-operative periapical radiograph, g) Post-operative buccal view and h) Post-operative lingual view.

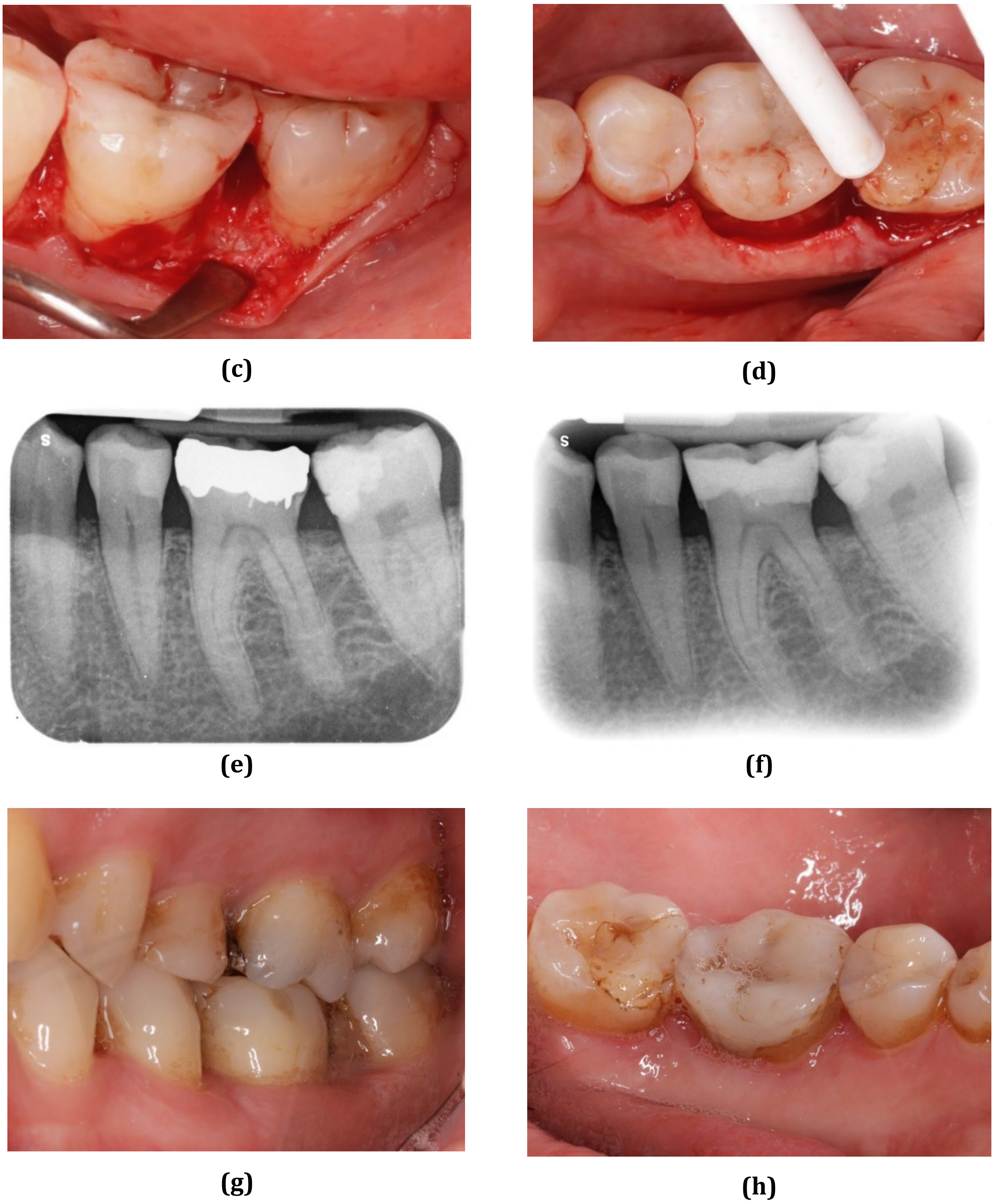


Figure 3. *(continued)*

The following periodontal clinical parameters of treated and adjacent teeth (adjacent tooth number 1 and number 2) were then recorded using a UNC - 15 University of North Carolina probe by an experienced and calibrated examiner (A.T.S). Periodontal probing depths, gingival recession, keratinised tissue height, plaque accumulation, gingival inflammation and crown height were measured at six sites per tooth: mesio-buccal (MB), mid-buccal (MidB), disto-buccal (DB), mesio-lingual(ML), mid-lingual (MidL) and disto-lingual (DL).

- Periodontal probing depth: measured in mm from the gingival margin to the bottom of the periodontal pocket.

- Gingival recession (GR): distance in mm from the cementoenamel junction (CEJ) or margin of the restoration to the gingival margin.

- Keratinised tissue height (KTH): distance in mm from the gingival margin to the mucogingival junction (buccal and lingual sites).

- Gingival phenotype (GP) was assessed at mid-buccal surfaces by inserting the periodontal probe within the gingival sulcus observing the periodontal probe shining through gingival tissue (Kan et al 2010):

    - Probe visible: thin (≤1 mm)
    - Probe not visible: thick (> 1 mm).

- Plaque accumulation: presence or absence after running periodontal probe (O'Leary et al., 1972).

- Gingival inflammation: presence or absence of bleeding after superficial gentle probing (Ainamo and Bay, 1975).

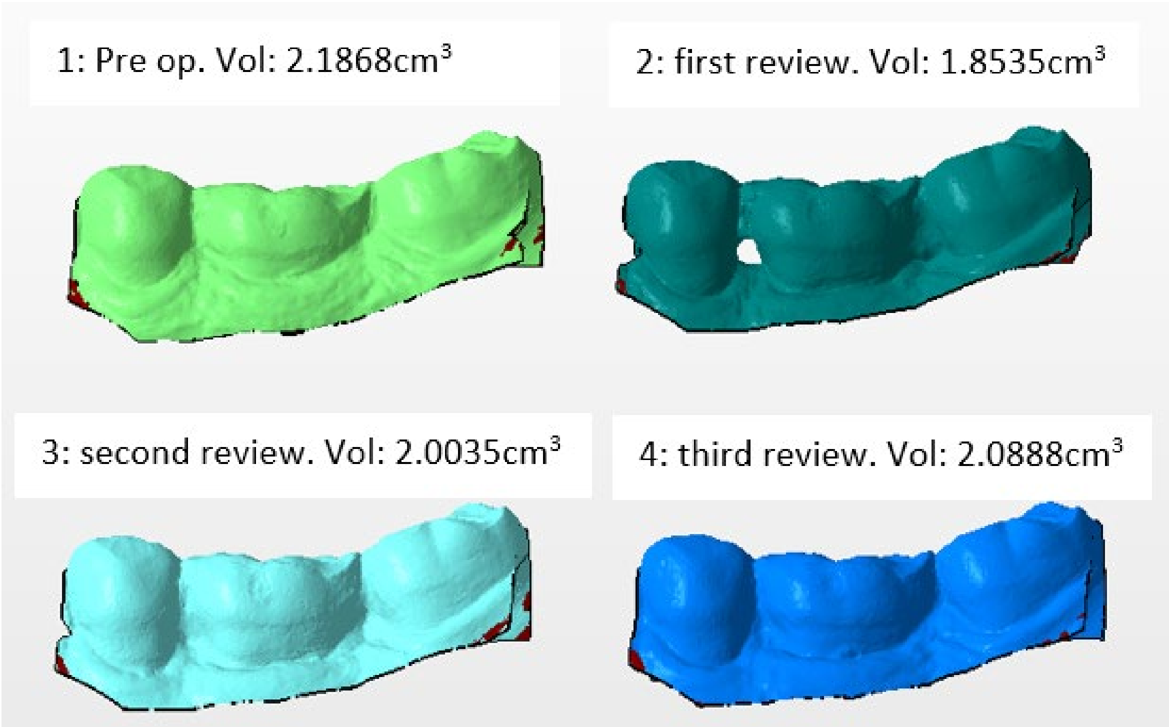
- Bone height: distance in mm from the CEJ to the most coronal aspect of the alveolar crest at mesial and distal sites of treated and adjacent sites were measured on periapical radiographs before and after crown lengthening.

- Crown exposure: distance in mm were measured for treated and adjacent sites in mm from the bottom edge of the customised stent to the gingival margin (GM).

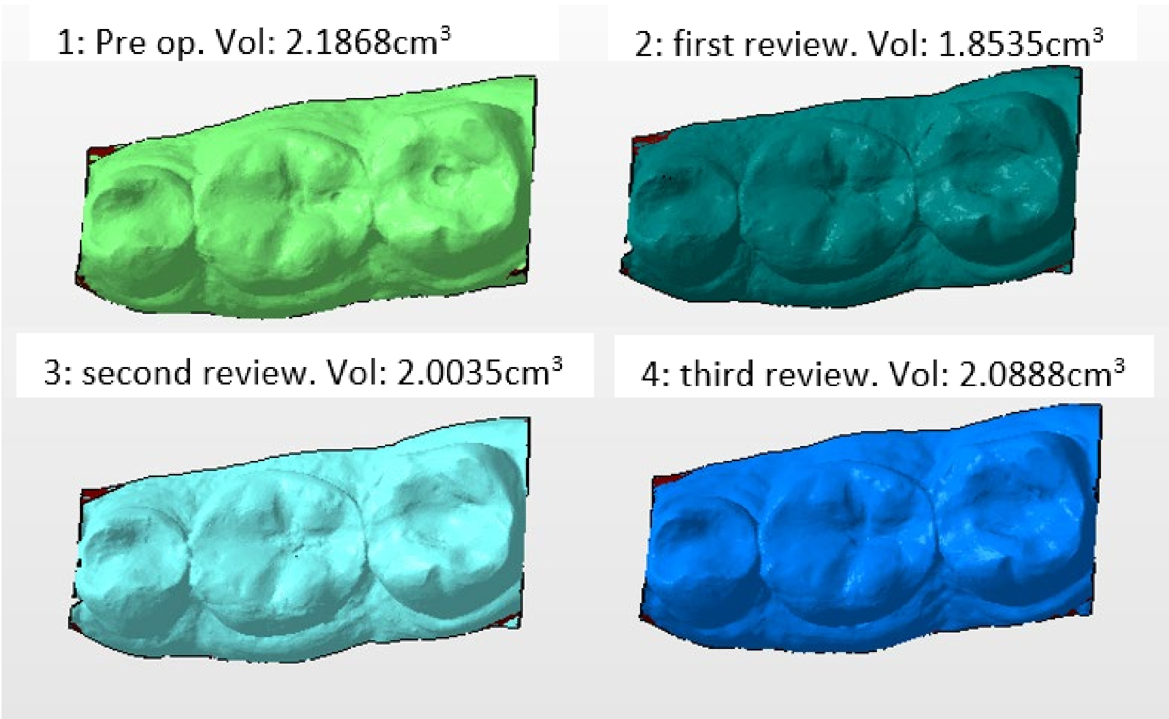
Three-dimensional volumetric evaluation: Digital intraoral impressions of participant's teeth and periodontium were obtained using a calibrated Trios 3 intraoral scanner (3Shape, Denmark), at baseline (before surgery), 1-, 3- and 6- months. The impressions were then exported and superimposed to measure the mean deviations (mm) and volumetric changes (mm^3) as seen in figure 3a – 3d (Autodesk Netfabb, United States).

Crown lengthening procedures were carried out by a DClinDent postgraduate student (R.G.) under the supervision of an experienced periodontist (A.T.S.). Four patients underwent surgery involving an apically repositioned flap and ostectomy while one patient had laser gingivectomy without osseous recontouring under local anaesthesia.

0.2% chlorhexidine mouthwash (Savacol, Colgate) was provided to the patients for two weeks following the procedure and the patients were advised not to use a toothbrush around the surgical area. Sutures were removed and the surgical area polished two weeks after the surgery and oral hygiene procedures reinstated.

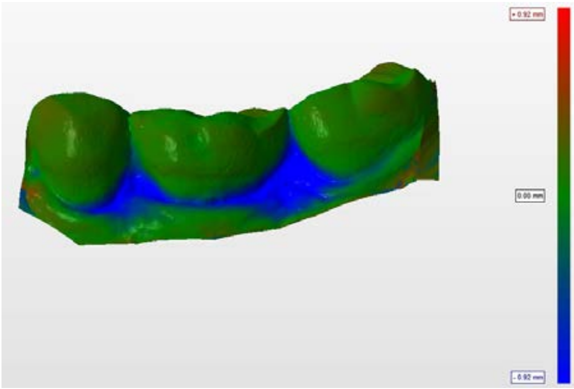


(a)

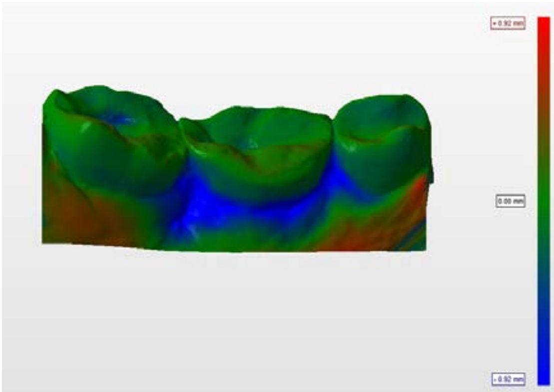


(b)

Figure 3. Comparison of digital volumetric changes between baseline and 1, 3, and 6-months (Colour images for different time points: Baseline: light green; 1-month: dark green; 3- month: light blue; 6-month: dark blue). a) Digital volumetric comparison (Buccal), b) Digital volumetric comparison (Occlusal), c) Digital volumetric comparison (Buccal), and d) Digital volumetric comparison (Lingual). Green areas in (c) and (d) indicate no difference in volume, blue areas indicate loss of tissue volume, while red areas indicate gain of tissue volume.



(c)



[d]

Figure 4. *(continued)*

Statistical Analysis

The Shapiro test was used to analyse normality. In the case of normal distribution, the student t-test will be used to determine the statistical difference on the set parameters. In case of non-normality, the non-parametric method of Wilcoxon signed-rank test was used using a significance level (2-tail) of P-value < 0.05. A one-way ANOVA was used to analyse differences among baseline (initial evaluation) and healing time (1-, 3- and 6-month), using F-test, the outcome of a significant difference, as a result, will lead to a post hoc analysis of Tukey test to identify the time of significant difference. Means were calculated for all parameters. Pearson correlation was used to determine association across parameters. The Statistical analysis was performed using R software (The R Foundation for Statistical Computing, Vienna, Austria, www.R-project.org), and RStudio (Boston, USA).

Results

Gingival recession changes were statistically significant following crown lengthening surgery for all sites apart from adjacent tooth 2 at 6 months. An initial increase in recession between baseline and 3 months followed by a gradual decrease was seen in all patients. The most significant changes occurred on the treated tooth between baseline and 1 and 3-month evaluation. The changes in probing depths were similar to recession. Adjacent tooth 1 and treated tooth had an initial decrease in probing depth, which continued to decrease over the observation period. Results for adjacent tooth 1 and treated tooth were all statistically significant when compared to baseline. The most significant changes occurred on the treated tooth (Table 1a, b).

The differences in crown exposure for adjacent tooth 1 and treated tooth were statistically significant throughout the study (table 2a). Overall, crown exposure increased at 1-month with minimal changes afterwards. The general trend of keratinised tissue levels, as shown by table 2b, showed a decrease from baseline to 1-month, followed by a slight increase at 3-months and then slightly more at 6-months. The only statistically significant results for keratinised tissue levels for all sites (adjacent tooth 1, treated tooth and adjacent tooth 2) were seen between baseline and 1-month.

Table 1. Changes in probing depths and gingival recession 1-, 3-, and 6-months compared to baseline following crown lengthening.

a. Probing depths (mm)					
		Mean ± (SD)	**Mean difference from baseline ± (SD)**	**95% CI**	**P-value**
Adjacent tooth 1	Baseline	2.77 ± (0.90)			
	1 month	2.20 ± (1.06)	0.57 (± 0.94)	0.22, 0.92	0.002
	3 months	2.37 ± (0.93)	0.40 ± (0.89)	0.07, 0.73	0.021
	6 months	2.50 ± (0.73)	0.27 ± (0.74)	-0.01, 0.54	0.058
Treated tooth	Baseline	2.83 ± (1.09)			
	1 month	2.10 ± (0.71)	0.73 ± (0.98)	0.37, 1,10	< 0.001
	3 months	2.20 ± (0.71)	0.63 ± (0.96)	0.27, 0.99	0.001
	6 months	2.37 ± (0.61)	0.47 ± (0.90)	0.13, 0.80	0.008
Adjacent tooth 2	Baseline	2.50 ± (0.94)			
	1 month	2.23 ± (0.77)	0.27 ± (0.69)	0.01, 0.52	0.043
	3 months	2.37 ± (0.67)	0.13 ± (0.73)	-0.14, 0.41	0.326
	6 months	2.27 ± (0.64)	0.23 ± (0.77)	-0.06, 0.52	0.109
b. Gingival recession (mm)					
		Mean ± (SD)	**Mean difference from baseline ± (SD)**	**95% CI**	**P-value**
Adjacent tooth 1	Baseline	1.03 ± (1.16)			
	1 month	1.80 ± (1.75)	-0.77 ± (1.45)	-1.31, -0.22	0.007
	3 months	1.67 ± (1.49)	-0.63 ± (1.30)	-1.12, -0.15	0.012
	6 months	1.43 ± (1.07)	-0.40 ± (0.86)	-0.72, -0.08	0.016
Treated tooth	Baseline	1.30 ± (2.02)			
	1 month	2.70 ± (1.66)	-1.40 ± (1.10)	-1.81, -0.99	< 0.001
	3 months	2.70 ± (1.64)	-1.40 ± (1.25)	-1.87, -0.93	< 0.001
	6 months	1.77 ± (1.50)	-0.47 ± (0.94)	-0.82, -0.12	0.011
Adjacent tooth 2	Baseline	0.57 ± (1.22)			
	1 month	0.90 ± (1.47)	-0.33 ± (0.76)	-0.62, -0.05	0.023
	3 months	0.83 ± (1.32)	-0.27 ± (0.52)	-0.46, -0.07	0.009
	6 months	0.70 ± (1.12)	-0.13 ± (0.57)	-0.35, 0.08	0.211
P-value compared to baseline, the unit of the analysis was the site not the patient					

Table 2. Changes in crown exposure and keratinised tissues (in mm) at 1-, 3- and 6-months compared to baseline following crown lengthening.

a. Crown exposure (mm)					
		Mean ± (SD)	**Mean difference from baseline ± (SD)**	**95% CI**	**P-value**
Adjacent tooth 1	Baseline	6.10 ± (2.59)			
	1 month	7.03 ± (2.57)	-0.93 ± (1.36)	-1.44, -0.42	< 0.001
	3 months	7.13 ± (2.92)	-1.03 ± (1.13)	-1.45, -0.61	< 0.001
	6 months	6.80 ± (2.72)	-0.70 ± (0.92)	-1.04, -0.36	< 0.001
Treated tooth	Baseline	6.13 ± (3.12)			
	1 month	7.50 ± (2.79)	-1.37 ± (1.33)	-1.86, -0.87	< 0.001
	3 months	7.47 ± (2.90)	-1.33 ± (1.27)	-1.81, -0.86	< 0.001
	6 months	6.93 ± (2.73)	-0.80 ± (1.06)	-1.20, -0.40	< 0.001
Adjacent tooth 2	Baseline	6.23 ± (2.78)			
	1 month	6.57 ± (2.50)	-0.33 ± (1.12)	-0.75, 0.09	0.115
	3 months	6.47 ± (2.73)	-0.23 ± (0.63)	-0.47, 0.00	0.050
	6 months	6.47 ± (2.87)	-0.23 ± (0.77)	-0.52, 0.06	0.109
b. Keratinised tissue (mm)					
		Mean ± (SD)	**Mean difference from baseline ± (SD)**	**95% CI**	**P-value**
Adjacent tooth 1	Baseline	4.77 ± (1.59)			
	1 month	4.50 ± (1.59)	0.27 ± (0.74)	-0.01, 0.54	0.058
	3 months	4.70 ± (1.70)	0.07 ± (0.64)	-0.17, 0.31	0.573
	6 months	4.77 ± (1.55)	0.00 ± (0.52)	-0.20, 0.20	1.000
Treated tooth	Baseline	5.23 ± (1.28)			
	1 month	4.93 ± (1.28)	0.30 ± (0.70)	0.04, 0.56	0.026
	3 months	5.03 ± (1.19)	0.20 ± (0.71)	-0.07, 0.47	0.136
	6 months	5.07 ± (1.08)	0.17 ± (0.53)	-0.03, 0.36	0.096
Adjacent tooth 2	Baseline	5.10 ± (1.12)			
	1 month	4.87 ± (0.86)	0.23 ± (0.50)	0.05, 0.42	0.017
	3 months	4.93 ± (0.94)	0.17 ± (0.53)	-0.03, 0.36	0.096
	6 months	5.10 ± (0.99)	0.00 ± (0.37)	-0.14, 0.14	1.000
P-value compared to baseline, the unit of the analysis was the site not the patient					

Throughout the observation period, both treated and adjacent sites maintained similar levels of plaque with low gingival inflammation levels. The results obtained for plaque and gingival indices showed no significant difference after crown lengthening surgery (Table 3).

The overall changes in the marginal bone levels associated with the surgical procedure were between 0.65 and 0.3 mm. The treated tooth had the highest bone level reduction when compared to the adjacent teeth. The treated site had significant bone level changes at 6 months (Table 4).

Table 3. Plaque and gingival indices recorded at adjacent and treated teeth.

	Adjacent tooth 1	Treated tooth	Adjacent tooth 2	*P*-value
Plaque index				
Baseline, n (%)				
Absent	15 (50.0)	13 (43.3)	15 (50.0)	0.837
Present	15 (50.0)	17 (56.7)	15 (50.0)	
One month, n (%)				
Absent	12 (40.0)	17 (56.7)	18 (60.0)	0.251
Present	18 (60.0)	13 (43.3)	12 (40.0)	
Three months, n (%)				
Absent	20 (66.7)	20 (66.7)	20 (66.7)	1.000
Present	10 (33.3)	10 (33.3)	10 (33.3)	
Six months, n (%)				
Absent	24 (80.0)	26 (86.7)	26 (86.7)	0.713
Present	6 (20.0)	4 (13.3)	4 (13.3)	
Gingival index				
Baseline, n (%)				
Absent	24 (80.0)	21 (70.0)	24 (80.0)	0.572
Present	6 (20.0)	9 (30.0)	6 (20.0)	
One month, n (%)				
Absent	21 (70.0)	24 (80.0)	24 (80.0)	0.572
Present	9 (30.0)	6 (20.0)	6 (20.0)	
Three months, n (%)				
Absent	25 (83.3)	25 (83.3)	26 (86.7)	0.919
Present	5 (16.7)	5 (16.7)	4 (13.3)	
Six months, n (%)				
Absent	27 (90.0)	27 (90.0)	29 (96.7)	0.538
Present	3 (10.0)	3 (10.0)	1 (3.3)	
Note: The unit of the analysis was the site and not the patient.				

Table 3. Bone level changes between baseline and 6-months following crown lengthening.

Bone level changes (mm)					
		Mean ± (SD)	**Mean Difference from baseline ± (SD)**	**95% CI**	**P-value**
Adjacent tooth 1	Baseline	3.12 ± (1.13)			
	6 months	3.64 ± (1.08)	-0.51 ± (0.79)	-1.08, 0.06	0.072
Treated tooth	Baseline	2.80 ± (2.02)			
	6 months	3.46 ± (1.96)	-0.65 ± (0.32)	-0.88, -0.43	< 0.001
Adjacent tooth 2	Baseline	2.50 ± (1.37)			
	6 months	2.87 ± (1.30)	-0.37 ± (0.51)	-0.73, 0.00	0.048
P-value compared to baseline; the unit of the analysis was the site not the patient					

Table 5 shows the volumetric changes at 1-, 3-, and 6- months compared to baseline. The results at 3 and 6 months were marginally statistically significant. A general trend was observed where volume tissue reduction was smaller at 1 month, and higher at 3 and

6 months. Although there was some tissue rebound at 1st month, volume changes were maintained after 3 months. Changes between 3 and 6 months were minimal, suggesting some degree of tissue stability. Superimposed digital impressions showed significant changes specially around the treated tooth and sites adjacent to the treated site. Greater changes are depicted with dark blue followed with sites with no change in green and rebound of tissue in red (Figure 3c and d).

Table 4. Volumetric changes at 1-, 3- and 6-months compared to baseline following crown lengthening.

Volumetric changes (cm^3)				
	Mean ± (SD)	**Mean difference from baseline ± (SD)**	**95% CI**	**P-value**
Baseline	2.184 ± (0.491)			
1 month	2.101 ± (0.540)	0.082 ± (0.149)	-0.103, 0.267	0.285
3 months	2.071 ± (0.445)	0.113 ± (0.092)	-0.002, 0.228	0.053
6 months	2.092 ± (0.458)	0.091 ± (0.072)	0.002, 0.181	0.047
Please note that due to the nature of the outcome, the unit of the analysis here is the patient.				

Discussion

Crown lengthening surgeries can affect the overall soft and hard tissue appearance. These volumetric changes may also extend to other sites. A systematic review published in 2017 reported that crown lengthening surgery can lead to clinical and aesthetic alterations on the adjacent/non-adjacent sites, which must be considered in the surgical planning phase (Nobre *et al.,* 2017).

Tissue stability changes after CLS has historically been evaluated using linear and radiographic measurements of treated and adjacent sites (Smith *et al.,* 2023). In line with the results of this study, other studies had also reported no statistically significant changes for plaque and gingival scores during the 6-month follow ups (Arora *et al.,* 2013; Brägger *et al.,* 1992). This is typically due to the strict inclusion criteria for these studies and for the surgery. In most cases, having stable periodontal health is required to undergo crown lengthening surgery as it provides the best surgical outcomes. As well as in this study, most other studies require plaque and gingival indices ≤1 (Arora *et al.,* 2013; Brägger *et al.,* 1992).

The initial increase in probing depth at treated and adjacent sites followed by a continual decrease over the observation period could be attributed to the healing and inflammation that took place within the first month. The result of this study is in line with other studies, which also showed decrease in periodontal probing between 3- and 6-months at both treated and adjacent sites (Arora *et al.,* 2013).

The results of the crown exposure from this study are also similar to other studies. The amount of crown exposure and tissue stability after CLS has been reported in various studies. Patient's age and sex, gingival phenotype, tooth type, location within the dental arch, postsurgical flap position, amount of bone reduction and surgical technique are factors that can influence tissue rebound (Arora *et al.,* 2013; Deas *et al.,* 2004; Lanning *et al.,* 2003). Most significant changes in tissue rebound are seen at 3 months; however, additional changes have been reported at 12 months (Deas *et al.,* 2004; Lanning *et al.,* 2003). There were minimal differences in tissue rebound between anterior and posterior teeth after crown lengthening (Arora *et al.,* 2013).

Most healing and tissue rebound occurred within the first 3 months with very little tissue alterations occurring between 3 and 6 months (Arora *et al.,* 2013). Tissue rebound was found to be directly correlated with the periodontal phenotype. Thicker and flatter phenotypes showed greater tissue rebound (Arora *et al.,* 2013; Pontoriero & Carnevale, 2001). Other studies have also reported that thin periodontal phenotypes are less resistant to trauma or surgical insult, which increase its susceptible to gingival recession (Joshi *et al.,* 2016).

The bone level changes reported in this case series are consistent with the findings published by Brägger and colleagues in 1992. Due to the nature of the surgery and exposure of the alveolar bone, further minor decrease in bone level is expected due to surgical trauma. Osseous reduction typically ranges between 1 and 3 mm at treated sites, and ideally, a distance of 3 mm is recommended to secure adequate space to avoid invasion of the supracrestal tissue attachment (Brägger *et al.*, 1992; Gargiulo *et al.,* 1961). Bone reduction of < 1.50 mm results in an average crown lengthening of 1.53 mm whereas bone reduction of more than 1.50 mm achieves a mean gain in crown length of 1.95 mm after CLS (Arora *et al.,* 2013). Soft tissues changes are closely related with bone levels, insufficient ostectomy or no bone recontouring can lead to relapse due to soft tissue rebound (Deas *et al.,* 2004).

To the authors' knowledge, this is the first study that utilized 3-dimensional analysis to assess volumetric tissue changes following crown lengthening surgery. The volumetric findings of this study correlated to the clinical outcomes accurately. As a result of the resective nature of crown lengthening procedures, there was an overall decrease in tissue volume after surgery, which was marginally significant at 3 and 6 months, but not at 1 month. The lack of statistical significance at 1 month could be due to swelling related to the surgical trauma (Arora *et al.,* 2013). The use of sequential digital impressions to evaluate volume changes have been evaluated and validated in other periodontal surgeries such as periodontal plastic surgery and surgical therapy of peri-implantitis (Galarraga-Vinueza *et al.,* 2020; Marques *et al.,* 2021). These studies found volumetric analysis to be a straight-forward and non-invasive method to objectively quantify periodontal outcomes. As digital technology continues to evolve in dentistry, volumetric results obtained from digital impressions will be even more precise.

Conclusion

In summary, the results obtained from this pilot study are comparable to other published studies. Most significant changes were observed in crown exposure, gingival recession, and bone levels, followed by probing depth reduction for treated sites. Volumetric changes were only significantly reduced after 6 months of healing while the width of keratinised tissues, plaque levels and gingival scores had minimal changes throughout the observation period. Volumetric changes measured through a series of digital impressions in this pilot study reflected on the clinical findings accurately. Future research with larger sample sizes and longer follow-up periods should be considered to further our understanding of the changes in the periodontium following crown lengthening surgery.

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CASE REPORT Open Access

Dentinogenic ghost cell tumour: A case report

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Abstract

A 23-year-old Malay female patient presented with a history of pain and swelling over right maxilla. Imaging showed a well-defined unilocular radiolucency with areas of radiopacity in the right maxilla. The lesion was initially thought to be a unicystic ameloblastoma. However, histopathology of the excised lesion proved otherwise with a final diagnosis given as dentinogenic ghost cell tumour. The clinical presentation of the case, subtypes of DGCT, similarities with ameloblastoma, and treatment modalities are discussed in this paper.

Keywords: *calcifying odontogenic cyst, dentinogenic ghost cell tumour, Gorlin cyst*

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Introduction

Dentinogenic ghost cell tumour (DGCT) presents as a rare invasive neoplasm characterized by islets of ameloblastoma-like epithelial cells in mature connective tissue. Aberrant keratinization can be found in the form of ghost cells in association with varying amounts of dysplastic dentin (Agrawal, *et al.,* 2017). This tumour makes up for only 2%-14% of all calcifying odontogenic cysts and less than 0.5% of all odontogenic tumours which owes to its rarity (Kumar, et al., 2010). It usually occurs in elderly persons with a male predilection (Pinheiro, *et al.*, 2019). The purpose of this article is to report a case of dentinogenic ghost cell tumour in a 23-year-old female, which is at a comparatively younger age.

Case Presentation

A 23-year-old Malay female was referred to Department of Oral and Maxillofacial Surgery, Segamat Hospital due to swelling over the anterior upper jaw which she noticed in the past one month. The lesion started as a small swelling at the upper sulcus of anterior teeth then gradually increasing in size. She also complains of intermittent throbbing pain over that region upon biting. Patient claims to have no known medical illness with no relevant family history. However, when vital signs were taken prior to biopsy, it was noted that patient had persistent tachycardia. Patient was then referred to Emergency Department and was later diagnosed as hyperthyroidism secondary to Graves' disease. Patient was then started on carbimazole and propranolol by medical team for her condition which has improved her symptoms.

Extraoral examination revealed right nasolabial fold obliteration secondary to swelling of right upper lip and philtrum region. Intraoral examination revealed swelling over right labial sulcus extending from 11 to 14 region with overlying mucosa appearing bluish, soft in consistency and non-tender on palpation (Figure 1a). Bony expansion was also noted on palatal region extending from 11 to 14 (Figure 1b). Teeth involved were firm, non-displaced, but non vital with electric pulp test. Upon aspiration from right labial sulcus, noted brown coloured fluid within lesion (Figure 1c). An incisional biopsy was performed and based on histopathological examination correlating with clinical and radiographical findings, differential diagnosis of ameloblastoma and calcifying odontogenic cyst were considered.

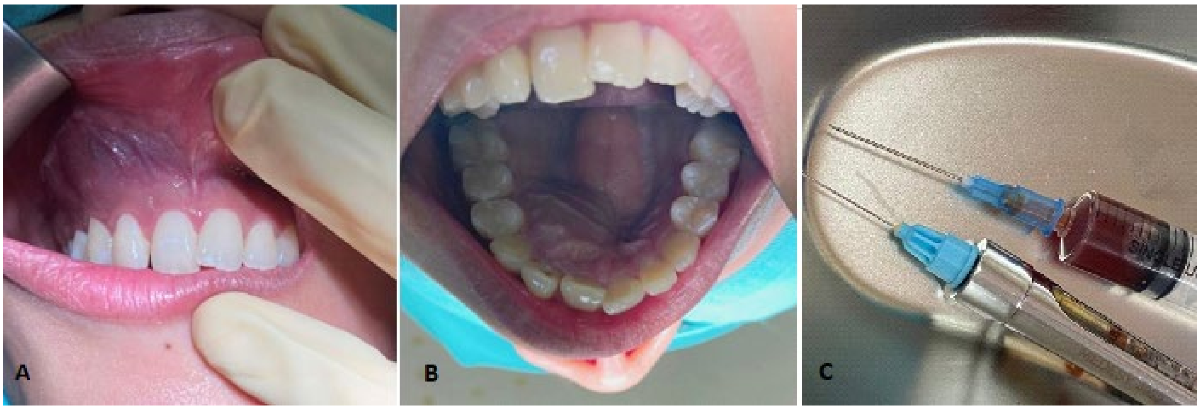


Figure 1. (A) Swelling over right labial sulcus extending from 11 to 14 region with overlying mucosa appearing bluish. (B) Bony expansion palate region extending from 11 to 14. (C) Brown coloured aspiration fluid.

A contrast enhanced computed tomography scan (CECT) showed a unilocular expansile intraosseous lesion measuring 2.5 x 2.8 x 2.7cm at the right paramedian anterior maxillary region with no significant enhancement. There are specks of hyper density within the lesion, suggestive of calcifications (Figure 2). There is associated root resorption of adjacent right upper incisors up to the first premolar. The lesion has caused abutment of anterior wall of maxillary sinus and right nasal cavity without invasion.

Figure 2. Contrast enhanced computed tomography showing unilocular expansile lesion with specks of calcification within (coronal and axial view).

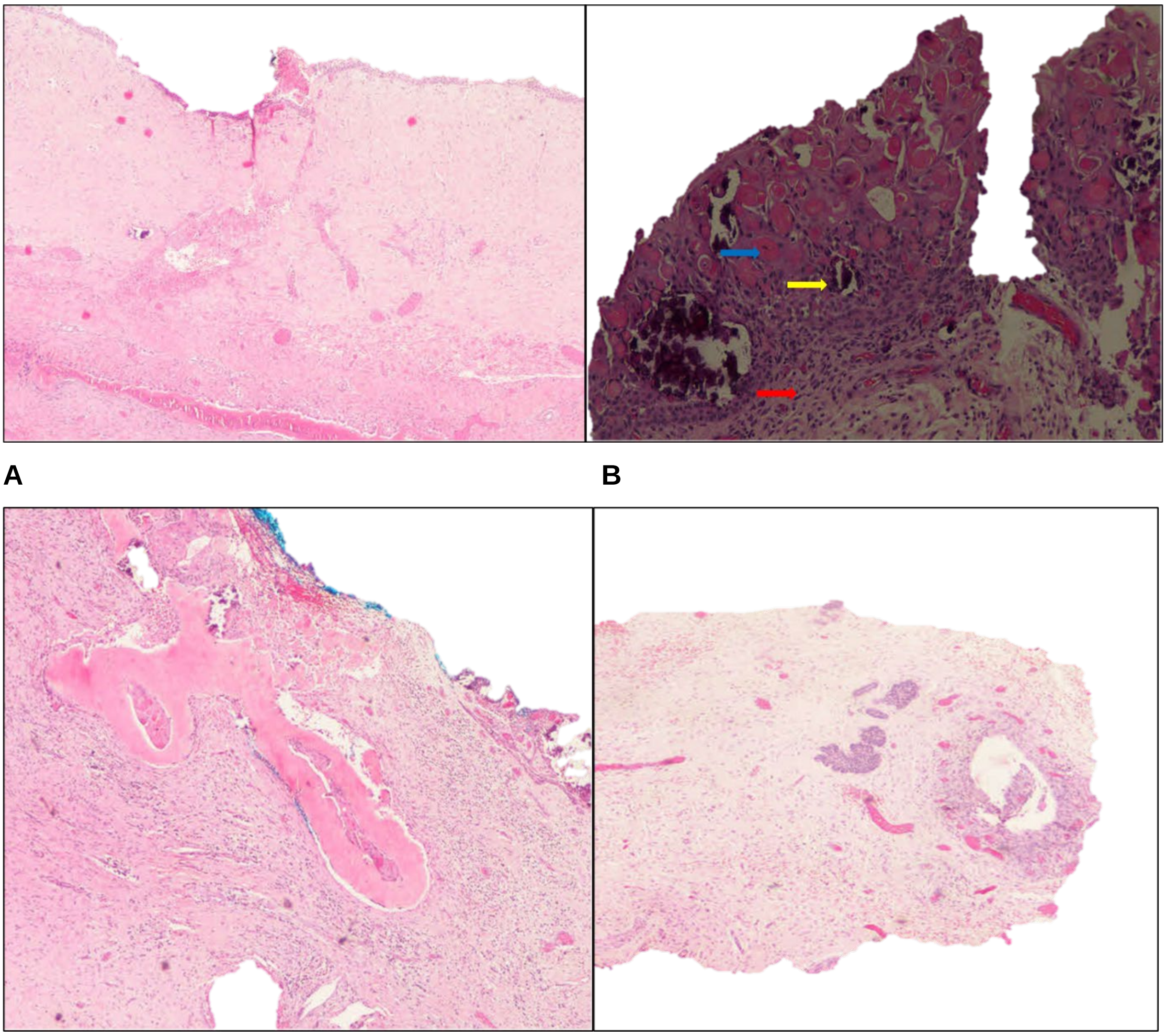
Enucleation of lesion (Figure 3) with ostectomy of approximately 1-2mm at the periphery margins of the lesion was done under general anaesthesia and the tissue was submitted for histopathological examination.

Figure 3. Enucleation of lesion with ostectomy under general anaesthesia.

Histopathological examination showed an odontogenic tumour lined by epithelium of varying thickness composed of loosely cohesive epithelium with palisading of basal cell layer with reverse polarity and areas having accumulation of ghost cell admixed with scattered calcifications (Figure 4a,4b). An ameloblastomatous island consisting of ghost cells and calcifications undergoing cystic degeneration and several small ameloblastomatous islands are seen in the fibrous tissue (Figure 4d).

Present within the fibrous tissue are dentinoid material (Figure 4c) adjacent to the lining and at the deeper aspects, the dentinoid material is seen associated with basaloid cells. Presence of dentinoid materials are also noted between sheets of cholesterol clefts with associated multinucleated giant cells.

Upon one year of follow-up examination, patient presented with well recontoured bone of right maxilla regaining normal morphology (Figure 5A, 5B) and did not show any signs of recurrence. Teeth involved with lesion, tooth 11 to 14, did not show any signs or symptoms clinically. Radiographic examination showed new bone formation filling the previous enucleated cavity while teeth involved with lesion previously did not show further resorption of root tips (Figure 5C). Electric pulp testing was done on involved teeth and noted tooth 13 and 14 regained vitality while tooth 11 and 12 remains non vital. All non-vital teeth were subjected for root canal treatment and apicoectomy with root end filling.



A B C D

Figure 4. (A) Fibrous wall containing bland odontogenic rests lined by loosely cohesive epithelium with focal areas having palisading of basal cell layer with reverse polarity. Parts of the cells near the luminal areas resemble stellate-reticulum like-cells. Presence of dystrophic calcifications and trabeculae of vital woven bone rimmed by osteoblasts is noted. [Hematoxylin and Eosin stain, ×4], (B) Odontogenic tumour lined by epithelium of varying thickness composed of loosely cohesive epithelium with palisading of basal cell layer (red arrow) with reverse polarity. Areas having accumulation of ghost cells (yellow arrow) admixed with scattered calcifications (blue arrow). [Hematoxylin and Eosin stain, ×10], (C) Dentinoid materials [Hematoxylin and Eosin stain, ×10], and (D) Ameloblastomatous islands [Hematoxylin and Eosin stain, ×10].

Figure 5. Progress of patient one-year post enucleation. (A) Slight scarring of upper buccal sulcus with no palpable bulge (B) Well recontoured palate, and (C) Upper occlusal radiograph showing new bone formation in the previous enucleated cyst cavity and resorption of root tips teeth 11 to 14 persist.

Discussion

Calcifying odontogenic cysts were first described by Gorlin and colleagues in 1962 as a separate entity of odontogenic origin. Calcifying odontogenic cysts account for 1–2% of all odontogenic tumours, in which 88.5% are cystic and the remaining 11.5% are solid tumours (Agrawal *et al*., 2017; (Singhaniya *et al*., 2009). As all lesions are not cystic, it is debatable, whether calcifying odontogenic cyst is a cyst or a neoplasm (Singhaniya *et al.*, 2009). Some have suggested that there could be a possibility of cystic degeneration taking place at the centre of proliferating epithelial islands rather than epithelial changes developing in a pre-existing cyst wall (Patankar *et al.*, 2019). Based on this dualistic concept, WHO termed all cystic lesions as calcifying cystic odontogenic tumours (CCOT) and the neoplastic entity as dentinogenic ghost cell tumours (DGCT) (Agrawal *et al*., 2017). In 2005, WHO defined DGCT as, "A locally invasive neoplasm characterized by ameloblastoma-like islands of epithelial cells in a mature connective tissue stroma. Aberrant keratinization may be found in the form of ghost cells in association with varying amounts of dysplastic dentin." (Bafna *et al*., 2016; Garcia *et al*., 2015; Patankar *et al*., 2019). The aetiology of this lesion is still unknown, but it has been suggested that missense mutation in β-catenin during odontogenesis disrupt the proper differentiation process coordinated in wingless integrated (Wnt) pathway, plays a crucial role in the formation of DGCT (Kim *et al.*, 2007).

DGCT may occur as an intraosseous central lesion (68%) and less commonly as an extraosseous peripheral lesion arising in the gingiva or alveolar mucosa (32%). The age may range from 7 to 82 years (mean 45 years) with strong male predilection. A majority of published DGCT cases were reported in the Asian population (65%) (Pinheiro *et al.*, 2019). Both the central and peripheral variant showed a greater predisposition to the mandible than the maxilla (Pinheiro *et al*., 2019). The tendency to occur at the canine to first molar region of the jaw (Agrawal *et al.*, 2017; Patankar *et al.,* 2019; Kumar *et al*., 2010). The behaviour of intraosseous DGCT is more aggressive than extraosseous DGCT (Kelleş *et al.*, 2012).

In this case, the lesion presented with both cystic and tumour characteristics. DGCT may appear radiographically as radiolucent, radiopaque, or mixed lesion amounting to the presence and extent of calcification. The radiopacity seen in the CECT taken was initially thought to be an impacted odontome or supernumerary tooth associated with the lesion. Lesions may appear unilocular or multilocular with either well-defined or ill-defined margins (Patankar *et al.*, 2019). Due to the mixed presentation of this lesion, the initial provisional diagnosis of the lesion was thought to be a cystic ameloblastoma as it showed clinical features such as resorption of adjacent tooth, association of impacted tooth, buccal and lingual cortical expansion with disruption of the buccal cortex those of which are found in cystic ameloblastoma (Cadavid *et al.*, 2019; Patankar *et al.*, 2019). Ameloblastoma represents approximately 11 to 18% of all odontogenic tumours, being the second most common after odontomas (Cadavid *et al.*, 2019), a much higher prevalence compared to DCGT. The characteristic features of DGCT that distinguish it from ameloblastoma and other odontogenic tumours are presence of numerous ghost cells and masses of dentinoid material (Singhaniya *et al.,* 2009; Martos-Fernández, *et al.*, 2014).

Among case reports reviewed, little has been mentioned regarding aspirated fluid from the lesion. *Agrawal et al* reported thin yellowish colour, blood-tinged aspiration fluid and on cytological examination only red blood corpuscles (RBC) were found (Agrawal *et al.*, 2017) while Kelles *et al.* reported aspiration fluid showed turbid brown fluid and on cytological examination revealed groups of degenerating cells with prominent cytoplasm with foamy histiocytes (Kelleş *et al.*, 2012). There is no conclusive evidence if the aspiration fluid of the cyst provides any diagnostic value.

Central DGCT is considered as locally aggressive neoplasm. Study showed a recurrence rate of 73% after conservative surgical treatment of enucleation or curettage compared with a recurrence rate of 33% after radical treatment of peripheral or segmental resection (Buchner *et al.*, 2016) where recurrence can occur up to 20 years after the initial surgery (Pinheiro *et al.,* 2019). Another author also finds similar finding with recurrence rate of DGCT at 71% and the recurrence tends to occur between 5 to 8 years post initial treatment (Alzaid *et al.*, 2022). Compared to the less aggressive extraosseous counterpart where no recurrence has been reported after conservative treatment (Pinheiro *et al.*, 2019). Although rare, it has been reported that recurrent DCGT have shown malignant potential and is diagnosed as ghost cell odontogenic carcinoma (GCOC) (Martos-Fernández *et al.*, 2014; Pinheiro *et al.*, 2019).

Some has proposed that DGCT should be treated as ameloblatomas as there are several histological features like ameloblatomas (Garcia *et al.*, 2015). It has been proposed that DGCT should be treated by resection with safety margin of at least 0.5cm, similar to recommendations for ameloblastoma (Buchner *et al.*, 2016; Garcia *et al.*, 2015; Pinheiro *et al.*, 2019). Some authors suggested initial conservative treatment of enucleation and meticulous curettage of the surrounding bony wall around 1 to 3mm for radiographic unilocular well-defined lesions. The initial radical treatment of peripheral or segmental resection is preserved for clinical and radiographic destructive lesions with ill-defined borders (Buchner *et al.,* 2016). Aggressive wide local excision such as en-block resection is proposed to be carried out in intraosseous subtype of DGCT (Patankar *et al.*, 2019) due to its high recurrence rate. However, it is important to note that some cases were treated with surgical enucleation without any recurrence (Pinheiro *et al.*, 2019).

For this patient, considering her young age, gender, and small size and location of the tumour, a more conservative approach was adopted. Radiographically, the lesion involves all right upper anterior teeth, with expansion into palatal region, abutting on right nasal floor and right anterior maxillary sinus. If a wider excision such as en-block excision was done, this patient will end up with large defect which required more complex rehabilitation process. Her appearance, speech and masticatory forces will be greatly impaired and leads to negative outcome on her quality of life. Based on the clinical and radiographical aspects, we decided on a much conservative treatment approach which is surgical enucleation with ostectomy of periphery margin up to 2mm. This treatment approach has been decided in the view of few authors who also practice more conservative approach in treating DGCT. The same approach also taken by other author who reported the enucleation of DGCT was carried out in a view of small tumour size with no local recurrence (Agrawal *et al.*, 2017).

Due to high risk of recurrence, this patient has been scheduled for a frequent long term follow up with imaging. Imaging is scheduled at least once a year to properly monitor for any recurrence of the lesion as the DGCT has malignant transformation for recurrent cases (Alzaid *et al.*, 2022). One year of follow-up examination on the patient did not show any signs of recurrence. The treatment of choice remains a dilemma for surgeons. However, it is consensus that all patients with DGCT should remain in long-term follow-up due to its high rate of recurrence and possible malignant transformation.

Conclusions

The mixed histopathology characteristic of dentinogenic ghost cell tumour leads to surgical dilemma on the best approach of the treatment. Therefore, a long term follow up is necessary to prevent the recurrence of the disease. The best approach of treatment should be tailored according to individual patient factoring clinical, radiographical, HPE and social impact to prevent unnecessary excision. Quality of life and rehabilitation of the patient should be considered in treatment planning.

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CASE REPORT Open Access

Prosthetic rehabilitation on patient with orbital defect: A customised approach

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Abstract

Loss of eye leads to significant psychological trauma which necessitate rehabilitation. Restoring eye defects with prostheses will uplift psychological status of such patients by re-establishing the facial structures and appearance, eventually returning them to their normal life. Even though prefabricated orbital prostheses are available, the lack of proper fitting indirectly affect comfort and aesthetics. Custom-made orbital prosthesis is still preferred due to its conformity which correspond to individual defect and the ability for shade personalization. This article elaborates the technique of fabrication of a custom-made orbital prosthesis in giving a life-like appearance to the patient.

Keywords: *customized orbital prosthesis, eye prosthesis, maxillofacial rehabilitation*

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Introduction

Eyes are most prominent features of the face to be noticed when people communicate. The unfortunate loss or absence of the eyes may be caused by congenital defect, irreparable trauma or tumors (Perman & Baylis, 1988). According to the severity, there are various surgical modalities of management for example; exenteration, evisceration or enucleation. By definition, evisceration is surgical removal of some portion of intraocular contents of globe, and leaving some portion of sclera conjunctiva, extraocular muscles and optical nerve tissue. While enucleation is surgical removal of globe and a portion of optical nerve tissue of globe. Exenteration is defined by an en bloc removal of entire orbit which involving partial or total removal of eyelids and most of the case is due to tumour (Croce *et al.*, 2008).

The facial disfigurement can cause significant physical and emotional problems. Some patient uses several accessories in order to hide the defect prior to rehabilitation; for example, usage of sunglasses, hat and facial mask in order to socialize with public and improve their self-confidence. Therefore, providing an artificial substitute to restore its form and functions is the mandatory reason for such disability. Prosthodontic rehabilitation has therefore become an option to restore aesthetics, comfort and also elevate psychological status of such patients (Taylor, 2000). Orbital prosthesis can be custom made or prefabricated. Custom-made orbital prosthesis demonstrates close contact with surrounding tissue, hence has capability of distributing pressure equally subsequently reducing the incidence ulceration as compared to prefabricated form. Customized prosthesis also possesses several advantages including improved fit, comfort, adaptation to facial contours, and enhanced aesthetics gained from the control over the size of the iris, pupil and colour of the iris, sclera and tissue to be replaced (Beumer & Zlotolow, 1996; Artopoulou, 2006; Ow & Amrith, 1997).

The procedure of prosthetic eye replacement presented with many challenges in determining the precise alignment of the pupil, balancing the interpupillary distance and positioning the prosthesis in regard to the contralateral eye (Doshi & Aruna, 2005). Many methods for locating the iris have been described; for example, using ocular locator, fixed calipers, grids, dividers, inverted anatomic tracings, and visual assessment (Babu *et al.*, 2016). Other than that, the remaining anatomical structures may affect the outcome of the treatment especially in improving prosthesis retention. Before embarking to planning and prosthesis designing, it is essential to assess the psychological element in order to gain the patients' confidence, in addition to a detailed medical history that includes the condition that led to the excision and enucleation in order to alert the possibility of recurrence (Cain, 1982). In this case report, a customised approach for fabrication of orbital prosthesis of an exenterated right orbital closed defect is presented. The objective of the proposed technique is to achieve predictable positioning of the iris to enhance the aesthetic effect.

Case Report

A 65-year-old female reported to the Prosthodontics postgraduate clinic requesting rehabilitation for her right orbital defect. She had undergone surgical exenteration due to malignant melanoma in August 2017. Two months later, she was then cleared from malignancy The patient presented with a favourable right eye defect sizing 7cm x 5cm (Figure 1). On examination, a well-healed orbital defect lined with split-skin graft was observed. The patient did not complain of pain or discomfort. An adhesive-retained orbital prosthesis was planned for complete prosthetic rehabilitation utilizing soft tissue undercuts.

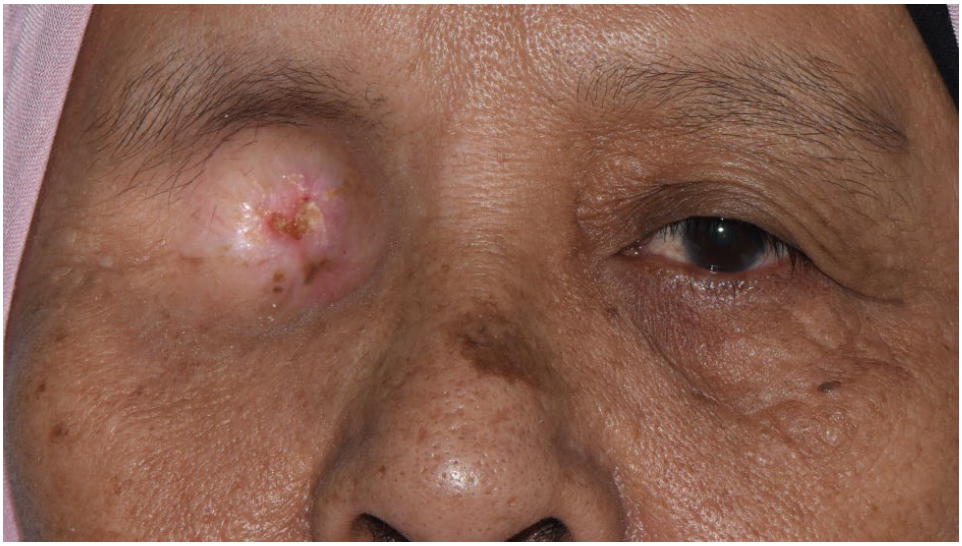

Figure 1. Frontal view of the patient with a favourable right eye defect.

Phases of fabrication of orbital prosthesis

a) Orbital impression making

During the first visit, facial and orbital impressions were made. Facial impression was taken for facial planning of prosthesis construction in the laboratory while orbital impression was used as working model. The patient was seated on the dental chair at an upright, relaxed position. Petroleum jelly was applied at the patient’s eyebrows and eyelashes. Gauze piece coated with petroleum jelly and ligated with floss was inserted into the nasal orifices to prevent the flow of impression material into nasal cavity. For the facial impression, two breathing tubes were inserted into the patient’s mouth to allow breathing. The facial tray was checked on the patient face; a hole made on the nasal area to minimize pressure and reduce tissue deformation while making the impression. The facial impression was made using irreversible hydrocolloid impression material (Kromopan, Lascod, USA). The material was spread on the patient's face; the facial tray was loaded with impression material and positioned on the face. The impression was detached from the face after complete set and was checked for any deformities or defect. For primary orbital impression, the impression was made using light-body and heavy-body vinyl polysiloxane impression material (VPS, Chemi-Sil, B&E, Korea) to capture texture and details of the defect for proper adaptation of the prosthesis. Light-body VPS was first injected into the defect followed by the heavy-body VPS into the rest of the right orbital region (Figure 2). After complete polymerization, impression was removed and inspected for any deformity or defect. Then, the patient was advised to sit in a relax position and to look straight ahead. The ocular and eye brow orientation points were defined and recorded.

b) Ocular component fabrication

Left iris shade was recorded. The iris for ocular component was fabricated utilising iris button painting technique (Fernandes *et. al*, 2009) (Figure 3). The contralateral iris diameter was measured. The size and shape of the ocular wax pattern was adjusted accordingly and processed to be inserted into the orbital prosthesis.

Figure 2. Orbital impression using light-body and heavy-body polyvinyl siloxane impression material.

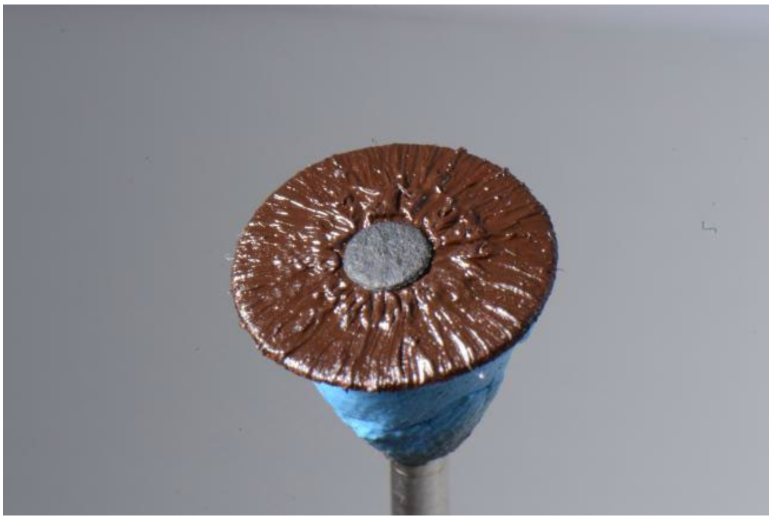

Figure 3. Iris button was produced using acrylic paint technique.

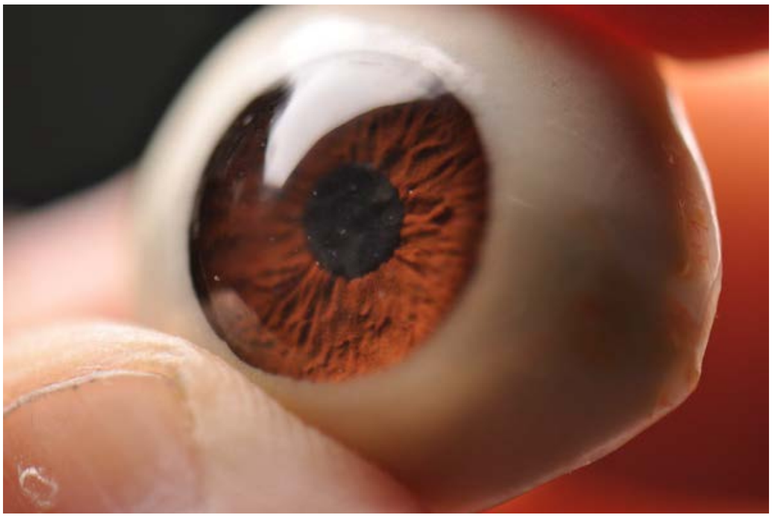

Figure 4. After completion of ocular prosthesis processing.

c) Orbital wax pattern fabrication and try-in stage

Both impressions were casted using Type 3 dental stone (Model Stone, Zhermack, Italy) (Figure 5), for orbital and facial cast. A framework was fabricated using light cured clear acrylic resin (Vertex™ Rapid Simplified, Vertex Dental) and customised with a mix of soft yellow intrinsic colour (P115 Intrinsic Staining, Technovent, UK). The framework was incorporated with perforations to reduce the weight of the prosthesis and to enhance silicone retention (Figure 6). The perforated framework was evaluated for fitting and retention while performing facial movements including opening and closing of mouth and raising the left eyebrow. A wax pattern of the orbital prosthesis was sculptured initially using modelling wax (Collegewax, Metrodent, UK) on the facial cast, to ensure the parallelism in relation to other facial landmarks (Figure 7). Then, soft wax was inserted into desirable undercuts of the orbital cast. The orbital wax pattern was attached to the soft wax on the orbital cast to ensure proper extension and fit of the prosthesis. Later, the ocular component was incorporated and evaluated chairside. The symmetry, contouring, and shape of the wax pattern were compared to the contralateral eye and its relation to other facial structures. The adaptation of the wax pattern was also assessed, especially on the borders. Texture and creases were created to match with patient's skin. It was examined from frontal, lateral, and 12 o'clock views. To evaluate prosthesis retention, the patient was asked to made several facial expressions. The skin base shade matching was determined by selecting the facial region that has a slightly lighter skin tone.

Figure 5. Facial model casted using Type 3 dental stone (Model Stone, Zhermack, Italy).

Figure 6. Positioning of ocular and acrylic framework on patient's face.

Figure 7. Wax pattern with ocular prosthesis on patient's face from frontal view.

d) Processing of orbital prosthesis

The wax pattern was sealed on the working cast to ensure good marginal adaptation. The sculpt was finally given a stippled surface using a bristle toothbrush. Ocular component was also indexed by attaching a plastic rod with cyanoacrylate resin to secure to the investment during dewaxing. The cast was invested in a two-piece dental flask (Figure 8). Dewaxing was performed and both flasks were left opened to dry. Separating medium was applied (Separating Fluid, Ivoclar Vivadent, Germany). Maxillofacial silicone elastomer was mixed according to the manufacturer's instructions (Platinum Silicone, Medical grade Technovent Co, UK). Shades of cream, light brown, and grey intrinsic stains (P115 Intrinsic Staining, Technovent, UK) were added with in combination with red (to mimic blood vessels) and yellow flocking to the mixed silicone. Then, the silicone elastomer was packed into a two-piece dental flask and polymerized for 60 minutes at 100°C following manufacturer's instructions.

Figure 8. Investment of orbital prosthesis.

e) Delivery of orbital prosthesis

The prosthesis was first fitted to the orbital defect area. Its engagement to the available undercuts was assessed (Figure 9). The prosthesis was be able to be inserted and removed easily without causing any pain or discomfort. Retention was evaluated by asking the patient to perform facial movement, including mouth opening, smiling, and whistling. She was also asked to move her head sideways and bending down. The retention was deemed acceptable. However, to improve her confident, a water-based adhesive (G609 Probond Adhesive, Technovent, UK) was prescribed. The margins and extension of the prosthesis were checked and adjusted. Silicone flash on the peripheries was left in-situ to ensure transitional blending to the skin. After chairside assessment, the external staining was painted using extrinsic stains (P702i extrinsic color, Technovent, UK) to complement patient's skin colour. Finally, extrinsic sealant (P799 extrinsic sealant, Technovent, UK) was applied and left for 60 minutes to set following manufacturer instructions. Artificial eyelashes were incorporated into the orbital prosthesis before delivery to the patient (Figure 10). The placement of the prosthesis was demonstrated to the patient. A non-prescription eyeglass was also prescribed to camouflage her prosthesis wearing. Maintenance care instructions regarding were provided to the patient and her spouse. Patient was asked to wipe clean the prosthesis with water and a clean cloth, avoid using soap. The patient was also instructed to avoid direct exposure to sunlight, and advocate the use sunglasses or umbrella as protection. She was aesthetically satisfied with the orbital prosthesis. She was also emphasized on the usage and of the prosthesis.

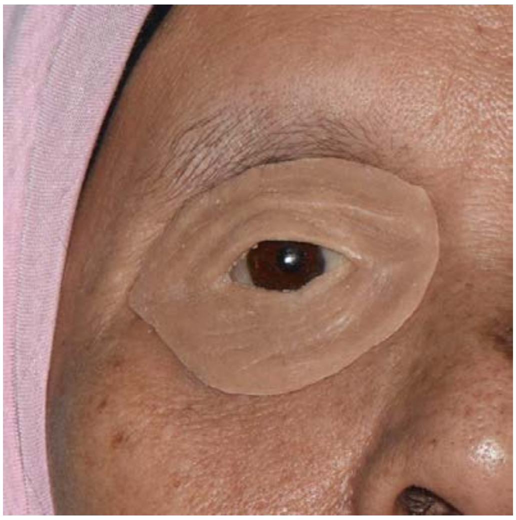

Figure 9: Processed silicone with ocular prosthesis without staining.

Figure 9. Extrinsic staining and Incorporation of artificial eyelashes prior to delivery.

f) Review and follow up

Patient was called for her first review after a week to check for patient satisfaction, retention of prosthesis, and adaptation of remaining soft tissue to the orbital prosthesis. Patient mentioned that the prosthesis is slightly loose and the chemical adhesive had helped a lot to retain the prosthesis. She was satisfied with her appearance wearing the prosthesis and to improve the look, she used spectacles to camouflage the prosthesis (Figure 11). Following three months review, patient was happy with her prosthesis. She would like to continue the usage of adhesive which gave her more confidence in public. The shade and fit of the prosthesis were checked and deemed satisfactory.

Then, her recall appointment was scheduled annually. Her prosthesis was assessed for the need of recolouring and the adhesive supply was replenished. Patient has been rehabilitated successfully for three years without any complications. At three years wearing, the margin of prosthesis was still good with excellent hygiene. Its shade was a tone lighter and external recolouring was attempted after three years insertion. The artificial eyelashes were also replaced.

Figure 10. Orbital prosthesis with spectacles to camouflage the prosthesis.

Discussion

Malignant melanoma of the eye is an uncommon disease but potentially life-threatening cancerous growth in the eye. It comprises about 2% of all eye tumors, about 5% of melanomas in the ocular region (Isager *et al.*, 2006) and 0.25% of all melanomas overall (Chang *et al.*, 1998). Enucleation and exenteration are radical treatments to eradicated the conjunctival melanoma especially in diffuse melanoma (Reese, 1966). However, such approaches have shown no improvement in survival and have the consequences of disfigurement and blindness (Paridaens *et al.*, 1994). These techniques are only performed as relief for tumors that invade the orbit or fully involve the entire conjunctiva (Shields *et al.*, 2011). The disfigurement associated with the loss of an eye can cause both emotional and physiological distress. Most patients experience significant stress primarily due to the function disability and also societal reactions towards facial impairment (Lubkin & Sloan, 1990). To improve appearance of this patients, orbital prosthesis was recommended. The requirements for orbital prosthesis should be aesthetic, light weight, economical, and retentive. Silicone material is the preferred material for orbital prosthesis as they provide better marginal adaptation and good appearance than acrylic (Guttal *et al.*, 2008). Attention to detail is mandatory in each and every step to bring out a satisfactory end result. For this case, minimal retention is obtained at the superior border of the orbit. Orbital prosthesis could be retained by multiple methods such as undercuts, facial accessories (such as spectacles), medical grade adhesive, or osseointegrated implants (Beumer *et al.*, 2011). Difficulty faced in attempting to restore the symmetry and to hide the margins of the prosthesis. For this case, implant was not an option due to patient's financial constraint and it will take longer treatment time. Retention using spectacles was also not considered due to difficulty of patient to adjust during insertion of prosthesis. The best treatment option would be retention of the prosthesis by means of utilizing available undercuts and skin creases, and usage of adhesive if needed. Prolonged usage of adhesive is not recommended due to the following reasons:

1. High level of dexterity to apply the adhesive,
2. More care for cleaning of the prosthesis and defect,
3. Possibility of tearing the borders while applying,
4. Financial burden to the patient.

During the fabrication of the prosthesis, perforated acrylic framework was incorporated to locate the prosthesis's position and also to reduce the weight of prosthesis. There were several limitations of constructing this prosthesis including difficulty in processing the silicone since the defect was quite large, incorporating and matching the ocular into orbital component, producing monotonous skin shade and hiding the margin of the prosthesis on the defect area. Regardless the difficulties, the advantages of this customize prosthesis were replicating individual and specific anatomical structure, skin and ocular shade were definite to the patient and last but not least were patient comfort and satisfaction. Special instructions were given to the patient to avoid washing the prosthesis with acidic or basic solutions that might cause fading to the extrinsic shades. There were several drawbacks with this such elastomeric prosthesis due to degradation of their colour and physical properties (Hatamleh & Watts, 2010; Kurunmaki *et al.*, 2008). Some article revealed this prosthesis needed to be replaced within 6-12 months and the main factors was colour changes due to exposure to ultraviolet radiation, humidity, cleansing agent and contact with body fluids (Andres *et al.*, 1992; Lemon *et al.*, 1995). However, for this patient, she was reviewed up to 3 years and the prosthesis was still able to adapt to the defect area and minimal colour changes noted. Patient was comfort to the current prosthesis and the longevity of the prosthesis might be due to good hygiene and handling care by the patient.

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