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Aims and Scope:

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

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CONTENTS

EDITORIAL

The Impact of COVID-19 Pandemic on Research

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Since the imposition of the Movement Control Order (MCO) by the government of Malaysia on 18th March 2020 due to the COVID-19 pandemic, local universities have been obedient and acted in line with the government's policy. The education sector at this moment was considered as non-essential activities, thus the university only operated at its minimal strength during the MCO. Most local university guidelines stated that staff was not allowed to be on campus except for those in critical services. In line with that, the postgraduate students or staff were eventually not allowed to enter the labs, with the exception only for those taking care of animal husbandry. With these, research activities were put on hold and thus research have been greatly impacted especially for those experimental-based researches that require physical experimentation in laboratory facilities. Similarly, community-based research was also being halted in this condition since no face-to-face data collection can be done during this period of time.

Reflecting on this situation, government funded-grants were given automatic extensions and this has certainly relieved the worries of most researchers. The Ministry of Higher Education (MOHE) has also come out with the initiative by inviting proposal submission through the Malaysia Greater Research Network System (MyGRANTS). This short-term grant should revolve around the public's lives during the MCO period and post-COVID-19, amounting to RM20,000 for each research (NewStraitsTimes, 2020). Some research facilities that work on COVID-19 from this point of time are then allowed to be opened, subject to the universities' approval.

MOHE in the press statement released on 27th May 2020 announced that the post-graduate students under research mode who require specific laboratory facilities or equipment to conduct research are allowed to resume their research on campus starting 1st June 2020. This decision was expected to affect 31,503 students in higher education institutions who need to carry out research in labs, design studios, or workshops using specialized equipment (Zolkepli, 2020). The research then has resumed back during the Recovery Movement Control Order (RMCO), but with stricter regulations and standard operating procedures.

Nevertheless, the COVID-19 pandemic has actually opened up doors for new research prospects, especially those revolving around COVID-19. Research groups on this research theme were immediately formed across the globe, and this has actually shows the concept of universal brotherhood and togetherness. The sharing of knowledge and findings since the pandemic outbreak has then occurred really fast. Databases like *'Wiley Covid-19 Resources'* (Wiley, 2020), *'NCBI SARS-CoV-2 Resources'* (National Center for Biotechnology Information, 2020), and the *'NIH Open-Access Data and Computational Resources to Address COVID-19'* (National Institute of Health, 2020) were created as open-sharing platforms specifically for resources pertaining COVID-19. In addition, reputable journal

publishers such as Elsevier (2020), Springer (2020), SAGE (2020), and PLOS (2020) have allowed fast track review process for those articles on this theme and this has actually helped the research progress on COVID-19.

The excellent progress of research made by researchers from all over the world has made potential vaccines' research and development much faster than usual. As of 28 October 2020, there are currently more than 100 COVID-19 vaccine candidates under development, and some are already in the human trial phase (WHO, 2020). In addition, the world has currently developed a significant number of diagnostic techniques employing the nucleic acid amplification tests or NAAT which include the reverse transcriptase-polymerase chain reaction (RT-PCR) to diagnose current COVID-19 infection as well as antigen testing (Caliendo & Hanson, 2020). From the expensive price of diagnostic technique with high reliability and accuracy, now the diagnosis can be made at a more affordable price with acceptable standard (BBC, 2020).

Researchers in the field of big data, public health, pure, applied, and medical sciences, as well as the social sciences from our local universities, government institutions, and also private companies, have made some excellent contributions to the research and innovations on COVID-19 (Aman, 2020; Arumugam, 2020). For instance, our Malaysian data scientist has created CoronaTracker, a one-stop platform that offers real-time data of confirmed COVID-19 cases and death across the globe, and at the same time, it provides global and local news updates on the COVID-19 (Arumugam, 2020). In addition, MY E.G. Services Bhd (myEG), the Malaysia's e-government service provider has developed a COVID-19 risk profiling system using the advanced artificial intelligence technology (Aman, 2020). Involvement of few government agencies including the National Security Council (NSC), the Ministry of Health (MOH), the Malaysian Administrative Modernisation and Management Planning Unit (MAMPU), the Malaysian Communications and Multimedia Commission (MCMC), and the Ministry of Science, Technology and Innovation (MOSTI) have innovated MySejahtera application software for monitoring the spread of COVID-19 ("MySejahtera," 2020). In addition, our local university has also made an excellent innovation by creating Hybrid Rapid Test Kit, acclaimed to have the same accuracy as the laboratory tests (Muzamir, 2020; Nasir, 2020).

Herein, until the world has resolved from this COVID-19 pandemic, there will always be uncertainties of the policies governing the conduct of research and this may greatly influence the trend of future research. We can perhaps speculate that the research on the COVID-19 theme will continue to be spearheaded in the future. We might also see an increase in the number of researches that will not involve physical experimental setup in laboratory facilities. It is also expected that the research that involves survey as the data collection instrument will be changed from the traditional face-to-face medium into the online medium. Regardless of whichever research designs that our local researchers advocate for in the future, hopefully, they can sustain in this challenging period.

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ORIGINAL ARTICLE

Pledge your teeth! The willingness of dental students to donate their teeth: A pilot study.

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Abstract

Extracted human teeth is the preferred choice for preclinical training as it simulates the clinical scenario as compared to artificial teeth. However, the increase in the number of dental undergraduates might cause an imbalance in the demand and supply of extracted human teeth. Not only that, extracted human teeth are also needed by researchers for the development of dentistry (e.g. dental pulp stem cells research). This study aimed to evaluate the socio-demographic and preclinical training factors associated with the willingness to donate teeth among dental students of a selected university in Malaysia. The results of this study show that the majority of students were willing to donate their teeth after extraction for educational purposes, followed by keeping their tooth, leaving it behind as clinical waste and donating it to research. Further research is recommended to determine the willingness of the community or public to donate their teeth to meet the demand for dental schools and researchers in Malaysia.

Keywords: teeth donation, preclinical training, Malaysia, dental students

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Introduction

The rise of dental schools in Malaysia has led to increasing demand for extracted human teeth required for preclinical training. There are currently thirteen government-funded and private universities in Malaysia offering either Bachelor of Dental Surgery (BDS) or Doctor of Dental Surgery (DDS). The local dental graduates in Malaysia has had a threefold increase in number throughout the years, from 208 graduates in 2007 to 654 graduates in 2016 as reported by the Malaysian Dental Council (2016).Therefore, there is no doubt that logically the demand for extracted human teeth has escalated tremendously over the years. Apart from dental students, researchers are also in need of human extracted teeth as there are a rising interest in dental pulp stem cells studies (Kabir *et al.*, 2014).

The preclinical training for dental students is crucial for the development of student's dexterity and comprehension of procedures needed to succeed in the clinic (Velayo *et al.*, 2014). Dental students commonly practice their preclinical skills on typodont teeth, artificial teeth or extracted human teeth (Smitha *et al.*, 2015). Extracted human teeth

are the preferred choice as compared to artificial teeth as it simulates the clinical situations (Kumar *et al.*, 2005; Lolayekar *et al.*, 2007). Typodont teeth pose no risk of cross-infection but are much more expensive (Smitha *et al.*, 2015). However, extracted human teeth is considered infective and classified as clinical specimens as they contain blood according to 'CDC Guidelines for infection control in dental health-care settings 2003', and should always be handled with precaution (Kohn *et al.*, 2003). Some studies recommended immersing teeth with 10% of formalin for five days, 5.25% of sodium hypochlorite for five days and autoclaving at 121°c, 15 lbs psi for 20 minutes as a successful method in disinfecting extracted human teeth (Dominici *et al.*, 2001; Sandhu *et al.*, 2012). Among resources for extracted human teeth in Malaysia include government dental clinics, private dental clinics or hospitals. To date, no studies has evaluated on the difficulty and the duration of time needed for dental students to acquire the total amount of extracted human teeth for their preclinical usage as this might highlight the necessity of promoting tooth donation to meet the demand for extracted human teeth.

The extraction of healthy teeth and their donation to dental pulp stem cells is well accepted by both patients and researchers alike (Le Breton *et al.*, 2015). Potential donors need to be adequately informed so that they can give consent freely (Le Breton *et al.*, 2015). When socio-demographic factors are taken into consideration among 500 patients at the Dental Care and Dental Teaching Centre in Jordon, it was found that those with higher education level are more likely to accept tooth donation. Other demographic variables such as age, gender, place of living and income have no statistically significant relationship with the willingness to donate teeth after extraction (Mortadi *et al.*, 2018).There are currently no known studies associating ethnic and religious beliefs to tooth donation.

Promoting teeth donation amongst the Malaysian citizens could be considered as an initiative to improve the imbalance of demand and supply of human extracted teeth for dental education and research. To create awareness on tooth donation in the Malaysian society or public, we think it is wise to determine whether the main stakeholders are willing to do the same. Our study aimed to evaluate factors involved in the acquisition of extracted human teeth for preclinical training and to determine whether or not dental students were willing to donate their teeth to meet the escalating demand of teeth for both research or preclinical usage.

Material & methods

A total of 257 dental students who had undergone preclinical training at the selected university participated in this study. Convenient sampling was used to include all students of the faculty from Year 2 to Year 5. Year 1 students were excluded as the preclinical training for most universities in Malaysia starts in Year 2. A response rate of 78% (n=257) out of 329 students was achieved. Ethics approval was granted by University Teknologi MARA Ethics Research Committee (REC/299/18).

The questionnaire consisted of 25 close-ended questions with three sections. Section 1 included socio-demographic characteristics such as gender, year of study, religion, ethnicity, and hometown. Section 2 were questions related to the acquisition of extracted human teeth for preclinical training. It investigated whether or not the students were aware of the need for finding extracted human teeth, the duration needed for teeth collection and the difficulty in obtaining a specific type of tooth. Section 3 was developed based on an existing validated survey instrument by Le Breton et

al. (2015) on thoughts of donation of teeth to science or research. Part of the questionnaire aimed to explore the students' attitude toward tooth donation, ownership of extracted tooth, and beliefs of students in donating theirs for research purposes. Furthermore, the questions were aimed to explore the students' ethical considerations in terms of obtaining consent for the tooth after extraction for research purposes. The questionnaire was then modified to include the thoughts of tooth donation after death. The survey underwent a content-validation process by two senior researchers to assess its suitability and to ensure the items could be understood and correctly interpreted by the intended respondents.

A questionnaire that used a multiple-choice grid was developed using Google Forms and emailed to the participants. The participants were informed about the aim of the study, and the participation of this study was voluntary.

The quantitative data were analysed using the Statistical Package for Social Sciences (SPSS) software program version 25.0 (IBM Corporation, Armonk, NY, USA). Further analysis was undertaken using the Chi-squared test (Phi and Cramer's) to determine the association between socio-demographic and preclinical training factors to the willingness to donate teeth. The significance value taken was $p<0.05$.

Results

Socio-demographic characteristics

A total of 257 responses (78%) out of 329 students were collected. The majority of responses belonged to female respondents (86.4%) and Year 4 dental students (31.5%). Socio-demographic characteristics of the participants were summarised in Table 1.

Table 1. Sociodemograhic characteristics of participants

Variable	n (%)
Year	
2	71 (27.6)
3	76 (29.6)
4	81 (31.5)
5	29 (11.3)
Gender	
Male	35 (13.6)
Female	222 (86.4%)
Hometown	
Johor	24 (9.3%)
Kedah	20 (7.8%)
Kelantan	29 (11.3%)
Melaka	17 (6.6%)
Negeri Sembilan	9 (3.5%)
Pahang	9 (3.5%)
Pulau Pinang	5 (1.9%)
Perlis	1 (0.4%)
Sabah	10 (3.9%)
Sarawak	6 (2.3%)
Selangor	68 (26.5%)
Terengganu	22 (8.6%)
Kuala Lumpur	14 (5.4%)
Labuan	1 (0.4%)
Putrajaya	-
Perak	22 (8.6%)
Ethnicity	
Malay	248 (96.5%)
Indian	-
Chinese	1 (0.4%)
Others	8 (3.1%)
Religion	
Muslim	251 (97.7%)
Hindu	-
Buddhist	-
Christian	6 (2.3%)
Others	-

Acquisition of extracted human teeth for preclinical training

Participants were asked questions relating to the awareness of the usage of extracted human teeth for preclinical training (Table 2). Students were made aware that they will be using extracted human teeth in their preclinical training before entering the dental course (73.2%) by their seniors (68.9%) in the faculty. Most of the students were informed during orientation week (37.4%) and the beginning of the semester 1 in Year 1 (36.6%). More than half of the students (52.1%) spent about 6 to 12 months to collect extracted teeth (Table 2).

Majority of students, 94.6% (n=243) experienced difficulty in finding a specific human tooth needed for their preclinical project (Table 3) and had their work delayed (95.7%) for not having the tooth necessary for a particular project especially molars (85.6%). Most of the extracted teeth collected were unsuitable for the preclinical projects (67.7%). To overcome their problem, most students (74.3%) asked their friends, seniors or juniors to donate some extracted teeth to them.

Attitude and belief towards ownership of extracted tooth and informed consent

When asked about the meaning of tooth to students, 97.7% of students believed their tooth as a part of them and 67.7% considered that their tooth still belongs to them after extraction (Table 4).

Table 2. Acquisition of extracted human teeth (n= 257)

Questions	Choices	n (%)
Did you know that extracted human teeth will be used as part of your preclinical training before entering this course?	Yes No	188 (73.2) 69 (26.8)
How did you get to know about the need of collecting extracted human teeth for your preclinical training?	Friend/s Family member/s Lecturer/s Senior/s in the faculty Relative/s Social media Other	32 (12.5) 15 (5.8) 18 (7.0) 177 (68.9) 4 (1.6) 4 (1.6) 7 (2.7)
Were you informed by any faculty member/lecturer on the need of extracted human teeth as part of your training?	Yes No	235 (91.4) 22 (8.6)
If Yes, when was this information conveyed to you?	During the interview (student selection process) During the orientation week Year 1 Semester 1.1 Year 1 Semester 1.2 Year 1 Semester 2.1	34 (13.2) 96 (37.4) 94 (36.6) 8 (3.1) 3 (1.2)
Duration of teeth collection	Below 6 months 6 months to 12 months 12 to 24 months No time at all. Senior dental students provided teeth collection	66 (25.7) 134 (52.1) 52 (20.2) 5 (1.9)

Table 3. Difficulties faced by students and how they managed to overcome the problem (n=257)

Questions	Choices	n (%)
Did you have any difficulty in finding a specific human tooth needed for your project?	Yes	243 (94.6)
	No	14 (5.4)
If Yes, which tooth of the below?	Incisors	20 (7.8)
	Canines	2 (0.8)
	Premolars	1 (0.4)
	Molars	220 (85.6)
Which of the following reasons contributed to the difficulty in finding teeth?	Limited dental clinics around my housing area.	12 (4.7)
	Many students were requesting for extracted human teeth from the same dental clinics.	67 (26.1)
	Friends/classmates refused to trade their collection of extracted teeth.	2 (0.8)
	Most of the extracted teeth collected were unsuitable for the preclinical projects.	174 (67.7)
	Did not bother to find the extracted human teeth myself and depend solely on other students' collection.	1 (0.4)
	Other	0 (0.0)
Does your work get delayed when you do not have the tooth needed for a particular project?	Yes	246 (95.7)
	No	11 (4.3)
If yes, how do you overcome this problem?	Ask from friends, seniors or juniors for extracted human teeth.	191 (74.3)
	Inform my lecturers, and hopefully, they could find a way to help.	1 (0.4)
	Just wait for the next batches of teeth collected from dental clinics.	13 (5.1)
	Offer money/cash to friends for an exchange of the tooth needed.	2 (0.8)
	Avoid attending the preclinical session provided.	0 (0.0)
	Work on other projects until the tooth needed is found.	38 (14.8)
	Other	1 (0.4)

Table 4. Students' attitude and belief towards ownership of extracted tooth and informed consent

Questions	Choices	n (%)
What does your tooth mean to you?	Nothing	6 (2.3)
	A part of me	251 (97.7)
Do you consider that your tooth still belongs to you after extraction?	Yes	174 (67.7)
	No	83 (32.3)
Would you like the practitioner to ask for your consent to conduct research on your extracted tooth?	Yes	227 (88.3)
	No	30 (11.7)
If yes, when?	Before surgery	203 (79.0)
	After surgery	24 (9.3)
Would you like to be informed about the research outcome?	Yes	223 (86.8)
	No	34 (13.2)
If yes, how?	On the phone	39 (15.2)
	Email	159 (61.9)
	By letter	25 (9.7)
If you give your tooth to science, do you consider that it still belongs to you or that it belongs to the researcher?	It will always be mine.	103 (40.1)
	It belongs to the researcher.	154 (59.9)
Who should inform you about the possibility of donating your tooth to science?	The surgeon.	176 (68.5)
	Other members of the health-care team.	81 (31.5)

Participants were also asked regarding the informed consent to research of their extracted tooth. More than half of the participants considered that the donated tooth belongs to the researcher (59.9%), whereas 40.1% of the respondents believed that the donated tooth belongs to them. The majority (88.3%) would like the practitioner to ask for their consent to conduct research on their extracted tooth. Of those who were willing to donate their extracted tooth for research, 79.0% participants preferred to sign the consent form before surgery, and they would like to be informed about the research involved (86.8%). An email was the preferred communication method to inform the donators about the research applied to their donated teeth. Moreover, 68.5% of participants preferred the surgeon to inform them about the possibility of donating their tooth to science.

Association of socio-demographic factors and willingness to donate teeth

Majority of students were willing to donate their tooth after extraction for educational purposes (49.8%) rather than for research (4.7%). However, most students were not willing to donate teeth after death (60%). There was no statistically significant association between gender, ethnicity and religion with the willingness to donate teeth after extraction and after death (Table 5 and 6).

Association between delayed preclinical work and difficulty in finding teeth with the willingness to donate teeth

About half the students (47.5%) who had difficulty in obtaining teeth for preclinical usage were willing to donate teeth for educational purposes after extraction but not after death (57.2%). Similar findings were noted for those who had their preclinical work delayed due to insufficient extracted human teeth. A total of 48.6% were willing to donate teeth for educational purposes followed by preferring to keep it (31.1%), leave it (11.7%) and donating it to science/ research (4.3%). Refer to Table 7 and 8.

Table 5. Association of socio-demographic variables and willingness to donate teeth after extraction (n=257)

Demo-graphic variables	Willingness to donate teeth after extraction						
	Prefer to keep it n (%)	Prefer to leave it n (%)	For science/ research n (%)	For educational purposes/ preclinical training n (%)	P-value	Chi-Square test	df
Gender							
Male	15 (5.8)	2 (0.8)	4 (1.6)	14 (5.4)	0.060	*7.396	3
Female	70 (27.2)	30 (11.7)	8 (3.1)	114 (44.4)			
Ethnicity							
Malay	79 (30.7)	32 (12.5)	12 (4.7)	125 (48.6)	0.433	**5.916	6
Chinese	1 (0.4)	0 (0.0)	0 (0.0)	0 (0.0)			
Others	5 (1.9)	0 (0.0)	0 (0.0)	3 (1.2)			
Religion							
Muslim	81 (31.5)	32 (12.5)	12 (4.7)	126 (49)	0.323	***3.483	3
Christian	4 (1.6)	0 (0.0)	0 (0.0)	2 (0.8)			

*2 cells (25%) have expected count less than 5. The minimum expected count is 1.63
** 8 cells (66.7%) have expected count of less than 5. The minimum expected count is .05
***4 cells (50.0%) have expected count of less than 5. The minimum expected count is .28

Table 6. Association of socio-demographic variables and willingness to donate teeth after death (n=257)

Demographic variables	Willingness to donate teeth after death				
	Yes	No	P-value	Chi-square test	df
Gender					
Male	13 (5.1%)	22 (8.6%)	0.703	*0.145	1
Female	90 (35%)	132 (51.4%)			
Ethnicity					
Malay	100 (38.8%)	148 (57.6%)	0.706	**0.697	2
Chinese	0 (0.0%)	1 (0.4%)			
Others	3 (1.2%)	5 (1.9%)			
Religion			0.733	***0.116	1
Muslim	101 (39.3%)	150 (58.4%)			
Christian	2 (0.8%)	4 (1.6%)			

*0 cells (0.0%) have expected count of less than 5. The minimum expected count is 14.03
**4 cells (66.7%) have expected count of less than 5. The minimum expected count is .40
***2 cells (50.0%) have expected count of less than 5. The minimum expected count is 2.40

Table 7. Association between delayed preclinical work and difficulty in finding teeth with the willingness to donate teeth after extraction of tooth

Delayed pre-clinical work	Willingness to donate teeth after extraction				P-value	Chi-square test	df
	Prefer to keep it	Prefer to leave it	For science/ research	For educational purposes/ preclinical training			
Yes	80 (31.1%)	30 (11.7%)	11 (4.3%)	125 (48.6%)	0.477	*2.488	3
No	5 (1.9%)	2 (0.8%)	1 (0.4%)	3 (1.2%)			
Difficulty in finding extracted teeth (pre-clinical)	Willingness to donate teeth after extraction						
Yes	78 (30.4%)	31 (12.1%)	12 (4.7%)	122 (47.5%)	0.484	**2.453	3
No	7 (2.7%)	1 (0.4%)	0 (0.0%)	6 (2.3%)			

*3 cells (37.5%) have expected count of less than 5. The minimum expected count is .51
**3 cells (37.5%) have expected count of less than 5. The minimum expected count is .65

Table 8. Association between difficulty in finding teeth and delayed preclinical work with the willingness to donate teeth after death

	Willingness to donate teeth after death				
	Yes	No	P-value	Chi-square test	df
Delayed preclinical work	99 (38.5%)	147 (57.2%)	0.797	*0.066	1
	4 (1.6%)	7 (2.7%)			
Difficulty in finding teeth	99 (38.5%)	144 (56.0%)	0.366	**0.816	1
	4 (1.6%)	10 (3.9%)			

* 1 cell (25.0%) 3 cells (37.5%) have expected count of less than 5. The minimum expected count is 4.41
**0 cells (0.0%) 3 cells (37.5%) have expected count of less than 5. The minimum expected count is 5.61

Discussion

Preclinical training is fundamental to ensure that dental students gain adequate experience before treating patients (Robberecht *et al.*, 2017). However, the rising number of dental schools in Malaysia might be contributing to the lack of supply of extracted human teeth. This study was designed to evaluate the socio-demographic and-factors associated with the willingness to donate teeth among students for education and research purposes. To date, there are limited studies on tooth donation hence the need to explore this particular area even further.

This pilot study involved a university which had the majority of Malay and Muslim students which could not have represented the actual scenario happening among Malaysian dental students. There was no statistically significant relationship between the socio-demographic characteristics such as gender, ethnicity, and religion with the willingness to donate teeth among dental students in Malaysia. However, from our findings, the Malay and Muslim students were more willing to donate their teeth after extraction for educational purposes (48.6%, 49%) as compared to after death (38.8%, 57.6%). As for other ethnicity and religion, they preferred to keep their tooth after extraction (2.3%, 1.6%) and were not willing to donate their teeth after death (1.2%, 0.8%). A study on organ donation in Malaysia reported that Malay and Muslim were unsure or unwilling to donate their organs as compared to Chinese and Buddhist (Abidin *et al.*, 2013). The Malays face the dilemma of being sceptical of whether their religion permits them to make organ donations (Noordin *et al.*, 2012). As the majority of students were Malay (96.5%) and Muslim (97.7%) in this study, it was quite challenging to justify whether the same scenario applies to tooth donation as there was a small number of students representing other ethnicities (3.5%) and religion (2.3%). A recent study by Mortadi *et al.* (2018) reported no significant relationship between age, gender, income, and place of living with the willingness to donate teeth after extraction. However, females (61.6%) were more likely to donate their teeth as compared to males (23.4%) (Mortadi *et al.*, 2018) which was similar to our findings. In future, this research should be extended to include more dental universities in Malaysia to omit the imbalance in terms of socio-demographic factors.

In general, most of the students were willing to donate their teeth after extraction for educational purposes (49.8%), followed by keeping their tooth (33.1%), leaving it behind as clinical waste (12.5%) and donating it to research (4.7%). Higher education level was associated with the likelihood of donating teeth (Mortadi *et al.*, 2018). Surprisingly, in this study, only a minority of students were willing to donate teeth for research despite considerable expansion on dental pulp stem cells (DPSC) research in recent years. The high percentage of students willing to donate their teeth for educational purposes might be due to their preclinical training experiences whereby 94.6% of students had difficulty in finding teeth, especially molars (85.9%). More than half of them (52.1%) spent a duration of 6 to 12 months for teeth collection, which contributed to the delay in completing their work.

Furthermore, most of the extracted human teeth collected were unsuitable for the preclinical projects (67.7%). To overcome this problem, students opted to ask friends, seniors, or juniors for extracted human teeth (74.3%). However, there was no statistically significant relationship between delayed preclinical work and difficulty in finding teeth with the willingness to donate teeth.

Nonetheless, students who faced these problems were likely to donate teeth for

educational purposes as compared to research. The lack of exposure to DPSC research among dental students might have contributed to the fact that most students did not consider donating teeth to research. With this finding, dental schools should start implementing into their curriculum the current research in dentistry so that students would appreciate it much better.

Furthermore, dental students should also be aware of the current development in dental research (e.g., dental pulp stem cells) to be able to promote tooth donation to the society. A study by Rumsey *et al.* (2003) reported that patients have a more positive attitude towards organ donation if they knew that the doctors were willing to donate organs themselves (Rumsey *et al.*, 2003). Although many students were willing to donate teeth after extraction, 67.7% of students still felt that they had ownership towards the extracted tooth. A study by Le Breton *et al.* (2015) reported that barely more than half of the patients (54.5%) considered the tooth was belonging to them after extraction (Le Breton *et al.*, 2015). Those living in rural areas contributed to the high percentage of refusing to sign the consent form for tooth donation. Thus, this could be related to the misunderstanding among participants on the rationale of the consent form; especially in older people and those with less formal education background (Mortadi *et al.*, 2018). Contrary to our findings, when research on the tooth was considered, 88.3% of students preferred to be asked consent before tooth donation. This might be due to the fact that our study population consisted of dental students who could relate well to the importance of signing consent before any procedures.

Nonetheless, it is best to separate the consent for tooth extraction in the context of routine dental care from the consent for extraction of teeth for research purposes (Le Breton *et al.*, 2015). Besides that, almost half of the students would like to be informed about the type of research that will be carried out on their teeth after donation similar to the report by Le Breton *et al.* (2015). Emails regarding the result of research on tooth were the preferred approach of contact for most participants in this current study. This similarity in preference is likely to be due to cultural similarity among dental students, where emails, are generally the preferred mode of communication in this faculty.

In this study, we were keen to know whether the dental students were willing to donate teeth after death which was not the case. Unlike organ donation, which has a well-established protocol in harvesting the organs, it does not apply to teeth. We decided that it is best to omit the idea as it is unfair to promote teeth donation after death to the public when the main stakeholders are resistant in doing so. However, promoting tooth donation after extraction is the way to move forward to meet the demand of extracted human teeth.

Conclusion

Promoting tooth donation to meet the demand for dental schools and researchers in Malaysia could be considered. The usage of extracted human teeth for preclinical training is still significant as it mimics the actual tooth in terms of its anatomy and structure of hard tissue. Further research is recommended to determine the willingness of the community in Malaysia to donate teeth before promoting tooth donation to the public. And perhaps with a higher number of participants, the association between the sociodemographic characteristics and willingness to donate teeth would differ from our findings. Besides that, the results from the public might guide us towards identifying the need of the developing a tooth bank to receive, disinfect and organise teeth from donors systematically and safely for the

usage of both dental students and researchers.

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ORIGINAL ARTICLE

Effect of phenotypic switching on the susceptibility of *Candida krusei* towards amphotericin B, nystatin and *Piper betle* aqueous extract

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Abstract

Candida krusei (*C. krusei*) is associated with oral candidiasis, particularly in immune-compromised patients. The objective of the study was to determine the effect of phenotypic switching to the susceptibility of *C. krusei* towards amphotericin B, nystatin and aqueous extract of *Piper betle* (*P. betle*). To induce phenotypic switching, *C. krusei* was inoculated in yeast extract peptone dextrose (YEPD) broth supplemented with 5 mg mL^{-1} phloxine B and incubated for five hours at 25 °C. Later, 100 µL of the suspension was inoculated on YEPD agar supplemented with 5 mg mL^{-1} phloxine B and incubated for five days at 25 °C. Disc diffusion and minimum inhibitory concentration (MIC) assays were conducted to determine the susceptibility of *C. krusei*. The results showed that all *C. krusei* switched generations were susceptible towards amphotericin B and nystatin with the 3rd and 4th generations significantly more susceptible than the un-switched, respectively ($P<0.05$). All *C. krusei* switched generations were also observed to be susceptible towards the aqueous extract of *P. betle*. The MIC of amphotericin B, nystatin and *P. betle* were determined at 10 µg mL^{-1}, 10 µg mL^{-1} and 12.5 mg mL^{-1}, respectively for all generations of *C. krusei.* In conclusion, the susceptibility of *C. krusei* was phenotypically switched generation dependent towards amphotericin B, nystatin, and *Piper betle* aqueous extract.

Keywords: phenotypic switching, disk diffusion test, microdilution assay, metabolic activity

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Introduction

Candida krusei (*C. krusei*) is associated with oral diseases such as oral candidiasis which is common in immuno-compromised patients (Muadcheingka & Tantivitayakul, 2015; *Mushi et al.,* 2016). The prevalence of *C. krusei* in patients with oral cancer has been reported to be higher than *Candida glabrata* and *Candida tropicalis* (Gravina *et al.,* 2007; Hautala *et al.,* 2007). *C. krusei* is an opportunistic microorganism that is resistant to fluconazole (Furlaneto-Maia *et al.,* 2008). Fluconazole is an antifungal agent that is usually prescribed for the treatment of candidal infection. Furthermore, *C. krusei* is also susceptible towards flucytosine, voriconazole, caspofungin, anidulafungin, micafungin and echinocandins. However, the sensitivity of the yeast towards these

antifungals has been reported to decrease (Hakki *et al.,* 2006; Pfaller *et al.,* 2008). Phenotypic switching is one of the important virulent factors of *Candida* species that enables the yeast to adapt in a stress environment (Jones *et al.,* 1994; Vargas *et al.,* 2004; Kang *et al.,* 2016; Palková & Váchová, 2016). It is a phenomenon that usually occurs in the oral environment of immuno-compromised patients (Morschhäuser, 2016). This mechanism of adaptation has been reported to associate with the alteration of genes that involve in the switching process such as metallothionein (*MT-2*), *SIR2* and mating-type loci (*MTL1)*, which may have a role in the decrease of sensitivity of *Candida* spp. towards antifungal agents (Brockert *et al.,* 2003; Low *et al.,* 2008; Arzmi *et al.,* 2012; Soll, 2014).

Amphotericin B and nystatin are used in the treatment of oral candidiasis. Amphotericin B is an antifungal agent that is prescribed in the treatment of primary oral candidiasis. This antifungal agent is also used as an adjunct to parenteral therapy in secondary candidiasis with systemic and topical manifestations on oral mucosal surfaces (Samaranayake *et al.,* 2009). Nystatin is another antifungal agent that is obtained from *Streptomyces noursei*, which is commonly used in the treatment of superficial fungal infection caused by *Candida* spp. (Silva *et al.,* 2013; Nenoff *et al.,* 2016).

Piper betle (*P. betle*) is a plant that belongs to the family of *Piperaceae* (Datta *et al.,* 2011). *Candida* has been reported to be susceptible to *P. betle* aqueous extract. However, there has been no study conducted with regard to the susceptibility of phenotypically switched *C. krusei*. (Himratul-Aznita *et al.,* 2011). In addition, *P. betle* oil-based extract has been reported to possess antibacterial and anti-protozoan that are effective in the treatment of *Salmonella typhi*, *Escherichia coli* and *Staphylococcus aureus* infections (Datta *et al.,* 2011; Himratul-Aznita *et al.,* 2011).

The objective of this study was to determine the effect of phenotypic switching to the susceptibility of *C. krusei* towards amphotericin B, nystatin and *P. betle* extract. It is hypothesised that the susceptibility of *C. krusei* towards amphotericin B, nystatin and *P. betle* extract is phenotypically switched generation dependent.

Materials and methods

Growth of microorganisms

C. krusei (ATCC 14243; Sigma-Aldrich, USA) was revived in yeast extract peptone dextrose (YEPD) broth (BD, USA) and incubated overnight at 37 ºC. Following that, 100 µL of the suspension was inoculated onto YEPD agar (BD, USA) and incubated at 37 ºC for 24 h.

Preparation of phenotypically switched *C. krusei*

C. krusei from YEPD agar was sub-cultured in YEPD broth supplemented with 5 mg mL^{-1} of phloxine B (Sigma-Aldrich, USA) to create a stress growth environment and incubated for 5 h at 37 ºC (Arzmi *et al.,* 2012). Following incubation, *C. krusei* cell density was standardised to 10^6 cells mL^{-1} that was equivalent to the optical density of 0.144 at 550 nm wavelength (OD_{550nm} of 0.144; Figure 1). The suspension was serially diluted, and 100 µL of the inoculum was pipetted on YEPD agar supplemented with 5 mg mL^{-1} of phloxine B. The suspension was spread evenly on the agar using a sterile glass spreader. The plate was incubated at 25 °C for five days. The plate that had approximately 50 colonies forming unit

(CFU) was examined for switched *C. krusei*. Colonies displaying morphological variation from the un-switched *C. krusei* were selected to represent the first switched generation (Arzmi *et al.,* 2012). Three colonies from the first switched generation that possessed similar morphology were collected and sub-cultured on to another set of YEPD agar supplemented with phloxine B to produce the 2nd, 3rd and 4th switched generations (Figure 2).

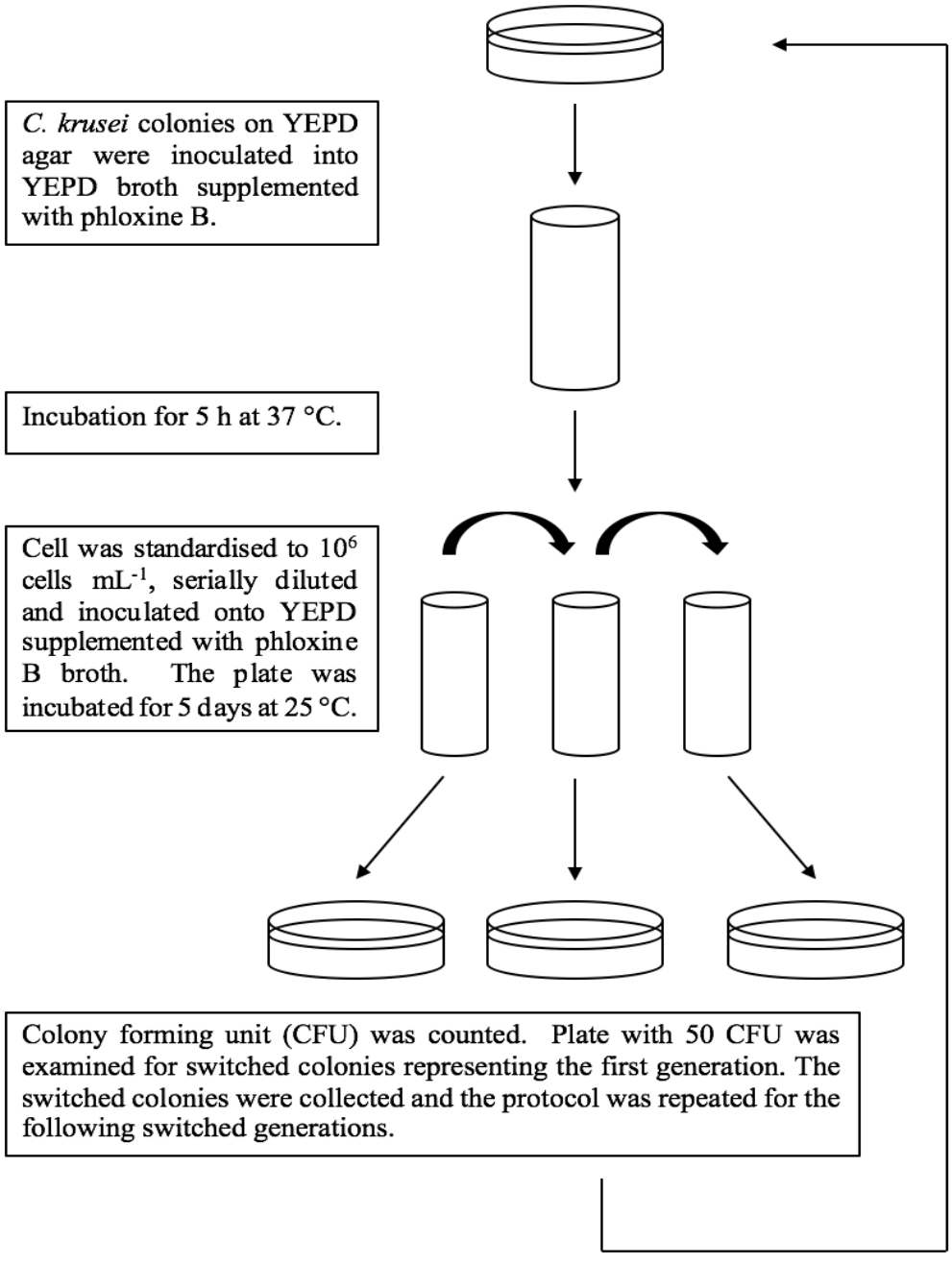

Figure 1. Illustration of protocol for phenotypic switching of *C. krusei*

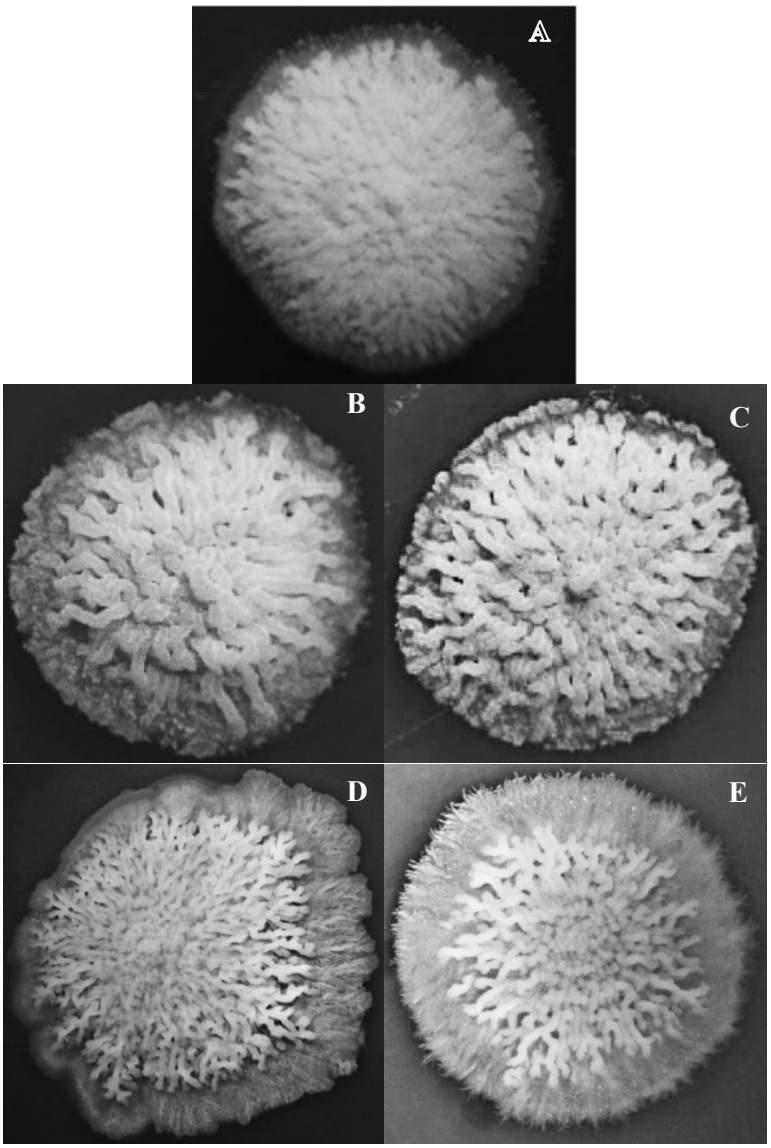

Figure 2. The colony of un-switched and switched *Candida krusei* at 10x magnification using a stereoscope. (A) Un-switched, (B) 1st switched generation, (C) 2nd switched generation, (D) 3rd switched generation, and (E) 4th switched generation (Arzmi *et al.*, 2012).

Determination of metabolic activity

The metabolic activity of un-unswitched and all switched generations of *C. krusei* was determined using BIOLOG YT MicroPlate (Hayward, CA). In brief, an overnight culture of *C. krusei* was suspended in 1 mL of sterile distilled water. Later, 100 µL of the suspension was inoculated in each well of YT MicroPlates following incubation at 25 °C for 24 h, 48 h and 72 h. The metabolic patterns were interpreted using BIOLOG's MicroLog 3 software and compared to the YT database.

Preparation of *P. betle* aqueous extract

P. betle leaves were freshly picked from Kampung Bukit Payong, Pokok Sena, Kedah, Malaysia. The leaves were cleaned with distilled water, and the total wet weight was recorded. The leaves were oven-dried at 60 ºC for 48 h, and the total dry weight was recorded. Following that, 100 g of the leaves were cut into small pieces and put into a conical flask. The leaves were boiled in 2 L of distilled water until the volume was reduced to half (Himratul-Aznita *et al.,* 2011). The decoction was filtered into a

500 mL beaker, and the filtrate was re-boiled to a final volume of 100 mL. Finally, the concentrated extract was freeze-dried to produce *P. betle* water-based extract powder.

Disc diffusion test

The susceptibility of *C. krusei* towards amphotericin B, nystatin and *P. betle* aqueous extract was assessed by using disc diffusion test and microdilution method. Disc diffusion test was conducted by following the protocol of the National Committee for Clinical Laboratory Standards (CLSI, 2015a). To prepare *C. krusei* inoculum, the yeast colonies that were grown on YEPD agar supplemented with 5 mg mL^{-1} of phloxine B were suspended into 5 mL of 0.85% (v/v) of sterile saline. The optical density of the cell suspension was then standardised to an OD_{550nm} of 0.144 to give 10^6 cells mL^{-1} of *C. krusei*. Following that, 100 µL of the suspension was pipetted out and evenly swabbed on Mueller-Hinton (MH) agar (BD, USA). Amphotericin B (25 µg), nystatin (20 µg) and *P. betle* (20 mg) impregnated paper discs were aseptically placed onto the agar plate and incubated overnight at 37 °C. Finally, the diameter of the growth inhibition zone was measured. A similar protocol was conducted for all switched generations of *C. krusei* in triplicates.

Minimal inhibitory concentration assay

Minimum inhibitory concentration (MIC) was determined using the microdilution method as recommended by the National Committee for Clinical Laboratory standard (CLSI, 2015b). Initially, seven wells of a sterile 96-well plate (Nunc, USA) were labelled W1 to W7 horizontally (Table 1). Later, 100 µL of YEPD broth was added to W2 through W7, and 100 µL of the antifungal agent was added into W1 and W2. The plate was slowly agitated to mix the content. Following that, 100 µL of suspension in W2 was transferred to W3. Following thorough mixing, 100 µL of suspension in W3 was transferred to W4, and the procedure was continued through W6. After mixing, 100 µL from W6 was discarded. Finally, 100 µL of the yeast inoculum that was standardised to 10^6 cells mL^{-1} in YEPD broth was added to W1 through W7 and incubated at 37 ºC for 24 h. W7 that received no antifungal treatment served as a negative control. The lowest concentration of antifungal agent that exhibited clear suspension was determined as the MIC. A similar protocol was repeated for all switched generations of *C. krusei* in triplicates.

Table 1. Illustration of minimum inhibitory concentration (MIC) determination.

Wells	W1	W2	W3	W4	W5	W6	W7
100 µL of YEPD broth		+	+	+	+	+	+
100 µL of antifungal agent	+	+	Serial dilutions were done until W6. Finally, 100 µL of suspension in W6 was discarded.				
100 µL of suspension	+	+	+	+	+	+	+

+ showed the presence of suspension in the well.

Statistical analysis

All data were statistically analysed using ANOVA post-hoc Tukey test on SPSS Statistic software version 25.0. The data were considered statistically significant if $P<0.05$.

Results

Metabolic activity of *C. krusei*

The un-switched an all switched generations of *C. krusei* were observed to ferment N-acetyl-D-glucosamine and α-D-glucose. However, only un-switched and 1st switched generation of *C. krusei* fermented γ-aminobutyric acid (GABA).

Susceptibility towards amphotericin B

Based on the disc diffusion test, the diameter of the inhibition zone of un-switched *C. krusei* when treated with amphotericin B was determined at 2.2 ± 0.1 cm (Table 2). The degree of susceptibility of the switched *C. krusei* towards amphotericin B increased gradually from the 1st, 2nd and 3rd generations with an inhibition zone of 2.3 ± 0.3 cm, 2.4 ± 0.1 cm and 2.6 ± 0.3 cm, respectively. The susceptibility was decreased in the 4th switched generation with an inhibition zone of 2.4 ± 0.1 cm. Finally, the MIC of amphotericin B towards un-switched and all switched generations of *C. krusei* were recorded at 10 µg mL^{-1}.

Susceptibility towards nystatin

The diameter of the inhibition zone for un-switched and the 1st switched generation of *C. krusei* were recorded at 2.4 ± 0.1 cm when treated with nystatin (Table 2). The susceptibility of *C. krusei* was decreased in the 2nd switched generation with an inhibition zone of 1.9 ± 0.2 cm, which was the least susceptible towards nystatin. In the 3rd and 4th switched generations, the susceptibility towards nystatin was observed to increase gradually with inhibition zones of 2.3 ± 0.1 cm and 2.6 ± 0.1 cm, respectively. The 4th switched generation was determined as the most susceptible among generations of *C. krusei*. The MIC of nystatin towards un-switched and all switched generations of *C. krusei* were determined at 10 µg mL^{-1}.

Susceptibility towards *Piper betle* aqueous extract

The un-switched *C. krusei* was susceptible towards *P. betle* aqueous extract with an inhibition zone of 2.2 ± 0.1 cm (Table 2). The degree of susceptibility of the 1st switched generation was increased compared to the un-switched *C. krusei*; however, no significant difference was observed ($P>0.05$). The inhibition zone of the 2nd switched geeration of *C. krusei* towards *P. betle* extract was recorded at 2.1 ± 0.1 cm, whereas the 3rd switched generation was determined at 2.1 ± 0.2 cm. The 4th switched generation was the least susceptible towards *P. betle* with an inhibition zone of 2.0 ± 0.2 cm. The MIC of *P. betle* aqueous extract was determined at 12.5 mg mL^{-1} for un-switched, and all switched generations of *C. krusei.*

Table 2. The effect of phenotypic switching on the susceptibility of *C. krusei* towards amphotericin B, nystatin and *Piper betle* extract.

Active ingredients	Amount of antifungal agent/extract	Growth generations				
		Un-switched	1st switched	2nd switched	3rd switched	4th switched
		Mean inhibition zone (cm)				
Amphotericin B	25 µg	2.2 (0.1)	2.3 (0.3)	2.4 (0.1)	2.6 (0.1)	2.4 (0.1)
Nystatin	20 µg	2.4 (0.1)	2.4 (0.1)	1.9 (0.2)	2.3 (0.1)	2.6 (0.1)
Piper betle	20 mg	2.2 (0.1)	2.3 (0.2)	2.1 (0.1)	2.1 (0.2)	2.0 (0.2)

Significantly more susceptible compared to un-switched ($P < 0.05$)
No significant different compared to un-switched ($P > 0.05$)
Significantly less susceptible compared to un-switched ($P < 0.05$)
The inhibition zones are the mean of three biological replicates. Standard deviation (SD) is given in the parenthesis.

Discussion

To our knowledge, this is the first study that emphasises on the effect of phenotypic switching to the susceptibility of *C. krusei* towards amphotericin B, nystatin and *P. betle* aqueous extract. The present study showed that the un-switched and all switched generations of *C. krusei* had different degrees of susceptibility towards amphotericin B with the 3rd generation exhibiting the least susceptible (Table 2). This finding supports the hypothesis of the study that the susceptibility of *C. krusei* towards amphotericin B is phenotypically switched generation dependent. Amphotericin B was reported to possess broad-spectrum fungicidal and fungistatic activities towards *Candida* spp. (Cuervo *et al.,* 2016). This antifungal has been shown to intercalate the membrane layer of *Candida* spp. to form channels that cause potassium ions to leak out, subsequently destroying the proton gradient (Kragelund *et al.,* 2016). Furthermore, it has been reported that amphotericin B can affect the sterol component of the yeast cell wall, which may have a role in the growth inhibition of *C. krusei* (Williams *et al.,* 2011).

The un-switched and all phenotypically switched generations of *C. krusei* were susceptible towards nystatin with the 2nd generation the least sensitive compared to other generations of *C. krusei*. This finding supports the hypothesis of the present study that the susceptibility of *C. krusei* towards nystatin is phenotypically switched generation dependent. The previous study has shown that nystatin can exhibit both fungistatic and fungicidal effects towards *Candida* spp. (Choudhary *et al.,* 2016). This antifungal agent can bind to the sterol component of the membrane, which subsequently affects the cell permeability of the yeast (Kragelund *et al.,* 2016). The previous study has shown that the interaction between nystatin and ergosterol component of the candidal cell membrane is responsible to the increase of cell porosity which leads to the loss of cytoplasmic substances (Williams *et al.,* 2011). It is postulated that the different degrees of *C. krusei* susceptibility towards nystatin is due to the phenotypic switching that may have altered the composition of ergosterol component in the cell membrane of the switched *C. krusei.* However, this warrants further study to ascertain this speculation.

Our study showed that *C. krusei* both un-switched and all switched generations were susceptible towards *P. betle* aqueous extract. However, no significant difference was observed between the switched generations (Table 1). This finding suggested that *P. betle* extract could be an effective antifungal agent towards *C. krusei*. *P. betle* extract is reported to contain hydroxychavicol which can destroy the cell membrane and reduce the cell viability of *Candida* spp. (Ali *et al.,* 2010). Furthermore, hydroxychavicol has also been reported as an effective antimicrobial agent towards *Microsporum gypseum* and *Streptococcus mutans* (Nalina & Rahim, 2007). Thus, further study to determine the specific and effective active compounds in *P. betle* aqueous extract using high-performance liquid chromatography (HPLC) would throw more light on its treatment of oral candidiasis.

The present study showed that only un-switched and 1st switched generation of *C. krusei* fermented GABA. GABA has been reported to be involved in the formation of succinate that is important in the energy production of a living cell (Bach *et al.,* 2009; Kregiel, 2012). The previous study has shown that the yeast cell wall stored a large portion of GABA indicating the potential role of this molecule in stress tolerance mechanism and as a nitrogen source (Coleman *et al.,* 2001; Bach *et al.,* 2009). This could explain the significant increase of susceptibility of the 3rd and 4th switched generations of *C. krusei* in the present study when treated with amphotericin B and nystatin, respectively (Table 2).

In conclusion, the present study confirmed that the susceptibility of *C. krusei* towards amphotericin B, nystatin and *P. betle* is phenotypically switched generation dependent that may have a role in the pathogenicity of the yeast in the oral cavity.

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ORIGINAL ARTICLE

Internet addiction among pharmacy undergraduate students in Malaysia

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Abstract

Internet addiction occurs due to excessive and uncontrolled use of the internet. It may hinder academic achievement and lead to reduction in quality of life of the students. This study aims to assess the prevalence of internet addiction (IA) among pharmacy students. A sample of 223 students from Kulliyyah of Pharmacy, International Islamic University Malaysia participated in this study. Chen Internet Addiction Scale (CIAS) was used to assess the prevalence of Internet addiction. The prevalence of IA among pharmacy students was 26.9% while 13.5% were at higher risk for addiction. Although the mean score was higher among male students but it was not statistically significant (P>0.05). IA is significantly higher among students who are spending more than 40 hours per week online. Students with poor social interaction had higher mean CIAS score than those who were socially active, however the difference was not statistically significant (P>0.05) In conclusion, Internet addiction need to be addressed early to prevent its effects on physical and mental wellbeing. Students need to be educated about controlling and managing time spent online to avoid progression into internet addiction.

Keywords: internet addiction, undergraduate, pharmacy, Malaysia

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Introduction

The Malaysian Communications and Multimedia Commission (MCMC) conducted the Internet Users Survey (IUS) which showed that 38.1% of internet users were between 20 to 29 years of age and the estimated number of internet users was 24.5 million people in 2016 (Internet Users Survey, 2017). Unrestricted unlimited access to internet services is generally available to undergraduates and it is envisioned to enrich education, communications and research.

The Kulliyyah of Pharmacy offers an undergraduate program of Bachelor of pharmacy which is four (4) years in duration. This program includes a significant amount of clinical practice in hospitals and other health care services together with on campus theory teaching and learning activities and so the use of internet is vital for the students to be updated with recent related knowledge and to solve assignments and many others.

However, prolonged and excessive internet use may result in serious adverse effects on academic achievement (Reed *et al.,* 2015), psychological well-being (Casale *et al.,* 2015), and quality of life (Cheng & Li 2014). The term addiction is now extended to be more broad and include addiction on behaviors that can cause problems and impairment other than addiction on substances (Van Rooij & Prause, 2014). Addiction to a substance and addiction to a behavior may look similar in their effects on behavioral patterns, emotions and physiology (Valentini & Biondi, 2016) Internet use, gambling, eating, sex, exercise, work and shopping are examples of behavioral addiction (Sussman *et al.*, 2011).

Internet addiction (IA), is a commonly used term to describe abnormal excessive internet use, it also describes the psychological problems that arise from prolonged heavy internet use (Chou *et al.*, 2005). IA is now considered a focus of many studies since it is becoming more prevalent all over the world. Although IA is not included in the Statistical Manual of Mental Disorders fifth edition (DSM-5), internet gaming disorder has been incorporated into section III of DSM-5 (Poli, 2017; American Psychiatric Association, 2013).

Many tools have been developed to detect and assess the severity of IA and it is very important to use a validated and reliable tool. An example of such a tool is the Chen Internet addiction scale (CIAS). It assesses five domains of Internet-related problems: compulsive use, withdrawal, tolerance, interpersonal and health consequences, and time management difficulties (Chen *et al.*, 2005).

Previous studies showed that emotional disturbances in the form of depression, anxiety and stress symptoms were known to occur among students during their university time study (Nayan *et al.*, 2017; Radeef & Faisal, 2018).

Since IA has impact on students' psychological well-being and academic performance, it is crucial to assess the occurrence of IA among undergraduate students therefore, our research aimed to assess the IA among pharmacy students.

Methodology

This a cross-sectional study conducted among undergraduate pharmacy students at the Kulliyyah (Faculty) of Pharmacy, International Islamic University Malaysia (IIUM). It was supported by a research grant from IIUM and approved by the Research Ethics Committee of the IIUM (approval number: IREC 662). Participation was entirely on a voluntary basis; students were ensured about confidentiality and consent was obtained prior to enrolment. In order to avoid the stressful effect of preparation for exams, the study was conducted early in the mid of the semester. The inclusion criteria are students who use the internet and agree to join in this study. Students who did not give consent or who could not understand English were excluded. The participant socio-demographic characteristics included in the study included nationality, age, marital status, gender, year of study, living accommodation during the studies and household income.

In this study, Chen Internet Addiction Scale (CIAS) was used to assess the prevalence of IA. CIAS questionnaire has been tested to have good psychometric properties. It has excellent internal consistency. Its test–retest reliability has been reported to be good: r = .83 and r = .88 (Chen *et al.*, 2005; Kesici & Sahin, 2010), and so we can consider CIAS as a good tool which can be used to accurately find the prevalence of IA in Malaysia (Radeef *et al.*, 2018). It is made of 26 items that can be self-answered by the

participant. The 26 items are rated on a 4-point Likert scale. It assesses five domains of Internet-related problems: compulsive use, withdrawal, tolerance, interpersonal and health consequences, and time management difficulties. The total score is from 26 at the lowest to 104 highest. The higher the score the more it indicated higher severity of internet addiction. The cutoff point of 63/64 and 67/68 of the CIAS were considered to be the best for screening and diagnosis of IA among college students respectively, meaning that 26-63 shows normal use, 64-67 indicates at risk use and need for screening and 68-104 indicates IA (Ko *et al.*, 2009).

Regarding assessing the purpose of using the internet, the students were asked to rate the different purposes of internet use (Social media, online media viewing, study purpose searching for information other than studying, E-mails) on a scale of 1-5, where 1=strongly disagree, 5=strongly agree.

Statistical analysis

Statistical Package for Social Sciences (SPSS) software version 24.0 was used in this study for descriptive and inferential analysis. Sociodemographic factors were presented in numbers and percentages. Independent T-test and ANOVA test were used to compare the mean score of Chen internet addiction scale with all demographic variables to assess the relationship between these factors with internet addiction .A value of $P < 0.05$ was considered significant. The mean score ± SD was used to analyze the purpose of internet use.

Results

A total of 223 pharmacy students participated in the study. The majority were females older than 21 years of age, Malaysian, single, living in a student hostel and with a family monthly household income of more than 5000 Malaysian Ringgit (Table 1).

Students with poor social interaction were having higher mean CIAS score than those who were socially active, however the difference was not statistically significant($P>0.05$) and participants with more than 40 hours of internet use per week had a significantly higher CIAS mean score ($P<0.05$). Internet addiction is not statistically different in relation to income, gender and marital status (Table 1).

This study showed that the prevalence of internet addiction among students is 26.9%, while 13.5% are considered at risk of addiction (Table 2). Regarding the purpose of internet use among students, the top five were social media (Facebook/ Instagram/ WhatsApp/ chatting), online media viewing (videos, songs, YouTube), study purpose, searching for information rather than for study purpose, and emails (Table 3).

Discussion

Although our study showed the prevalence of IA to be 26.9%, however it is still lower compared to other previous study conducted among medical students in Malaysia using internet addiction test where the prevalence was 36.9% (Ching *et al.,* 2017) this may be due to the difference in the nature of the course of study between pharmacy and medicine. The effect of the type of course on IA can also be displayed through the results of studies among allied health and dental students were the rate of IA is 31.8% and 29.2% respectively (Othman & Lee, 2017; Radeef & Faisal, 2019).

Still, there are some studies among medical students that show results lower than ours (Siraj, 2015; Radeef & Faisal, 2018). This could be explained by the different tools used to detect IA.

Table 1. Socio-demographic factors and its association with internet addiction

	Total, n (%)	CIAS mean score	P-value
Gender			
Male	32(14.3)	64.2	0.11
Female	191 (85.7)	60.6	
Age (years)			
≤ 21	87 (39)	60.2	0.341
>21	136 (61)	61.7	
Family monthly income			
≤ RM1500	45 (20.2)	63.5	0.290
RM 1501-5000	76 (34.1)	60.8	
>RM 5000	102 (45.7)	60.3	
Marital status			
Married	3 (1.3)	55.7	0.568
Single	220 (98.7)	61.2	
Social interaction			
Socially active	123 (55.2)	60.2	0.220
Socially inactive	100 (44.8)	62.2	
Time of internet use per week			
> 40 hours	70 (31.4)	63.7	0.001
20-40 hours	115 (51.6)	61.6	
< 20 hours	38 (17.0)	55.0	

Table2. Prevalence of internet addiction (IA) among pharmacy student

Status of IA	Number (%)
No IA	133 (59.6)
At high risk	30 (13.5)
Have IA	60 (26.9)
Total	223 (100)

Table 3. The purpose of internet use

Purpose of internet use among students	Mean score ± SD
Social media (Facebook/Instagram/WhatsApp/chatting)	4.61 ± 0.731
Online media viewing (videos, songs, YouTube)	4.46 ± 0.792
Study purpose	4.43 ± 0.725
Searching for information rather than studying	4.32 ± 0.808
E-mails	4.13 ± 0.942

We can observe from the results of previous studies that there are no consistent results of IA among students of the same course and that can be due to the availability of different tools that can assess IA and also the effect of sample size and sampling method among different studies. The prevalence of IA can also be affected by cultural differences. A previous review article showed a wide range of IA prevalence from 0.8% to 26.7% (Kuss *et al.*, 2014). Another example on cultural effect on the prevalence of IA is the results from two different studies on university students, one in Pakistan and the other in Taiwan revealed that the prevalence was 28% and 17.9% respectively (Saleem *et al.*, 2015; Tsai *et al.*, 2009).

Internet addiction can also be associated with gender, however the results in different studies around the world showed varied results. In some studies, IA is associated with male gender (Ching *et al.*, 2017; Tsai *et al.*, 2009; Salehi *et al.,* 2014) but in a study done in Japan the rate of IA

was more among females (Mihara *et al.*, 2016). However in our study there was no significant gender difference which is consistent with previous studies in Malaysia (Radeef & Faisal, 2019; Haque *et al.*, 2016; Usman *et al.*, 2014), Vietnam (Tran *et al.,* 2017), India (Malviya *et al.*, 22014) and USA (Beavers *et al.,* 2015).

Defects in social interaction play a role in encouraging people to use the internet more to fulfill their needs to interact with people and studies have found that poor social interaction is significantly associated with increased prevalence of internet addiction (Radeef & Faisal, 2019; Van der *et al.*, 2009; Liu, 2007). However, in this study, although the students with poor social interaction were having higher mean CIAS score than those who were socially active but the difference was not statistically significant (P >0.05). This can be explained by the current trend of increasing use of social media for social interaction instead of real socialization and so a student who is socially active might also have a high mean score for IA. Which results in both socially active and inactive students being heavy users of internet; students with poor social interaction spend more time online to replace the time spent on social interaction, while socially active students spend more time online to socialize with their friends through social media.

Conclusion

Internet addiction is prevalent among pharmacy students. Although female participants were more than males, however, there were no statistically significant gender differences in the prevalence of IA. Students using the internet for more than 40 hours per week have higher levels of IA. Internet addiction need to be addressed early to prevent its effects on physical and mental wellbeing. Students need to be educated about controlling and managing time spent online to avoid progression into internet addiction.

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CASE REPORT

Odontogenic cutaneous sinus tract - misdiagnosis and follow-up in a seven-year-old boy

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Abstract

Cutaneous sinus tracts of dental origin are relatively rare, but frequently misdiagnosed. In this case report, we present a seven-year-old patient with a cutaneous lesion in the left submandibular region misdiagnosed by a physician as an abscess secondary to suppurative lymphadenitis, and thus incorrectly treated with surgery and systemic antibiotics. Following a detailed dental examination, the patient was correctly diagnosed with an odontogenic sinus tract from a periapical abscess of tooth 36. Treatment of the immature tooth was initiated with apexification combined with nonsurgical endodontic treatment. The cutaneous and the periapical lesions were all resolved after the treatment and there has been no recurrence during an eight-year follow-up.

Keywords: cutaneous sinus tract, cutaneous fistula, odontogenic, apexification, immature permanent tooth

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Introduction

Odontogenic cutaneous sinus tracts (OCSTs), uncommon and therefore frequently misdiagnosed, are generally caused by chronic endodontic infections (Chen *et al.*, 2016; Chouk & Litaiem, 2020). Patients presenting with OCSTs are often treated incorrectly with systemic antibiotics or surgical procedures by physicians because the dentition is often asymptomatic. When the infection persists, patients are often referred to a dental clinician to determine a dental etiology (Gimenez-Garcia *et al.*, 2015; Yadav *et al.*, 2014). Necrotic teeth are frequently overlooked because the offending tooth manifests little symptoms after perforation of the periosteum. In addition, the stoma of the sinus tract may be located at a distant site from the infection, for instance, at a submental cutaneous sinus tract of mandibular second molar origin, making the diagnosis very challenging (Bai *et al.*, 2014; Ghazali & Ngeow, 1996; Ong & Ngeow, 1999). Moreover, the variable symptoms of OCSTs also lead to diagnostic confusion and mismanagement (Lee *et al.*, 2016).

The mechanism of cutaneous sinus tract formation depends greatly on anatomic pathways. Odontogenic infection spreads from the apex of the necrotic tooth and penetrates the alveolar bone and soft tissues through the path of least resistance, limited only by muscle attachments and fascial planes (Kaban, 1980). As a result, OCSTs may occur when the infection is superior to the maxillary attachment of the buccinator muscle and inferior to the mandibular attachments of the buccinator, mylohyoid, and mentalis muscles (Laskin, 1964). Odontogenic infections in children and adolescents are theoretically easier to develop into extraoral sinus tracts because teeth are often partially erupted with immature roots and the alveolar process is less developed than in adults, with a higher chance of the infection to cause a perforation beyond the muscular attachments (Swales *et al.*, 2016). However, OCSTs in children are infrequently reported.

This article presents a case of a cutaneous sinus tract misdiagnosis that was successfully treated as an endodontic periapical infection with nonsurgical root canal therapy of the left mandibular first molar (tooth 36) in a seven-year-old male patient, with an eight-year follow-up.

Case report

On clinical examination, a cutaneous lesion in the left submandibular region was noted. The patient's medical history was examined, which indicated that two months prior to the referral, the patient was diagnosed with suppurative lymphadenitis subsequent to swelling with pain in the left submandibular region and odynophagia with fever. His physicians commenced treatment with incision and drainage along with a systemic antibiotic regiment of erythromycin ethyl succinate 100mg/2.5ml, four times daily. The symptoms disappeared and the incision healed after two weeks. However, a month later, swelling reoccurred with intermittent discharge of pus. Incision and drainage was repeated along the lesion, which presented as an abscess of size 11× 8mm. Nevertheless, the lesion was not resolved in a month, and discharge of pus still persisted (Figure 1A, 1B).

A seven-year-old Chinese boy was referred to our clinic at the Department of Pediatric Dentistry, Guanghua School of Stomatology, Hospital of Stomatology, Sun Yat-sen University, Guangzhou, P.R. China. His chief complaint was of “severe pain lasting two days on the lower left side when chewing”.

An intraoral clinical examination revealed a slight discoloration in the mesial marginal ridge of the left mandibular first molar (tooth 36). The tooth was positive to percussion and pain on biting, however tested negative to vitality and cold tests. A panoramic radiograph was taken, which revealed a radiolucent lesion on the mesial aspect of the crown of tooth 36 that extended to the mesial pulp horn, while the occlusal aspect of the crown was intact (Figure 1C). An intraoral periapical radiograph showed an extended periapical radiolucency at the distal root of tooth 36, along with immature apices (Nolla stage 9) (Figure 1D). From the radiographic findings, a diagnosis of periapical abscess with secondary cutaneous sinus tract formation was made.

Treatment was commenced with standard access cavity preparation by removal of caries under rubber dam on tooth 36. The four canals: mesiobuccal (MB), mesiolingual (ML), distobuccal (DB), and distolingual (DL) canals were cleaned and shaped. There was discharge of pus, and the tooth was left open for drainage. Two days later, on the follow-up visit, there were no signs of acute inflammation. A rubber dam was placed after administration of an inferior alveolar nerve block using 2% Lidocaine 1:100,000

epinephrine and further canal debridement was performed. A non-setting calcium hydroxide paste (LELE®, Shanghai Eryi & Zhangjiang Biomaterial Co., Ltd., Shanghai, China) was applied as an intracanal antimicrobial dressing for two weeks. On the third visit, the patient was asymptomatic and healing of the cutaneous sinus tract had occurred (Figure 2A). Apexification treatment was initiated by applying a premixed calcium hydroxide paste with iodoform (Vitapex®, morita, Osaka, Honshu, Japan) to the root canal system (Figure 2B). Between appointments, the coronal cavity was provisionally sealed with glass ionomer cement (Fuji IX GP®, GC Co., Tokyo, Japan).

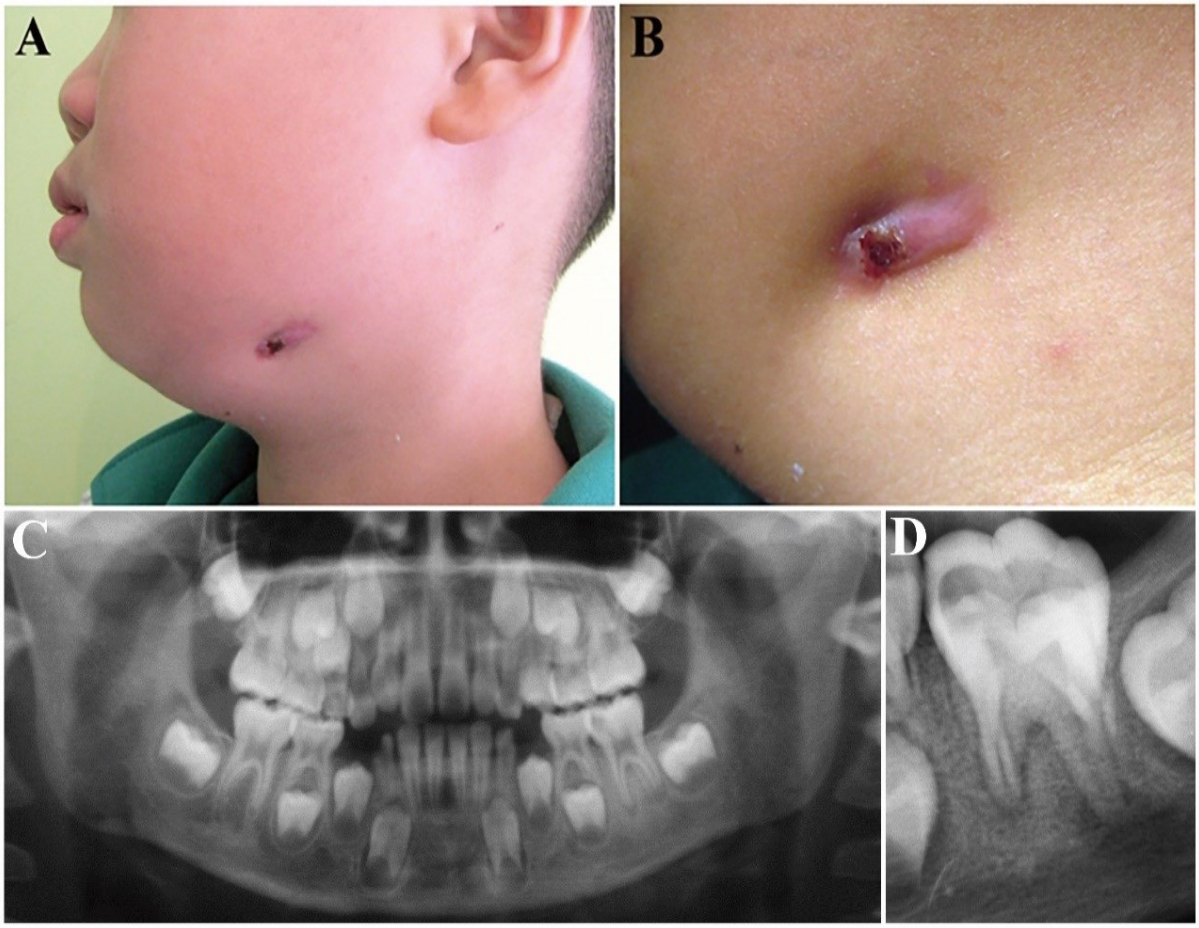

Figure 1. (A) Pre-operative view of the cutaneous sinus tract. Repetitive drainage and antibiotic therapy did not resolve the condition. (B) Magnified view of the cutaneous sinus tract. (C) Pre-operative panoramic view, showing a radiolucent lesion on the mesial aspect of the crown of tooth 36 extending to the mesial pulp horn, while the occlusal aspect of the crown was intact. (D) Periapical radiograph of tooth 36, revealing immature apices and an extended periapical radiolucency at the distal root.

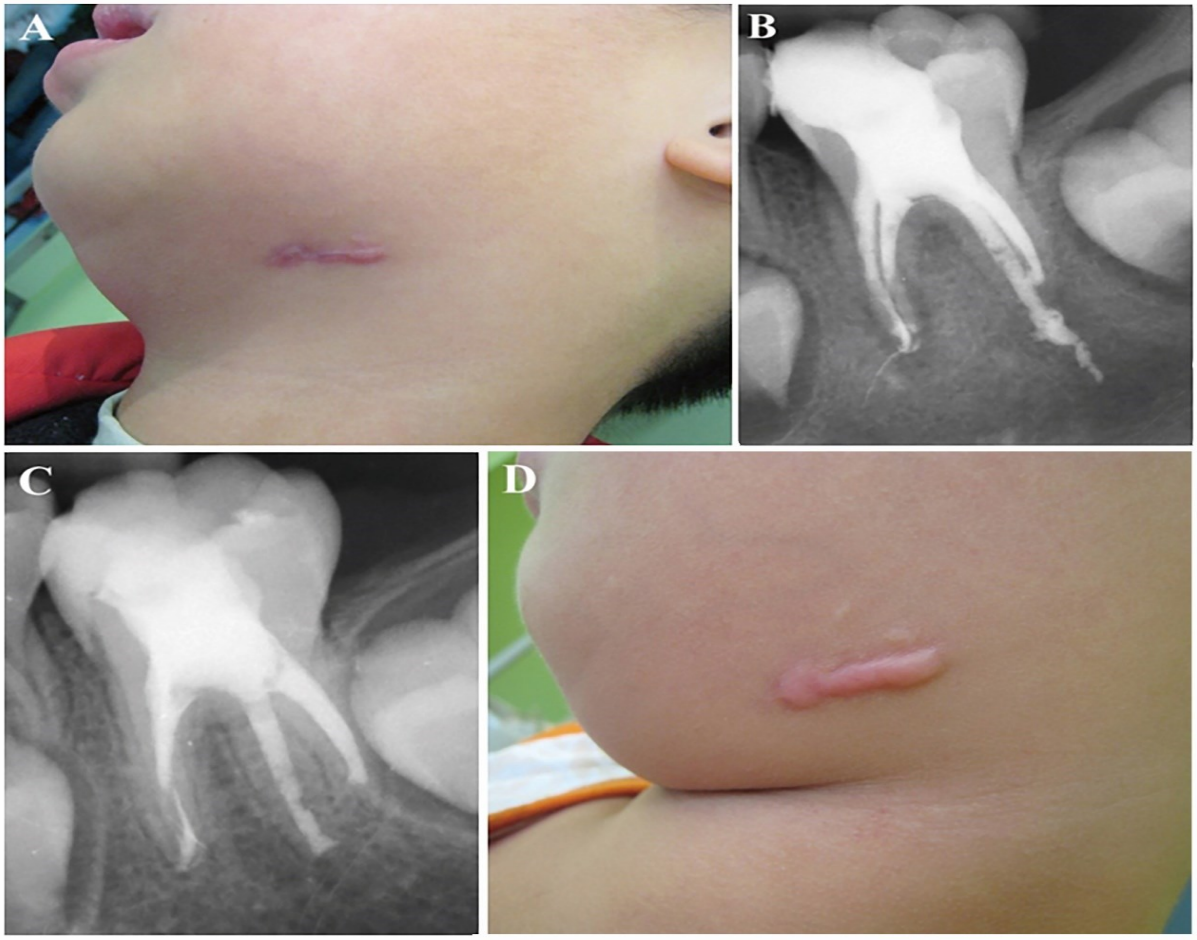

Figure 2. (A) After 2-week intracanal dressing with calcium hydroxide paste, healing of the cutaneous lesion had occurred. (B) Radiograph after applying a premixed calcium hydroxide paste with iodoform to the root canals. (C) Radiograph showing resolution of the periradicular radiolucency, formation of the calcified barrier at the mesial root apices and initiation of apexification. (D) Extraoral photograph after 7 months, showing hypertrophic scar formation.

Radiographic examination after seven months demonstrated resolution of the periradicular radiolucency and the emergence of a calcified barrier at the mesial root apices (Figure 2C). An extraoral view at this time exhibited the formation of a hypertrophic scar (Figure 2D). Eleven months after the apexification treatment, a periapical radiograph demonstrated the absorption of the root canal dressing (Figure 3A). Nickel-titanium rotary files (ProFile®, Dentsply Maillefer, York, PA, US) were used through the application of a crown down technique to shape the MB, ML, DB, DL canals to a size #35.04. Concurrently, all the canals were irrigated copiously with 2.5% sodium hypochlorite, 3% hydrogen peroxide, and normal saline, respectively. The canals were obturated using Cortisomol sealer (Morita, Osaka, Honshu, Japan) by way of a warm gutta-percha technique. A final postoperative radiograph revealed a homogeneous and dense root filling (Figure 3B). The access opening was restored with composite resin.

After an eight-year follow-up, the tooth was visibly unremarkable, with no radiographic and clinical symptoms (Figure 3C). The cutaneous lesion resolved with minimal scarring, except for a slight hyperpigmentation of the area (Figure 4A, 4B).

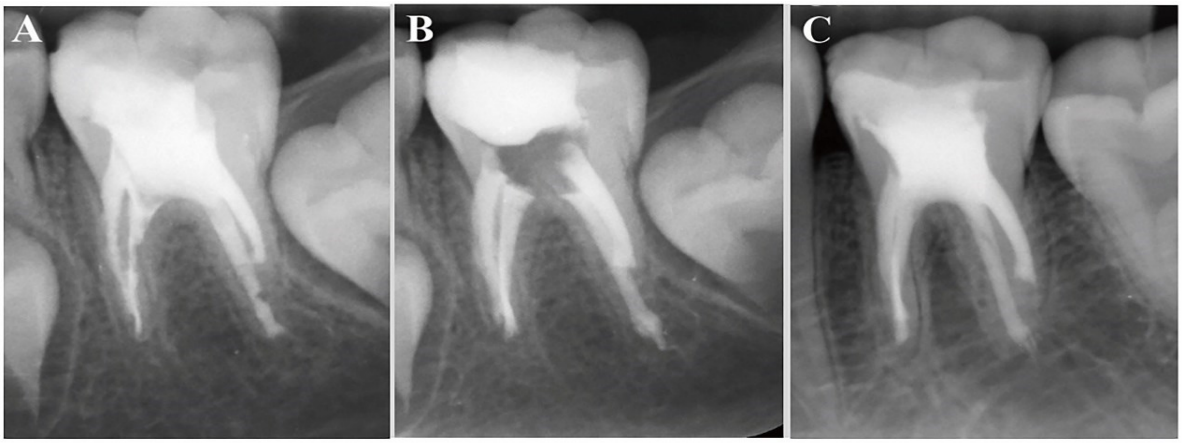

Figure 3. (A) Radiograph 11 months after apexification treatment, presenting the absorption of the root canal dressing. (B) Post-operative radiograph of tooth 36, revealing homogeneous and dense root filling. (C) Radiographic follow-up after 8 years.

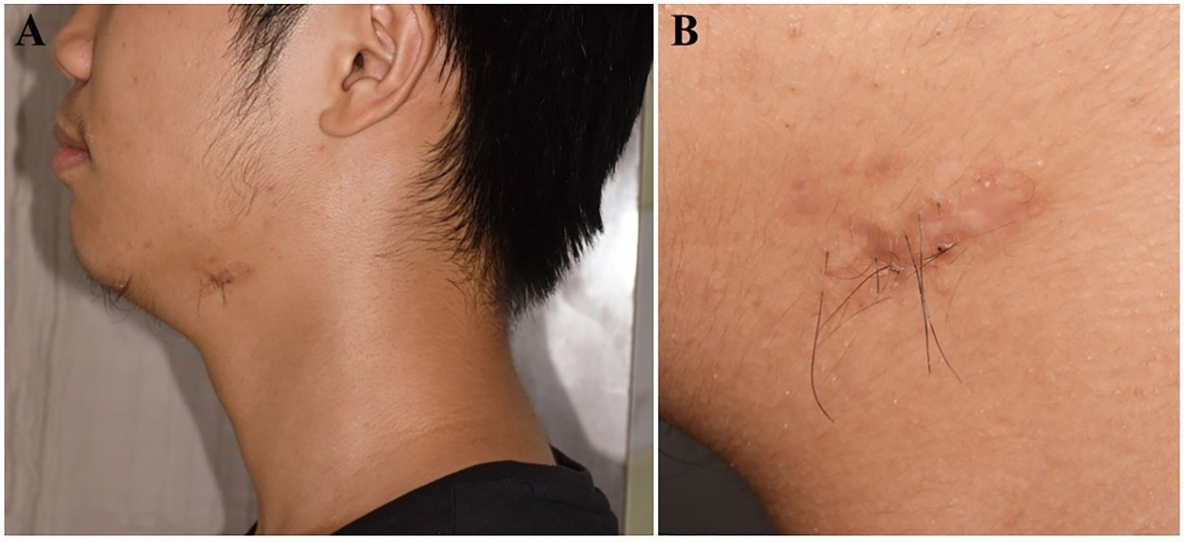

Figure 4. (A) Clinical examination follow-up after 8 years. (B) Magnified view of the area.

Discussion

In this case, based on the patient's age and radiographic examination, the enamel calcification of tooth 36 was incomplete. As permanent teeth erupt, an additional period of two years is needed to complete the calcification process by exposure to saliva, thus the dentition is susceptible to caries formation during this time (Chin *et al.*, 2016). In addition, acute caries occurs frequently at this age, which progress faster and can consequentially cause endodontic infection. Acute carious lesions often present as light brown or grey in color, penetrating from the occlusal or interproximal aspect leading to early diagnostic challenges. Histologically, the region of the sinus tract constitutes a conglomerate of inflammatory cells and granulation tissue. The luminal region is comprised of granulomatous tissue along with purulent exudates containing polymorphonuclear leukocytes and chronic inflammatory cells. After treatment is initiated with root canal therapy or extraction, healing begins, resulting in minimal scar formation (Yi *et al.*, 2017).

As reported in the literature, OCSTs are frequently misdiagnosed and as a

consequence often mismanaged (Bashar *et al.*, 2019; Swales *et al.*, 2016). In addition, differential diagnosis is paramount in making an efficient constructive treatment plan. In this case report, differential diagnosis should include traumatic lesion, epidermal cyst, furuncle, carbuncle, pyogenic granuloma, salivary gland fistula, and actinomycosis (Cantatore *et al.*, 2002; Gulec *et al.*, 2001; Jamshidi *et al.*, 2015; Tavee *et al.*, 2003). Therefore, detailed history taking and comprehensive examination is crucial in determining an accurate diagnosis. Furthermore, dental and medical clinicians should be aware that often odontogenic lesions can be a common cause of cutaneous sinus tract infection. Once the etiology is clear and the causative factors are removed, the stoma and the sinus tract will close spontaneously within several days.

In this report, tooth 36, which was determined to be nonvital and immature, was successfully treated with apexification using calcium hydroxide to induce formation of a calcified barrier at the root terminus. The time needed to form a barrier of a root canal apex varies, which is dependent upon the size of the apical foramen, as well as the severity of the periapical lesion.

Alternatively, mineral trioxide aggregate (MTA) can be used as a rapid apexification protocol. However, both MTA and calcium hydroxide do not promote continued development of the root, leading to a thin root dentin and a large canal lumen, thus predisposing the tooth to possible fractures (Cvek, 1992). Recent developments in revascularization and regeneration therapy in apexogenesis to yield thicker and longer root development using 3Mix-MP paste (including ciprofloxacin, metronidazole, minocycline with propylene glycol, and macrogol) have shown to be an effective protocol for management of immature permanent molar teeth with pulpal necrosis (Sonmez *et al.*, 2013).

Furthermore, when accessing healing, the duration of lesion plays a critical role. Bodner *et al.* evaluated cases of 28 children with odontogenic cutaneous sinus tracts and found rapid healing occurs in lesions with shorter duration, whereas extraoral scarring occurred in lesions with longer duration (Bodner *et al.*, 2012). In the case report, the duration of the origination of the lesion up to the date of the correct diagnosis was estimated to be less than 3 months and the lesion healed with minimal scarring. Moreover, location of the lesion should also be taken into consideration. As presented in our case, the lesion was located in the submandibular region with relaxed skin tension and the scar is parallel to existing skin-tension lines, consequently yielding a better condition for wound remodeling (Son & Harijan, 2014).

Conclusions

Patients exhibiting non-healing cutaneous sinus tracts in the maxillofacial region need a thorough medical and dental evaluation. Once the infection of the offending tooth is removed by endodontic treatment or tooth extraction, healing of the cutaneous lesion will occur. Early diagnosis and accurate treatment are paramount for healing of OCSTs without scar formation.

Acknowledgements

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CASE REPORT

Orthodontic treatment of an adult patient with aggressive periodontitis –A case report

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Abstract

A 26-year-old man with an aggressive periodontitis sought for orthodontic treatment to improve the appearance of his smile. He presented with generalised anterior spacing, missing lower left central incisor and deep traumatic bite. He was treated successfully with a combination of orthodontic and periodontal treatment. After 18 months of orthodontic treatment and follow up by the periodontist, his alignment of teeth was improved, a stable occlusion was achieved, and occlusal trauma was prevented. As a result, the patient's smile appearance and self-confidence were improved. Orthodontic tooth movement is not only to correct the alignment of his teeth but also to improve the bone level especially at the anterior region. This case report shows the successful treatment outcome in aggressive periodontitis patient which requires good collaboration between the orthodontist and the periodontist.

Keywords: aggressive periodontitis, bone level, orthodontist and periodontist, occlusal trauma, traumatic bite

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Introduction

Awareness for orthodontic treatment among periodontic patients is increasing due to pathologic dental migration, which compromise the facial aesthetics (Brunsvold, 2005; Feng *et al.*, 2005). Periodontitis is the inflammation of the supporting tissues of the teeth, caused by specific microorganisms, which leads to progressive destruction of the periodontal ligament and alveolar bone with either pocket formation, recession, or both (Gyawali & Bhattarai, 2017). Interdisciplinary approach by the orthodontist and periodontist is required in the orthodontic management of cases with compromised periodontium (Vinod *et al.*, 2012).

Aggressive forms of periodontal disease have been defined based on the following primary features (Lang *et al.*, 1999): non-contributory medical history, rapid attachment loss and bone destruction, and familial aggregation of cases. The general secondary features of aggressive periodontitis is: amounts of microbial deposits inconsistent with the severity of

periodontal tissue destruction. The other secondary features were laboratory features such as elevated proportions of *Aggregatibacter actinomycetemcomitans* (previously named *Actinobacillus actinomycetemcomitans*) and, in some populations, *Porphyromonas gingivalis*; phagocyte abnormalities; hyper-responsive macrophage phenotype, including elevated production of prostaglandin and interleukin in response to bacterial endotoxins (Lang *et al.,* 1999).

Aggressive periodontitis affects adolescents, and the percentage of adolescents is highest among orthodontic patients (Bagga, 2010). Prevalence of aggressive periodontitis varies widely among various races and ethnicities from 0.1% to 15% (Albandar *et al.*, 2007). Besides, genetics, age, and environment may also influence it. Females are found to be more affected than male (Hormand & Frandsen, 1979).

Patients with an aggressive periodontitis may lose the interproximal attachment and this is the main factor for the pathological migration of teeth (Martinez-Canut *et al.*, 1997). Changes the position such as proclination, rotation, spacing and extrusion the anterior teeth may compromise aesthetics (Towfighi *et al.*, 1997). Orthodontic treatment can facilitate improvement by light intrusive orthodontic forces to correct the pathological extrusion and migration of teeth (Garat *et al.*, 2005; Panwar *et al.*, 2010). Nonetheless, orthodontic treatment should be started only after the clinician is convinced that the patient is well motivated and can follow the oral hygiene instructions well (Gyawali & Bhattarai, 2017).

During the treatment, it is important to reinforce good oral hygiene. Clinicians should inform the patients the consequences of poor oral hygiene on the teeth and supporting structures. Orthodontic patients with aggressive periodontitis also require a separate periodontal appointment with a periodontist once every 3 months (Levin *et al.*, 2012). Good communication and understanding between the orthodontist and periodontist are essential to achieve successful results and avoid unwanted complications. This case described the management of aggressive periodontitis through the interdisciplinary approach for improving the aesthetics and fulfilled the patient expectation. The correction of extruded upper central incisors with controlled intrusion led to a decrease in the clinical crown length, better access for oral hygiene procedures, better gingival form, and a more suitable distribution of occlusal forces (Rabie *et al.*, 1998).

Diagnosis and etiology

A 26 year old male presented in October 2015 at the Orthodontic – Periodontic - Restorative Joint Specialist Clinic in Klinik Pergigian Cahaya Suria, Kuala Lumpur. His chief complaint was “spacing and forward position of the upper teeth”. He was very keen to improve his esthetics, which affected his self-confidence. Patient’s medical history was non-contributory. He was a light smoker. He had mentioned during history taking interview that his mother’s siblings had lost their teeth at early age and wearing dentures. Pre-treatment records indicated that the patient had full mouth plaque score of 51% and bleeding score of 38%. The presence of plaque was noted to be at supragingival areas and thin in thickness. These scores had improved following completion of initial periodontal therapy to 23% and 36%, respectively, prior to referral to orthodontist.

He presented with a symmetrical face and Class I skeletal profile (Figure 1). Intraorally, he had Class II division 1 malocclusion with an overjet of 10mm. The molar relationship on the right and left side were in Class I and

Class III, respectively. In addition, the canine relationships on the right and left side were in Class II and III, respectively. The posterior segment appeared acceptable occlusion, but the patient's main concern was at the anterior region.

Anteriorly, he presented with a 90% deep bite and traumatic to the palate on mandibular left lateral incisor. His mandibular right central incisor impinged on the cervical area of maxillary right central incisor, with proclination of the maxillary right central incisor and extrusion of both maxillary and mandibular incisors. He lost his mandibular left central incisor in his early 20's due to mobility. He did not replace the mandibular left central incisor and unfortunately, the mandibular incisors migrated forward to fill an empty space area. Spacing in the upper and lower arch were 8mm and 5mm, respectively. The curve of Spee was 4mm. Gingival recession was noted at the anterior region with obviously exposed 1/3 of the root length of maxillary left central incisor. The periodontal probing depths were more than 6mm mainly at maxillary and mandibular incisors and molars; and right mandibular canine. Deepest probing depth was 9mm at mesial aspects of left maxillary central incisor. Mobility of grade 2 was recorded for maxillary incisor.

Figure 1. Pre-treatment extraoral and intraoral photographs

The panoramic radiograph (Figure 2) showed that all teeth were present except the mandibular left central incisor. It revealed a generalized horizontal bone loss of ≥ ⅓ of root length to ≥ ½ of root length, and more severe bone loss at first molars (≥ ½ of root length). A severe vertical bone loss of ≥⅔ of root length was obvious on periapical radiograph at maxillary central incisors (Figure 7(a)). There was pathological displacement of the upper right and left central incisors. The upper right and left central incisors appeared extruded and upper left central incisors displaced distally. Lateral cephalometric radiograph (Figure 2) showed a Class I skeletal pattern with proclined upper incisors. The mandibular plane angle within the normal range (28.7 degrees) and lower facial height ratio was normal. More cephalometric values pre-treatment as stated in Figure 6 and based on these findings, the patient was diagnosed with a Class II division I malocclusion on a Class I skeletal base with aggressive periodontitis.

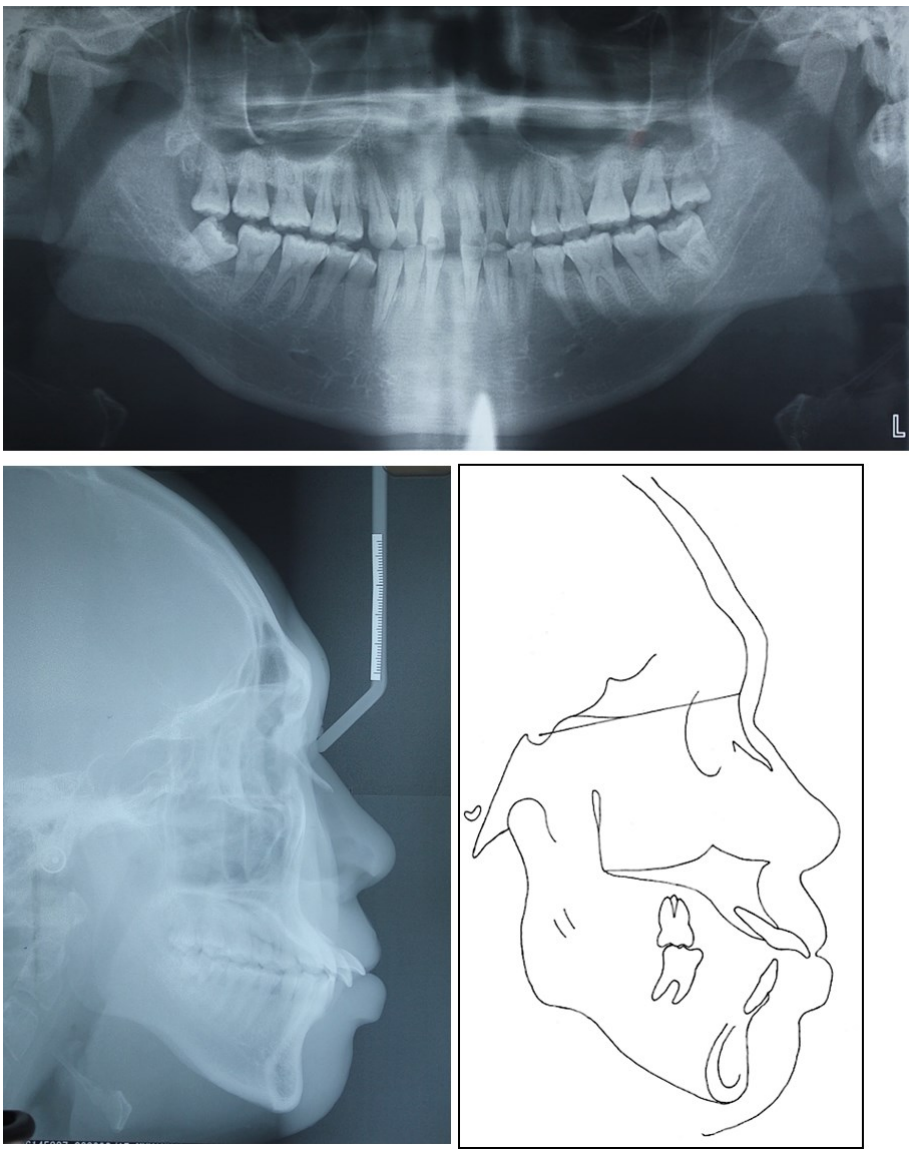


Figure 2. Pre-treatment panoramic radiograph and lateral cephalometric radiograph and tracing.

Treatment options

The treatment options that were given to the patient in the interdisciplinary Orthodontic-Periodontic-Restorative Joint Clinic are as follows:

1. Comprehensive periodontal treatment until maintenance phase, followed by orthodontic treatment and later with Prosthodontic treatment. The patient needs to start with 2-3 years orthodontic treatment with regular follow up (every 6-8 weeks) with the orthodontist. In addition to that, patient have to be committed to the periodontal health care maintenance and attends regular review (every 3 months) with the periodontist throughout the active orthodontic treatment. After the course of the orthodontic treatment, the missing tooth was planned for replacement with a prosthesis, followed by night time (life time) upper and lower retainers. The patient was warned of potential loss of vitality of the upper central incisors and thus, needed close monitoring.
2. Endodontic treatment for upper right and left central incisors followed by crown placement. This treatment option saved cost and time for the patient. However, the traumatic overbite would not be corrected and it may cause continued trauma on the palatal mucosa.
3. Extraction of the upper central incisors and fabrication of prosthesis with either fixed prosthesis or partial removable prosthesis on upper central incisors. This treatment option may cause the patient to lose two sound teeth, which would be replaced with prostheses. The overbite would be not be corrected and the lower incisors may occlude on the fixed prosthesis or acrylic and may cause mobility of the lower incisors.
4. Orthodontic extrusion of upper centrals incisors to create bone for further prosthesis treatment. This treatment option require orthodontic treatment and the overbite would be not corrected.

After the discussions and considering the risks and complications, the patient decided to proceed with combined orthodontic, periodontic and prosthodontic treatment which was the first option given to him. The patient agreed with the explained treatment and signed the written consent. The patient was referred to Periodontist prior to Orthodontic treatment.

The orthodontic treatment objectives were built on the complete treatment objectives for periodontal health (Xie *et al.*, 2014).

The complete periodontal treatment objectives for this patient consisted of:

i) The medical problem was non-contributory and the patient was instructed to stop the smoking habit (Azouni & Tarakji, 2014).
ii) Motivation and customized oral hygiene instructions were given in order for him to maintain good oral hygiene. The initial periodontal therapy was directed towards elimination or suppression of the infecting microorganisms and providing an environment conducive to long-term maintenance, of which include full mouth scaling and root planing (subgingival debridement). The subgingival debridement with combined systematic antibiotics as an adjunctive (Guerrero *et al.*, 2005). Reassessment was made for all the periodontal parameters and ensured stable. The full-mouth plaque index was targeted to be within 25%, the full-mouth percentage of positive bleeding on probing sites less than 30%, and no residual pockets deeper than 5 mm (Xie *et al.*, 2014).
iii) Patient was referred to the orthodontist and restorative specialist to achieve stable occlusion and restore aesthetics (Azouni & Tarakji, 2014).

iv) Once orthodontic treatment was finished, the maintenance phase began. The patient was required to be reviewed every 3 to 6 months to prevent reinfection and recurrence (Xie *et al.*, 2014).

After the initial phase of periodontal therapy, the patient was referred to Orthodontist. The Orthodontic treatment objectives were to:

i) Secure the optimum oral hygiene before starting orthodontic treatment.
ii) Ensure the vertical control in the reduction of overbite.
iii) Eliminate dental crowding, intrusion of upper centrals, level and align the teeth.
iv) Retract upper incisors to close the spaces and maintain the space for lower left central incisor.
v) Obtain ideal overbite and overjet.
vi) Achieve a mutually protective functional occlusion.
vii) Retain the corrected results and referred to prosthodontist for the replacement of the missing teeth.

Treatment progress

Patient was instructed to do his full medical examination to exclude the systemic diseases. Once patient notified his medical condition was clear, the patient was seen by Periodontist for the oral hygiene instructions and increased his motivation to maintain good oral hygiene. The scaling and subgingival debridement with combined systemic antibiotics regime as an adjunctive. This treatment approach has been thoroughly validated in randomized controlled clinical trials (Aimetti *et al.*, 2012; Guerrero *et al.*, 2005; Mestnik *et al.*, 2010; Mestnik *et al.*, 2012): achievement of adequate supragingival plaque control (<25% of tooth sites with detectable plaque); rigorous subgingival instrumentation with a combination of hand and ultrasonic instruments completed within 2 days; and an adjunctive systemic antibiotic regime.

After three months the patient was referred to orthodontist when the periodontal status satisfied the referral criteria, which were proper infection control, full-mouth plaque index within 25%, the full-mouth percentage of positive bleeding on probing sites less than 30%, and no residual pockets deeper than 5 mm (Xie *et al.*, 2014). Patient was able to maintain satisfactory periodontal parameters throughout active orthodontic treatment as required.

Orthodontic treatment started in December 2015 and finished in June 2017. It took 18 months to achieve the stable and good occlusion. In order raise the bite for the placement of upper and lower fixed appliances, the upper removable appliance with an anterior bite plane and plint clasp was constructed. After two weeks, pre-adjusted edgewise brackets (0.022x0.028-in, MBT prescription) were bonded to all the teeth except the upper second molars (Figure 3).

Upper and lower 0.012-in nickel titanium archwires were placed and treatment progressed up to 0.019x0.025-in stainless steel archwires. Initial alignment followed by levelling in the upper and lower arches was achieved in 6 months. Upper and lower 0.019x0.025-in stainless steel archwires were maintained for 2 months in order to fully express the torque. The anterior bite plane was removed and en-masse retraction of the upper arch was done with elastic chains (150g) to close the remaining spaces. In the lower arch, same mechanics was used as an upper in order to retract lower arch. The space for lower left central incisor was maintained with dead coil spring.

Figure 3. Upper fixed appliances combination with anterior bite plane and plint clasp to facilitate the placement of fixed appliance in the lower arch.

After 16 months of active orthodontic treatment, all the spaces closed. Two months of finishing and detailing were done, the brackets were debonded and the patient was given the Hawley retainer. The patient was instructed to wear the retainers for 24 hours throughout 6 months and to continue wearing the retainers at night indefinitely. The patient was referred to prosthodontist for the construction of partial denture in the lower arch. The partial denture were planned to be replaced by resin bonded bridge. In addition, the vitality of upper centrals regularly monitored. Even though the orthodontic treatment was on the retention phase, the patient need to have regular review by periodontist for every 6 months to ensure the maintenance of good periodontal health and prevention of reinfection. It was reported that after a year after he was debonded, the patient was able to maintain good oral hygiene; the plaque and bleeding indexes were kept below 30%; all probing depths were $\leq$5mm, and improved mobility of the maxillary incisor teeth.

Treatment results

Figures 4 to 6 show the final outcome of the case. The post-treatment frontal photograph showed that there was significant improvement of his facial aesthetics. He appeared more confident with his smile compared to the pre- treatment frontal photograph. His facial profile showed more balanced with competent lips due to significant retraction of upper and lower lips, as shown in the general superimposition (Figure 6). After the treatment the occlusion was stable with acceptable alignment of the teeth, normal overjet and overbite. The traumatic occlusion was corrected with the proclination of lower teeth as shown in the mandible superimposition (Figure 6). In addition, the pathological migrated upper central incisors were successfully intruded and retracted. The adequate space for lower left central incisor was prepared for future prosthesis. The mobility of upper anterior teeth improved from grade 2 mobility in pre-treatment to grade 1 mobility in post-treatment.

The post-treatment panoramic radiograph (Figure 5) showed minimal root resorption especially in the anterior region. Periapical radiograph (Figure 7) of upper centrals showed some sign of bone deposition from 1/3 the root tip in pre-treatment and improved to nearly 1/2 of the root tip in post-treatment.

Figure 4. Post-treatment facial and intraoral photographs

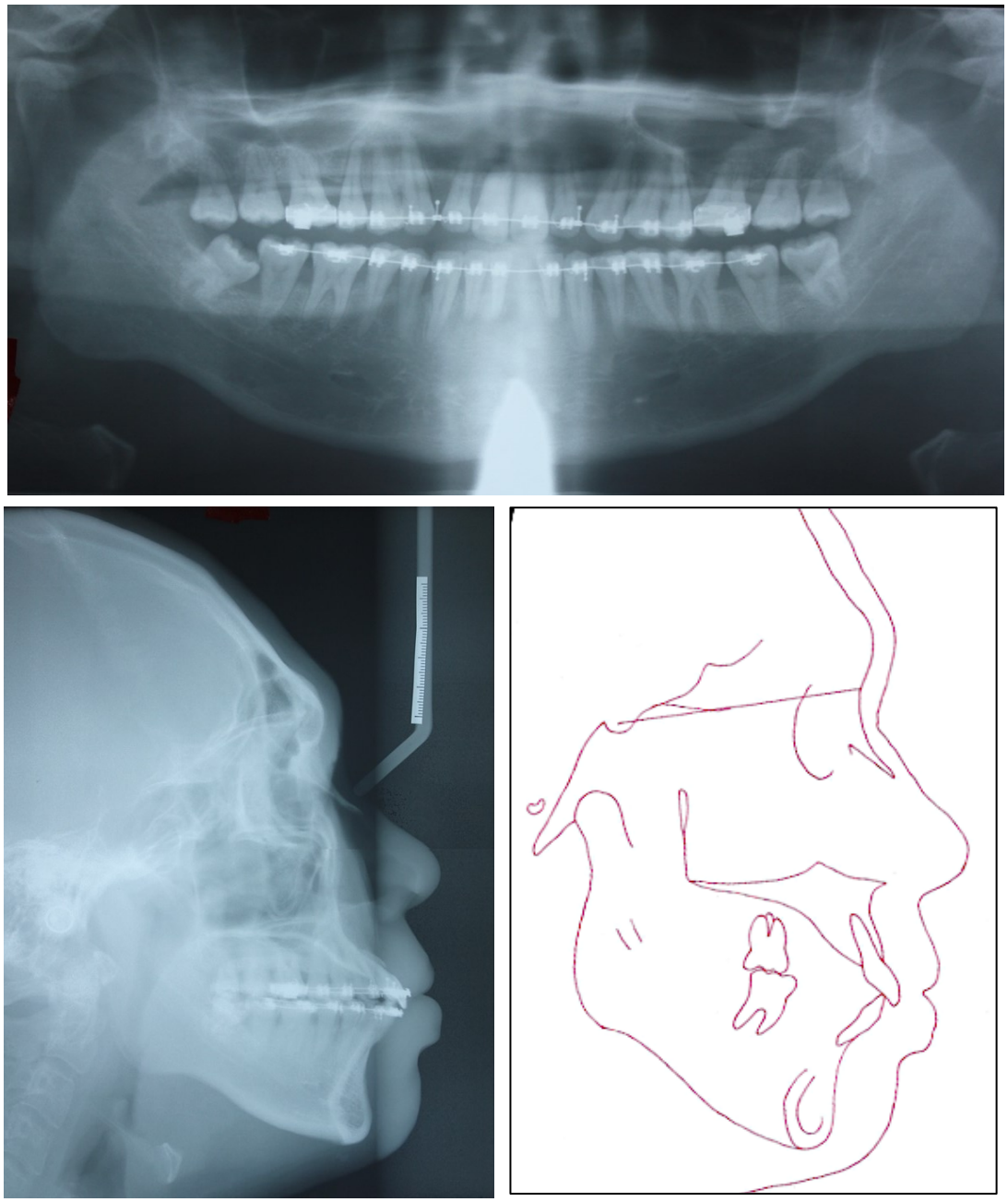
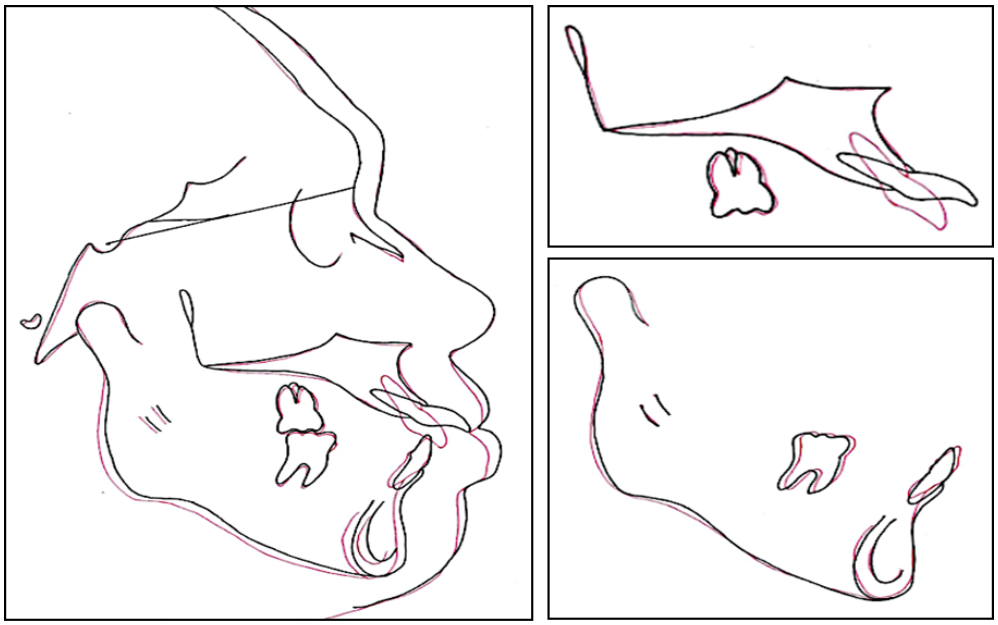


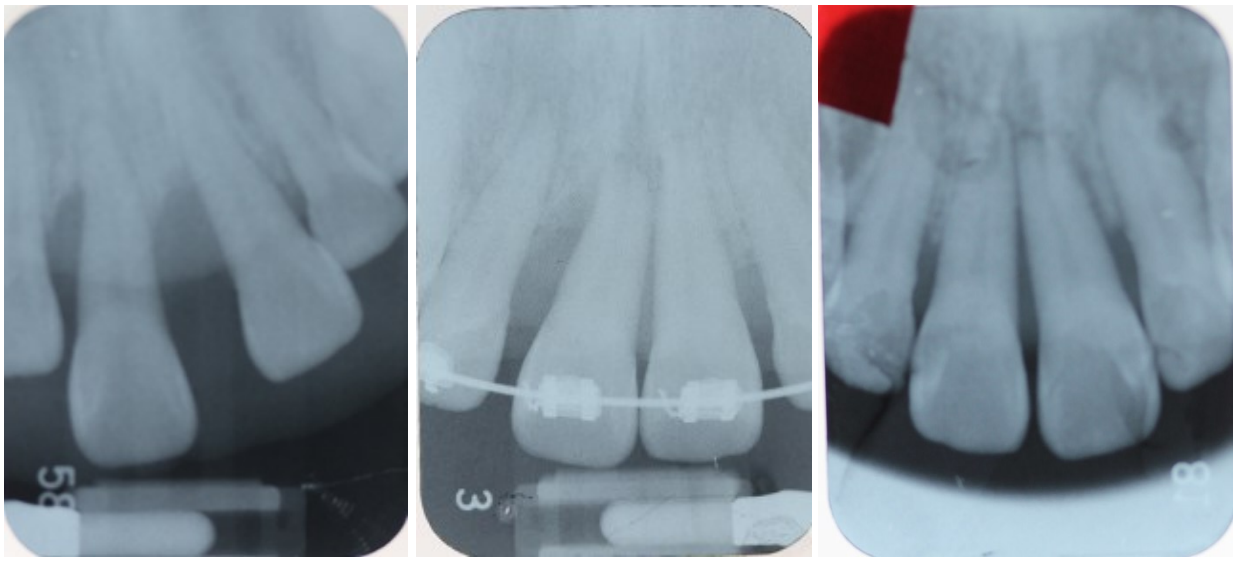
Figure 5. Pre-treatment panoramic radiograph, lateral cephalometric radiograph and tracing, Lateral cephalometric radiograph measurements



Cephalometric Measurements			
	Pre-treatment	**Post-treatment**	**Difference**
Skeletal			
SNA (°)	85°	85°	0°
SNB (°)	80°	78°	-2°
ANB (°)	5°	7°	+2°
SN-Mx (°)	7°	7°	0°
FMA (°)	34°	35°	+1°
Dental			
U1 to NA	+14.0mm	+5.0mm	-9.0mm
U1 to Mx	143°	115°	-28°
L1 to NB	+10.0mm	+11.0mm	+1.0mm
L1 to MP	90°	97°	+7°
IIA	94°	114°	+20°
Facial			
E-line to UL	+2.5mm	+1.0mm	-1.5mm
E-line to LL	+7.0mm	+4.0mm	-3.0mm
LFH ratio	57.0%	58.0%	+1.0%

U1, maxillary incisor; L1, mandibular incisor; UL, upper lip; LL, lower lip; LFH, lower facial height; Mx, Maxillary Plane; MP,Mandibular plane; IIA, Interincisal Angle

Figure 6. The overall superimposition, maxillary and mandibular superimpositions with the measurements are also shown. Pre-Treatment (Black), Post-Treatment (Red)



(a) Pre-treatment (b) Mid-treatment (c) Post-treatment

Figure 7. Evidence of bone deposition on the mesial and distal root of 11 and 21 respectively. The bone level was about ⅓ of alveolar bone crest at the root tip (in pre-treatment periapical radiograph) had improved to nearly ½ in post-treatment radiograph. Pre-treatment periapical radiograph from the left (a) Post-treatment periapical radiograph; (b) Mid-treatment periapical radiograph; (c) Post-treatment periapical radiograph.

Discussion

Aggressive periodontitis is described based on age‐dependent terminology such as early‐onset periodontitis or juvenile periodontitis (FH, 2018). In the 1999, Classification Workshop of the American Academy of Periodontology (AAP), a consensus report adopted the term aggressive periodontitis as a new name for this unique disease classification, replacing the term early-onset periodontitis (Lang *et al.*, 1999; Tonetti & Mombelli, 1999). This proposed terminology was to the greatest extent based on clinical presentation and the AAP committee concluded that all periodontal diseases were infectious in nature but could be categorized as either slowly progressing (chronic) or rapidly progressing (aggressive) disease (Armitage, 1999, 2000; Fine *et al.*, 2018). The prevalence of aggressive periodontitis varies significantly between populations and different racial/ethnic origins (Susin *et al.*, 2014). The pool prevalence of aggressive periodontitis was 1.6% and relatively higher prevalence was found in Africa (4.2%) (Bouziane *et al.*, 2020). In a small population study in Malaysia, the prevalence of aggressive periodontitis was 5.3%(Yee *et al.*, 2016). However, the most recent periodontal classification 2017 was just launched during EuroPerio9 in Amsterdam in 2018. This new classification had regrouped the chronic and aggressive periodontitis as a single category. Since the classification is fairly new to most clinician, the terminology of periodontal disease within this paper follows the previous classification.

In this patient, the diagnosis of aggressive periodontitis was based on the patient history, clinical examination, and radiographic assessment (FH, 2018). It is important to note that the patient was a healthy patient without systematic disease and this is an primary feature of aggressive periodontitis (Albandar, 2014). Another

primary feature that defines the aggressive form of periodontal disease is rapid attachment loss and bone destruction in young age with genetic component (Lang *et al.*, 1999). The definitive diagnosis of aggressive periodontitis for this patient was also made based on the secondary features whereby the amounts of microbial deposits inconsistent with the severity of periodontal tissue destruction (Lang *et al.*, 1999). This case was sub-classified as generalized aggressive periodontitis as the patient exhibited generalized interproximal attachment loss affecting at least three permanent teeth other than first molars and incisors (Lang *et al.*, 1999; Tonetti & Mombelli, 1999).

Pre-treatment records indicated that the patient had full mouth plaque score of 51% and bleeding score of 38%. The presence of plaque was at supragingival area and thin in thickness. These scores had improved following completion of initial periodontal therapy to 23% and 36%, respectively. Although scores for bleeding index were similar, the post treatment bleeding was only minute bleedings (pin points-like). Plaque accumulation was noted on the pre-treatment photos and this indicated possibility that the patient has some form of local aetiologic factor contributing to the periodontal disease for aggressive periodontitis (Albandar, 2014). It was reported that throughout the active orthodontic treatment period and after a year after he was debonded, the patient was able to maintain good oral hygiene; the plaque and bleeding indexes were kept below 30%; all probing depths were ≤5mm, and improved mobility of the maxillary incisor teeth.

After the initial periodontal phase, the patient was referred to the orthodontist for the corrective phase. In the corrective phase, the aesthetic concern was managed by orthodontist and Prosthodontist. Orthodontic treatment with a proper force and good oral hygiene may avoid further damage of the periodontal tissue because with plaque, the same force can cause bone defects and attachment loss (Garat *et al.*, 2005). In this patient, it was extremely important for both periodontist and orthodontist to work together to eliminate inflammation before, during, and after the orthodontic treatment. In the retention phase of orthodontic treatment, the maintenance phase begin and the patient need to be reviewed every 3 to 6 months to prevent recurrence (Xie *et al.*, 2014).

Orthodontic treatment plan and accurate biomechanics in a three dimensions need to be considered before starting the orthodontic treatment. The main factor of reduced bone level and loss of periodontal support made the orthodontist treat this case differently like other normal cases. In a sagittal dimension, placement of upper removable appliance with stopper on the mesial of upper canines was an advantage of this case. Upper removable appliance covered the palatal mucosa and palatal surface of the upper teeth acted as anchorage unit for the upper arch. Initial placement of light force with upper 0.012-in Nickel Titanium (Hazan-Molina *et al.*, 2013) in combination with an anchorage reinforcement by upper removable appliance used to move upper centrals with reduced bone support (FH, 2018). In these case, the center of resistance was moved apically, and large moments are needed to control the root movement given the increased moment of the applied force (Nanda, 2012).

In a sagittal direction, a large moment created for upper right central controlled by the other anterior teeth and upper anterior bite plane. Once the 0.012-in archwire ligated on the upper anterior teeth, the crown of upper right central incisor tipped palatally. However, the palatal movement of upper central incisor limited by the labial movement

of upper right lateral incisor and upper left central incisor. Transient increased in overjet happened and was reduced by the retraction of upper labial segment. However, this round tripping movement caused the incisors root to resorb as evident in the periapical radiograph (Figure 7) (Alexander, 1996; Krishnan, 2017). In addition, other advantage of using upper removable appliance covering the palate in this case could reduce the mesial migration of molars, termed the "row-boat effect" (Kim *et al.*, 2014). In the lower arch, the curve of Spee flattened by the proclination of lower incisors and extrusion of lower posterior teeth with the placement of lower fixed appliances.

In the vertical direction, placement of upper archwire into a high upper right central incisor deformed the general arch form and could cause canting of the occlusal plane (Nanda & Tosun, 2010). However, this side effect was reduced by the use of the upper removable appliance and the placement of upper 0.019X0.025 -in stainless steel archwire, which corrected the root angulation and occlusal canting. In the lower arch, the proclination of lower incisors with placement of lower fixed appliances corrected the overbite. In a transverse direction, placement of the fixed appliance in the high position of upper right central and distal angulation of upper left central caused correction of the upper left central distal angulation and moved the of the upper midline towards to the left due to the larger moment for the right central incisor and later the upper midline would be corrected by the placement of elastics.

Anterior bite plane played an important role in the case because it eliminated of occlusal force and facilitated correction of deep bite. The gentle force with step by step manner in changing the archwire allowed correction of the position of upper central incisors. In the finishing stage, the stiff archwire 0.019X0.025-in stainless steel was used to control the roots of the teeth. Excellent control of torque facilitates the stabilization of tooth roots in the central cancellous bone of the alveolar bone and contributes to reconstruction of the bone (Xie *et al.*, 2014). The newly formed bone is shown in the periapical x-ray films (Figure 8).

Correction of Class II division I malocclusion usually required large amount of space of about 14mm in order to reduce the overjet, which is commonly managed with extractions of premolars. In this case, the periodontal health was compromise and did not favour extraction to reduce the overjet. However, the overjet correction was facilitated by proclination of lower incisors, retroclination and intrusion of the upper incisor. The facial profile was improved as the lip changes followed the supporting incisors. Overall, a downward and backward displacement of the mandible was observed due to the side effect of extrusion of the upper and lower molar as shown in the superimpositions (Figure 6).

In terms of retention of the orthodontic results and long term follow up, removable retainers were used in the upper and lower arch because removable retainers aided periodontal health maintenance but the poor compliance by the patient may result in relapse (Gyawali & Bhattarai, 2017). Fixed retainers was not in option after active orthodontic treatment because it may contribute to plaque retention (Levin *et al.*, 2008). In addition, periodontal examination should be done for each follow up session because for the patient who have not developed aggressive periodontitis till the end of active orthodontic therapy may develop during the retention phase (Gyawali & Bhattarai, 2017). Regular periodontal follow-up and combine radiographic examination once a year along with strict oral hygiene measures contribute to long term maintenance of the achieved result (Harpenau & Boyd, 2000; Levin *et al.*, 2012).

Conclusion

Management of patients with an aggressive periodontitis is a challenge for orthodontist and periodontist. The importance of having good oral hygiene before, during and after orthodontic treatment significantly improve the function, esthetic and periodontal health of the patient. Therefore, a good collaboration between orthodontist and periodontist are important to ensure the successful treatment outcome in patient with aggressive periodontitis.

Acknowledgement

The authors would like to thank all staff at Orthodontic Unit, Klinik Pergigian Cahaya Suria (Sementara), Kuala Lumpur for assistance and contribution to this case report. Special gratitude to the Director General of Health Malaysia and the Principle Director of the Oral Health Division, Ministry of Health Malaysia for permission to have this case report published.

Declaration of patient consent

The authors certify that they have obtained all appropriate patient consent forms. In the form the patient(s) has/have given his/her/their consent for his/her/their images and other clinical information to be reported in the journal. The patients understand that their names and initials will not be published, and due efforts will be made to conceal their identity, but anonymity cannot be guaranteed.

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TECHNICAL REPORT

Safe dental practice during the COVID-19 pandemic

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Abstract

Coronavirus disease 2019 (COVID-19) has caused widespread public health concerns and many disruptions in our daily life. Dental professionals may encounter patients with COVID-19 infections in their practice. This technical report provides suggestions and recommendations for implementing infection control in the dental office during COVID-19 pandemic.

Keywords: COVID-19, SARS-CoV-2, dental, infection control

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Introduction

An unknown pneumonia-like virus outbreak was first reported in Wuhan City, Hubei Province, China in late December 2019, originating from the Hunan South China Seafood Market (Guo *et al*., 2020). On the 12 January 2020, the World Health Organization (WHO) named the disease as 2019-nCoV, and later COVID-19 in February 2020 (Li *et al*., 2020; Sun *et al*., 2020). This highly infectious disease is caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) (Gorbalenya *et al*., 2020). Genome sequencing revealed that the coronavirus has 96.3% genetic similarity to Yunnan bats (Hu *et al*., 2018; Zhou *et al*., 2020) and 99% genetic similarity to pangolins (Wahba *et al*., 2020), albeit the source of the virus remains unknown. The infection spread rapidly to other provinces in China and later to over 211 countries worldwide (Phelan *et al*., 2020), with the virus infection being classified as pandemic by the WHO on 12 March 2020. As of 16 November 2020, there are over 54.5 million confirmed cases worldwide with 1,318,884 deaths (approximately 2.42% mortality rate) (Dong *et al*., 2020).

Routes of transmission

The virus is transmitted via direct inhalation of respiratory droplets when an infected individual coughs or sneezes in close proximity within one meter radius (Rodriguez-Morales *et al*., 2020), or indirectly by touching an infected surfaces and then contacting the nasal, oral or eye mucous membranes (Lu *et al*., 2020; Santarpia *et al*., 2020). Studies have also revealed the disease may be transmitted via saliva (Li *et al*., 2020; To *et al*., 2020) and feces *(Zhang et al*., 2020). Hoffmann *et al.* (2020) showed that SARS-CoV-2 could bind to human angiotensin-converting enzyme 2 (ACE-2) receptors which are concentrated in salivary glands, thus suggesting that saliva could serve as reservoir for the virus transmission. New evidence has also suggested that the virus is transmissible through asymptomatic

patients (Chan *et al*., 2020; Rothe *et al*., 2020). The incubation period was found to be up to 14 days before any symptoms appear (Backer *et al*., 2020; Guan *et al*., 2020; Huang *et al*., 2020), therefore transmission of SARS-CoV-2 is possible before any symptoms are evident. Backer and colleagues estimated that the mean incubation period to be 6.4 days, ranging from 2.1 to 11.1 days (Backer *et al*., 2020). Studies have also revealed that the virus can stay active for more than six days on different surfaces, such as plastic, metal and glass (Otter *et al*., 2016; Kampf *et al*., 2020).

Symptoms

Symptoms of a patient infected with SARS-CoV-2 may include dry cough, fever, loss of smell and/or taste, muscle weakness, hemoptysis, headache, sputum production and diarrhea (Chen *et al*., 2020; Giacomelli *et al*., 2020; Huang *et al*., 2020). More than 80% of the infected individuals can recover on their own since they have only mild symptoms that resemble flu-like symptoms (Wu & McGoogan, 2020). Individuals over 70 years old with medical problems such as diabetes, hypertension, cardiovascular disease and immunosuppression typically develop severe acute respiratory symptoms (Guan *et al*., 2020) which may require ventilator support; about 1 in 10 patients of aged 70 and over die *(*Chen *et al*., 2020; Huang *et al*., 2020; Sun *et al*., 2020).

Transmission in dental practice

Dental profession is in a high risk group for contracting the virus as the saliva (Li *et al*., 2020) and aerosol (Srirengalakshmi *et al*., 2020) can potentially transmit the disease if appropriate preventive measures are not taken. The risk of infection is high due to the close physical proximity of dentists to their patient's oropharyngeal region during the entire process of dental treatment, the long duration of close proximity and aerosol generation associated with many procedures in dentistry. There have been reports of SARS-CoV-2 spread to health care professionals given the high transmissibility of the disease (Lan *et al*., 2020; Wax & Christian, 2020). Typically in a dental setting, SARS-CoV-2 transmission occurs through four major routes: (1) direct inhalation of respiratory droplets or direct exposure to infected saliva or blood; (2) indirect contact with contaminated fomites; (3) inhalation of suspending airborne viruses; and (4) mucosal contact with contaminated droplets/aerosols (Harrel & Molinari, 2004; Liu *et al*., 2011; Chen, 2020; Cleveland *et al*., 2016; Kampf *et al*., 2020). Therefore, all dental team members must take appropriate measures for prevention and management to mitigate the further spread of SARS-CoV-2 in the dental environment.

The following recommendations and information are current up to October 2020. It is anticipated that new information and guidelines will emerge as this novel disease is evolving and new knowledge surfaces. Dental practitioner may use this report as a starting point and continue updating themselves with the latest information as this outbreak continues.

Patient triage

Prevention can start even before the patient arrives at the dental practice. Following the recommendations published by the Mayo Clinic (https://www.mayoclinic.org/covid-19), standard questions can be asked by the receptionists / nurses over the phone for initial screening to establish their COVID-19 risk (patient triage by telephone). The three most pertinent questions for triaging can include (Ather *et al*., 2020):

- Do you experience any flu or flu-like symptoms such as fever with temperature more than 37.5°C, cough, sore throat, runny nose, shortness of breath, wheezing, headache, tiredness, muscle aches and chills of sudden onset?
- Have you travelled overseas or to any red zone areas in the past fourteen days?
- Have you come in contact with anyone confirmed with COVID-19 / anyone under quarantine / person under investigation (PUI)?

Patients can also fill up health and travel declaration form on the arrival or the form can be emailed to them prior to their appointments. Patient triage is strongly recommended either before or upon patient's arrival at the clinic (Meng *et al*., 2020). Based on Centers for Disease Control and Prevention (CDC) 2020 and American Dental Association (ADA) 2020 guidelines for dentists on the coronavirus disease, should the answer to any of these questions is yes, these high-risk patients must be postponed for non-emergency dental appointments. Meng *et al*. (2020) suggested for postponing the non-emergency dental treatments in an asymptomatic patient up to fourteen days following close contact with an infected subject, or those individuals who have recently traveled to a red zone area owing to the incubation period of SARS-CoV-2. These individuals must self-quarantine and contact their local health authorities through telephone (Wang *et al*., 2020).

As per the recommendations by ADA (2020), genuine dental emergencies must be dealt with and treated appropriately, for example patients with severe dental pain, swelling, soft tissue infection, longstanding ulcers, soft tissue damage, dentoalveolar trauma, intra-oral trauma from denture, orthodontic appliances, broken bracket/tubes/bands with possibility of foreign body inhalation. Waiting and treatment time must be kept short, with patient seen on the last appointment of the day. Treatment is carried out in a dedicated separate room with limited staff in order to reduce the number of people exposed (Lockhart *et al*., 2020). Other requirements are similar to aerosol generating procedures (AGPs) guidelines which will be discussed later.

With regards to facility, points of entry need to be limited. This includes separation into staff and patient's entrance if feasible. Everyone coming into the building must be screened, with history taken and temperature check performed (Srirengalakshmi *et al*., 2020). The health status of all dental staff must be ascertained. Staff with flu symptoms need to be quarantined at home or visit a doctor.

Body temperature check should be performed at the reception on patient's arrival, preferably with a contact-free forehead thermometer (Li & Meng, 2020; Peng *et al*., 2020) or temperature sensor mounted on tripods (Srirengalakshmi *et al*., 2020). Hand sanitizer is provided for patients. Patients must be requested to wear a surgical mask in the waiting area and physical distancing of at least one meter must be observed at all times to minimize spread of the disease (Dorst, 2020). The flowchart in Figure 1 provides a simplified pathway from the time the patient arrives at the clinic until treatment completion for the new normal practice (Fallahi *et al*., 2020).

Hand hygiene

It is extremely important to have good hand hygiene in the dental practice, since oronasal route is one of the transmission routes of COVID-19 (Meng *et al*., 2020;

Peng *et al*., 2020). The principle of 'bare below elbow' should be practiced by all dental personnel: no wristwatch, no jewelry and short sleeves (Li & Meng, 2020). The recommended seven-step hand washing time by WHO is 40 to 60 seconds using water and soap (WHO, 2020) (Table 1).

Likewise, alcohol hand rub of at least 60% ethanol or 70% isopropanol must be adopted by dental health practitioners to reduce the risk of SARS-CoV-2 transmission (Lotfinejad *et al*., 2020). Hand rubbing time should be between 20 and 30 seconds as recommended by WHO (WHO, 2020) (Table 2).

In principle, good hand hygiene must be practiced before any patient contact, before any aseptic task, after body fluid exposure, after patient contact and after contact with patient surroundings (Li & Meng, 2020; Peng *et al*., 2020).

Personal Protective Equipment (PPE) for dental professionals

PPE must be worn for dental staff on duty as per the advice given by Occupational Safety and Health Administration (OSHA) (2020). PPE is not only just for clinicians, but also for all the other supporting staff in the dental clinic. There may be slight variations in certain dental clinics in terms of the level of PPE required. Many of the suggestions here are based on *'International Islamic University Malaysia (IIUM) Medical Centre: Infection Prevention & Control Orientation Checklist'*. These can be divided into level 1, level 2 (droplet), level 2 (airborne) and level 3 as illustrated in Table 3. It is advisable to always refer to your local setting for PPE recommendation.

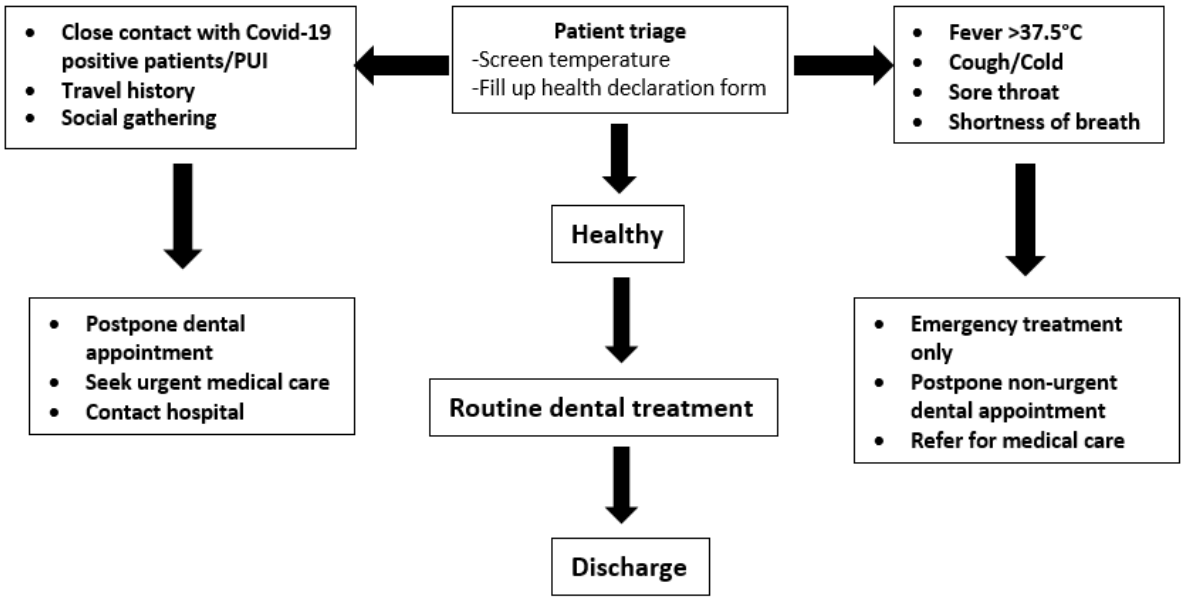


Figure 1. Dental clinic workflow during the COVID-19 pandemic (Fallahi *et al*., 2020)

Table 1. Seven steps of hand washing techniques

	Step 1 Hands are wet with liquid soap. Hands are rubbed from palm to palm.
	Step 2 The back of both hands is rubbed.
	Step 3 Palm to palm with fingers interlaced.
	Step 4 Back of fingers to opposing palm, with fingers interlocked.
	Step 5 Rotational rubbing of left thumb clasped in right palm, and vice versa.
	Step 6 Rotational rubbing backward and forward with clasped fingers of right hand in left palm and vice versa.
	Step 7 Right hand wrapped over left wrist using rotational movements up to mid forearm and vice versa.

Table 2. Seven steps of hand rubbing techniques

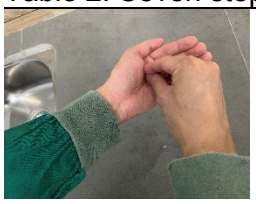
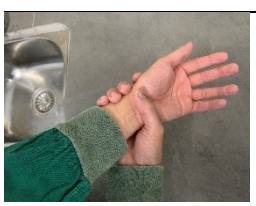
	Step 1 Adequate hand sanitizer is poured onto left palm. All fingers of the right hand are dipped onto the left palm and vice versa.
	Step 2 Hands are rubbed from palm to palm.
	Step 3 Right palm over left dorsum with interlaced fingers and vice versa.
	Step 4 Palm to palm with fingers interlaced.
	Step 5 Back of fingers to opposing palms with fingers interlocked.
	Step 6 Rotational rubbing of left thumb clasped in right palm and vice versa.
	Step 7 Rotational rubbing of left wrist clasped in right palm and vice versa.

Table 3. PPEs required and the different levels of infection exposures

Level 1 No suspected infectious agent Suspected exposure to blood and / or other body fluids	• 3-ply surgical mask / Face shield / Goggles • Plastic apron • Gloves
Level 2 (Droplet) Suspected or confirmed infectious agent spread by the droplet route e.g. respiratory viruses	• 3-ply surgical mask / Face shield / Goggles • Plastic apron • Full body gown • Double gloves (Verbeek *et al*., 2019)
Level 2 (Airborne) (Fig 2) ➢ Suspected or confirmed infectious agent spread by the airborne route e.g. pulmonary TB, measles ➢ Aerosol-generating procedures	• N95 / Face shield / Goggles • Plastic apron • Full body gown • Double gloves • Head cover • Shoes cover
Level 3 For suspected or confirmed infectious diseases of high consequence spread by direct/indirect contact/airborne route e.g. SARS, MERS-CoV, Ebola virus, Avian influenza	• N95 / Face shield / Goggles • Jumpsuit • Plastic apron • Double gloves • Shoes cover

Adapted from 'Infection Prevention & Control Orientation Checklist IIUM Medical Centre'

PPE required depends on the risk of procedure, that is whether the procedure is aerosol generating (AGPs) or non-aerosol generating (non-AGPs). Aerosols are produced when air current moves across the surface of a film of liquid. The greater the force of the air, the smaller the particles that are produced (Jones & Brosseau, 2015). AGPs are defined as any procedure that results in the production of airborne particles (aerosols) (Gralton *et al*., 2011; Judson & Munster, 2019). These are relevant to COVID-19 transmission since this may occur via both direct air-borne infection (Judson & Munster, 2019) and indirect spread via contact with contaminated surfaces (Ferretti *et al*., 2020). Restriction of AGPs is, therefore, an important control measure. In essence, AGPs present high risk of virus transmission compared to non-AGPs. Examples of dental AGPs include the use of high/slow-speed handpieces, use of sonic/ultrasonic scalers, use of 3 in 1 air syringes, air polishing and air abrasion (Leggat & Kedjarune, 2001; Cleveland *et al*., 2016). Figure 2 illustrated the PPE required when performing an AGP.

There should be adequate time between each appointment and extra disinfection time must be taken into account between patients. As mentioned earlier, AGPs are scheduled towards the end of the day, and preferably it is performed in a separate treatment room. The room should have a good ventilation system with limited number of attending staff (Lockhart *et al*., 2020). Air should be exhausted directly to the outside building or filtered through a High Efficiency Particulate Air (HEPA filter) (Mousavi *et al*., 2020). A HEPA filter must remove at least 99.95% of particles in which the diameter is equivalent to 0.3 micron according to European Standard. Following AGPs, aerosol takes up to 60 minutes to settle in neutral pressure room (van Doremalen *et al*., 2020). Therefore, the treatment room door should be closed and windows facing out the building are opened (Izzetti *et al*., 2020).

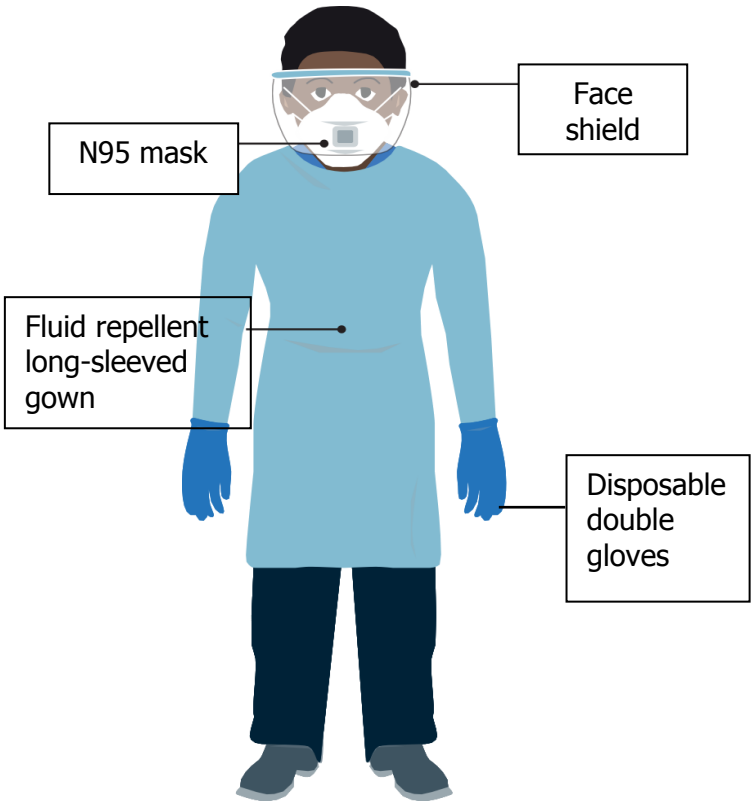


Figure 2. PPE required when involved in aerosol generating procedures (AGPs)
(Figure adapted from 'Public Health England: A visual guide to safe PPE')

Studies have proven the effectiveness of N95 masks in the prevention of viral respiratory infections among the health care workers (Radonovich *et al*., 2019; Long *et al*., 2020). N95 mask has the feature of good air tight and close facial fit during AGPs (Desai & Mehrotra, 2020), with high fluid resistance which is suitable when large amount of aerosols are being produced (Loeb *et al*., 2009). Mask filtration efficiency is measured by particle filtration efficiency (PFE) and bacterial filtration efficiency (BFE), and N95 mask can block more than 95% of 0.3 micron particles according to Food and Drug Administration (FDA). For fit checking of N95 mask, it should collapse slightly on inhalation and expand slightly upon exhaling. There should be no air leaking around the mask. Mask should be fitted firmly under the chin and conformed to the bridge of the nose and across the cheekbones. Straps must be positioned at the base of the neck and on the crown of the head. It is imperative that a fit check is performed every time the mask is applied before entering the surgery area to protect oneself from airborne diseases (Grinshpun *et al*., 2009). The mask should not be reused and must be changed after every patient. It should also be changed when the mask becomes wet or damaged during patient care. Hand hygiene must be performed before applying N95 mask, before and after its removal.

For 3-ply surgical mask, it must cover the nose and mouth to inhibit droplet transmission of viral or bacterial diseases (Desai & Mehrotra, 2020). The side of the mask that has a stiff bendable edge is the top and is molded to the shape of the nose, whilst the colored side is the front and the white side touches the face. If using surgical mask with ties, the upper tie is secured with a bow first before the bottom

tie. On removal, the bottom bow is untied first before the top bow. For face mask with ear loops, the loop is placed around each ear, and both ear loops are held and taken off gently on mask removal. Hand wash and hand rub must be performed before putting on and removing surgical mask. Mask must neither be hung under the chin, nor kept in the pocket. Face mask must be changed after every dental procedure (Izzetti *et al*., 2020). Used mask must be disposed of into clinical waste bin.

Goggles must fit well with a good seal and must cover the eyes. Some goggles come with elastic band. Anti-fog goggles are available, or anti-fog spray can be applied onto goggles. Goggles with holes or improper seal are unacceptable in dental setting. Head cover must cover the hair, whilst hijab cover should cover the hair, ears and neck. For plastic apron or gown, they should be waterproof. Plastic apron must be changed in between patient. Full body gown must fully cover torso from the neck to the knees, from the arms to the end of wrists, and wrap around the back. Hand rub must be performed after doffing the plastic apron and gown. They must also be disposed of into the clinical waste bin.

Gloves must be changed when torn or heavily contaminated. They should not be recycled and should not be regarded as substitute for hand hygiene. Gloves must extend to cover the wrist and over the gown to prevent wrist exposure during movement (Verbeek *et al*., 2019). Hand hygiene must be performed before and after wearing gloves. Meanwhile, the used gloves must be disposed of into clinical waste bin. The sequence of donning and doffing for level 2 (droplet), level 2 (airborne) and level 3 PPE material are outlined in Table 4 and Table 5 respectively. This recommendation is based on *'International Islamic University Malaysia (IIUM) Medical Centre: Infection Prevention & Control Orientation Checklist'*. Hand rub must be performed every time an item of PPE is removed assuming that the hands are contaminated.

Pre-operative mouth rinse

Although scanty information is available on the effectiveness of pre-operative mouth rinse, pre-treatment oxidizing mouth rinse, for example using 0.5-1% hydrogen peroxide (for 15-30 seconds) (Kampf *et al*., 2020) or 0.2% povidone-iodine (for 15-30 seconds) can be considered (Kariwa *et al*., 2004; Peng *et al*., 2020), since these chemicals contain oxidative agents that reduce the salivary load of oral microorganisms (Carrouel *et al*., 2020). In the past, chlorhexidine mouth rinse was used preoperatively but this may not be effective against virus, as the mouthwash is only effective against bacteria (Fehr & Perlman, 2015).

Use of rubber dam

Rubber dam isolation when possible, for example during simple restorative procedures, can reduce the aerosol at close distance (Al-Amad *et al*., 2017). A study by Samaranayake *et al*., (1989) showed that bacterial aerosol reduction was greatest at one meter from the headrest with the use of rubber dam, thereby minimizing the inhalation of infective aerosols by the dental personnel significantly up to 88%. The study also suggested procedure needed high volume suction following rubber dam placement to prevent aerosol spread. It has been reported that more than 90% of the aerosols were evacuated in the dental setting using high volume suction (Devker *et al*., 2012).

Table 4. Sequence of donning for level 2 (droplet and airborne) and level 3

Level 2 (Droplet)	Level 2 (Airborne)	Level 3
Hand wash & hand rub	Hand wash & hand rub	Hand wash & hand rub
↓	↓	↓
Surgical mask	N95 mask	N95 mask
↓	↓	↓
Head cover	Head cover	Jumpsuit
↓	↓	↓
Shoes cover	Shoes cover	Shoes cover
↓	↓	↓
Hand rub	Hand rub	Hand rub
↓	↓	↓
1st layer of glove	1st layer of glove	Plastic apron / Gown
↓	↓	↓
Full body gown	Full body gown	1st layer of glove
↓	↓	↓
Plastic apron	Plastic apron	2nd layer of glove
↓	↓	↓
2nd layer of glove	2nd layer of glove	Face shield
↓	↓	
Face shield	Face shield	

Anti-retraction handpiece

Anti-retraction handpiece is paramount to reduce the backflow of debris and fluids into the dental units. Backflow may have the consequence of microbial contamination of the air and water tubes resulting in potential cross infection from the dispersion of aerosol and droplets (Samaranayake & Peiris, 2004; Hu *et al*., 2007). Therefore, anti-retraction dental handpiece with anti-retractive valves is strongly recommended in the current climate of pandemic.

Surface disinfection

All surfaces must be cleaned and disinfected regularly especially at the frequently touched surfaces such as door handles, light handles, switches, chairs and lift buttons, in view of the long persistence of SARS-CoV-2 on surfaces (Kampf *et al*., 2020). WHO recommended the usage of ethanol 70-90%, hydrogen peroxide (H_2O_2) of more than 0.5%, or sodium hypochlorite 0.1% for environment and 1% for blood and body fluid for disinfection (Fallahi *et al*., 2020). Ultraviolet light can disinfect small surface area, but its use can potentially harm the eyes if not protected (Srirengalakshmi *et al*., 2020). Atmosphere is kept dry to mitigate the virus spread, as evidence has suggested that coronaviruses have significantly higher activity at 50% humidity than 30% (Kampf *et al*., 2020). Staff performing decontamination should wear appropriate PPE and follow strictly the doffing procedure at the end of the process.

Table 5. Sequence of doffing for level 2 (droplet and airborne) and level 3

Level 2 (Droplet)	Level 2 (Airborne)	Level 3
Outer layer of glove	Outer layer of glove	Outer layer of glove
↓	↓	↓
Hand rub	Hand rub	Hand rub
↓	↓	↓
Plastic apron	Plastic apron	Plastic apron
↓	↓	↓
Hand rub	Hand rub	Hand rub
↓	↓	↓
Full body gown	Full body gown	Face shield
↓	↓	↓
Hand rub	Hand rub	Hand rub
↓	↓	↓
Face shield	Face shield	Head cover of jumpsuit
↓	↓	↓
Hand rub	Hand rub	Jumpsuit along with shoes
↓	↓	cover
Head cover	Head cover	↓
↓	↓	Hand rub
Hand rub	Hand rub	↓
↓	↓	N95 mask
Shoes cover	Shoes cover	↓
↓	↓	Hand rub
Hand rub	Hand rub	↓
↓	↓	Inner layer of glove
Surgical mask	N95 mask	↓
↓	↓	Hand wash
Hand rub	Hand rub	
↓	↓	
Inner layer of glove	Inner layer of glove	
↓	↓	
Hand wash	Hand wash	

Management of medical waste

Every dental practice should follow standard infection control protocol. All dental equipment must be sterilized if possible, with disposable wraps used for non-sterilizable items. Used items must be disposed of properly, including disposable PPE. Dental waste generated from the patient treatment must be regarded as infectious waste and be transported to the temporary storage area of the dental facility in a timely manner. Double layer yellow-colored medical waste bags with 'gooseneck' ligation should be used in accordance with the CDC Guidelines for environmental infection control in health-care facilities (2003).

Summary

In summary, COVID-19 is a highly contagious disease. Dental professionals must be familiar with how SARS-CoV-2 is spread, how to identify patients with SARS-CoV-2 infection and what protective measures that should be adopted during dental practice to prevent the transmission of the disease. The underpinning evidence and sources regarding safe practice of dentistry amidst COVID-19 are highly variable. In view of the different guidelines and recommendations, clinicians should always follow local or institutional guidelines whenever appropriate. All dental personnel would need to adopt this 'new norm' to protect oneself, patients and the loved ones from contracting this novel coronavirus infection which is likely to stay for a long time.

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