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GUEST EDITORIAL

Are we learning from the pandemic?

Over a decade ago, in a retrospective review, of the Severe Acute Respiratory Syndrome (SARS), which struck the World in 2003, in a leading article to the Journal of the American Dental Association (JADA) Professor Malik Peiris and I wrote '*... the dental community cannot let down its guard and must be constantly aware of impending infectious threats in various guises, as well as the recrudescence of disease, that may challenge the current infection control regimen*`.[1] Unfortunately, with the pandemic of coronavirus disease 2019 (COVID-19), this portentous prediction became true, as the organism mutated into a newer, more infectious, and a much more vicious variant in the guise of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and engulfed the globe with the devastating outcomes of COVID-19. Moreover, it is further evolving and mutating into further variants, such as Delta and Omicron that exhibit even higher infectivity, contagiousness, and immune resistance.[2]

COVID-19 pandemic was a wake-up call for the entire world. The inadequate preparedness of humanity for such a devastating disease was evident early in the pandemic and now, with the somewhat dysfunctional vaccine programs, particularly in many developing regions of the world, perhaps, including Sri Lanka. Although the dental profession was one step ahead in dealing with COVID-19 patients due to the universally used, rigorous infection control measures, the profession has to be forearmed not only for a constantly recurring and possibly endemic disease with various SARS-CoV-2 viral variants but also new emerging diseases that are predicted to arrive periodically in various guises. Indeed, these emerging and reemerging pandemics behooves the profession to think and act out of the traditional framework of conducting dentistry. We cannot and should not carry on with the same

`old way` of our predecessors. So, what are the lessons we could learn from the pandemic?

One glaring exception during the current period of extraordinary crisis was the negligible role played by the dental profession in serving the community in health care institutions except in a few jurisdictions such as Singapore. Despite our competence to practice needle infiltration and immediate aftercare, sterile surgical techniques, and primary life support emergency care, it is correct to say, that dentists played a little, if any, role in this unprecedented health care crisis There might be several reasons for this, but going forwards, the profession, the dental educators in particular, needs to take the lead by broadening the curriculum and overall educational programs to include voluntary medical care, in community dentistry programs. This could also be implemented on a firmer footing through robust interprofessional education systems and collaborative practices, thus extending the repertoire of dentistry beyond just dental/oral care.[3]

Additionally, the profession and the educators need to embrace novel technologies and solutions by fostering digital literacy in the future dental workforce. Last but not least, policymakers and regulatory bodies in each jurisdiction need to take a broad and deep look at how the pandemic has impacted the profession and review and modify the current guidelines and recommendations across the whole spectrum of dental practice and training.

It is true to say that the contemporary epidemics/ pandemics beginning with the HIV pandemic have modulated dentistry beyond recognition, now with assiduous and robust infection control measures in place. Nevertheless the fact that COVID-19 may become an endemic disease, particularly due to emerging variants, behoves the dental community to further reinforce and not only to adopt modified infection control measures, but also new technology such as teledentistry, and point-of-care (POC) diagnostics, to mention a few. Hence the profession has to be forearmed and battle ready, as it were, with the necessary tools to combat the elusive pathogens that periodically confront humanity.

However, there is a silver lining in this dark cloud as it appears that technological advances in vaccine production and other predictive tools that forecast the next great pandemic may save humanity from such recurrent perils. For instance, forecasting how various pathogens evolve, how they change their surface antigenic structure, and then priming the human immune system for the next wave of combatants is a sure way of winning this contest. One such approach called `deep mutational scanning` is already in the pipeline, and it observes the pathogens` surface antigen `drifts and shifts` under *in silico* using artificial intelligence and machine learning.[4]

This means that vaccines for the next viral variant or indeed a new pathogen could be predicted far in advance and the infection annihilated in the bud, prior to becoming a ravaging pandemic. These silver linings in the dark pandemic-laden clouds may provide some comfort to the profession as well as the public, but act we must now, to begin changing the dental curricula and how we conduct the practice of dentistry on a daily basis and be forearmed for the next pandemic which is predicted to arrive soon!

Selected References:

1. Samaranayake LP, Peiris M. Severe acute respiratory syndrome and dentistry: a retrospective view. *Journal of the American Dental Association* 2004; 135(9):1292-302.

2. Samaranayake LP SARS-CoV-2 omicron variant and the future of the pandemic *Dental Update* 2022; 49 (5), 432-435.

3. Samaranayake, L; Fakhruddin K.S. Pandemics past, present, and future: their impact on oral health care *The Journal of the American*

Dental Association 2021; 152 (12), 972-980.

4. Fowler DM, Fields S. Deep mutational scanning: a new style of protein science. *Nature Methods* 2014;11(8):801-07.

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Knowledge and Attitudes towards COVID-19 vaccine among Sri Lankan dentists and dental undergraduates

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Abstract

Background: Efficacious vaccines are now available for prevention of Coronavirus Disease 2019 (COVID-19), however, the dental professionals' knowledge and attitude towards these vaccines are not clear.

Aim: To assess the knowledge, perception, and attitudes of Sri-Lankan dental professionals towards COVID-19 vaccines.

Methods: Ananonymous web-based survey of Sri Lankan dental practitioners and clinical dental students was conducted using a pre-tested, self-administered questionnaire, to elicit data on COVID-19 vaccine uptake, attitudes, and general knowledge related to vaccination.

Results: A total of 590 dental practitioners (n=341)and clinical undergraduates (n=249) (39.2% males, 60.8% of females) participated in the study. The vast majority (72.7%) of the respondents' perceived that they have an adequate knowledge of the vaccine (99.5% CI= 68.9 - 76.3), and were prepared to be vaccinated or already vaccinated (93.2%; 95%CI = 93.2-90.0).Approximately, one half of the respondents (51.0%; 95% CI= 52.8- 61.4) preferred the Astra Zeneca vaccine to the Sinopharm, and 40% preferred the Pfizer variant, though unavailable at the time. Only 51.0% perceived that COVID-19 vaccines possess an acceptable level of efficacy.

Conclusions: In general, the knowledge, perception and attitudes of Sri Lankan dental professionals on COVID-19 vaccines appear satisfactory, however, the gaps in their knowledge need to be addressed. The respondent's main concerns were the side-effects of the vaccines, perception of their poor supply, and delivery chain logistics, and lack of an option to select the preferred vaccine.

Key words: COVID 19; COVID-19 vaccines; Knowledge; Dental Undergraduates; Dental Practitioners.

1.0 Introduction

The emergence of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) in late December 2019[1] resulted in a global pandemic with a devastating impact on human morbidity and mortality, social behaviour, the global economy and its healthcare systems. Dental professionals are uniquely exposed to high levels of virus-laden aerosols and oral fluids, particularly

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due to the frequent aerosol generating procedures (AGPs), including high speed instrumentation[2, 3]. As a result, during the pandemic, dental treatment has been limited in many countries, to emergency hand instrumentation with management of trauma, pain, acute head and neck infections, and malignant tumours[4].

As an integral part of the fight against coronavirus disease 2019 (COVID-19) pandemic, an earnest search for an efficacious vaccine ensued which led to the development of a number of efficacious vaccines for COVID-19in a record period of 9 to10 months[5]. The consensus is that upto 70 to 80 per cent of the population should be successfully vaccinated to achieve herd immunity of a community to prevent further disease spread[6].

There are however, a number of major obstacles for this elusive goal, one of which is the relatively widespread vaccine hesitancy and the associated anti-vaccination movements as well as the poor global vaccine supply, and distribution[7]. Indeed, the World Health Organisation has recently declared that vaccine hesitancy is one of ten major threats to global health[8]. Echoing these fears, one recent study found that 76% of COVID-19 vaccine hesitancy is due to safety concerns[9].

The global response to COVID-19 vaccines has been mixed. Several studies have reported confusion, unfounded fears and hesitancy towards the COVID-19 vaccines[10-12]. For an instance, a study in Sri Lanka identified only 54% of the general population and 57.4% of the health care workers planning to receive the vaccine[13]. As opposed to the above, a recent survey of college students in USA noted that 92% intended to receive the vaccine[14].

There is no data on the attitudes and awareness of COVID-19 vaccines, amongst the dental professionals in Sri Lanka. Such data are essential to establish not only the intention of vaccine uptake, but also to ascertain the attitudes of a key group of health care professionals with a remit of public advocacy on COVID-19 and related issues. Hence, we conducted the current, web-based questionnaire survey amongst dental practitioners and dental undergraduates (who are handling patients)with the aim of assessing their awareness, attitudes and the perception of COVID-19 vaccines and practitioners' awareness of the vaccination received by the ancillary staff.

2.0 Methods

An online web-based, cross-sectional questionnaire survey was conducted amongst dental practitioners and dental undergraduates in Sri Lanka. All the dental surgeons both in the government service and in private practice, dental undergraduates who are handling patientswith access to the World Wide Web were invited to participate and were included in the study. The data collection was carried out during the time period of May 2021 to July 2021.

A self-administered pre-tested Google form was used as the study instrumentwhich comprised close-ended questions and 5-point Likert responses for the questions assessing the attitudes (Appendix 1). Questionnaire assessed the attitude of the respondents on different vaccines, their supply and distribution for health care workers, efficacy and side effects. Further, they were asked about the type of the vaccine they would select if the options were given with the reason for selection. The questionnaire, formulated by the authors, was first pre-tested among a randomly selected group of 20 involving both dental practitioners and dental undergraduates. The pre-tested group was not included in the final study. The questionnaire distributed via social media and email included demographic data, specific questions to assess the respondents' views, knowledge and attitudes on the vaccine and the vaccination programme for COVID-19 in Sri Lanka. A cover letter describing the purpose of the study, its outline, filling instructions was included and confidentiality and anonymity of the data provided were assured. A statement was included following the cover letter to obtain

consent. The survey link was then disseminated as a Uniform Resource Locator (URL).

The data management and statistical analyses were performed using the statistical software SPSS version 21.0. Frequencies and percentages were obtained for categorical data, and a Chi-square test was used to determine the association between variables. A logistic regression was performed to ascertain the effect of age, professional category, state of contraction of COVID-19 and its symptomatic nature, perceived knowledge of COVID-19, state of being afraid for short term and long -term side effects and perceived efficacy of the vaccines on likelihood that participants obtain the vaccine. Ethical clearance (ERC/FDS/UOP/I/2021/33) for the study was obtained from the Ethics Review Committee of the Faculty of Dental Sciences, University of Peradeniya.

3.0 Results

A total of 590responded, from a distribution list (email list obtained from the Sri Lanka Dental Association) of 1704 dental practitioners and dental undergraduates yielding a response rate of 34.6%. All the dental students belonged to the age group of 20-30 years; while the breakdown of different age categories is depicted in table 1.

Interestingly, only 1.5% (9) respondents had contracted the disease at the time of the survey, while a quarter 25.3% (149) said they were unlikely to contract the disease in the past.

Of thenine participants who were previously diagnosed with COVID-19, eight (88.87%) had experienced symptomatic disease.

3.2 Uptake and preference of COVID-19 vaccine types

At the time of the distribution of the questionnaire, a vast majority of the dental practitioners(n= 550, 93.2%) accepted the COVID-19 vaccine (Table 2), and the majority (n= 534; 97.1%) received the Oxford AstraZeneca vaccine, and could not opt for the alternate, Sinopharm vaccine available at the time. However, given such an opportunity over a third of the respondents (38.3%) would opt for another type with the most preferred being Pfizer (78.4%), Sputnik V (11.5%), and Moderna (2.9%), in the given order.

Table 1. Characteristics of the study (N = 590)

Attribute	Number	Percentage (%)
Type of dental practitioner		
Academics	45	7.6%
Government Dental surgeons	274	46.2%
Full time private practitioners	22	3.7%
Dental students	249	42.2%
Gender		
Male	231	39.5%
Female	359	60.5%
Age category		
20 to 30 years (Dental Students)	249	42.2%
20 to 30 years(Dental Surgeons)	67	11.3%
31 to 40 years	106	18%
41 to 50 years	95	16%
51 and above	73	12.4%

Table 2. Percentage of the Dental Surgeons and Dental students accepting the COVID-19 vaccine in Sri Lanka.

	Yes (%)	No (%)	Total (%)
Vaccine Accepted			
Dental students	241 (96.7)	08 (3.3)	249 (100)
Dental surgeons	309 (90.6)	32 (9.4)	341 (100)

Table 3.2 ***Reasons for not taking the COVID-19 vaccine among dental practitioners and dental students in Sri Lanka (N =54).***

Attribute	**Number**	**%**
worries of side effects	37	67.5
Comorbidities	9	17.5
preference for natural immunity	8	15

As revealed by the study participants, more than 90% of the dental ancillary staff in the government sector were offered the vaccine, and of these only 70.8% accepted the vaccine as opposed to the private sector employees, where 47.5%were offered the vaccine, of whom 70.6% accepted the vaccine.

Most respondents had no choice in the vaccine selection process due to the extant regulatory policies in Sri Lanka. Yet, 38.3%of the respondents preferred another vaccine brand given the opportunity, the majority preferring them RNA technology-based Pfizer vaccine. However, the study participants were unable to provide a reason why they would choose a different vaccine rather than the given one.

3.3 Perceived Knowledge of COVID-19 vaccines

The respondents' level of perceived knowledge of COVID -19 vaccines, were assessed on a 5-point-Likert scale.

The data on perceived knowledge level was assessed for normality and the data were more or less normally distributed and therefore they were used for calculating z- scores after standardization. The level of z=0.00 was taken as having an acceptable level of knowledge and the z scores were dichotomized. Accordingly, for the perceived level of knowledge, 72.8% of dental practitioners perceived that they possess an acceptable level of knowledge on CoVID-19 vaccines.

The study participants were asked whether they knew that the success or failure of the vaccine need to be checked by a blood (serology) test about 4 weeks after the second dose.This fact was known by only 39%.

When their awareness of the serological verification to count on immunity was assessed related to the standardised dichotomised level of perceived knowledge and there was a statistically significant difference (X2= 6.273, df=1, Sig= 0.049) (Table 3). Out of those who had an acceptable level of perceived knowledge on vaccines, 76% were aware that the serological verification is necessary.

When the dichotomised perceived knowledge

Table 3. Level of Knowledge on CoVID-19 Vaccine Perceived by the Dental Practitioners in Sri Lanka by their awareness of the need of a serological test to count-on immunity (N=590).

Level of Knowledge / Serology awareness	Serology YES (%)	test	Serology NO(%)	test	Grand Total	
Basic	40	(6.78)	27	(4.58)	67	(11.36)
Average	46	(7.80)	48	(8.14)	94	(15.93)
Better	180	(30.51)	107	(18.14)	287	(48.64)
Very good	78	(13.22)	43	(7.29)	121	(20.51)
Excellent	15	(2.54)	6	(1.02)	21	(3.56)
Total	359	(60.85)	231	(39.15)	590	100.00

of COVID-vaccines was assessed across males and females, the former perceived they had a better level of vaccine knowledge than their counterparts (P=0.001). When this item was assessed across professional categories, we noted that dental students perceived their vaccine knowledge to be less than acceptable ($p< 0.05$). Approximately 60% of the 359 respondent dental surgeons were aware of the desirability for a serological test to assess the degree of seroconversion after completing the vaccine schedule. On further analysis, it was noted thata majority 76% of the latter group had the perception of having an acceptable level of knowledge of COVID-19 vaccines($p= 0.013$).When questioned on the knowledge of the efficacy of COVID-19 vaccines, the majority(51%) surmised the acceptable level of efficacyof a vaccine should range from 80% to 90%. The latter correlated and corresponded well with those who had a good knowledge on COVID vaccines (Pearson's Correlation=0.273; $p < 0.05$) (Fig 1).

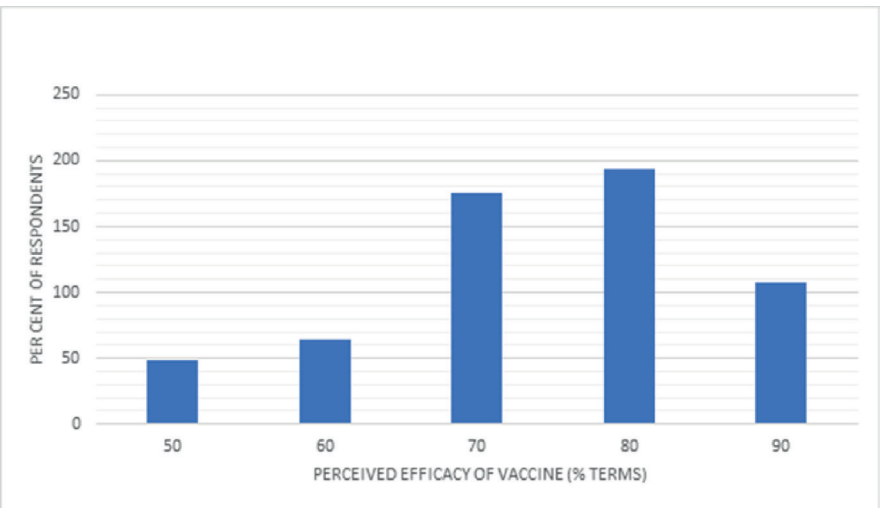

Figure 1. Efficacy of the COVID-19 vaccine as perceived by the Dental Practitioners/Dental students in Sri Lanka (N=590)

3.4 Perception and attitude to COVID-19 vaccines

In terms of the level of anxiety on side effects of COVID-19 vaccines, over one half of dental practitioners were mildly worried about both the short-(53.4%), and long-term (50.3%) side effects the COVID-19 vaccine in comparison to dental undergraduates who were significantly more anxious about both the short- and long- term side effects (P= 0.005 for both). As for the major vaccine adverse effects, 54.2% of the respondents were unaware of severe and rare side effect of thrombosis due to the AstraZeneca vaccine. Only 60 (10.2 %) of the respondents correctly identified that the blood clots occur approximately one in one million AstraZeneca vaccine recipients.

3.5 Adherence to infection control guidelines

According to the study participants majority (92.1%) of the dental practitioners in Sri Lanka are following the infection control guidelines promulgated by the government of Sri Lanka for COVID-19, with varying degrees of compliance, and only a small minority (<1.0%) did not do so. Following infection control protocols to a greater extent has a positive correlation with the ascending age of the dental practitioner (sig-= 0.42, Pearson correlation= 0.89) which means that the older respondents appeared to be more compliant than the younger respondents, in this context (Fig 3).

In terms of vaccine delivery, approximately a third of the respondents (38.6%) perceived that the distribution of COVID-19 vaccines by the government was a biased process, and was unequally distributed amongst high and low risk groups at risk of contracting the infection.

Further, the respondents noted that over two thirds of the ancillary dental staff (70.3%) in the government sector were offered the COVID-19 vaccine as opposed to only a fifth of private sector workers (22.9 %; p = 0.000). The respondents were aware that virtually almost all of the government (95.5%),and private sector (93.3%) ancillary co-workers who were so offered, accepted the vaccine.

The logistic regression model was not significant X2= 5.37, Sig=0.718. The model explained only 19.4% (NagelkerkeR2) of variance in obtaining the COVID-19 vaccine and correctly classified in 93.4% of cases. Thus, there was not enough evidence to conclude that the model fits the data.

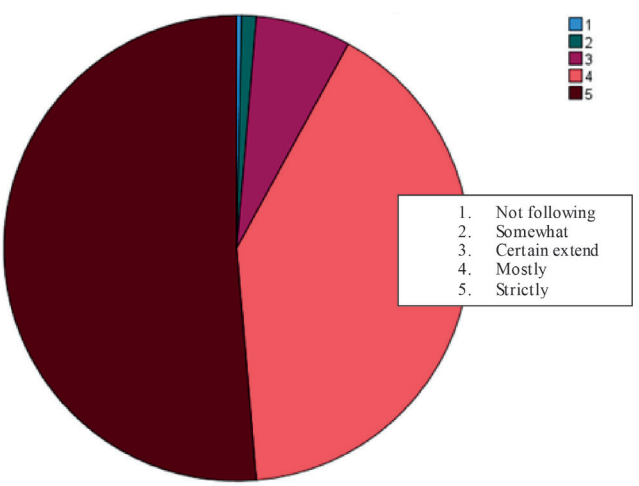

Figure 3. Participants compliance with the infection control protocols (N = 590)

4.0 Discussion

There are currently over five different vaccines approved by WHO against COVID-19, produced on several platforms, across the world[15].Sri Lanka, facing its third wave of the infection, initiated its COVID-19 vaccination program, with two different vaccines, Sinopharm and AstraZeneca, immediately prior to the current survey. The Sinopharm vaccine is an inactivated SARS-CoV-2 virus, while the AstraZeneca is aviral vector vaccine[15]. There are, however, multiple shortfalls in the COVID-19 vaccination process currently implemented in Sri Lanka particularly due to the lack of vaccines, and issues with their distribution logistics in a resource-poor nation. These, coupled with the vaccine hesitancy of a significant proportion of the population have hampered the overall vaccine uptake by of the population. Given this scenario, eliciting theviewsof dental professionals was important not only because of the high-risk SARS-CoV-2 infection poses to the profession but also to ascertain their knowledge and attitudes towards COVID-19 vaccines, as a key group contributing to health education in the community.

Although there were study reports which described the efficacy of each type of vaccine, they were not available at the time of our study[16,17]. However, a vast majority, (80.8%), of our respondents were generally inclined to take the vaccine and believed that the efficacy of the vaccine could be as high as 70-90%. This is almost identical to the results of a survey of 248 dental students, from three separate dental schools in USA, where 78.8% of responded said that vaccination was important to them[18].

The majority of the respondents (72.7%) also perceived that they possessed an acceptable knowledge of COVID-19 vaccines, and also surmised that a serological test is required to confirm the immunity following the vaccination. Although, establishing post-vaccination seroconversion is important with other vaccines such as hepatitis B[19], Food and Drugs Administration (FDA), USA has recently stated that such antibody testing is not necessary, until further data are available on the immunological correlates for protection against SARS-CoV-2[20].These findings highlight the need fora regular update of the awareness of this evolving pandemic, particularly for the dental professionals, through continuing professional development programs.

The AstraZeneca vaccine has been deployed against COVID-19 in many countries, and its efficacy approaching 70% is fairly well known[21]. Yet, given the option, 38.3% of our respondents preferred another vaccine, and the majority voted for the mRNA based, Pfizer vaccine as the alternative. This is likely to be due to the rare blood clotting disorder, associated with the AstraZeneca vaccine[22] as 45.6% of the respondents were aware of this rare complication, as well as the well-known, extremely high (91.3%) efficacy and minimal side effects of mRNA vaccines[21]. A study similar to the present study conducted amongst dental personnel in Italy, reported the main reasons for vaccine hesitancy wereinadequate information on the safety of the vaccines (37%), and possible adverse effects (58%)[23]. A number of other surveys have reported similar observations. Thus, El-Elimatet al.,[24] noted that the 49%of public in a Jordanian survey, and Pogue et al., observed 63% of public in a US survey were mainly concerned about the vaccine side effects[25]. Another contemporaneous study of public and the health care workers in Sri Lanka revealed that the majority was very concerned about the vaccine side effects including allergies[13].

Nevertheless, the fact that over 93% of the respondents in our survey were already vaccinated implies that the acceptance of the vaccine has taken precedence over the expressed concerns. This is far greater than the data from a recent Sri Lankan survey where only 57.4% of the health care workers planned to obtain the vaccine[13].

Even during a pandemic, dentists will have no choice but to treat patients presenting with

emergencies, such as pain, bleeding and sepsis[26]. Therefore, it is crucial that basic infection control measures including special disinfection and transmission-based precautions (TBP) such as second-tier precautions including contact, droplet and airborne types be practiced in the dental setting to prevent dentists, patients or other dental staff from becoming infected during delivery of dental procedures as a result of aerosols and droplets which may carry the SARS-CoV-2 virus[27, 28]. As for the adherence to infection control practices in dentistry, a previous study of dental professionals in Sri Lanka reported, that they were aware of these protocols and the vast majority were planning to implement these measures during the pandemic period[29]. In the present study participants have reported (92.1%) that they have implemented the guidelines declared by the health authorities. However, it is worrying that a small minority (2 %) of the respondents appear to have ignored these protocols to a greater or lesser extent (Fig. 2). However, given the resurgence of the COVID-19 regular awareness programmes need to be conducted to emphasize the importance of infection control with special reference to dental setting.

The binary logistic regression model was used to assessed the factors that has influenced thelikelihood that participants obtain the vaccine was not concluded with good model fit. The possible reason for this may be the fact that Aztrazenica was the vaccine only available for a vast majority of dental professionals and they were not offered with a choice of vaccine.

5.0 Limitations of the study

Because the present study has used only the dental professionals it limits the generalizability of the survey findings to the population at large, particularly the attitude to the COVID-19 vaccines. Second, there have been explosions of new data on the vaccines, their efficacy and side-effects, since we conducted the survey and the dynamics of this rapidly moving landscape may not be truly reflected in the findings. Although, the participants were asked about the choice of vaccine, they did not have opportunity of selecting the type as only one type was available in the country at the time of this study. There were no reports available on the efficacy of different types of vaccines at the time of our study and if available, it could have changed the respondents'

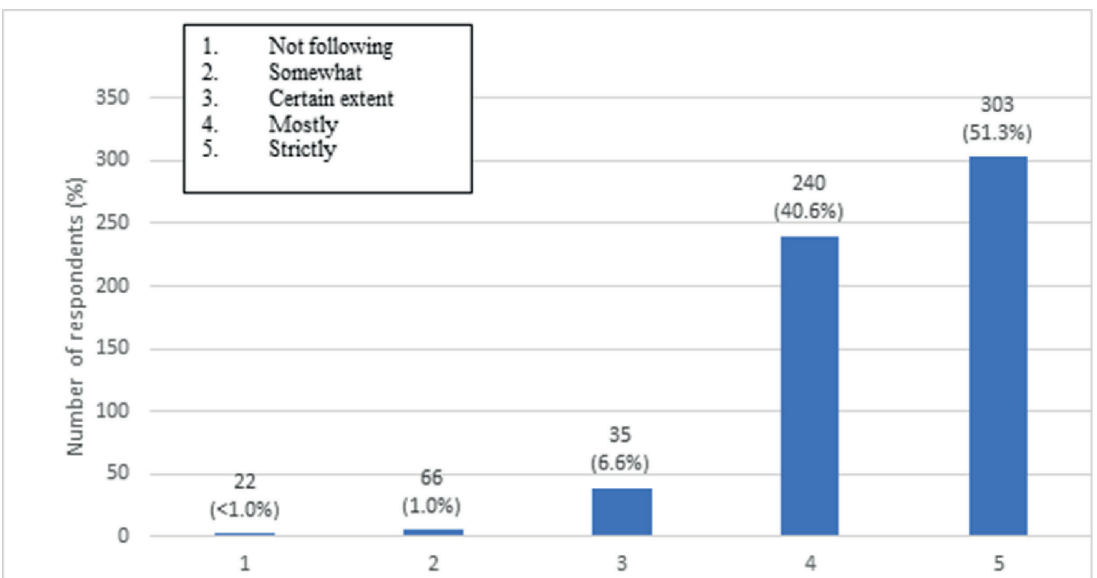

Figure 2. The self-reported adherence to infection control protocols by study participants (N = 590)

attitude on the efficacy of the vaccines. Finally, authors would like to acknowledge since the questioner was delivered via a google form using social media such as WhatsApp and email, it could have omitted the dental practitioners who have no access to internet. Even though this is an inherent limitation of the study poor motivation could have limited the response rate rather than the lack of facilities. Nevertheless, our study findings was the first form the South Asian region, therefore, should provide insights for health care administrators on how best to address the tacit concerns of the dental health professionals in Sri Lanka on COVID-19 vaccines.

6.0 Conclusions

In general, the knowledge, perception and attitudes of Sri Lankan dental professionalsand dental studentsonCOVID-19 vaccination appear satisfactory, but there are significant gaps in their perceived knowledge that need to be addressed. The respondents' main concerns highlighted were the side-effects of the vaccines, the perception of their poor supply and delivery chain management logistics, the vaccine distribution amongst the risk-population groups, and the lack of an option to select a specific vaccine brand. These concerns should be taken into consideration when continuing the COVID-19 vaccination program in the future. Our study also emphasizes importance of continuing educational programs on COVID-19 and its prevention for health care workers in Sri Lanka given the resurgence of the disease.

References

1. Wu Y, Ho W, Huang Y, Jin D-Y, Li S, Liu S-L, et al. SARS-CoV-2 is an appropriate name for the new coronavirus. Lancet. 2020;395(10228):949–50.

2. Bentley CD, Burkhart NW, Crawford JJ. Evaluating spatter and aerosol contamination during dental procedures. J Am Dent Assoc. 1994;125(5):579–84.

3. Checchi V, Bellini P, Bencivenni D, Consolo U. COVID-19 dentistry-related aspects: A literature overview. Int Dent J. 2021;71(1):21–6.

4. Samaranayake L. Dental Practice during the Coronavirus Disease 2019 (COVID-19) Pandemic: Two Pathfinder Documents Point the Way. Dental Update. 2020; 47(10): 878-882.

5. Callaway E. The race for coronavirus vaccines: a graphical guide. Nature. 2020;580(7805):576–7.

6. McNeil DG Jr. How Much Herd Immunity Is Enough. The New York times. 2021 Apr 1; Available at; https://www.nytimes.com/2020/12/24/health/herd-immunity-covid-coronavirus.html. Accessed on 17th July 2021.

7. Dubé E, Vivion M, MacDonald NE. Vaccine hesitancy, vaccine refusal and the anti-vaccine movement: influence, impact and implications. Expert Rev Vaccines. 2015; 14:99–117.

8. Ten threats to global health in 2019 [Internet}. Who.int. [cited 2021 Jul 21]. Available from: https://www.who.int/vietnam/news/feature-stories/detail/ten-threats-to-global-health-in-2019.

9. Dror AA, Eisenbach N, Taiber S, Morozov NG, Mizrachi M, Zigron A, et al. Vaccine hesitancy: the next challenge in the fight against COVID-19. Eur J Epidemiol. 2020;35(8):775–9.

10. V. LJ, S R, A P, Lo G, Hj L, K R. Hesitant or not? A global survey of potential acceptance of a COVID-19 vaccine. medRxiv. 2020;08 23 20180307.

11. Lin Y, Hu Z, Zhao Q, Alias H, Danaee

M, Wong LP. Understanding COVID-19 vaccine demand and hesitancy: A nationwide online survey in China. PLoSNegl Trop Dis. 2020;14(12):e0008961.

12. Di Gennaro F, Murri R, Segala FV, Cerruti L, Abdulle A, Saracino A, et al. Attitudes towards anti-SARS-CoV2 vaccination among healthcare workers: Results from a national survey in Italy. Viruses. 2021;13(3):371.

13. Wijesinghe MSD, Weerasinghe WMPC, Gunawardana I, Perera SNS, Karunapema RPP. Acceptance of COVID-19 Vaccine in Sri Lanka: Applying the Health Belief Model to an Online Survey. Asia Pacific Journal of Public Health [Internet]. 2021; Available from: http://dx.doi.org/10.

14. Graupensperger S PhD, Abdallah DA, Lee CM. Social norms and vaccine uptake: College students' COVID vaccination intentions, attitudes, and estimated peer norms and comparisons with influenza vaccine. Vaccine. 2021;39(15):2060–7.

15. Samaranayake LP, Seneviratne CJ, Fakhruddin KS. Coronavirus disease 2019 (COVID-19) vaccines: A concise review. Oral Dis [Internet]. 2021;(odi.13916). Available from: http://dx.doi.org/10.1111/odi.13916.

16. X. Cai C, Peng Y, Shen E, Huang Q, Chen Y, Liu P, Guo C, Feng Z, Gao L, Zhang X, Gao Y, Liu Y, Han Y, Zeng S, Shen H. A comprehensive analysis of the efficacy and safety of COVID-19 vaccines. Mol Ther. 2021 Sep 1;29(9):2794-2805. doi: 10.1016/j.ymthe.2021.08.001. Epub 2021 Aug 6. PMID: 34365034; PMCID: PMC8342868.

17. Ghasemiyeh P, Mohammadi-Samani S, Firouzabadi N, Dehshahri A, Vazin A. A focused review on technologies, mechanisms, safety, and efficacy of available COVID-19 vaccines. Int Immunopharmacol. 2021 Nov;100:108162. doi: 10.1016/j.intimp.2021.108162. Epub 2021 Sep 17. PMID: 34562844; PMCID: PMC8445802.

18. Mascarenhas AK, Lucia VC, Kelekar A, Afonso NM. Dental students' attitudes and hesitancy toward COVID-19 vaccine. J Dent Educ [Internet]. 2021;(jdd.12632). Available from: http://dx.doi.org/10.1002/jdd.12632.

19. Samaranayake L. Essential Microbiology for Dentistry. "Elsevier"; 2018, 5 Edition.

20. Center for Devices, Radiological Health. Antibody testing not recommended to assess immunity after vaccination [Internet]. Fda.gov. 2021 [cited 2021 Jul 21]. Available from: https://www.fda.gov/medical-devices/safety-communications/antibody-testing-not-currently-recommended-assess-immunity-after-covid-19-vaccination-fda-safety.

21. Samaranayake L, A S. Understanding COVID-19 vaccines and immunity Dental Update. Vol. 48. p. 157–160.

22. Mueller B, Grady D. AstraZeneca vaccine and blood clots: What is known so far. The New York times [Internet]. 2021 Apr 10 [cited 2021 Jul 21]; Available from: https://www.nytimes.com/2021/04/10/health/astrazeneca-vaccine-blood-clots.html Accessed on 30th March 2021.

23. Belingheri M, Roncalli M, Riva MA, Paladino ME, Teruzzi CM. COVID-19 vaccine hesitancy and reasons for or against adherence among dentists. J Am Dent Assoc [Internet]. 2021; Available from: http://dx.doi.org/10.1016/j.adaj.2021.04.020.

24. El-Elimat T, AbuAlSamen MM, Almomani BA, Al-Sawalha NA, Alali FQ. Acceptance and attitudes toward COVID-19 vaccines: A cross-sectional study from Jordan. PLoS

One. 2021;16(4):e0250555.

25. Pogue K, Jensen JL, Stancil CK, Ferguson DG, Hughes SJ, Mello EJ, et al. Influences on attitudes regarding potential COVID-19 vaccination in the United States. Vaccines (Basel). 2020;8(4):582.

26. Panesar K, Dodson T, Lynch J, Bryson-Cahn C, Chew L, Dillon J. Evolution of COVID-19 Guidelines for University of Washington Oral and Maxillofacial Surgery Patient Care. J Oral Maxillofac Surg. 2020; 1–11. pmid:31888859.

27. Ge Z, Yan L, Xia J, et al. Possible aerosol transmission of COVID-19 and special precautions in dentistry. J. Zhejiang Univ. Sci. B (Biomed &Biotechnol) 2020 21(5):361-368.

28. Tran K, Cimon K, Severn M, Pessoa-Silva CL, Conly J. Aerosol generating procedures and risk of transmission of acute respiratory infections to healthcare workers: a systematic review. PLoS One. 2012; 7: e35797. Epub 2012 Apr 26. pmid:22563403.

29. Jayasinghe RD, Jayasinghe RM, PVKS Hettiarachchi, Samaranayake LP Sri Lankan dental professionals' knowledge of the Corona Virus Disease-19 (COVID-19): a questionnaire survey. STOMATOL EDU J. 2021;8(1):7–17.

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Evaluation of the morphology and position of Mental Foramen in a Sri Lankan population using Digital Panoramic Radiograph: A Retrospective cross-sectional study

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Abstract

The present study was conducted to evaluate the position, symmetry and shape of mental foramen (MF) using dental panoramic radiographs in a cohort of Sri Lankans. Six hundred and nineteen digital panoramic radiographs (DPRs) were evaluated using an established method which used mandibular premolar crowns and apices to score the position. The position of MF was also evaluated using the various reference points of the body of the mandible. A Chi-square test was used to analyze the data. The sample consisted DPRs of 258 (41.68%) males and 361 (58.31%) females. According to the method which used mandibular premolars as a reference point, MF was frequently found between the 1st premolar and 2nd premolar cusp tips (crown score 3) (52.7 %) followed by under the 2nd premolar cusp tip (40.55%) (Crown score 4). A similar pattern was observed with the apex score. The most common shape of the MF was oval (48.9%), followed by round (33%) and irregular (18.1%) shapes. Accessory canals were observed in 8.1% and 6.5% on right and left sides, respectively. The present study has demonstrated that MF was commonly located in between mandibular 1st and 2nd premolars accompanied by beneath the 2nd premolar. The oval shape of the MF was frequently found in the study cohort while the prevalence of accessory canals was between 6.5 % - and 8.1%. MF is an important landmark, and its preservation is vital during periapical, implant and orthognathic surgery highlighting the importance of identifying its precise location.

Introduction

The mental foramen is found in the premolar region of the anterolateral region of the body of the mandible and usually assumes a funnel-like opening. Inferior alveolar nerve (IAN) branches give rise to the mental nerve along with its vascular counterparts leaving the mental canal through the mental foramen to innervate and vascularize the ipsilateral mental region of the face. It serves as a crucial anatomical landmark, especially during the administration of mental and incisive nerve block (MINB). MINB is commonly used in the diagnostic, endodontic, and surgical treatment of anterior mandibular soft and hard tissues[1]. MINB has been reported to be more effective in anaesthetizing premolar teeth than IAN block (IANB)[2]. Furthermore, accurate identification of its position and preservation is of utmost importance in periapical surgery, implant surgery, construction of complete dentures, and management of maxillofacial trauma and orthognathic procedures because MF cannot be visualized clinically or palpated[3–5]. In rare situations, the MF may not be visible on the radiographs while the appearance of clear MF

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in radiographs has been documented to range from 77.3% to 100%[6]with a slight predominance on the left side[5]. According to the literature, accessory mental foramina are found in 6.62% of the mandibles[7]. Since MF is commonly found inferior or between mandibular premolars as a radiolucent area, sometimes that might make them undergo unintended trauma[8]. The MF also aids in interpreting anatomical landmarks in oral pathology and forensic dentistry[9]. For example, the position of MF is considered an important landmark in sexual dimorphism in the latter case[2].

Scientific evidence has demonstrated the position, symmetry, and shape of the MF by anatomical as well as radiographic methods. These studies show that the location and appearance may vary depending on sex, age, and ethnicity. According to the studies done using dry mandibles, MF is commonly located between the 1st and 2nd premolar apices (position 3) or beneath the 2nd premolar apex (position 4)[10]. MF was commonly found at position3 in Chinese, British, Asian Indians, East Africans, Kenyans, and Nigerian populations while it was found at position4 among North Americans, North Indians, Turks, Zimbabweans, and Malawians[10]. However, few studies conducted using dry mandibles as well as by radiographic methods demonstrated that MF maybe located anywhere between position1 and position6, anterior to the 1st mandibular premolar and mesiobuccal root of 1st mandibular molar, respectively[1,10–16] Similarly, a comparative study by Green in 1987 using dry mandibles of various ethnic groups demonstrated that the longitudinal axis of the 2nd premolar tooth can be considered the most common location of MF[17]. Few reports have been published in Sri Lanka which analyzed the position of the MF using dry skulls as well[18–20]. These studies have all found similar findings, including the presence of bilateral MF, accessory foramina, and the MF being positioned on the longitudinal axis of the mandibular 2nd premolar tooth[18,19]. However, a significant limitation of the aforementioned studies is the small sample sizes (52, 51 and 26, respectively)[18–20].

Even though dry mandibles provide valuable information on morphological features and morphometrics of mental foramen with reference to surrounding anatomical landmarks[21]clinicians rely on panoramic radiographs for identifying the position of the mental foramen, clinically. Digital Panoramic Radiograph (aka DPR, PAN, OPG, and DPT) is carried out under a low radiation dose and is a simple imaging technique. Other advantages of DPR include the low-cost and speedy output. DPR is commonly employed in dentistry for diagnosis and planning before surgical procedures. Though the two-dimensional nature of DPR may give rise to some inaccuracy, studies have demonstrated that 100% detection of MF can be obtained with DPR[6]. Since DPR provides a view of the mandible in its entirety, horizontal and vertical axes can be used to locate the MF accurately. Moreover, DPR is cheaper than other radiological methods such as CBCT, and it is easy to perform and interpret the data. Studies done using panoramic radiograph has also shown that MF is frequently found in between mandibular premolars[3,6,22-28] and along the longitudinal axis of mandibular 2nd premolar[1,8,12–15,29,30]. However, up to date, no published data was found on the localization of MF using DPRs in Sri Lankans. To our knowledge, this is the first study which evaluated the mental foramen position in a Sri Lankan cohort using digital panoramic radiographs.

Hence, the objectives of the present study are to identify the position of the MF using an established method and then compare the data based on gender, shape, size, and presence of accessory canals. Further, the anterior-posterior and superior-inferior position of MF to hard-tissue structures were evaluated. The data obtained from this study was compared with similar studies done on different ethnic populations.

Materials and methods

A study has revealed that a sample size of 100 is adequate to yield a statistical difference[6]. Hence, the present retrospective cross-sectional

study included six hundred and nineteen dental panoramic radiographs obtained during 2014-2018 from the Department of Oral Medicine and Periodontology, Faculty of Dental Sciences. All panoramic radiographs of the patient, with erupted 1st and 2nd premolars and 1st molars, were selected. Radiographs were excluded if any pathology or trauma was present in the mental region and the presence of impacted teeth. The study was approved (ERC/FDS/UOP/I/2019/08) by the Ethics Review Committee of the same institution. The following criteria were used to select the DPRs as described elsewhere[6] with some modifications. Panoramic radiographs, which clearly showed MF on both sides and contained all premolars in patients between the ages of 18 and 45, were obtained. The randomized panoramic radiographs were deidentified by extracting the data such as patient age, gender and ethnicity to make the data extraction process anonymous. The position of the MF was recorded as described elsewhere[6] by using premolar cusp tips (crown score) and apices of premolars (apex score) (Table 1 and Figure 1).

Table 1. Crown and apex score used in the study.

Score	Tooth	Position
Crown Score 1	First premolar	Anterior
Crown Score 2	First premolar	Directly inferior
Crown Score 3	First and second premolar	In between
Crown Score 4	Second premolar	Directly inferior
Crown Score 5	Second premolar	Posterior
Apex Score 1	First premolar	Mesial
Apex Score 2	First premolar	Directly below
Apex Score 3	First and second premolar	In between
Apex Score 4	Second premolar	Directly below
Apex Score 5	Second premolar	Distal

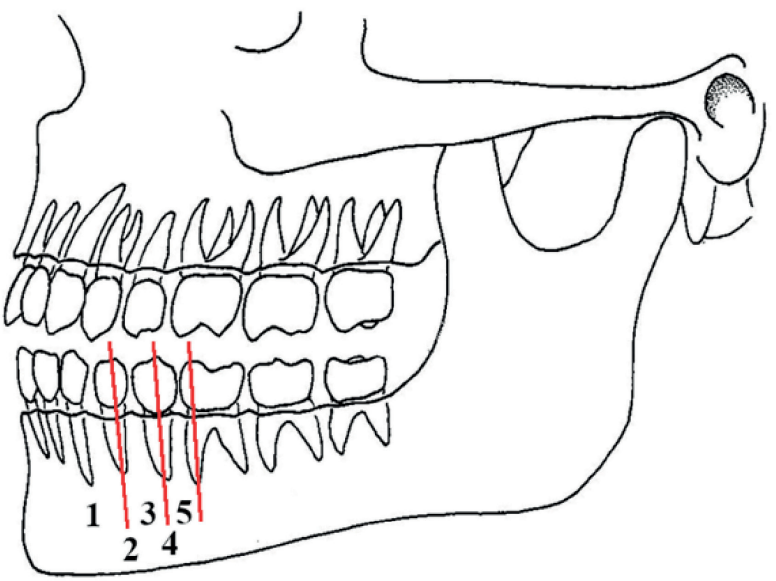

Figure 1. The position of the MF by using premolar cusp tips (crown score) and apices of premolars (apex score)

Shape and the horizontal and vertical distances were measured as described elsewhere[31]. The horizontal distance was taken between the anterior border of the MF to the symphysis menti(MF-A). The vertical distance between the upper edge of the MF to the upper edge of the alveolar crest (MF-B) and the distance from the lower edge of the MF to the inferior border of the mandible was measured (MF-C). SPSS® v. 25.0 was used to analyze the data. The shape of the MF was also recorded. The Chi-square test was used for categorical variables and is presented as frequencies and percentages. The difference was considered statistically significant when P<0.05.

Results

Six hundred and nineteen (619) radiographs which fulfilled the inclusion criteria were collected from the archives corresponding to 258 males (41.7%) and 361 females (58.3%) with a female predominance (F: M 1.39). The mean age was 27.65 years with a range of 18-45 years. The group consisted of Sri Lankans consisting of Sinhalese (n=559, 90.3%), Muslims (n=51, 8.2%) and Tamils (n= 9, 1.5%). According to the crown score which used the tip of the crowns of 1st and 2nd premolars as the landmark, MF was commonly found between mandibular premolars as denoted by crown score 3(52.7%) followed by crown score 4, directly below 2nd premolar (40.55%)(Table 2) with these two positions making an overall prevalence of 93.25%. The analysis did not reveal a difference in the frequency pattern of crown scores between the right side and left side (Table 2). According to the apex score, which used apices of premolars, MF was located most frequently at apex score 3, in between the premolar apices (50.4%), followed by apex score 4, beneath the 2nd premolar apex (37.88%) (Tables 2) with these two positions making an overall prevalence of 88.28%. Not a single DPRs were found corresponding to score 1 (Table2). Crown scores revealed the symmetrical positioning of MF in 84.5% of DPRs (Table 3) and asymmetrical in 15.5%. Regarding apex score, symmetrical positioning was observed in 82.4% and asymmetrical positioning in 17.6% of DPRs (Table 3).

Table 2. Position of the mental foramen as described by Currie et al.,[6].

	Right N (%)		Total (%)	P Value	Left N (%)		Total (%)	P Value
Crown Score	**Males**	**Females**			**Males**	**Females**		
Score 1	0	0	0	0.178[a]	0	0	0	0.320[a]
Score 2	3 (1.2)	6 (1.7)	9 (1.5)		3 (1.2)	7 (1.9)	10 (1.6)	
Score 3	123 (47.7)	201 (55.7)	324 (52.3)		127 (49.2)	201 (55.7)	328 (53)	
Score 4	113 (43.8)	136 (37.7)	249 (40.2)		115 (44.6)	138 (38.2)	253 (40.9)	
Score 5	19 (7.4)	18 (5.0)	37 (6.1)		13 (5.0)	15 (4.2)	28 (4.5)	
Apex score								
Score 1	0	0	0	0.145[a]	0	0	0	0.01[b]
Score 2	1 (0.4)	4 (1.1)	5 (0.8)		0	5 (1.4)	4 (0.8)	
Score 3	118 (45.7)	193 (53.6)	311 (50.2)		117 (45.3)	196 (54.4)	313 (50.6)	
Score 4	109 (42.2)	122 (33.9)	233 (37.6)		109 (42.2)	126 (35.0)	236 (38.1)	
Score 5	30 (11.6)	40 (11.1)	70 (11.3)		32 (12.4)	33 (9.2)	65 (10.5%)	

[a] statistically not significant P > 0.05. [b] Statistically significant; P < 0.05.N = number of mental foramen on right and left sides.

When the symmetrically positioned mental foramina were evaluated, position 3 (n = 285, 54.5%) was found more frequently with the position 4 (n = 209, 40%) being second. Among asymmetric MF, the most common position was score 4 (43.75%), followed by score 3 (42.7%) and score 5 (12%). The Monte Carlo chi-square test was employed to analyze any differences between right and left sides and could not detect any significant difference for both crown and apex scores. When the data were evaluated based on gender, it was revealed that score 3 was the most common position irrespective of gender, and there was no statistical difference between the genders (Table 2). However, data revealed that apex scores might vary on the left side depending on gender (Table 2). When we analyzed the data based on ethnicity the MF was more commonly found in position 4 in Tamils (n=9) where as it was both in position 3 and 4 (1:1 ratio) in Muslims (data not shown).

The oval shape has been identified as the most prevalent shape (n=605, 48.9%), followed by a round and irregular shapes accounting for 33%and 18.1%, respectively (Table 4). No differences were found based on sides and gender (Table 4). However, the shape may vary on the right side depending on gender (Table 4).When the data was analyzed for the symmetry of shape, 537

Table 3. Symmetrical positioning of MF between the right and left side crown and apex scores.

Mental Foramen position Score - Crown		Right side				Total
		2	3	4	5	
Left side	2	8	2	0	0	10
	3	0	285	36	7	328
	4	1	34	209	9	253
	5	0	3	4	21	28
Total		9	324	249	37	619
Mental Foramen position Score - Apex						
Left side	2	4	1	0	0	5
	3	0	271	33	9	313
	4	1	35	187	13	236
	5	0	4	13	48	65
Total		5	311	233	70	619

Table 4. Shape of the mental foramen on both sides by gender.

Shape	**Right N (%)**		**Total**	**P Value**	**Left N (%)**		**Total**	**P Value**
	Males	**Females**			**Males**	**Females**		
Oval	110 (42.6)	187 (51.8)	297 (48)	0.0001[b]	116 (45)	192 (53.2)	308 (49.8)	0.038[a]
Round	110 (42.6)	100 (27.7)	210 (33.9)		97 (37.6)	101 (28)	198 (32)	
Irregular	38 (14.7)	74 (20.5)	112 (18.1)		45 (17.4)	68 (18.8)	113 (18.3)	
Total	258 (100)	361 (100)	619 (100)		258 (100)	361 (100)	619 (100)	
Accessory foramina	17[a] (2.74)	33[b] (5.33)	50 (8.07)	0.0001[b]	10[a] (1.61)	30[b] (4.84)	40 (6.45)	0.020[a]

[a] Not statistically significant P >0.0001. [b] Statistically significant, P < 0.0001. N = number of mental foramen.

Table 5. Summary of studies done in Sri Lanka.

Author	Study type	Side	Position (%)						MF-A (mm)	MF-B (mm)	MF-C (mm)	MF-D (mm)	Shape (%)		
			I	II	III	IV	V	VI					Oval	Round	Irregular
Nanayakkara et al., 2018	Dry skulls	R	0	0	22.8	63.6	13.6	0	25.65	65.01	13.23	13.34	96.1	3.9	-
		L	0	0	18.2	45.4	36.4	0	25.5	64.58	13.47	12.89	92.3	7.7	
Ilayperuma et al., 2009	Dry skulls	R	-	23.53	11.76	58.82	0	5.88	24.87	-	-	-	59	41	-
		L		29.41	11.76	47.06	0	11.76	24.77						
Prabodha and Nanayakkara, 2006	Dry skulls		-	-	-	-	-	-	26.52	65.38	-	12.25	66.67	33.33	-
Present study	DPR	R	0	1.5	52.1	40.3	6.1	-	30.5	-	16.34	15.3	48	33.9	18.1
		L		1.5	52.6	41.7	4.3		31.2		16.4	15.4	49.8	32	18.3

MF-A - distance between the anterior border of the MF and the symphysis menti; MF-B - distance between the upper edge of the MF and the upper edge of the alveolar crest; MF-C - distance between the lover edge of the MF and inferior border of the mandible.

(86.75%) showed symmetry, while 82 (13.25%) were asymmetrical. Interesting symmetrically shaped MF was found more in females (50.4%) than males (36.34%) though it did not reach statistical significance. MF-A distance from the anterior border of the MF to the symphysis menti were 30.486 mm and 31.161 mm for the right and left sides of the mandible, respectively (Table 5).

The mean vertical distance from the upper edge of the alveolar crest to the upper edge of the MF (MF-B) was 16.341 mm and 16.368 mm for the right and left sides, respectively (Table 5). And the mean distance of MF-C was15.3 mm and 15.371mm on the right and left sides, respectively (Table 5). Accessory canals were present in 6.5% and 8.1% on the left side and right side, respectively (Table 4). Females recorded statistically significant accessory foramina than males ($P<0.0001$).

Discussion

MF is an important anatomical landmark for diagnostic and clinical procedures. Identification of the precise location of MF is important to avoid untoward damage as a result of surgical procedures in this region. Digital panoramic radiographs (DPRs) offer many advantages over conventional panoramic radiographs and intraoral radiographs (IOPAs). It has been demonstrated that intra-observer difference is less with DPRs[3,15,32]. In addition, DPRs provide a very clear image of MF in a wide area of hard and soft tissues. Since the image is observed as a continuous one the horizontal and vertical positioning of MF with known hard tissue landmarks can be obtained very easily. Certain limitations of IOPAs such as the inability to capture MF if it is below the edge of the film do not occur with DPRs.

There are many studies, which evaluated the position of MF in various ethnic groups using both dry skulls and radiography. The seminal work by Green[17] 1987 in which he reanalyzed data from 45 reports (including his study) which were carried out either using dry skulls

or radiographs showed that MF was commonly found just below the apex of the 2nd premolar tooth. A similar comparison was carried out by Igbigbi et al.[10] had shown that in seven studies out of twelve, the position of MF lies in between mandibular 1st and 2nd premolars whereas in five studies MF was positioned below the 2nd premolar apex. According to the current study, MF was commonly found between the apices of the 1st and 2nd premolars (score3) accompanied by lying beneath the 2nd premolar apex (score 4) and distal to the 2nd premolar apex (score 5) irrespective of the side. This is consistent with data reported from similar studies on various ethnic groups such as Nepalese, Indians, Chileans, Iranians, Malaysians and Caucasians[6,22–26,28,31,33]. However, many studies done using DPRs have found that the modal position can also lie in a longitudinal axis passing through the 2nd premolar as demonstrated in Iranians, Senegalese, Indians, Bangladeshi, Moroccans, Sudanese, Kurdish, Malaysians and Saudi.[1,8,9,11,14–16,26,30,32–36] However, our findings are not in agreement with the study by Nanayakkara et al.,[19] , who showed that the most frequent position of the MF in dry skulls is beneath the 2nd premolar on both sides. However, the second common position of MF on the right and left side were different while it between the 1st and 2nd premolars on the right side (22.8%) and between the 2nd premolar and the 1st molar on the left side (36.4%). Interestingly, in their study using dry skulls they could not find a single MF lying mesial to the 1st premolar and beneath the 1st premolar. In the present study though MF was not found mesial to the 1st premolar, it was found beneath the 1st premolar apex in nine DPTS (1.5%) irrespective of the side. Few studies have demonstrated that the MF shifts posteriorly with ageing indicating that in young individuals it maybe present between the apices of 1stand 2nd premolars with subsequent positioning beneath the apex of 2nd premolar[22]. This may explain the different in the present study and the study by Nanayakkara et al.,[19]. A comprehensive search of the electronic databases (Pubmed, Scopus and Embase) was conducted for articles published from 1988-2018 in the English language using DPRs and the characteristics of the studies are summarized in Table 6. The sample size of those studies varied between 100 and 3788 whereas in the present study sample size was 619. However, the age of the sample in the present study was relatively young with a mean age of 27.65 ranging between 18 - 45 years whereas the majority of studies showed a wide age range with very young (12 years) and older subjects (79 years).

Two different landmarks have been used in the measurement of the superior-inferior position of the MF either by relating to teeth or the body of the mandible. When the data was analyzed using premolar teeth as an anatomical landmark as described by Curie et al.,[6] it showed that MF was symmetrical in 84.5% for crown scores which is similar to the findings of other studies in different ethnic populations and include Indians 81.7%[8,14], Iranians 80% - 85.7%[27,30], Kurdish 82.7%[34] and Turkish 85.8%[26]. The symmetry reported by Bello et al.,[3] and Gada et al.,[24] were 48.8% and 54.66%, respectively which is much less than the present study. When the horizontal positioning of MF was compared between the genders, we could not find a statistically significant difference in agreement with the other similar studies done using different ethnic groups such as Asians, Iranians[32], Chileans[23], Iraqi[35] and Moroccans[11]. Interestingly in contrast to the aforementioned observations, the study by Haghanifar and Rokouei[27] demonstrated that the horizontal position of the MF in males and females lie beneath 2nd premolar and between 1st and 2nd premolars, respectively. However, the opposite scenario was observed in the study by Al-Khateeb[33]. The current study reported that the most frequent shape of the MF is oval which is in agreement with other studies done using dry mandibles[36] and panoramic radiographs[3]. We have observed more irregularly shaped MF than the studies published in Sri Lanka using dry skulls[19]. The study by Bello et al.[3] also demonstrated a high percentage of irregularly shaped MF (32.8%). On the other hand, the most prevalent shape among Jordanian[35], Indians[33] and Iraqi[1] subjects were

oval. A study with a cohort of Jordans showed that up to 10% of accessory mental foramina were found in their group[33] while in the present study it was 8.1% and 6.6% on right and left sides, respectively. A similar study has shown a prevalence of 7.3 % in the Iraqi population[35].

Measurements were done on dry human mandibles were considered the gold standard in determining the position of MF. Therefore, the data obtained with panoramic radiographs must be evaluated against the gold standard. The study by Olasoji et al.,[37] using panoramic radiographs of northern Nigerian adults and dry mandibles could not find any significant difference between both methods for locating the MF. On the other hand, a study has revealed that proper patient positioning will provide a more accurate position of MF than dry skulls[34]. However, the MF positions in the present study and two studies done in Sri Lanka using dry mandibles were not in agreement (Table 5). Since the age range (and mean) of these subjects were not reported, it may be possible that the dry mandibles used in those studies were from elderly patients.

Conclusion

The present study has demonstrated that the position of MF is more commonly found between 1st premolar and 2nd premolar teeth by both crown and apex scores. Though sample size is adequate to come to a conclusion the sample size of various ethnic groups needs to be increased to evaluate the effect of ethnicity on the position of MF. Additionally, the study consists of patients who have visited the Dental Hospital, Sri Lanka. Therefore, a broad generalization of the findings for the whole population may not be justifiable, and further studies with a nationally representative samples are necessary to come to a firm conclusion.

Table 6. Summary of various population groups reviewed with regard to the position of MF.MF-A - distance between the anterior border of the MF and the symphysis menti; MF-B - distance between the upper edge of the MF and the upper edge of the alveolar crest; MF-C - distance between the lover edge of the MF and inferior border of the mandible.

Reference	Population	Age range (mean)	N	Symmetry (%)	Position (%)						MF-A		MF-B		MF-C		Shape (%)		
					I	II	III	IV	V	VI	M	F	M	F	M	F	oval	Round	Irregular
Shrestha et al.2019	Nepalese	>18 (29.4)	170		0.3	0.9	54.7	35.9	7.6	0.6			15.53		11.43				
Bello et al. 2018	Nigerians	18 – 64 (38.5)	320	48.8	0.48		65.9		33.6		-	-	-	-	-	-	55.2	12	32.8
Ghimire and Gupta. 2018	Nepalese	20-70 (28)	417	-	0	10.8	39.1	35.5	13.9	0.7	-	-	-	-	-	-	-	-	-
Carteset al. 2018	Chilean	18-34 (23)	336	-	2.65	3.15	51.8	36.72	5.6	-			R-12.85 L-13.10	11.33 11.37	17.19 16.80	15.83 15.76	-	-	
Rezaei et al. 2018	Iranian	-	500	73	0.1	1	34.7	53.4	10.8	0	-	-	-	-	-	-	-	-	

Ndiaye et al. 2018	Senegalese	18-73 (32)	187	-	0	0	18.2	57.8	18.7	5.3	-	-	-	-	-	-	-	-	-
Fuentes et al. 2017	Chilean	(34.65)	163	-	0.63	2.51	61.96	29.61	5.3	-	24.57				12.49	11.40	-	-	-
Alok et al.2017	Indians (Bareilly)	18–65	750	81.7	-	0.15	19.3	74.4	6.15	0.05	-	-	-	-	-	-	-	-	-
Srinivas et al. 2017	Indians Karnataka Kerala	16-45	 50 50	-	 3 3	 16 14	 58 56	 22 24	 0 1	 1 3	-	-	-	-	-	-	-	-	-
Thakareet al.2016	Indians	15-59	200	-	2.25	4.75	30.5	46.7	13	2.75	-	-	-	-	R-13.02 L-12.97	11.31 11.26	-	-	-
Currie et al. 2016	UK-based	18-30	100	62	0	1.8	51	43	4.2	-	-	-	-	-	-	-	-	-	-
Al-Shayyab et al. 2016	Iraqi	18-79	518	78.4	0	2.6	48.6	43.7	4.9	0.2	-	-	-	-	-	-	41	51	8
Zaman et al (2016)	Bangladeshi	14 -50	3788	75.9	0.2	1.8	35.6	58.4	3.5	0.5	-	-	-	-	-	-	-	-	-
Mohammad et al.2016	Palestinians	>18	368		3.2	7.6	40.6	38.4	9.2	0.8		-	-	-	-	-	8.3 10.5	51.6 51.6	40.1 43.6
Chowdhury et al. (2015)	Bangladeshi	15-70	101	-	5.4	9.4	35.1	37.6	9.4	3.0	-	-	-	-	-	-	-	-	-
Parnami et al.2015	Indians	15-59	582	86.8	0.09	3.01	28.7	61.0	5.7	1.5	-	-	-	-	-	-	-	-	
Verma et al. 2015	Indians	18-25	120	62	5	8.75	35.41	43.33	7	0.42	-	-	-	-	-	-	27	56	17
Swamy et al. 2015	Indians	15-50	100	-	1	3.5	23.0	63.5	9.0	0									
Gada et al. 2014	Indians	18-66	300	54.66	3.67	2.33	63	10.33	20.67	-	-	-	-	-	R-15.52 L-15.26		-	-	-
Kumar et al. 2014	South Indians North Indians	>18	380	85	0 0	2.4 1.9	31.1 59.2	62.8 36.9	3.7 3.2	0 0	-	-	-	-	-	-	-	-	-
Kquiku et al 2013	Kosovarian	18-70	500		-	4.4	44.4	43.2	8		R- 24.76 L-24.92		20.59 20.17		14.58 14.77		-	-	-
Chkoura et al. 2013	Moroccans	18.2-63.9	377	79	0.45	1.3	30	63	4.5	0.8	-	-	-	-	-	-	-	-	-
Ayad et al.2013	Sudanese	18-45	113		-	-	29.2	70.8	-	-	28.8	28.5	-	-	14.8	14.6	-	-	-
Afkhami et al.2013	Iranian	18-40	100	80	-	6	24	63	3	-	28.2	27.53	-	-	12.01	10.02	-	-	-

Al-Juboori et al. 2013	Malaysians	>25	376	-	-	4.2	54	33.2	9.5	-	-	-	-	-	-	-	-	-	-
Shah et al. 2013	Indians	20-50	184	65.7	-	7.68	63.86	26.36	1.36	0.82	-	-	-	-	-	-	35.6	48.37	16.03
Rupesh at al.2011	Indians	Mean 31.67	500	57	3.5	1	47.6	33.5	11.4	3	-	-	-	-	-	-	-	-	-
Haghanifar and Rokouei (2009)	Iranian	>18	400	85.7	0	1.6	47.2	46	5.1	0.1	-	-	-	-	-	-	-	-	-
Talabani et al.2008	Kurdish	18–40	110	82.7	-	1.81	35.9	55	7.27	0			R-23.50 L-23.50	21.43 21.64	15.80 15.96	14.01 14.14	-	-	-
Al-Khateeb et al. 2007	Jordanian	12- 77	860	67	1	3	47	40	10		-	-	-	-	-	-	42	47	11
Gungor et al.2006	Turkish	14-57	361	85.8	1.2	3.2	71.5	22.4	1.7	0			-	-	-	-	-	-	
Kim et al. 2006	Korean	12-69	112	-	-	8.9	28.6	62.5	-	-	-		-	-	16.98	15.69	-	-	-
Ngeow et al. 2003	Malaysians	14-43	161	67.7	0	3.4	19.6	69.2	6.8	0.9	-	-	-	-	-	-	-	-	-
Al Jasser and Nwoku. 1998	Saudi	14-64	397	80	0.6	5.3	42.7	45.3	5.2	0.9	-	-	-	-	-	-	-	-	-
Neo J. 1989	Malays Indians	-	158		-	2.4 5.3	16.1 19	67.75 47	6.45 21.7	7.2 6.87	-	-	-	-	-	-	-	-	-
Present study	Sri Lankans	18-45 (27.65)	619	85.3	0	1.5	52.3	41	5.2	-	R-31.24 L-31.97	29.95 30.58	17.12 16.89	15.78 15.99	16.23 16.084	14.63 14.86	48.86	32.95	18.17

References

1. Zaman S, Alam M, Yusa T, et al. Mental Foramen Position Using Modified Assessment System: An Imperative Landmark for Implant and Orthognathic Surgery. *Journal of Hard Tissue Biology*. 2016;25:365-370. doi:10.2485/jhtb.25.365.

2. Alsoleihat F, Al-Omari FA, Al-Sayyed A, Alasmar A, Khraisat A. The mental foramen: A cone beam CT study of the horizontal location, size and sexual dimorphism amongst living Jordanians. *HOMO*. 2018;69. doi:10.1016/j.jchb.2018.11.003.

3. Bello SA, Adeoye JA, Ighile N, Ikimi NU. Mental Foramen Size, Position and Symmetry in a Multi-Ethnic, Urban Black Population: Radiographic Evidence. *Journal of Oral & Maxillofacial Research*. 2018;9(4):2. doi:10.5037/JOMR.2018.9402.

4. Gherghiţă OR, Csiki IE, Bordea EN, et al. Morphometric study for determining the anteroposterior position of the mental foramen in dentate human subjects. *Romanian Journal of Morphology and Embryology*. 2021;62(2):517-523. doi:10.47162/RJME.62.2.18.

5. Deleu M, Dagassan D, Berg I, et al. Establishment of national diagnostic reference levels in dental cone beam computed tomography in Switzerland. *Dentomaxillofac Radiol*. 2020;49(6):20190468. doi:10.1259/dmfr.20190468.

6. Currie CC, Meechan JG, Whitworth JM, Carr A, Corbett IP. Determination of the mental foramen position in dental radiographs in 18-30 year olds. *Dentomaxillofac Radiol*. 2016;45(1). doi:10.1259/DMFR.20150195.

7. Shankland WE 2nd. The position of the mental foramen in Asian Indians. *J Oral Implantol*. 1994;20(2):118-123.

8. Alok A, Singh I, Panat S, Singh S, Kishore M, Jha A. Position and Symmetry of Mental Foramen: A Radiographic Study in Bareilly Population. *Journal of Indian Academy of Oral Medicine and Radiology*. 2017;29:16-19. doi:10.4103/jiaomr.JIAOMR_69_16.

9. Neo J. Position of the mental foramen in Singaporean Malays and Indians. *Anesth Prog*. 1989;36(6):276-278.

10. Igbigbi PS, Lebona S. The position and dimensions of the mental foramen in adult Malawian mandibles. *West Afr J Med*. 2005;24(3):184-189. doi:10.4314/WAJM.V24I3.28195.

11. Chkoura A, el Wady W. Position of the mental foramen in a Moroccan population: A radiographic study. *Imaging Science in Dentistry*. 2013;43(2):71. doi:10.5624/ISD.2013.43.2.71.

12. Chowdhurya TG, Rahmanb D, Jannat TA, et al. Radiographic position of mental foramen in selected urban population of Bangladesh. *Update Dental College Journal*. 2015;5(1):3-9. doi:10.3329/UPDCJ.V5I1.25820.

13. Mohamed A, Nataraj K, Mathew VB, et al. Location of mental foramen using digital panoramic Radiography. *Journal of Forensic Dental Sciences*. 2016;8(2):79. doi:10.4103/0975-1475.186365.

14. Parnami P, Gupta D, Arora V, Bhalla S, Kumar A, Malik R. Assessment of the Horizontal and Vertical Position of Mental Foramen in Indian Population in Terms of Age and Sex in Dentate Subjects by Panoramic Radiographs: A Retrospective Study with Review of Literature. *Open Dent J*. 2015;9(1):297-302. doi:10.2174/1874210601509010297.

15. Thakare S, Mhapuskar A, Hiremutt D, Giroh V, Kalyanpur K, Alpana KR. Evaluation of the Position of Mental Foramen for Clinical and Forensic Significance in terms of Gender in Dentate Subjects by Digital Panoramic Radiographs. *The Journal of Contemporary Dental Practice*. 2016;17:762-768. doi:10.5005/jp-journals-10024-1926.

16. Verma P, Bansal N, Khosa R, et al. Correlation of Radiographic Mental Foramen Position and Occlusion in Three Different Indian Populations. *The West Indian Medical Journal*. 2015;64(3):269. doi:10.7727/WIMJ.2014.143.

17. Green RM. The position of the mental foramen: a comparison between the southern (Hong Kong) Chinese and other ethnic and racial groups. *Oral Surg Oral Med Oral Pathol*. 1987;63(3):287-290. doi:10.1016/0030-4220(87)90191-5.

18. Ilayperuma I, Nanayakkara G, Palahepitiya N. Morphometric Analysis of the Mental Foramen in Adult Sri Lankan Mandibles. *International Journal of Morphology*. 2009;27(4):1019-1024. doi:10.4067/S0717-95022009000400010.

19. Nanayakkara D, Sampath H, Manawaratne R, et al. Positional variation and localization of the mental foramen. *MOJ Anatomy & Physiology*. 2018;Volume 5(Issue 1). doi:10.15406/MOJAP.2018.05.00162.

20. Elsakr 11, Da J, Oldfield YZ. The position, dimensions and morphological variations of mental foamen in mandibles. *Galle Medical Journal*. 2009;11(1):13. doi:10.4038/GMJ.V11I1.1111.

21. Mangla M, Rajput L, Kumar A, Sharma P, Rathi V, Kumar S. Orthopantomogram study of mental foramen in Muradnagar-Ghaziabad Population. *Journal of Dental Specialities*. 2017;5:138-141. doi:10.18231/2393-9834.2017.0031.

22. Cartes G, Garay I, Deana NF, Navarro P, Alves N. Mandibular Canal Course and the Position of the Mental Foramen by Panoramic X-Ray in Chilean Individuals. *BioMed Research International*. 2018;2018. doi:10.1155/2018/2709401.

23. Fuentes R, Flores T, Dias F, et al. Localization of the Mental Foramen Through Digital Panoramic Radiographs in a Chilean Population Localización del Foramen Mental a Través de Radiografías Panorámicas Digitales en una Población Chilena. *Int J Morphol*. 2017;35(4):1309-1315.

24. Ketan Gada S, Nagda SJ. Assessment of position and bilateral symmetry of occurrence of mental foramen in dentate asian population. *J Clin Diagn Res*. 2014;8(2):203-205. doi:10.7860/JCDR/2014/7257.4060.

25. Ghimire, B., and Gupta, S. (2018). Location of Mental Foramen in Dentate Adults using Orthopantomogram. JNMA J Nepal Med Assoc 56, 791–795. - Google Search. Accessed July 21, 2022. https://www.google.com/

Research Article

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Characterization of alveolar bone loss and osteoclast activity in a modified LPS-induced periodontitis murine model

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Abstract

Periodontitis induced by bacterial toxins such as lipopolysaccharide (LPS) gives rise to alveolar bone resorption due to the activation of the bone resorbing osteoclasts. Experimental models using LPS is well described in many animal models including rodent and murine models. However, most of them have used a large amount of LPS with longer duration to develop the models. In the present study alveolar bone loss was induced by instituting 2ml of *Escherichia coli* LPS (10 mg/ml) into mousemaxillary gingiva between first and second molars three times per week. Animals were sacrificed on day 7 and maxillae were isolated, fixed and decalcified for histological analysis. On day 7, downwards migration of junctional epithelium was observed, a significant trend for interdental alveolar bone loss in both ipsilateral and contralateral sides of the maxilla, due to the activation of osteoclasts following LPS injection. This behoves an easy and reproducible animal model of periodontal bone loss which could be used in studies to evaluate therapeutic agents in the management of periodontitis.

Key words: LPS, periodontitis, murine model.

Introduction

Periodontitis is considered as one of the most prevalent chronic inflammatory diseases that involved periodontium giving rise to progressive loss of the soft and hard tissues such as gingiva, periodontal ligament, and the alveolar bone. Periodontitis commonly occurs due to the periodontopathic bacteria in the biofilm or dental plaque that builds up adjacent to the teeth and is considered a major cause of tooth loss in adults [1,2].The biofilm constitutes gram negative anaerobic commensals as well as opportunistic pathogens such as *Porphyromonas gingivalis (P. gingivalis), Treponema denticola,* and *Tannerella forsythia*[3]. In addition *Aggregatibacter actinomycetemcomitans* (earlier *Actinobacillus actinomycetemcomitans* - Aa) is also implicated in periodontal diseases especially in young adults and in severe periodontitis[1]. As a result of release of various enzymes and toxins, these bacteria induce a host inflammatory response leading to tissue destruction. Moreover, production of cytokines such as tumour necrosis factor alpha (TNF-α), interleukin (IL)-1, and lipopolysaccharide (LPS) has been shown to play a major role in host modulation of periodontal disease[4]. Bone resorption is an important sequela of periodontitis that leads to tooth loss[1]. Periodontitis is also implicated in many systemic diseases such as cardiovascular diseases and rheumatoid arthritis. Host susceptibility may also play a role in the

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initiation and progression of periodontitis in addition to the pathogenic bacteria[5].

Animal experiments are commonly employed to complement the *in-vitro* experiments before evaluating new treatment modalities. This necessitates the use of pertinent animal models in the development of novel methods to prevent, diagnose, and treat diseases and to see whether the new therapies or drugs are safe and effective. As a result, experimental animal models such as murine and rodent models have been developed to reproduce periodontitis of humans and to establish pre-clinical relevance[6]. Though cell cultures using humans cells provide some insight, they were not adequate to provide the overall effect indicating that animal models are still pivotal in the analysis of periodontal disease and development of improved treatments[5].

Periodontal diseases are induced in animal models by using dietary manipulation[7],oral gavage[8,9], ligatures [10], calvarial model [11,12] or injection of bacterial toxins such as LPS[13] which require several weeks to produce measurable periodontal bone loss[8]. LPS is identified as a cell wall constituent of subgingival gram-negative organisms and to induce polymorphonuclear leukocyte infiltration, oedema and vascular dilatation in inflamed periodontal tissues. It has been demonstrated that *E.coli* LPS induces expression of MMP-9 and may contribute to periodontal tissue destruction[14]. Hard tissue destruction by LPS is brough by the production of inflammatory mediators such as IL-1,-6 and -8 and TNF-α and the activation of osteoclasts (OCs) which are the main cells of bone resorption[15]. LPS-induced periodontitis model is commonly employed with murine and rodent models using LPS of many bacterial species such as *E.coli*[14,16,17], *P.gingivalis*[8] and *A. Actinomycetemcomitans*[18,19].

Though LPS-induced periodontitis model provides a suitable animal model a considerable time is taken to establish the model. In addition, there are several drawbacks such as slow-disease progression, cost and technical challenges pertaining to certain models. In the present study a previously described experimental model of periodontitis in the rat has been modified and characterised in a murine model using LPS from *E.coli*. A simple, reproducible method of induction of bone loss after OC activation is described in order to use this model in studies to evaluate therapeutic agents in the management of periodontitis.

Materials and methods

Induction of periodontal bone loss in mice

The present animal experiment was carried out in accordance with the ethical guidelines pertaining to animal studies and ethical clearance for the study was obtained from the ethics committee of Faculty of Veterinary Medicine and Animal Science, University of Peradeniya, Sri Lanka. Mice were obtained from the Medical Research Institute (MRI), Sri Lanka and they were housed in the Faculty of Medicine, University of Peradeniya animal facility. Food and water were provided *ad libitum* throughout the experiment. Mice were under the supervision of a Veterinary surgeon who handled all the *in-vivo* animal experiments of this research and had taken good care of the mice. LPS-induced periodontitis in mice was induced as described by Nogueira, *et al.* (2014)[20] with some modifications. A total of eight 12 weeks old C57BL6 mice were used to develop periodontitis. The mice were anesthetized with a mixture of 10% ketamine and 2% xylazine. To induce periodontal inflammation and bone loss, 2 µL (10 µg/µL) of *E. coli* LPS (strain O111: B4- Sigma Chem Co., St. Louis, MO, USA) were injected into the palatal gingiva between the right maxillary first and second molars using a Hamilton micro syringe. LPS was released slowly and the needle was held in place for several seconds following injection to avoid loss of LPS through the needle track as explained elsewhere[13]. A 2 µL of PBS vehicle (PBS) wasinjected into the palatal gingiva between the right maxillary first and second molars in control mice. 2 µL of *E. coli* LPS (10 µg/ µL) was injected on day 3 and day 5

as well (a total of 60 µg of *E. coli* LPS per mouse). The weight of all mice was measured daily throughout the experiment. On day 7, mice were sacrificed under overdose of general anaesthesia followed by cervical dislocation. The skin and the soft tissues of the maxilla were removed, and the maxillary bones were collected. Then the bones were fixed in 4% paraformaldehyde in PBS for 48 hours and decalcified with 10% EDTA at 4°C as described elsewhere [21].The maxillae were sectioned, and the left and right sides were processed in an automated tissue processor and embedded in paraffin wax. 4-5 µm thick serial sagittal sections were prepared using a rotary microtome. Some of the sections were stained with haematoxylin and eosin (H&E) to see the gingival tissue, inflammatory infiltrate, and alveolar bone loss. TRAP staining was done to visualize the OCs as described elsewhere[21].

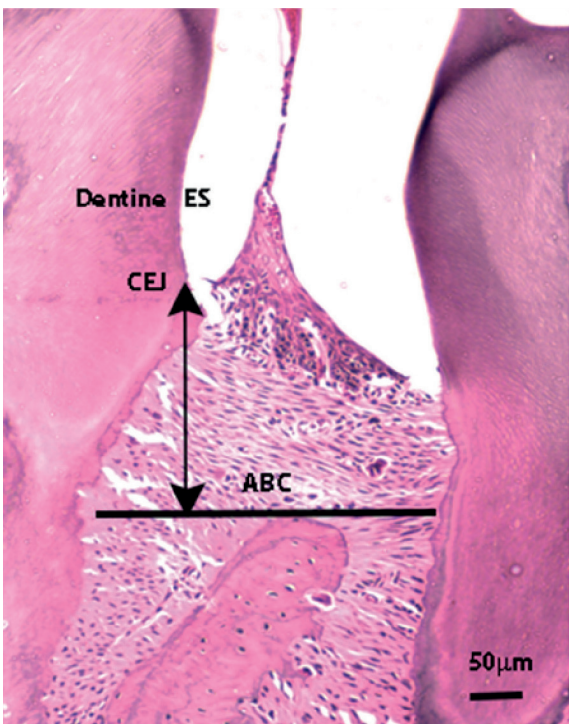
Measurement of alveolar bone loss

Histological sections were analyzed to measure the interseptal crestal bone loss between the first and second maxillary molars as described elsewhere[13]. In brief, vertical distance between the first and second molars, from cemento-enamel junction (CEJ) to alveolar bone crest (ABC) (Figure. 1) was measured in five random sections, and the mean cemento-enamel junction to alveolar crest height was calculated. Mean ABC-CEJ height was then calculated for each group of animals. The amount of alveolar bone loss induced by LPS injection was the difference between the bone loss in LPS-injected animals compared to controls.Image analysis was performed as described elsewhere[13]. Briefly, the images were captured on an image analysis system. Analysis was carried out using the program Image J (developed at the US National Institutes of Health and available on the Internet at http://rsb.info.nih.gov/nih-image/) which is available in the public domain as described by Dumitrescu et al., 2004[13].

Measurement of osteoclast number

OC swere detected fromTRAP-stained sections and TRAP-positive ($TRAP^+$) multinuclear cells containing more than two nuclei were considered as OCs. OCs were counted along the mesial surface and the distal surface of the alveolar bone of interdental septum between maxillary first and second molars in both sides of the maxilla. Data was presented as the number of osteoclasts.

Figure 1. Schematic diagram shows the measurement of distance between CEJ to ABC on a decalcified, H&E stained section of PBS-injected control periodontium between first and second molars of the maxilla. The horizontal line shows the top of the ABC and the CEJ-ABC distance is the vertical distance from ABC to the CEJ (double headed arrow). ES = enamel space; D = dentine; ABC = alveolar bone crest; CEJ = cementum-enamel junction.



Statistical analysis

All results were expressed as mean ± SEM. The statistical significance of differences among groups was assessed using one-way ANOVA (analysis of variance). *P* values <0.05 were

considered significant.

Results

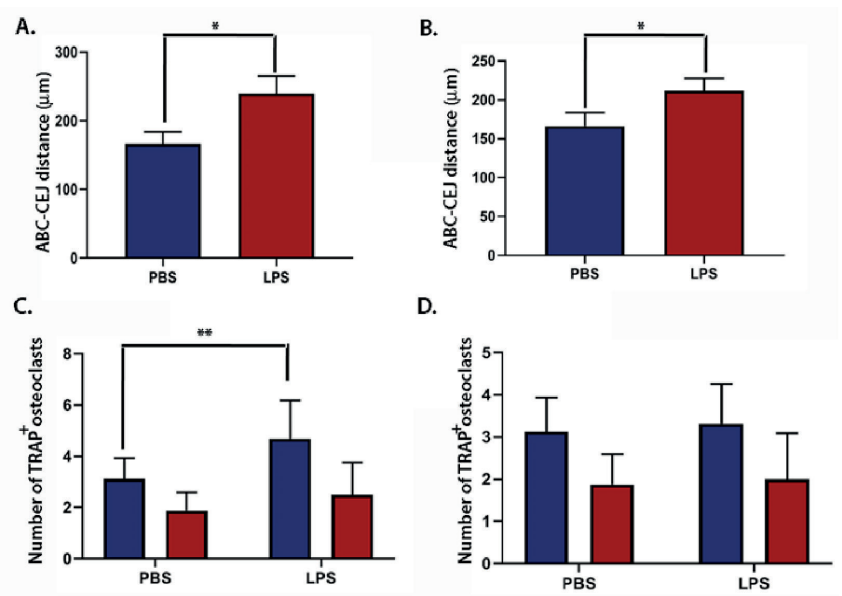
LPS-induced alveolar bone loss

During the study period mice were active and did not show any signs of systemic illness. The weights of all animals were not changed during the study period. All LPS-injected mice demonstrated a breakdown of the interdental papilla and migration of junctional epitheliumapically. Since we did not observe a difference between ABC-CEJ distance on both left and right sides of the maxilla of PBS-injected control mice, the results were pooled and analysed for control group as described elsewhere[13]. The ABC-CEJ distance was significantly high ($P < 0.001$) on ipsilateral and contralateral side of the maxilla in LPS-injected animals compared to control animals (Figure 2A and Figure 2B).

Osteoclast activity

$TRAP^+$ multinucleated cells were observed on the mesial surface of the alveolar bone in LPS-injected animals. The number of OC son both the mesial (adjacent to first molar) and distal (adjacent to second molar) surfaces of the interdental septum of the alveolar bone were calculated as described elsewhere [13]. In LPS-injected animals, the numbers of $TRAP^+$ multinucleated cell son the mesial surface of the interdental septum were increased significantly ($P< 0.05$)on ipsilateral side of the maxilla (Figure 2C) whereas the number of $TRAP^+$ multinucleated cell son the distal surface of the interdental septum was not increased significantly (Figure 2C). However, the $TRAP^+$ multinucleated cells were not increased significantly on both mesial and distal surfaces of the contralateral side (Figure 2D) of the maxilla in LPS-injected animals compared to control animals.

Figure 2. Alveolar crestal bone loss due to osteoclast activation in PBS-injected and LPS-injected mice. A. A significant increase was observed in ABC-CEJ distance between the first and second molar interseptal area in LPS-injected animals on ipsilateral and B. contralateral sides.C. Active osteoclast numbers were significantly increased on the mesial surface (blue filled bars)of the alveolar bone at the site of LPS injection on day 7 but not on distal surface (red filled bars). D. The OC numbers were not increased significantly on both mesial and distal surfaces of the contralateral side of the maxilla in LPS-injected animals compared to control animals.* $P < 0.001$ and ** $P< 0.05$ compared to control.



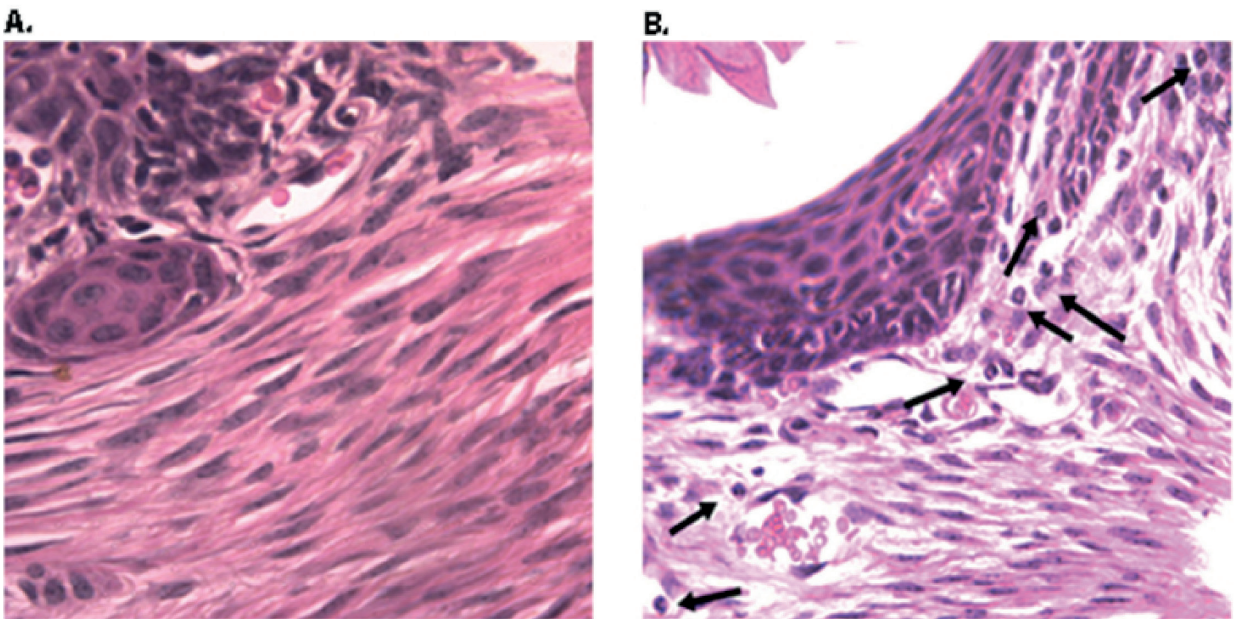
LPS-induced inflammatory infiltrate

The decalcified sections of maxillae were stained with H&E to observe the inflammatory infiltrate induced by LPS. Histological sections of LPS-injected mice demonstrated a marked inflammatory cell infiltrate proximal to the junctional epithelium and the surrounding alveolar crest within the connective tissue compared to PBS-injected controls (Figure 3). These findings indicates that inflammatory cells such as neutrophils were available in the LPS-injected tissues, while fewer inflammatory cells were observed in the PBS-injected mice.

Discussion

Experimental animal models which mimic periodontal diseases are commonly employed to clarify the pathogenesis and molecular mechanisms of such diseases. A murine model has many attributes which makes it a suitable model to delineate the underlying pathogenesis and treatment modalities in periodontal diseases. Studies have shown that the anatomy of the periodontium and the histopathological presentation of periodontitis in mice share many similarities to that of humans[10,22]. In addition, low cost, easy handling of animals, known genetics, and a well-characterized immune system favour using mice for periodontitis studies [23]. Varying methods have tested to trigger LPS-induced periodontitis however, most of these methods require considerable time for periodontal lesions to develop[24]. In these models, disease progression is very slow without the placement of ligatures around the teeth. It has been demonstrated that even in a simple ligature-induced periodontitis model it takes 18 days for the full development of periodontitis [25]. In the present study, we have developed a simple murine model of periodontitis by local injection of *E-coli*-LPS within 7 days, which is much easier and required only a few days compared to that of bacterial infection experimental models. Moreover, this model avoids the technical challenges of placing ligatures around murine teeth consistently.In this study, the models of LPS-induced periodontitis described by Dumitrescu, *et al.,*[13] and Nogueira, *et al.,*[20] using rodents were modified and carried out in a mouse model using *E. Coli* LPS similar to the latter study. *E. Coli* LPS which is a Toll-Like Receptor (TLR)-4 agonist induces pro-inflammatory cytokines in PDL cells, though

Figure 3. Inflammatory infiltrate of A. PBS-injected and B. LPS-injected mice. Arrows show the inflammatory cells such as neutrophils.



it has some limitations such as that it is not found in the oral environment[20]. However, many LPS-induced periodontitis models have been developed using *E. Coli* LPS in rats and mice.

In the study by Dumitrescu, *et al.,*[13], a single injection of 10 µg of *S. Typhimurium* (1 µL) has been used to induce periodontal inflammation in mandibular gingiva of rats at the buccomesial aspect of the second molar. Other than the aforementioned study most of the studies in published literature have used considerable amounts of LPS. For example, in the study by Nogueira, *et al*.,[20] 3µL of a 20µ/µ*l E. coli* LPS was delivered into the palatal gingivaebetween both maxillary first and second molars 3 times perweek (60 µ per injection). In the present study, the injection was made into the palatal gingiva between the right maxillary first and second molars using *E. coli* LPS (60 µ per mouse for the whole study). The study by Baker *et al*.,[8] has shown that several mouse strains including C57BL/6J mice are more resistant to *P. gingivalis*-induced alveolar bone loss while mouse strains such as DBA/2J and BALB/cJ mice are more susceptible. However, there are studies which have used C57BL/6J mice to induce periodontitis using LPS[10]. Similarly, as we used C57BL6J mice which are relatively resistant to periodontitis we used thrice a week injection of 2 µ of *E.coli* LPS (10µ/µ) but much less than the amount used by Nogueira, *et al*.,[20]. Moreover, our study has demonstrated that LPS-induced alveolar bone resorption takes place in C57BL6J mice suggesting that this model can be used in evaluating therapeutic agents to ameliorate bone resorption due to periodontitis. Our study indicates that thrice a day injection of 2 µ of LPS (10µ/µ) is adequate to induce OC activity and alveolar bone resorption in mice akin to the studies which used LPS to induce periodontitis.

There is plethora of reports to show that a larger amount of LPS with more duration have been used to induce periodontitis in mice. A study using a rat model has demonstrated that thrice a week injection of 30 µg of LPS from *E. coli* has induced inflammation and bone loss which increased from day 7 to day 15 and sustained until day 30[26]. Another study has shown that LPS induces greater bone and PDL loss in SPARC-null mice by developing periodontal disease by injections of 20 µL (10 µg/µL) of LPS from *A. Actinomycetemcomitans*[19]. Another study has established a murine periodontitis model by local injection of 10 µL (10 µg/µL) of LPS from *P. gingivalis* four times at 2-day interval into the gingival sulcus of mandibular left incisor. Marked periodontal bone and ligament loss with abundant inflammatory cell infiltrate was observed histopathologically in the LPS-injected side[27]. Nishida, *et al*.,[21] demonstrated an active bone resorption following the 4th injection of *A. actinomycetemcomitans* LPS whereas bone loss was observed after the 7th injection of *P. gingivalis* LPS. In this study the authors have administered 5 µg of LPS dissolved in 3 µL of PBS into the gingiva on the mesial side of the left lower first molarof seven-week-old male BALB/c miceevery 48 hour.

Scanu, *et al.,*[28] have performed a study with CD1 mice and have shown that the distance between CEJ and ABC was 0.29±0.08 mm in the LPS-injected mice compared to control mice which is 0.17±0.05 mm ($p<0.001$). The amount of alveolar crestal bone loss brought by gavage model is relatively low (0-0.03 mm) compared to that of the ligature model (0.1- 0.2 mm bone loss). Ligature model could induce, on average, ten times more bone loss than the gavage model[8,9]. The reported bone loss with Aa-LPS model is much more compared to other models (0.634mm[18] whereas in the present study the difference was 0.083 mm somewhat less than the ligature model but higher than gavage model .

Histopathological analysis of decalcified sections of maxillae demonstrated the inflammatory features of periodontitis by day 7 following administration of LPS. In addition, there was an apical migration of the junctional epithelium,

significant alveolar bone resorption, and OCs activation at the site of LPS injection compared to control group. These observations suggest that studies of bone loss are best carried out during this time period. A study by de Aquino *et al*.,[24] has demonstrated that LPS and ligatures induce an obvious inflammatory reaction with general histological characteristicssimilarly in both models.

There are several studies[29] which have used the contralateral side as the control in LPS-induced periodontitis. The study of Dumitrescu, *et al.,*[13] showed that there is a significant increase in alveolar bone resorption and a tendency to increase OC number on contralateral side of the mandible (but not significant) by day 7 of periodontitis induction with LPS similar to the present study indicating that contralateral side should not be taken as a control when inducing periodontitis in ipsilateral side.

Taken together the present study provides a simple reproducible murine model of periodontitis using a local injection of LPS into maxillary interdental area. This model demonstrated $TRAP^+$ multinucleated cells and alveolar crestal bone loss at day 7 following LPS injection. Similar changes were also observed in the contralateral maxilla as well.Since LPS-induced periodontitis murine model mimics features of periodontitis in humansit can be easily employed in the studies to evaluate bone loss in periodontitis and to investigatedifferent therapeutic strategies.

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References

1. Pihlstrom, B. L., Michalowicz, B. S. & Johnson, N. W. Periodontal diseases. *Lancet***366**, 1809–1820 (2005). http://doi.org/10.1016/S0140-6736(05)67728-8.

2. Soysa, N. S., Waidyarathne, H., Ranaweera, M. & Alles, C. N. R. A. Clinical efficacy of local application of sustained-release metronidazole in periodontal therapy. *Dentistry Review***1**, 100006 (2021).https://doi.org/10.1016/j.dentre.2021.100006.

3. Holt, S. C. & Ebersole, J. L. Porphyromonas gingivalis, Treponema denticola, and Tannerella forsythia: the 'red complex', a prototype polybacterial pathogenic consortium in periodontitis. *Periodontol 2000*38, 72–122 (2005). https://doi/10.1111/j.1600-0757.2005.00113.x.

4. Salvi, G. E. & Lang, N. P. Host response modulation in the management of periodontal diseases. *J. Clin. Periodontol.*32 Suppl 6, 108–129 (2005). https://doi/10.1111/j.1600-051X.2005.00785.x.

5. Oz, H. S. & Puleo, D. A. Animal models for periodontal disease. *J. Biomed. Biotechnol.*2011, 754857 (2011).https://doi: 10.1155/2011/754857.

6. Struillou, X., Boutigny, H., Soueidan, A. & Layrolle, P. Experimental animal models in periodontology: a review. *Open Dent J*4, 37–47 (2010).https://doi: 10.2174/1874210601004010037.

7. Blasco-Baque, V. *et al.* High-fat diet induces periodontitis in mice through lipopolysaccharides (LPS) receptor signaling: protective action of estrogens. *PLoS ONE***7**, e48220 (2012). https://doi: 10.1371/journal.pone.0048220.

8. Baker, P. J., Dixon, M. & Roopenian,

D. C. Genetic control of susceptibility to Porphyromonas gingivalis-induced alveolar bone loss in mice. *Infect. Immun.*68, 5864–5868 (2000).https://doi: 10.1128/IAI.68.10.5864-5868.2000.

9. de Molon, R. S. *et al.* Long-term evaluation of oral gavage with periodontopathogens or ligature induction of experimental periodontal disease in mice. *Clin Oral Investig*20, 1203–1216 (2016).https://doi: 10.1007/s00784-015-1607-0.

10. Li, C. H. & Amar, S. Morphometric, histomorphometric, and microcomputed tomographic analysis of periodontal inflammatory lesions in a murine model. *J. Periodontol.*78, 1120–1128 (2007).https://doi: 10.1902/jop.2007.060320.

11. Soysa, N. S., Alles, N., Takahashi, M., Aoki, K. & Ohya, K. Defective nuclear factor-κB-inducing kinase in aly/aly mice prevents bone resorption induced by local injection of lipopolysaccharide. *J. Periodont. Res.*46, 280–284 (2011).https://doi: 10.1111/j.1600-0765.2010.01333.x.

12. Alles, N. *et al.* Suppression of NF-κB Increases Bone Formation and Ameliorates Osteopenia in Ovariectomized Mice. *Endocrinology*151, 4626–4634 (2010). https://doi.org/10.1210/en.2010-0399.

13. Dumitrescu, A. L., Abd-El-Aleem, S., Morales-Aza, B. & Donaldson, L. F. A model of periodontitis in the rat: effect of lipopolysaccharide on bone resorption, osteoclast activity, and local peptidergic innervation. *J. Clin. Periodontol.*31, 596–603 (2004).https://doi: 10.1111/j.1600-051X.2004.00528.x.

14. Buduneli, E. *et al.* Matrix metalloproteinases, tissue inhibitor of matrix metalloproteinase-1, and laminin-5 gamma2 chain immunolocalization in gingival tissue of endotoxin-induced periodontitis in rats: effects of low-dose doxycycline and alendronate. *J. Periodontol.***78**, 127–134 (2007).https://doi: 10.1902/jop.2007.050451.

15. Soysa, N. S. & Alles, N. Osteoclast function and bone-resorbing activity: An overview. *Biochem. Biophys. Res. Commun.*476, 115–120 (2016).https://doi: 10.1016/j.bbrc.2016.05.019.

16. Ukai, T., Hara, Y. & Kato, I. Effects of T cell adoptive transfer into nude mice on alveolar bone resorption induced by endotoxin. *J. Periodont. Res.*31, 414–422 (1996).https://doi: 10.1111/j.1600-0765.1996.tb00510.x.

17. Hara, Y., Ukai, T., Yoshimura, A., Shiku, H. & Kato, I. Histopathological study of the role of CD4- and CD8-positive T cells on bone resorption induced by Escherichia coli endotoxin. *Calcif. Tissue Int.*63, 63–66 (1998).https://doi: 10.1007/s002239900490.

18. Rogers, J. E. *et al.* Actinobacillus actinomycetemcomitans lipopolysaccharide-mediated experimental bone loss model for aggressive periodontitis. *J. Periodontol.*78, 550–558 (2007).https://doi: 10.1902/jop.2007.060321.

19. Trombetta-Esilva, J. *et al.* LPS induces greater bone and PDL loss in SPARC-null mice. *J. Dent. Res.*90, 477–482 (2011). https://doi: 10.1177/0022034510391800.

20. Nogueira, A. V. B. *et al.* HMGB1 Localization during Experimental Periodontitis. *Mediators of Inflammation*2014, 1–10 (2014). https://doi: 10.1155/2014/816320.

21. Nishida, E. *et al.* Bone resorption and local interleukin-1alpha and interleukin-1beta synthesis induced by Actinobacillus actinomycetemcomitans and Porphyromonas

gingivalis lipopolysaccharide. *J. Periodont. Res.*36, 1–8 (2001). https://doi: 10.1034/j.1600-0765.2001.00637.x.

22. Klausen, B. Microbiological and immunological aspects of experimental periodontal disease in rats: a review article. *J. Periodontol.***62**, 59–73 (1991). https://doi: 10.1902/jop.1991.62.1.59.

23. Genco, C. A., Van Dyke, T. & Amar, S. Animal models for Porphyromonas gingivalis-mediated periodontal disease. *Trends Microbiol.***6**, 444–449 (1998). https://doi: 10.1016/s0966-842x(98)01363-8.

24. de Aquino, S. G. *et al.* Differential regulation of MMP-13 expression in two models of experimentally induced periodontal disease in rats. *Arch. Oral Biol.***54**, 609–617 (2009). https://doi: 10.1016/j.archoralbio.2009.03.007.

25. Marchesan, J. *et al.* An experimental murine model to study periodontitis. *Nat Protoc***13**, 2247–2267 (2018). https://doi: 10.1038/s41596-018-0035-4.

26. Souza, J. A. C. de *et al.* Suppressor of cytokine signaling 1 expression during LPS-induced inflammation and bone loss in rats. *Braz Oral Res***31**, e75 (2017). https://doi: 10.1590/1807-3107BOR-2017.vol31.0075.

27. Ma, Z. *et al.* Macrophage-mediated nanoparticle delivery to the periodontal lesions in established murine model via Pg-LPS induction. *J. Oral Pathol. Med.*44, 538–542 (2015). https://doi: 10.1111/jop.12269.

28. Scanu, A. *et al.* OP0172 Lps-induced periodontitis promotes arthritis development in mice. in *THURSDAY, 14 JUNE 2018* 136.1-136 (BMJ Publishing Group Ltd and European League Against Rheumatism, 2018). doi:10.1136/annrheumdis-2018-eular.2522. https://doi: 10.1007/s10753-019-00975-6.

29. Lopes, D. E. M. *et al.* Inhibition of 5-Lipoxygenase (5-Lo) Attenuates Inflammation and Bone Resorption in Lipopolysaccharide (Lps)-Induced Periodontal Disease. *Journal of Periodontology* 1–18 (2017) doi:10.1902/jop.2017.170210. https://doi: 10.1902/jop.2017.170210.

Clinical Update

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Pathway of care in osteonecrosis of the jaw

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Abstract

This article is aimed to inform health care professionals in the prevention, diagnosis and management of the osteonecrosis of the jaw (ONJ). The content of this article is based on clinical experience and evidence found in the medical literature. ONJ is a site-specific osseous pathology with many possible etiologies which include head and neck radiotherapy and medication such as bisphosphonate or denosumab. Predisposing factors are bone involving invasive dental procedures. It is a clinical diagnosis which can be painful or asymptomatic. Plain radiography, cone beam computer tomography and magnetic resonance imaging may play a role in definitive diagnosis. Periodic dental examination with dental panoramic radiograph may be recommended for patients before embark on antiresorptive, antiangiogenic drugs or head and neck radiotherapy. In such a patient conservative dental treatments like endodontics are more preferred instead of extractions. The management includes minimal surface bony debridement and appropriate antimicrobial therapy to prevent secondary bacterial infection and osteomyelitis. If surgery is indicated in severe osteonecrosis of the jaw, suspension of bisphosphonate should be considered by assessing risk versus benefits.

Key words: Osteonecrosis of the jaw, radiotherapy, bisphosphonate, denosumab, radiotheraphy, invasive dental procedures.

Introduction

Osteonecrosis of Jaw a clinical entity with many possible etiologies. It is a site-specific osseous pathology that has been described in the literature since the 19thcentury[1].The possible etiologies of the ONJ include head and neck radiotherapy and prolonged treatment with either bisphosphonate or denosumab[1,2]. The risk or predisposing factors include dental extractions, periodontal disease, edentulousness, trauma from ill-fitting dentures and bone involving invasive dental procedures[1]. In addition, malignancies, concomitant chemotherapy or corticosteroids and infections also play a role in the causation of this condition[1, 3].In 2014 the American association of oral maxillofacial surgeons (AAOMS) recommended changing the name of bisphosphonate related ONJ to medication related ONJ due to the increasing number of cases resulting from medications such as antiresorptives (bisphosphonates and denosumab) or antiangiogenic (bevacizumab) treatment[4].The pathogenesis of ONJ involves the ramifications of hypercoagulability, diminished arterial flow, increased intravenous pressure and ultimate osseous hypoxia[1].In contrast, radiation therapy causes endothelial cell damage and direct killing of bone cells leading to radiation induced osteonecrosis known as osteoradionecrosis. Studies have shown that the combination of risk factors, compared to a single risk factor, leads to a higher incidence for ONJ[5,6]. ONJ has been seen in patients with malignancies including multiple myeloma, breast, prostate and thyroid

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cancer[7]. Among those multiple myeloma has the highest risk.

Denosumabis a new antiresorptive used in the treatment of skeletal related malignancies and osteoporosis. Denosumab related ONJ is more aggressive compared to bisphosphonate related ONJ[7]. Denosumab related ONJ can occur following dental extractions or merely due to periodontal disease. Within weeks the ONJ progresses fast with widespread suppuration and tooth mobility and images reveal bone destruction in combination with periosteal reaction due to bony infection.

Diagnosis

ONJ is a clinical diagnosis made after visual inspection when a non-healing extraction socket with exposed necrotic bone is seen in the maxilla or mandible for at least 8 weeks[2, 8]. The condition can be painful or may be asymptomatic. The lesions becomes symptomatic when there is a concurrent infection or trauma to the adjacent bone or tissue opposing the lesion.[1] ONJ usually manifests with pain, soft tissue swelling and inflammation, loosening of previously healthy teeth and purulent discharge from the exposed bone in the mandible or, much less often, the maxilla with intraoral or extra oral fistulae[8]. Lesions appear commonly at the site of previous tooth extractions or any other bone involving dental procedures, but may occur spontaneously. Heaviness, numbness and dyesthesia in the affected area are not uncommon[1]. Plain radiography, Cone beam computed tomography , Computed tomography and Magnetic resonance imaging can confirm the diagnosis of ONJ[9, 8, 10]. Bone lesion of ONJ may appear less or more radiodense than healthy bone, mimicking bone metastasis[1].Dental panoramic tomogram (DPT),intra oral periapical radiograph (IOPA) can demonstrate subtle bone alterations. The initial bone changes are difficult to identify, but in advanced stages the surface bone breaks down with radiolucent changes. When malignancy cannot be ruled out by imaging, biopsy should be performed. Microbial cultures are recommended to identify secondary infections and actinomyces has been detected in many instances[1,11].

The osteoradionecrosis of the jaw is detected when bone in the radiation field is exposed for at least 2 months in the absence of local neoplastic disease[12]. It is characterized by devitalized and exposed bone through the overlying skin or mucosa, most common in the mandible due to less vasculature. The majority (70- 94%) of cases occur within the first 3 years after radiation therapy due to hyper- fractionated irradiation regimen (high total dose 6000-7000cGy)[12]. The risk factors of osteoradionecrosis of the jaw include pre–irradiation or post–irradiation dental extractions, poor oral hygiene with periodontitis, long standing smoking and alcohol intake.

Prevention

Patients planning to be on either bisphosphonates or denosumab are recommended to have periodic dental examinations with DPT to detect periodontial health[1].Health care workers should be educated to carry out conservative dental treatment like root canal treatments instead of tooth extractions, whenever possible. When bisphosphonate therapy can be delayed, oral hygiene should be optimized by implementing full mouth scaling, restoration, correction of ill-fitting prostheses and extractions and sufficient time should be allowed for healing before initiating bisphosphonates. Risk versus benefits of delaying bisphosphonate therapy has not been proven to date. The decision to delay treatment before optimizing oral health should be taken jointly by treating physician or oncologist in consultation with oral and maxillofacial surgeon or other dental specialist[1].Routine prophylactic antibiotics are not recommended for patients undergoing routine dental treatment before starting bisphosphonate. However, patients previously identified to have prophylactic antibiotics before invasive dental procedures for other reasons such as indwelling venous catheters, previous endocarditis, cardiac value abnormalities or artificial heart valves should

be treated accordingly[1, 13]. Patients should be educated to undergo regular dental assessments, follow proper oral hygiene practices and seek timely medical advice. The frequency of dental assessments should be 3- 4months, but may vary based on the dental status of the patient.

Patients currently on either bisphosphonates or denosumab must maintain proper oral hygiene to minimize the risk of dental and periodontal infections. Full mouth scaling should be performed carefully without injuring the adjacent gingival tissue. Removable dentures should be examined to prevent possible trauma to underlying soft tissue or risk of infections due to poor denture hygiene. Steps should be taken to avoid invasive treatment involving bone and take conservative measures such as endodontic treatments, whenever possible. Coronal amputation after root canal treatment can be established to prevent tooth extractions in patients who are planning to undergo radiotherapy or bisphosphonate therapy. Elective surgeries like dental implant should be avoided while on treatment. Dental therapies and procedures which require bone healing should be completed before initiating bisphosphonate therapy[1,8,2]. Instructions should be given to have regular dental checkups and maintain good oral hygiene. Frequent dental assessments and conservative dental management are suggested for patients who are vulnerable to get ONJ.

Management

The management of ONJ involves nonsurgical approaches like minimal surface bony debridement. It should be performed to minimize sharp, rough bone surfaces and reduce risk of trauma to the surrounding soft tissue. Biopsy is not recommended as it can aggravate the bone damage. If a biopsy is performed to detect metastasis, a specimen should be submitted for microbial analysis to detect infections [11]. Thin vinyl vacuum formed mouth guard or thin acrylic stent can be used to keep the necrotic area hygienically and protect from further damage[1]. Well-fitting dentures can be worn under supervision and instruction should be given to clean and remove the dentures at night. Temporary soft tissue liners can be used to adjust ill-fitting dentures. Antibiotics can be used to protect from secondary infections and osteomyelitis. Appropriate cultures and antibiotic sensitivity tests should do to determine appropriate antimicrobial therapy.

Suggested pharmacologic treatment (until cultures and sensitivity studies are available).

- Amoxicillin - 500 mg every 8 hours for 7 to 10 days, then bd for maintenance OR
- Patients with penicillin allergy-Clindamycin 150 to 300 mg qds, vibramycin100 mg qds, erythromycin 500 mg tds OR
- Azithromycin 500 mg PO × 1 on day 1; 250 mg PO qds on days 2 to 5
- Antifungals[1]

Nystatin oral suspension - 5 to 15 mL qds or 100,000 IU/mL
Clotrimazole - 10 mg tds 5×/day for 7 to 10 days
Fluconazole - 200 mg initially, then 100 mg qds

Oral rinses such as 0.12% chlorhexidine gluconate or minocycline hydrochloride in periodontal pockets has a proven efficacy. Patients should be reviewed every 3 months or more frequently according to the severity of disease. Temporary soft tissue liners should be monitored once in 3 months for potential risk of tissue damage. Hyperbaric oxygen is not proven to be effective in managing ONJ according to the new studies[15]. Some studies, however, have demonstrated good prognosis with hyperbaric oxygen in patients with osteoradionecrosis and also beneficial as a prophylactic treatment prior to dental extractions for the patients who have received previous radiotherapy[5,15]. Medical management with pentoxyfylline and tocopherol has been shown to be effective in osteoradionecorsis according to the literature[12]. Pentoxyfylline is a hemorrheologic agent which increases blood flow to the affected microcirculation and enhance tissue oxygenation.

Tocopherol has activity of vitamin E, which is a fat soluble antioxidant.[12] If surgery is indicated in severe ONJ, suspension of bisphosphonate should be considered by assessing risk versus benefits. The rate of success of conservative management varies between 20-50% and only some cases progress to chronic ONJ with complications[7]. Some physicians recommend interrupting bisphosphonate therapy in those receiving such therapy and requiring a dental procedure[2]. The discontinuation of denosumab gives significant improvement of symptoms, in contrast to a bisphosphonate. Severe cases can be managed with sequestrectomy, saucerization and segmental resection and free flap reconstruction[1, 2].

Conclusion

Bisphosphonates are beneficial in cancer patients reducing morbidity and bone metastasis. The overall risk of developing ONJ is low in both cancer patients and those with osteoporosis. Optimizations of oral health before commencing treatment and close supervision during treatments are recommended in order to prevent and early diagnosis of ONJ. Close coordination between treating physician and oral surgeon/dental specialist is required to prevent this condition.

References

1. Ruggiero BS, Gralow J, Marx RE, Hoff AO. Practical Guidelines for the Prevention , Diagnosis , and Treatment of Osteonecrosis of the Jaw in Patients With Cancer. *J Oncol Pract*. 2006;2(1).

2. Khan A, Morrison A, Cheung A, Hashem W, Compston J. Osteonecrosis of the jaw (ONJ): diagnosis and management in 2015. Published online 2015. doi:10.1007/s00198-015-3335-3.

3. Assouline-dayan Y, Chang C, Greenspan A, Shoenfeld Y, Gershwin ME. Pathogenesis and Natural History of Osteonecrosis. 2002;32(2):94-124. doi:10.1053/sarh.2002.33724.

4. Dibart JP. Osteonecrosis of the Jaw. *Pract Osseous Surg Periodontics Implant Dent*. Published online 2013:17-18. doi:10.1002/9781118785652.ch3.

5. Surg JOM. New Foundations in Understanding Osteonecrosis of the Jaws. Published online 2004:125-126. doi:10.1016/j.joms.2003.11.009.

6. Cancer ACOF. Selected topics. Published online 1982:251-253.

7. Kyriakidou E, Badr M, Atkins S, Harrison S. Case Series Denosumab-associated Osteonecrosis of the Jaw ; A Case Series and Literature Review. 2016;9(4).

8. Aliya A Khan, Archie Morrison, David A Hanley, Dieter Felsenberg, Laurie K McCauley et al, Diagnosis and Management of Osteonecrosis of the Jaw: A Systematic Review and International Consensus. https://asbmr.onlinelibrary.wiley.com/doi/10.1002/jbmr.2405.

9. Remy H Blanchaert, Jr, DDS M. No TitleBisphosphonate-Related Osteonecrosis of the Jaw. https://emedicine.medscape.com/article/1447355-overview.

10. Berg BI, Mueller AA, Augello M, Berg S, Jaquiéry C. Imaging in patients with bisphosphonate-associated osteonecrosis of the jaws (MRONJ). *Dent J*. 2016;4(3):1-14. doi:10.3390/dj4030029.

11. Ruggiero SL. Guidelines for the diagnosis of bisphosphonate-related osteonecrosis of the jaw (BRONJ). *Clin Cases Miner Bone Metab*. 2007;4(1):37-42. https://pubmed.ncbi.nlm.nih.gov/22460751.

12. Fan H, Kim SM, Cho YJ, Eo MY, Lee SK,

Woo KM. New approach for the treatment of osteoradionecrosis with pentoxifylline and tocopherol. Published online 2014:1-10.

13. Hellstein JW, Adler RA, Edwards B, et al. Managing the care of patients receiving antiresorptive therapy for prevention the American Dental Association Council on Scientific NAME GENERIC NAME DOSAGE MANUFACTURER. *J Am Dent Assoc*. 2011;142(11):1243-1251. doi:10.14219/jada.archive.2011.0108.

14. Nicolatou-Galitis O, Schiødt M, Mendes RA, et al. Medication-related osteonecrosis of the jaw: definition and best practice for prevention, diagnosis, and treatment. *Oral Surg Oral Med Oral Pathol Oral Radiol*. 2019;127(2):117-135. doi:10.1016/j.oooo.2018.09.008.

15. Cancer N. The Use of Hyperbaric Oxygen for the Prevention and Management of Osteoradionecrosis of the Jaw : A Dana-Farber / Brigham and Women ' s Cancer Center Multidisciplinary Guideline. Published online 2017:1-8.

Case Report

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Comprehensive oral care to a child with continuation of early childhood caries: A case report

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Abstract
Early childhood caries (ECC) is a major health concern that affects the oral health of infants and children negatively. Maxillary incisors are affected first followed by molars, and due to the protective nature of the saliva, the mandibular incisors are often spared. When left untreated, the quality of life of the child could be seriously affected by severe pain and discomfort. The physical appearance and structural loss caused by ECC affects aesthetics, leads to compromised mastication, mispronunciation of labiodental sounds, and also causes psychological trauma to the child. Furthermore, untreated caries in primary dentition have an impact on the permanent dentition and would result in enamel defects of the permanent successor teeth. The following case describes comprehensive management of a child patient with continuation of early childhood caries.

Key words: Early childhood caries, preventive strategies, rehabilitation.

Introduction

Dental caries is a global public health challenge and the most common chronic infectious disease among young children[1]. It is affecting 60-90% of school children and the vast majority of adults in most industrialized countries[2]. Early Childhood Caries (ECC) is defined as the presence of one or more (non cavitated or cavitated) decayed, missing (due to caries) or filled tooth surfaces in any primary tooth in a child 71 months of age or younger. Children with atypical, progressive, acute or rampant patterns of dental caries are referred to as severe ECC (S-ECC)[3].

ECC is a multifactorial disease and risk factors are population specific. In different populations ECC is associated with low parental education, low socioeconomic status, dietary and feeding habits, prolonged breastfeeding, delay in starting oral hygiene practices and not using fluoridated toothpaste. ECC is largely preventable by early detection. Identification of individual risk factors, parental counseling and education, preventive care procedures such as topical fluoride application may diminish the progressive nature of the disease and improve the quality of life of the individuals.

The present case report describes the task of treating a patient reported to the Division of Pediatric Dentistry, Faculty of Dental Sciences, University of Peradeniya, Sri Lanka suffering from severe pain on posterior right lower teeth presenting with multiple decayed teeth.

Case Report

A 6 1⁄2-year- old boy presented with the complaint of pain in relation to lower right back teeth and lingually erupting lower anterior permanent teeth. According to his mother intermittent episodes of pain had been present for the past couple of months and it was relieved when gargling with

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salt water, but its severity has progressively increased and night pain also developed recently. His past medical history revealed tricuspid regurgitation (TR) in echocardiogram but he was clinically fit and SABE (SubAcute bacterial Endocarditis) prophylaxis was not recommended prior to dental treatment. He had no experience in dental treatments and this was his first experience at a dental clinic. Fluoridated dentifrice was being used since the boy was two years of age and he brushes his teeth twice a day before breakfast and after dinner. In the anamnesis and dietary diary, the patient is a frequent chocolate consumer but neither toffee nor biscuits. He had bottle fed at night until the age of three years. He is the youngest in the family and his father is a businessman while mother is a housewife.

On general examination his physical and mental development was age appropriate. Intra oral examination revealed that he had full complement of primary teeth except 71 with newly erupting mandibular permanent central incisors lingual to the primary predecessors. Maxillary anterior teeth 51, 52, 61 and 62 had moderately deep dentinal caries. All maxillary and mandibular deciduous molars showed different levels of caries. Caries of 54, 84, and 85 were extended into the pulp while initial dentinal decay could be identified in 55, 64, 65 and 74. The root portions of badly damaged 75 were retained. Pulp polyp was present on 85 while gingival polyp was found extending from distal side of 84. On percussion 54, 84 and 85 showed tenderness. Accordingly his dmft index (decayed, missing, filled teeth) received a high value of 12. Following Clinical and radiographic evaluation the patient was diagnosed with continuation of severe early childhood caries (S-ECC) (Figure 1, 2 and 3).

Figure 1. Pre-operative intra oral view of anterior teeth in occlusion

As the emergency management, pain and infection in relation to 84 and 85 were controlled. Following that, habit intervention, oral prophylaxis with performing full mouth scaling was performed. Application of fluoride gel (1.23% Acidulated phosphate fluoride) was included into the preventive measures.

Extraction of over retained 81 was performed and facilitated proper alignment of the lingually erupting permanent successors. Stabilization of dental caries with resin composite, pulp therapies for 54, 84 and 85 were completed followed by

Figure 2. Pre-operative occlusal view of upper and lower teeth

stainless steel crowns. Retained root of 75 was extracted and crown and loop space maintainer was provided (Figures 4 and 5). Finally, to improve the anterior aesthetics 51, 52, 61 and 62 were restored with light cure composite (LCC) resin and patient and parents were highly satisfied with the final aesthetic outcome. (Figure 6)

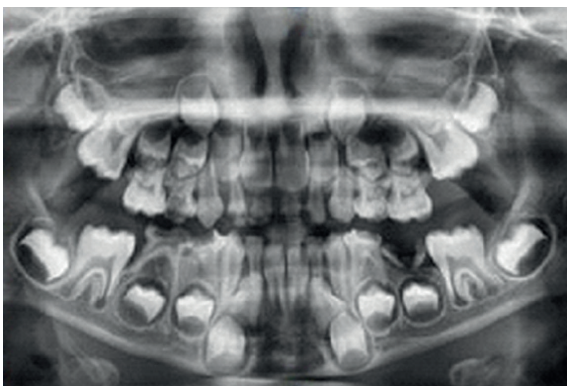

Figure 3. Preoperative dental panoramic radiograph

Maintenance phase was initiated with subsequent review appointments in 1 month and 3 months' time period and each and every recall visits, and oral hygiene measures were reinforced. Dietary advice was given and assessed the success of restorations and risk of development of new caries.

Discussion

Dental caries is a worldwide epidemic, multifactorial, transmissible, chronic infectious oral disease caused primarily by the complex interaction of cariogenic oral flora with fermentable dietary carbohydrates on the tooth surface over time[4]. It is a preventable disease with evidence-based preventive methods. In fact, the WHO oral health report-2005[2] noted that the prevalence and incidence of dental caries can be

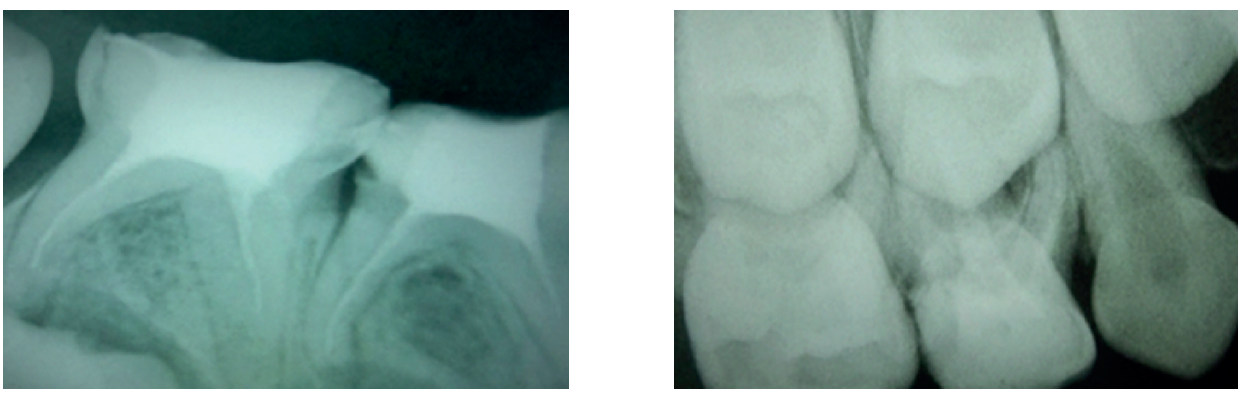

Figure 4. Post operative IOPA radiographs of 84, 85 and 54

Figure 5. Intraoral view of upper and lower arches following cementation of SSC on 54, 84, 85 and crown and loop space maintainer on 74

Figure 6. post operative view of upper and lower teeth in occlusion

controlled by the joint action of the community, professionals and individuals. The incidence and prevalence of dental caries among children have declined in industrialized countries over the past two decades, while developing countries have experienced an increasing trend[5]. Therefore, dental caries has become a significant public health problem among children especially in developing countries like Sri Lanka and lack of programs to promote oral hygiene among pre-school children and wrong feeding factors may be contributing for the high prevalence of caries in Sri Lanka[6].

Early Childhood Caries (ECC) is a virulent type of dental caries that develops on smooth surfaces, progressing rapidly in those who are at high risk. Its effects on primary dentition begin soon after the eruption of teeth. Diet and feeding practices play an important role in acquisition of infection and development of ECC[7]. Factors such as frequent high sugar consumption and prolonged bottle feeding may be the causative factors for ECC in this case. ECC has socio-economic implications and many parents underestimate the importance of the primary dentition and neglect getting treatment until the lesions cause pain and discomfort[8]. The late presentation of this child with continuation of ECC also may be due to the same reason mentioned above.

Safe and effective treatment for dental caries require proper understanding and modification of the child's and family's response and attitude towards oral care. Hence, behavior management plays a main role in this aspect and main goals are to establish communication, alleviate fear and anxiety, deliver quality dental care, build a trusting relationship between clinician and child/ parent and promote the child's positive attitude towards oral health care[9].

Dental caries is a preventable disease and it can be controlled and even potentially reversed during its early stage. Preventive input should be customized according to the age, behavior of the child, risk status and extent of the lesions as well as degree of parental education and cooperation. Preventive methods include plaque control, sensible diet with healthy food, limiting starchy and sugary snacks, use of fluoridated dentifrices, fluoride gel application on regular intervals and fissure sealing of unaffected posterior teeth. Professional treatment for ECC ranges from diet counseling to the prosthetic rehabilitation of the patient. Restorations are accomplished by glass Ionomer cement (GIC), composites, pulp therapy, followed by stainless steel crowns, while grossly decayed teeth were extracted followed by space maintainers[10].

Restorative management primarily depends on the extent of destruction of tooth structure, child's cooperation, available facility, aesthetic requirement and affordability for the formulated treatment plan. When the caries extends to pulp or close to pulp, symptoms of reversible or irreversible pulpitis may develop and such situations pulp therapy is indicated. Diagnosis of the status of the pulp should be confirmed by high quality radiographs as pulp sensibility tests have little value in children.

Deep carious lesions approximating the pulp but without signs or symptoms of pulp degeneration can be treated with indirect pulp capping technique[11]. Pulpotomy is recommended in a

primary tooth with a carious or mechanical pulp exposure but without evidence of radicular pathology. Pulpectomy is indicated when pulpal tissue is irreversibly infected or necrotic due to caries or trauma. Stainless steel crowns are useful following pulp therapy, teeth with developmental defects, fractured teeth, teeth with extensive wear and as abutments for space maintainers[12]. Following pulpectomies on 54, 84 and 85 preformed stainless steel crowns were cemented and achieved full coronal coverage on badly broken down teeth in this high caries risk patient. Space management can minimize the development of crowding in the permanent dentition. However the best space maintenance treatment is the preservation of an intact deciduous molars until its normal time of exfoliation[11]. Crown and loop space maintainer was preferred over band and loop as 74 had occlusal dentinal caries already restored with composite resin restoration and prevent risk of secondary caries in this patient. The maxillary anterior teeth were the last to be restored in this patient, because those were symptom free and their impaired aesthetic was a great source of motivation for the patient as well as parent to attend until the treatment was complete. The successful overall management depends mainly on reassessment at recall visits and maintenance phase. The importance of recall appointments were emphasized to the parents and cooperation and motivation may contribute to the successful outcome of the treatment and recall appointments were arranged in future according to the patient's risk for future diseases.

Conclusion

Early childhood caries is a multifactorial disease that has numerous biological, psychosocial, and behavioral risk factors. Although ECC can be arrested, early detection is of paramount importance. Treatment plans of ECC can be accomplished through preventive measures, intervention, recall and maintenance care. Also, a closer cooperation between health-care professionals and pediatric dentists is required for successful achievements.

References

1. Colak H, Dulgergil CT, Dalli M, Hamidi MM. Early childhood caries update; A review of causes, diagnosis and treatment. Joun of Nat Bio Med 2013; 4: 29 – 38.

2. Petersen PE, Bourgeois, Ogawa H, Estupinan-day S, Ndiaye C. The global burden of oral diseases and risks to oral health. Bulletin of the World health organization 2005; 83: 9.

3. American Academy of Pediatric Dentistry. Policy on early childhood caries (ECC) Classification, consequences and preventive strategies. The reference Manual of Pediatric Dentistry Chicago. American Academy of Pediatric Dentistry, 2020:79-80.

4. Roberson TM, Heyman HO, Swift EJ. Studivant's Art and Science of Operative Dentistry. 5th edition: Mosby Elsevier 2008.

5. Petersen PE. The World Oral Health Report 2003: continuous improvement of oral health in the 21st century-the approach of the WHO Global Oral Health Programme. Community Dent Oral Epidemiol 2003; 31(1): 3-23.

6. Ministry of Health and Indigenous Medicine, Sri Lanka. National Oral Health Survey of Sri Lanka (2002/2003). Dental caries 28-39.

7. Berkowitz RJ. Causes, treatment and prevention of early childhood caries. A microbiological perspective. Journal of Canadian Dent Asso 2003; 69: 304 – 307.

8. Misra S, Tahmassebi JF, Brosnan M. Early childhood caries – A review. Dent update 2007; 34: 556 – 564.

9. American Academy of Pediatric Dentistry. Guideline on Behavior Guidance for the pediatric dental patient. Reference Manual of Pediatr Dentistry 2014; 36: 179 – 191.

10. Mishra A, Pandey R, Pandey N, Jain E.A pedoprosthetic rehabilitation in patients with severe Early childhood caries (S-ECC).BMJ Case Reports 2013.;DOI: 10.1136/bcr-2013-010269.

11. Cameron AC, Widmer RP. Handbook of Pediatric Dentistry. 4th edition. Mosby Elsevier. USA, 2014.

12. Randall R. Preformed metal crowns for primary and permanent molar teeth: Review of the literature. Pediatric Dentistry 2002; 24: 489 – 500.

Mini Review

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Clinicopathological spectrum of oral myofibroma: A series of 15 cases with a literature review

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Abstract

Myofibroma is a tumour that occursmainly in children, involving any site in the bodywith a predilection to head and neck region.Studies that analyse clinicopathological presentations of oral myofibromas are sparse. Thus, the aim of the study was to present the clinicopathological spectrum of 15 new myofibromas involving the oral cavity.All the oral myofibromas of the present series were solitary lesions which occurred in children and adults with a mean age of 37 years at presentation. Pure intra-osseous involvement was observed in 14% of the cases. When soft tissues were involved, gingiva was the commonest site of occurrence (35%). Though majority of the tumours were indolent lesions that were clinically diagnosed as benign tumours or reactive lesions, there was a tendency of clinically diagnosing the lesion as a malignancy in the presence of ulceration. Histopathological and immunohistochemical profile of oral myofibromas were similar to those described for lesions elsewhere in the body. In conclusion, oral myofibromas may occur in adults as well as children and involve both soft and hard tissue. Larger myofibromas in the presence of ulceration may mimic malignancy and thus incisional biopsy is recommended for such lesions in order to avoid mismanagement.

Introduction

According to the WHO classification of soft tissue tumours, the term myofibroma refers to the solitary occurrence of benign neoplasms composed of contractile myoid cells arranged around thin-walled blood vessels. When these neoplasms occur multicentrically, the condition is termed myofibromatosis[1]. Comprehending the existing literature on myofibroma/ myfibromatosis is tedious as the lesionshave been reported previously under a variety of terms such as inflammatory myofibroblastictumour, infantile myofibromatosis, congenital generalized fibromatosis and congenital mesenchymal harmatomas; some of which are now considered as separate entities[1,2].

Back in the day myofibroma was considered as a lesion that occurred in infants. However, later it was found out that it can affect individuals in a wider age range. No matter the pattern of presentation (either, infantile, adult, solitary or multicentric), it is observed that this condition shows a predilection to oral and peri-oral structures with approximately one third of myofibromasoccuring in the head and neck region[3,4]. However according to previous literature, the epidemiology of myofibroma when seen in the oral mucosa shows some contrasting features with that occurring in other parts of the body. Generally, age at time of diagnosis of oral mucosa myofibroma ranged from birth to 70 years (mean 21.7 years), considerably higher than myofibroma in other parts of the

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body. Male:female ratio was 1:1.6, contrary to the male predominance in other parts of the body[2,5,6]. According to Brasileiro et al., 2010 myofibroma has a predilection to the submucosal or intramuscular tissue of the tongue, buccal mucosa and the lips[7].

This entity is diagnosed based on histopathological findings with immunohistochemical confirmation. Features including multinodular proliferation with a zoned configuration under low power, light- stained myofibroblasts arranged in short fascicles or whorls can be observed in both solitary and multicentric variants[2]. Immunohistochemistochemical investigations with smooth muscle actin (SMA) can aid in diagnosis, by confirming the tissue of origin. Histopathological differential diagnoses for myofibroma include neurofibroma, benign fibrous histiocytoma, leiomyoma, leimyosarcoma and haemangiopericytoma[8]. Myofibromas are managed with excision of the lesion. Less than 10% of solitary lesions are known to recur even with complete excision. However, factors that are predictive of recurrence have not been identified [1].

Method and Materials

Fifteen cases of myofibromasdiagnosed between 2007-2020 were retrieved from the archives of the Department of Oral Pathology, Faculty of Dental Sciences, University of Peradeniya, Sri Lanka to be reported in this case series. The cases selected accordingly are summarized in the following table (Table - 01)

Among these 10 cases are from females while 5 are males, with the age of occurrence ranging from 6 to 70 years. The male to female ratio in this data set is 1:2. And the mean age of presentation is 37 years. Allthese cases were presented as well-defined lesions with a firm consistency. Majority of the intraosseous cases presented demonstrated involvement of the mandible while some lesions were confined to the soft tissues.

Case	Clinical Presentation					Treatment	Follow up / Comments
	Age	Sex	Site	Size	Duration		
1	6	M	mandibular region	5	4	Excision	NED
2	15	F	36 region of gingiva	3	2	Excision	NED
3	15	F	Palate	2	0.5	Excision	NED
4	16	F	Angle of the mouth	1	1	Excision	NED
5	18	M	MI		MI	Excision	NED
6	25	F	Posterior mandible with intraosseous involvement		MI	Excision	NED
7	26	F	zygomatic buttress	1.5	MI	Excision	NED
8	34	M	lower lip	0.9	3	Excision	NED
9	44	F	Lower anterior gingiva	1	MI	Excision	NED
10	45	F	MI		MI	Excision	NED
11	55	F	floor of the mouth	3	6	Excision	NED
12	60	M	mandible	2	MI	Excision	NED
13	65	F	retromolar region	2.5	MI	Excision	The lesion recurred and was re-excised
14	65	F	gingiva	1	6	Excision	NED
15	70	M	buccal mucosa	0.6	5	Excision	NED

* MI - missing information; NED-No evidence of Disease

The largest size reported was 5 cm and the average size of a lesion based on this data set is 1.95cm. Information on the duration of these lesions were lacking, most probably owing to the fact that their discovery depends on the patient's awareness. All the lesions have been treated with excision. Only one case among the 15 cases mentioned have reported with a recurrence, which was also re-excised.

Histopathologic descriptions were provided for all 15 cases. They were reported to be unencapsulated in two of the cases while another two reported superficial ulceration (Fig1). Spindle cells with vesicular nuclei and eosinophilic cytoplasmsarrangedinto fascicles were observed (Fig 2). Cytological atypia was not seen. Scattered vascular spaces were noted in all cases (clefts like vasculature / staghorn like vasculature). Additionally, focal stromal hyalinization, dense collagen fibre bundles and myxoid regions (Fig 3) have been observed in few of the cases. Further, immunohistochemical investigations have revealed SMA (Smooth muscle actin) positivity (Fig 4) in all caseswhile, Desmin, CD34, CD99 and S100 were negative. Negative stains were done only for a few cases where neurofibromas and solitary fibrous tumour were considered in the histopathological differential diagnosis. All cases were managed with excision and only one reported recurrence following excision.

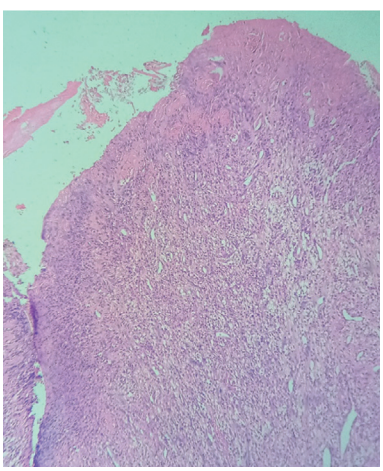

Figure 1. Myofibroma presenting with superficial ulceration (H&E x10)

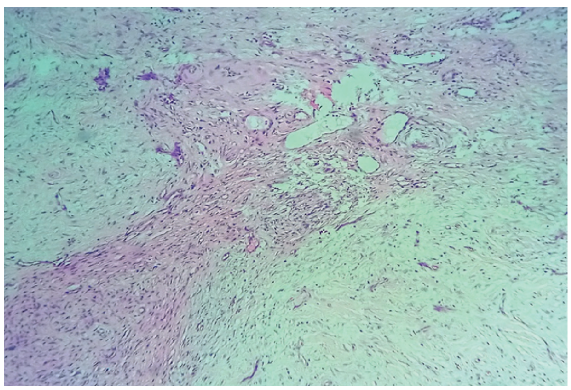

Figure 2. Myofibroma showing spindle cells arranged into short fascicles with myxoid change elsewhere contributing to zonal arrangement (H&E x10)

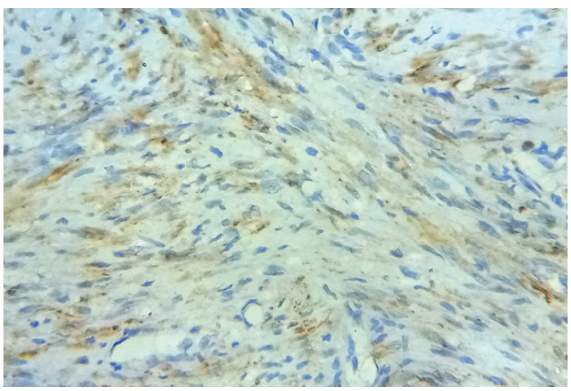

Figure 3. Myofibroma presenting with smooth muscle actin positivity with immunohistochemistry (IHC-SMA x10)

Discussion

The aim of this case series is to identify the clinicopathological spectrum of cases reported as myofibroma/ myofibromatosis by the Department of Oral Pathology, Faculty of Dental Sciences, University of Peradeniya and comparethose clinical and histopathological features with the existing literature. Published reports in literature is quite vivid in this regard and most case series included myofibroma/ myofibromatosis occurred in other parts of the body along withhead and neck area making the analysis of the oral counterpart difficult. However, in this literature review we haveconsidered only the single case reports

published in relation to the oral cavity and some of the case series confined to the oral cavity (table 2).

Earlier, myofibroma/myofibromatosis was considered as a pathological entity restricted to infancy. However, the present case series reveals that it can appear later in life, although 46% of the lesions were reported in the first three decades. It also must be noted that the duration of these lesions cannot be commented upon considering the indolent clinical appearance. They usually do not present with pain nor any other alarming symptoms. Therefore, the initial presentation of the lesion is biased with patient's awareness.

Generally, it is considered that myofibromatous lesions show a predilection to male gender. Male to female ratios ranging from 1.5:1 to 2:1 have been reported in literature. Nevertheless, studies with a female predilection also have been published (1.6:1)[2,(9-16]. Similarly, in the present case series, a female predilection with a ratio of 2:1 is observed. This could be a result of higher health awareness among females, considering the Sri Lankan general population. Based on these demographic findings, perhaps it is safer to expect myofibromairrespective of the age group nor a predilection to the sex of the patient.

The main clinical features observed in this case series include; well circumscribed lesion with a firm consistency. Although the colour of the overlying mucosa resembled that of a normal mucosa, there were instances where superficial ulceration was reported. With the clinical findings, differential diagnoses such as giant cell granuloma, fibrous epulis, gingival epulis, minor salivary tumour, dermoid cyst and squamous cell carcinoma (when ulceration was present) were considered.

The site of involvement can be intra osseous or soft tissues, and tongue was the most frequently affected site[2]. Therefore, myofibroma should be considered in the clinical differential diagnosis of masses of the oral soft tissues, especially in the tongue and buccal mucosa of children and adolescents[2]. The clinical differentials considered in majority of the soft tissue cases include, either a fibroma, salivary gland tumour or a neurofibroma and on a rare occurrence a squamous cell carcinoma[14].

Histopathology together with immunohis to chemistry is the current gold standard to diagnose a myofibroma. Histopathologicallymyofibroma presents as a biphasic spindle cell tumourarranged into short fascicles which necessitate it to be differentiated from other benign and low grademalignant spindle cell tumours such as neurofibroma, solitary fibrous tumour, leiomyoma, inflammatory myofibroblastic

Table 2. Summary of cases reported in the literature

Location	Mean age	Age range	Gender	Immunohis to chemistry performed				Follow-up period	Recurrences
				SMA +	Vimentin	HHF-35 +	Other positivity		
Maxilla (9,13)	14	01-57	F-8 M-1	8	`4	3	Ki 67 CD 34	`19.75	None
Soft tissues (14,17,18)	25.6	23-56	F-3	2				24	None
Mandible (10-13,15, 19-23)	13.8	3 weeks to 53	F-7 M-5	10	4		Calponin Ki 67 P53	14.33	None

tumour and low grade myofibroblastic sarcoma. Myofibromas are invariably positive for SMA. Histopathological characteristics of biphasic pattern and hemangiopericytomatous vasculature are clues to differentiate a myofibroma from leiomyoma despite both tumours showing SMA positivity. CD34 and CD99 are two markers used to differentiate solitary fibrous tumour from myofibroma as former tumour is generally positive for the immunohistochemical markers mentioned. S-100 is the marker used to differentiate tumours of neural origin from myofibroma. Thus, immunohistochemical investigations play a main role in differentiating myofibroma from other spindle cell lesions.

There are no substantial histopathological differences between myofibroma in the oral soft tissues and rest of the body. However, it is noted that necrosis and calcification is less commonly seen in oral myofibromatous lesions. It is a feature that is known to associate with spontatneous regression by apoptosis. Therefore, the absence of necrotic features may point out a reduced chance of spontaneous regression.The treatment of choice in majority of the cases was surgical excision if not regressed clinically. Prognosis is excellent in solitary myofibromas. However, only the myofibromatosis with involvement of internal organs/visceral involvement show poor prognosis[11].The summary table indicates that the follow-up period of the studies included were ranging from 14 to 24 months, and these cases were disease free during this period[9,11-15,18-20,22]. However, the recurrence rates for myofibromas are quite low, ranging from 0% to 12.5%. Recurrences can be attributed to tumors with difficult surgical access or incomplete removal. Smaller lesions can regress spontaneously, and observation might be warranted in those cases[24].

In conclusion, oral myofibromas may occur in adults as well as children and involve both soft and hard tissue. Larger myofibromas in the presence of ulceration may mimic malignancy and thus incisional biopsy is recommended for such lesions in order to avoid mismanagement.

References

1. Teng XD. World Health Organization classification of tumours, pathology and genetics of tumours of the lung. Zhonghuabing li xue za zhi Chinese journal of pathology. 2005;34(8):544–6.

2. Vered M, Allon I, Buchner A, Dayan D. Clinico-pathologic correlations of myofibroblastic tumors of the oral cavity. II. Myofibroma and myofibromatosis of the oral soft tissues. Journal of Oral Pathology and Medicine. 2007;36(5):304–14.

3. Gardner DG. Oral a n d maxillofacial pathology. Pathology. 1996;82(6):660–9.

4. Chung, E. B. Enzinger FM. Infantile Myofibromatosis. 1980;(August 1979).

5. Rubin BO, Bridge JA. Myofibroma/ myofibromatosis. In: Fletcher CD, Unni KK, Mertens F, eds. WHO classification of tumors. Pathology and genetics. Tumours of soft tissue and bone. Lyon: IARC Press, 2002; 59–61.

6. Myofibroma, solitary and multicentric (myofibromatosis). In: Kempson RL, Fletcher CD, Evans HL, Hendrikson MR, Sibley RK, eds. Atlas of tumor pathology. Third series, Fascicle 30. Tumors of the soft tissues. Washington, DC: Armed Forces Institute of Pathology, 2001; 64–7.

7. Brasileiro BF, Martins-Filho PRS, Piva MR, Da Silva LCF, Nonaka CFW, Miguel MCDC. Myofibroma of the oral cavity. A rare spindle cell neoplasm. Medicina Oral, Patologia Oral y CirugiaBucal. 2010;15(4).

8. Beham A, Badve S, Suster S, Fletcher CDM. Solitary myofibroma in adults:

clinicopathological analysis of a series. Histopathology. 1993;22(4):335–41.

9. Cunha JLS, Rodrigues-Fernandes CI, Soares CD, Sánchez-Romero C, Vargas PA, Trento CL, et al. Aggressive Intraosseous Myofibroma of the Maxilla: Report of a Rare Case and Literature Review. Head and Neck Pathology. 2021 Mar 1;15(1):303–10.

10. Mathew PC, Kanitkar R, Ramaiah A. Gossypiboma after Le Fort I osteotomy that manifested as a lesion of the maxillary sinus. Vol. 57, British Journal of Oral and Maxillofacial Surgery. Churchill Livingstone; 2019. p. 706–7.

11. Narayen V, Ahmed SA, Suri C, Tanveer S. Myofibroma of the gingiva: A rare case report and literature review. Vol. 2015, Case Reports in Dentistry. Hindawi Publishing Corporation; 2015.

12. Dhupar A, Carvalho K, Sawant P, Spadigam A, Syed S. Solitary Intra-Osseous Myofibroma of the Jaw: A Case Report and Review of Literature. Children. 2017 Oct 24;4(10):91.

13. Lopes RN, de Abreu Alves F, Rocha AC, Suassuna TM, Kowalski LP, de Castro JFL, et al. Head and neck solitary infantile myofibroma: Clinicopathological and immunohistochemical features of a case series. Acta Histochemica. 2015 May 1;117(4–5):431–6.

14. Sharifi A, Sen P, Lonsdale R, Pawaroo D. A rare case of a myofibroma presenting on the tongue. Annals of the Royal College of Surgeons of England. 2015;97(4):e57–60.

15. Satomi T, Kohno M, Enomoto A, Abukawa H, Fujikawa K, Koizumi T, et al. Solitary myofibroma of the mandible: an immunohistochemical and ultrastructural study with a review of the literature. Medical Molecular Morphology. 2014 Sep 1;47(3).

16. Haspel AC, Coviello VF, Stevens M, Robinson PG. Myofibroma of the mandible in an infant: Case Report, Review of the Literature, and Discussion. Journal of Oral and Maxillofacial Surgery. 2012;70(7):1599–604.

17. Andreadis D, Epivatianos A, Samara A, Kirili T, Iordanidis F, Poulopoulos A. Myofibroma of the oral mucosa: A case report. Medical Principles and Practice. 2012 Apr;21(3):288–91.

18. Brasileiro BF, Martins-Filho PRS, Piva MR, da Silva LCF, Nonaka CFW, Miguel MCDC. Myofibroma of the oral cavity. A rare spindle cell neoplasm. Medicina Oral, Patologia Oral y CirugiaBucal. 2010 Jul;15(4).

19. Atarbashi-Moghadam S, Lotfi A, Shahrabi-Farahani S, Atarbashi-Moghadam F, Author C. Myofibroma as a Rapidly Growing Gingival Mass in a 4-year-old Boy: a Case Report. Vol. 19, J Dent Shiraz Univ Med Sci. 2018.

20. Chattaraj M, Gayen S, Chatterjee RP, Shah N, Kundu S. Solitary myofibroma of the mandible in a six-year old-child: Diagnosis of a rare lesion. Journal of Clinical and Diagnostic Research. 2017 Apr 1;11(4):ZD13–5.

21. Chinta M, Sai Sankar AJ, Gantha SN, Kanumuri PK, Kanumuri K. Tumour that challenged diagnosis: mandibular myofibroma. 2016; Available from: http://group.bmj.com/group/rights-licensing/permissions.

22. Aiki M, Yoshimura H, Ohba S, Kimura S, Imamura Y, Sano K. Rapid growing myofibroma of the gingiva: Report of a case and review of the literature. Vol. 72, Journal

of Oral and Maxillofacial Surgery. W.B. Saunders; 2014. p. 99–105.

23. Daniel É, Rodrigues R, Ferreira De Oliveira EM, da Costa Ribeiro R, Passador-Santos F, Rodrigues De Matos F, et al. Myofibroma of the oral cavity mimicking a non-neoplastic inflammatory process: an unusual report. Journal of oral Diagnosis. 2020;

24. Yong-Moon Lee, Seung-Myoung Son, Kyoung Won Kim1, Ok-Jun Lee. Solitary Myofibroma of the Adult Mandible: A Case Report and Review of Literature. *J PatholTransl Med.* 2014;48 (4): 307-310.

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These include reports on current topics, modified techniques. New materials, practice management etc. Interesting results from routine, clinical work or laboratory investigations also may be accepted. They should not exceed 1500 words (excluding the abstract and references) and 10 references.

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Acknowledgements
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Few examples of correct forms of references are given below.

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Bartlett IG, O'Keefe P. The bacteriology of the perimandibular space infections. J Oral Surg

1979; 37(6): 407-9.

Electronic journal article
Lemanek K. Adherence issues in the medical management of asthma. J Pediatr Psychol [Internet]. 1990 [cited 2010 Apr 22];15(4):437-58. Available from: http://jpepsy.oxfordjournals. org /cgi / reprint / 15 / 4 / 437 (If available, mention the DOI number at the end of the URL)

Unpublished article
Barker DS. Lucas RB. Localised fibrous growth of the oral mucosa. J Dent Res 1965: in press.

Books and other monographs
Becker A. Orthodontic treatment of impacted teeth. 3rd ed. Oxford, United Kingdom: Wiley-Blackwell;2012

Chapter in an edited book
Boyde A. Amelogenesis and the structure of enamel. In: Cohen B. Kramer KH (eds). Scientific Foundations of Dentistry. London: William Heinemann Medical Books Ltd.; 1976.p 335-352.

Chapter in a non-edited book
Speroff L, Fritz MA. Clinical gynaecologic endocrinology and infertility. 7th ed. Philadelphia: Lippincott Williams and Wilkins; 2005. Chapter 29, Endometriosis; p.1103-33.

No author given
International statistical classification of diseases and related health problems, 10th revision, vol J. Geneva: World Health Organisation, 1992; 550--564.

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References: 1. Communication from TGA on 27 May 2005. Comparative dissolution data for Panadeine.
2. Boureau F, Joubert J, Lasserre V, *et al.* Double-Blind Comparison of An Acetaminophen 400 mg-Codeine 25 mg Combination Versus Aspirin 1000 mg and Placebo in Acute Migraine Attack. *Cephalalgia.* 1994;14(2):156-161. **3.** Adam EI. A Treatment for the Acute Migraine Attack. *Int J Med Res.* 1987;15:71-75.

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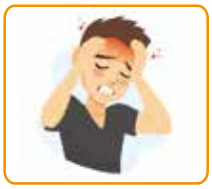

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