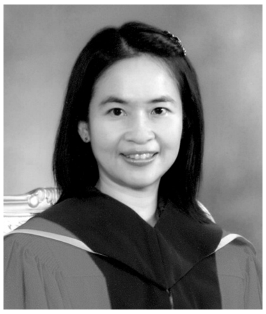
Volume 14

November 2018

THE INTERNATIONAL JOURNAL OF ORAL HEALTH

Welcome Message



Dear friends and colleagues,

On behalf of the Board of Executives of Asian Academy of Preventive Dentistry (AAPD) and the local organizing committee (LOC), we are delighted to welcome you to the 13th International Conference of Asian Academy of Preventive Dentistry (13th AAPD) in Khon Kaen, Thailand, during November 21-23, 2018. The main theme of the Conference is "Improving Health through Oral Health", emphasizing that oral health is an essential component of human health. It also highlights the values of oral health promotion and preventive dentistry in the improvement of health outcomes for patients and population.

The AAPD International Conference has been held every 2 years in participating Asian countries. This is the second time for Thailand to host the AAPD; the first was held in Chiang Rai in 1998, with Professor Prathip Phantumvanit being the 3rd President of the AAPD. Twenty years later, Khon Kaen city at the heart of in northeastern region was selected as the venue for the 13th Conference. The Conference is co-hosted by Khon Kaen University Faculty of Dentistry and the Thai Society of Public Health Dentistry. The Japanese Society for Oral Health has also joined force to co-organize t he symposium on "Future Geriatric Oral Health for Asia".

The conference program includes 5 keynote lectures, 8 symposia (23 lectures), and 117 poster presentations. We have an honor from distinguished speakers and renowned researchers from Asia, Europe and America to share their experiences and knowledge. We are grateful to all invited speakers and presenters who contribute to the state-of-the-art scientific program of the conference.

The International Journal of Oral Health (IJOH) is published once a year as the official voice of AAPD. The Editorial Board of IJOH would like to invite all professionals and scholars in Preventive Dentistry, Dental Public Health and related areas to contribute to our journal. My sincere thanks to all who have contributed in this journal.

Yours sincerely,

Waranuch Pitiphat, DDS, MPHM, MS, SD (Epidemiology), FRCDS (Dental Public Health)
13th President of the Asian Academy of Preventive Dentistry
Editor-in-Chief, The International Journal of Oral Health
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Proceedings of the
13th International Conference of
Asian Academy of Preventive Dentistry

21-23 November 2018

Khon Kaen, Thailand

Improving Health through Oral Health

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Proceedings of the 13th International Conference of the Asian Academy of Preventive Dentistry

Contents **Pages**

Original Article

Outcomes for Tobacco Cessation Counselling by Dental Students in Oral Health Suite, University Teknologi MARA (UiTM)

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Abstract

Assisting tobacco users to quit proved to be the most cost-effective investment in healthcare. Faculty of Dentistry, UiTM is one of the higher institutions in Malaysia that embeds tobacco cessation counselling (TCC) in the curriculum and train the dental students to deliver TCC to their patients who smoke. **Objectives:** The aim of this study was to evaluate the outcomes for student-delivered tobacco cessation counselling in Oral Health Suite, UiTM. **Methods:** Data collection of 100 patients who completed three visits of the tobacco cessation counselling (TCC) with dental students from year 2012 to 2016 was analysed. The outcomes measured included quitting status, number of cigarettes, exhaled carbon monoxide (CO) level and nicotine dependency scores (FTND) which were extracted from the records. Subsequently, a follow-up telephone survey was conducted for follow up of the quitting status (quit or relapse) of the participants. **Results:** Twenty-five percent of the participants quit smoking during the third visit. The participants' CO level and FTND scores showed a significant reduction after attending TCC ($p< 0.001$). There was a positive correlation between number of cigarettes and CO level ($r=0.65$, $p<0.001$), as well as CO level and FTND scores ($r=0.54$, $p<0.001$). The participants who had low CO level (<6ppm) were 3 times more likely to quit smoking than participants with higher CO level (>6 ppm) with an odds ratio of 3.19 (95% confidence interval, 1.25, 8.12). **Conclusion:** Tobacco cessation counselling delivered by the students to patients attending Oral health Suite in UiTM has shown favourable outcomes in helping the patients to quit smoking.

Key words: tobacco cessation, dental students, evaluation, outcomes

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Introduction

Tobacco use is a major preventable cause of premature death and diseases killing 6 million people worldwide annually. Smoking is still prevalent in many parts of the world and about 80% of 1 billion smokers live in low and middle-income countries.[1,2] Smoking rates have decreased in developing and industrialized countries including Malaysia.[3] Assisting tobacco users to quit is one of the most cost effective and egalitarian investments in healthcare. Twenty-five percent of Malaysian adults smoked tobacco in 2015.[4] Only 9.5% of Malaysians have successfully quit among those who have smoked on a daily basis and 14.3% of the smokers plan to or are thinking about quitting within the next 12 months.[2] It is very rare to see smokers who stop smoking for good, the possibility of relapse is high and the cycle continues from quitting to continuing smoking and vice versa.[6]

Prochaska and Goldstein suggested that people who smoke go through a five stage tobacco addiction cycle that leads them from being non-smokers to new smokers, then to committed smokers, to smokers trying to stop and to finally reformed smokers.[7] However, only a few former smokers stay in the final stage of completely quitting the behaviour. In March 2017, the Ministry of Health Malaysia announced its aim to reduce smoking prevalence to 15% or less by 2025, and to achieve a Smoke-free Malaysia (the End Game of Tobacco) by 2045, as stated in the National Strategic Plan for Tobacco Control 2015-2020.[8] Their aim is to reduce consumption and availability of tobacco in the society to minimal levels through full, effective and accelerated implementation of all policy measures recommended by WHO.[9]

The role of dental practitioners in tobacco cessation is important as there are regular interactions between the patients and practitioners. Studies have shown that dental treatment plans that included tobacco cessation increase the likeliness of patients to quit smoking.[10-12]

In order to prepare the next generation of oral healthcare providers, a few studies proposed a paradigm shift on how tobacco use prevention and cessation may be incorporated into existing curricula.[13,14] Faculty of Dentistry, UiTM has introduced and implemented tobacco cessation counselling as a part of the dental undergraduates' curriculum since 2007. All Year Three and Year Four students need to assist two smoking patients in at least three visits (with an interval of two weeks for each visit) of tobacco cessation counselling as part of their academic requirements. The students used the 5A's (Ask, Advice, Assess, Assist, Arrange) technique as an aid in the tobacco cessation counselling supervised sessions with trained oral health preventive lecturers. For smokers who are not interested in quitting smoking, we used the 5R's approach (Relevance, Risks, Rewards, Roadblocks, Repetition). The counselling session takes about 30 minutes each. Students were introduced to the tobacco cessation module (includes lectures on Introduction to Nicotine Addiction, Stages of Behaviour Change, 5A's and 5R's approach, Motivational Interviewing, Non-pharmacological and Pharmacological Aids in Tobacco Cessation) early in their Year Three training.

The students provided correct information and realistic tobacco use quitting plan to achieve sustainable positive outcomes among the smokers. Counselling combined with prescribed pharmacotherapy (nicotine gum or patches) were also provided whenever necessary. It has been proven to increase the rate of tobacco cessation 2 to 3 times higher compared to only advice or behavior modifications.[15] Smokers who quit by the end of the third visit, who self-reported a 24-hour abstinence from smoking, were measured as successfully quit.[16]

In order to achieve abstinence and maintaining the status of a former smoker, self-efficacy or self-confidence plays an important role in tobacco cessation.[17,18] With improving the smokers' self-efficacy, the dental students or practitioners who assist smokers to quit, can enhance their success by helping them to sustain their behavior change and preventing relapse.[17] In our centre, we help patients by providing ways to curb their withdrawal symptoms and suggest ideas on how to cope as a former smoker to increase their self-efficacy.

The aim of this study was to evaluate the outcomes of student-delivered tobacco cessation counselling (TCC) in Oral Health Suite, UiTM. The hypotheses tested in this study was that the TCC is ineffective and there is no association between number of cigarettes, exhaled carbon monoxide levels and nicotine dependence with quitting status of the patients attending TCC in UiTM.

Materials and Methods

The study was granted human ethics approval from the Ethics Committee, UiTM on 26 May 2017 (Ref. No. 600-IRMI (5/1/6). Secondary information gathering involved a non-arbitrary review of all patients' records in UiTM Dental Centre. Only patient records that indicated that they received tobacco cessation counselling were pulled out for the survey. The charts were then checked to verify that there was complete documentation of tobacco cessation counselling data that we needed.

Out of 412 tobacco users that were registered in the tobacco cessation registry from 2014 to 2017 in the Oral Health Suite UiTM, we calculated the sample size to be 196 participants according to the formula by Krejcie and Morgan[19]. The inclusion criteria were all dental patients who claimed to be smokers in UiTM Dental Centre who attended and completed the three-visit TCC sessions with at least two weeks' interval for every visit from 2014-2017. All of them must have had a set of complete data including all three-visit TCC sessions' number of cigarettes, exhaled carbon monoxide (CO) level and Fagerström Test Nicotine Dependency (FTND) scores. We excluded records of tobacco users with incomplete data (contact number and tobacco history records) and if they did not complete the three-visit TCC sessions. All of the patients who were included in this study had completed TCC by the Year Three and Year Four dental students supervised by oral health preventive lecturers. The three outcomes that were measured in this study were all three-visit TCC sessions' number of cigarettes, CO level and FTND scores. Information was obtained from the tobacco history records in the students' logbook and crosschecked with the records in the patients' folders. Each student was required to fill in a standardized tobacco history form (which included all the three outcomes) for every visit and kept in the folder. During every visit of TCC, the number of cigarettes that the patient smoked was asked by interview and recorded in the logbook and records.

Patients were required to breathe out the carbon monoxide by using the piCO* Smokerlyzer®, as it displayed the measurement of CO in parts per million (ppm) and carboxyhaemoglobin (%COHb). These two measurements are compatible and convertible as the CO reading relates to gas in the lungs and in the breath (i.e. the amount of CO that has been inhaled), while the COHb reading relates to the percentage of vital oxygen that has been replaced in the bloodstream.[20] Hung et al. conducted a research of correlation between exhaled CO level and daily cigarette consumption in 150 chemical manufacturer workers in Taiwan.[21] The cut-off point between smoker and non-smoker has been found to be 6 ppm CO. The readings from piCO* Smokerlyzer® defines a non-smoker as 0-6 ppm, a low-dependence smoker as 7-15 ppm and a strongly addicted smoker to be over 15 ppm.[20-22]

The FTND scores were acquired from the cumulative score each patient achieved using a validated questionnaire of a six-item scale designed to measure physical dependence with five score-levels: very high dependence (scores of 8-10), high dependence (scores of 6-7), medium dependence (score of 5), low dependence (scores of 3-4) and very low dependence (scores of 0-2).[23] Each item has its own individual response scale that varies by item. The FTND is a revision of the original Fagerström Tolerance Questionnaire and has fair internal consistency.

After compiling the information from the patients' records, a brief telephone survey was done by two Year 4 Students using a standardized scripted interview based on the study done by Bolt et al[24] to further follow up the current smoking behaviour of the participants who has completed the three-visit TCC. The initial review revealed that 50 out of the 100 participants could not be contacted due to incorrect or unreachable contact numbers. Participants were contacted via messages, online messages and telephone calls. The survey was conducted using a scripted telephone interview based on The Wisconsin Predicting Patients' Relapse Questionnaire (7-item) and Fagerström Test Nicotine Dependency Questionnaire (7-item).[24] Participants were assured that their involvement was voluntary, confidential and kept anonymous. After four consecutive times through the list, contact attempts were stopped and they were excluded from the telephone survey.

Statistical analyses were performed using SPSS version 22.0. The number of cigarettes, CO level and FTND scores, from every visit of the TCC were compared using paired t-test. The correlation between quit smoking status and the outcomes were performed by Pearson Correlation test. Risk estimation was done by calculating the odds ratio (OR) of the outcomes to the quitting status of the participants. Descriptive analysis was done for the telephone survey using GoogleForms.

Results

Out of 412 tobacco users that were registered in the tobacco cessation registry from 2014 to 2017 in Oral Health Suite UiTM, only 100 of the tobacco users had complete data including all three-visit sessions' number of cigarettes, exhaled CO level and FTND scores. The average age of the participants was 32 years (standard deviation, SD=12.8) and all of them were male. Most of the participants belonged to the age group 19 to 29 years (59%). The participants smoked an average of 10 cigarettes per day (SD=6.9). Twenty-five percent of the participants quit smoking during the third visit.

The participants' number of cigarettes, CO level and FTND scores showed a significant reduction from the first visit after attending TCC as illustrated in Table 1. From these findings, it is evident that tobacco cessation could significantly reduce the number of cigarettes, CO level and FTND scores of the participants after attending three visits of TCC in UiTM.

Table 1 Mean differences of number of cigarettes, CO level and FTND scores comparison of Visit 1 and Visit 3.

	N	Mean	SD	Mean difference 95% Confidence Interval	p-value
Visit 1 no. of cigarettes	100	9.9	7.0	5.4 (4.1, 6.7)	<0.001
Visit 3 no. of cigarettes	100	4.5	4.7		
Visit 1 CO level	100	11.18	9.4	4.1 (2.6, 5.6)	<0.001
Visit 3 CO level	100	7.04	6.1		
Visit 1 FTND score	100	2.7	2.3	1.4 (1.1, 1.8)	<0.001
Visit 3 FTND score	100	1.3	1.7		

SD = standard deviation; CO = exhaled carbon monoxide; FTND = Fagerström Test Nicotine Dependency

We analyzed the data to find any association between CO level, FTND score and number of cigarettes using Pearson correlation test. The r value was 0.645 (p<0.001), which was more than 0.3. Hence, there was a positive association between CO level and number of cigarettes. We also analyzed for any association of FTND score and CO level. There was a positive association with FTND score and CO level (r=0.473, p<0.001). We also analyzed for any association of FTND score and number of cigarettes. There was also a positive association (r=0.542, p<0.001).

We tabulated CO level visit three and quitting status as shown in Table 2. Out of the 100 participants, 60 participants had 0-6 ppm CO level. Forty percent successfully quit tobacco use. Only one participant who had CO level more than 6 ppm successfully quit tobacco use.

Table 2. Cross tabulation of CO level Visit 3 and quitting Status (quit or not quit)

		Quitting status (*n*=100)		**Total**
		Quit, *n* (%)	**Not quit, n (%)**	
CO level (*n*=100)	0-6 ppm	24 (40.0)	36 (60.0)	60
	7-10 ppm	0 (0.0)	20 (100.0)	20
	11-15 ppm	1 (8.3)	11 (91.7)	12
	16-25 ppm	0 (0.0)	6 (100.0)	6
	26-35 ppm	0 (0.0)	2 (100.0)	2
Total		25 (25.0)	75 (75.0)	100

CO = exhaled carbon monoxide; ppm = parts per million

FTND score visit three and quitting status was also tabulated (Table 3). Seventy-eight participants had very low to low FTND score (score 0-2). Out of this, 32.1% had successfully quit smoking. For participants who had FTND scores of 3-10 (medium, high and very high nicotine dependency) no one successfully quit in this category.

Table 3. Cross tabulation of FTND Visit 3 and quitting status (quit or not quit)

		Quitting status		Total
		Quit	Not quit	
FTND score	1-2 (very low)	25 (32.1)	53 (67.9)	78
	3-4 (low-moderate)	0 (0.0)	16 (100.0)	16
	5-7 (moderate)	0 (0.0)	5 (100.0)	5
	8+ (high)	0 (0.0)	1 (100.0)	1
Total	Count	25 (25.0)	75 (75.0)	100

We also did risk estimation on CO level and quitting status. The participants who had low CO level (<6 ppm) were 3 times more likely to quit smoking than participants with higher CO level (>6 ppm) with an odds ratio of 3.19, 95% CI (1.25, 8.12) (Table 4).

Table 4. Cross tabulation and risk estimate of CO level Visit 1 and quitting status

		Quitting Status *(n=100)*		Odds Ratio (95% confidence interval)
		Quit	Not quit	
CO level	0-6 ppm	15	24	3.19 (1.25, 8.12)
	7 ppm and above	10	51	
Total		25	75	

CO = exhaled carbon monoxide; ppm = parts per million

Telephone Survey Follow-up Results

Of the 100 subjects, only 27% (n=27) responded to the telephone survey. Seventy-three participants were unable to be contacted and/or not interested to participate.

From the telephone survey, 52% of the participants claimed to relapse back to their smoking habits. Twelve respondents resumed their smoking habit less than three months after completing the tobacco cessation counselling. We also asked the reasons for the relapse. Most of the participants answered stress (32%) and bored (32%). The least likely factor to cause regress of smoking abstinence was addiction (5%).

Six respondents (43%) said that they did not need tobacco as much even after hours of not smoking. They admitted to smoke after 60 minutes from waking up. Likewise, twelve participants (86%) felt burdened for not smoking in the non-smoking areas. Another twelve participants (86%) purported to need only 10 or less cigarettes per day. Moreover, eleven individuals concurred that they did not want to smoke when they were so sick.

The patients' opinions regarding the tobacco cessation program were documented as well. Majority (86%) advocated for the program and would recommend it to their relatives. All of them rated the program as satisfactory or better.

Table 5. Responses to survey questions, by percentage and frequency of each question

Questions	**Answers % (n= respondents)**						
1.What is the highest grade or school that you completed?	Never attended	Elementa-ry school	Secondary school	Diploma	Degree	Master	PHD
	-	-	19% (n=5)	33% (n=9)	48% (n=13)	-	-
2.Have you received quit smoking coun-seling from UiTM Dental Faculty?	Yes			No			
	100% (n=27)			-			
3.Do you currently smoke after the tobacco cessation counselling?	Yes			No			
	52% (n=14)			48% (n=13)			
4.After how long have you started smoking again after receiving the tobacco cessation counselling?	Less than 3 months		Between 3 to 6 months		More than 6 months		
	86% (n=12)		-		14% (n=2)		
5.Why do you start smoking again?	Stress	Peer influence	Loss weight	Fi-nan-cial problem	Bored	Addic-tive	Other (enviro-ment)
	32% (n=6)	11% (n=2)	-	-	32% (n=6)	5% (n=1)	21% (n=4)
6.If someone in your household wants to smoke, does he/she have to leave in order to smoke?	Yes			No			
	79% (n=11)			22% (n=3)			
7.Which of the statement best describes your place of work's smoking policy for work areas?	Smoking is not allowed in any work areas	Smoking is allowed in all work areas		Smoking is allowed in some work areas		I do not work outside the home	
	36% (n=5)	36% (n=5)		21% (n=3)		7% (n=1)	
8.I am surrounded by smokers much of the time.	1 (not true at all)	2	3	4	5	6	7 (extremely true)
	-	-	7% (n=1)	-	50% (n=7)	14% (n=2)	29% (n=4)
9.When I have not been able to smoke for a few hours, the craving gets intolerable.	1 (not true at all)	2	3	4	5	6	7 (extremely true)
	14% (n=2)	14% (n=2)	14% (n=2)	43% (n=6)	7% (n=1)	-	7% (n=1)
10.How soon after you wake up do you smoke your first cigarette?	Within 5 minutes	5-30 minutes		30-60 minutes		More than 60 minutes	
	-	21% (n=3)		14% (n=2)		64% (n=9)	

Table 5. Responses to survey questions, by percentage and frequency of each question (continued)

Questions	Answers % (n= respondents)				
11.Do you find it difficult to refrain from smoking in the places where it is forbidden, eg; in church, at the library, in the cinema?	Yes			No	
	14% (n=2)			86% (n=12)	
12.Which cigarette would you hate the most to give up?	The first in the morning			Any other time	
	43% (n=6)			57% (n=8)	
13.How many cigarettes per day do you smoke?	10 or less		11 to 20	21 to 30	31 or more
	86% (n=12)		14% (n=2)	-	-
14.Do you smoke more frequently during the first hours after waking than the rest of the day?	Yes			No	
	36% (n=5)			64% (n=9)	
15.Do you smoke when you are so ill that you are in the bed most of the day?	Yes			No	
	21% (n=3)			79% (n=11)	
16.Will you recommend the tobacco cessation counselling program to your friends or relatives who smoke?	Yes			No	
	85% (n=23)			15% (n=4)	
17.Will you rate the tobacco cessation counselling program?	Very good	Good	Satisfactory	Poor	Very poor
	37% (n=10)	44% (n=12)	19% (n=5)	-	-

Discussion

The tobacco cessation program is an intervention to help smokers achieve smoking abstinence. A well-constructed evaluation should be done to determine the effectiveness of the program for further improvement. This research was done by analyzing three outcomes which are number of cigarettes, CO level and FTND scores from the first visit to the third visit. The two-week interval three-visit sessions implemented for every patient who attended TCC in UiTM that was carried out by the dental students were based on Cochrane Reviews.[25] The reviews found that smokers who were followed up at least once after the initial counselling had better outcomes in terms of increased success rates in quitting tobacco.

From the telephone survey, we intended to follow up all the patients who had undergone TCC and asked about their abstinence from smoking and reason of relapse. Currently this is not the current practice in our centre to review patients (by appointment visit or phone call) after the third visit. If a patient fails to quit, the patient is asked if he wants to be referred to a Preventive Specialist to continue TCC. However, according to Stead et al., with additional telephone counselling or follow up, quit rates were reported to be higher.[26]

In this study, the most reliable outcome is the CO level wherein patients were required to exhale into the smokerlyzer to get the CO level reading. The device is an inexpensive, mobile carbon monoxide monitor that was previously shown to be effective in detecting carbon monoxide level from the expired breath.[27] It can also be used to assess the blood COHb concentration from the exhaled CO level accurately.[28]

We expected to obtain less precise data from the other outcome measures; number of cigarettes and FTND scores. Number of cigarettes can be manipulated by the patients as they might underestimate or overestimate the amount of their tobacco use. This can lead to information bias wherein information on the amount of tobacco use is not as accurate as the CO level reading. As for FTND scores, the patients were required to answer a 6-item questionnaire interview by the students. This may lead to information bias and interviewer bias wherein scores might underestimate or overestimate the nicotine dependence of the patient during counselling session. It should be noted that one of the problems relating to validation of FTND is the lack of either biological measures of dependence or a universally agreed upon set of criteria.[29] On the other hand, the FTND has acceptable levels of internal consistency, and is closely related to biochemical indices of heaviness of smoking.[23]

From this study, 25% of the 100 participants successfully quit smoking (at least 24-hour abstinence) during the third visit of TCC. However, out of these patients, only 27 are contactable for follow-up for the purpose of this study. Out of these 27 participants, 52% of them relapsed back to being smokers after at least three months. This is similar to other studies where more than 50% of the smokers who undergo any form of tobacco cessation counselling will relapse.[30] The outcome has affirmed the non-effectual methods while executing the program excluding the patients' internal factors of self-efficacy in maintaining the quitting status[17]. In clinical studies designed to report the success rates achieved by smoking cessation program, it was stated that the percentage of participants who were still abstinent was relatively high (>50%) around 3 months after quitting. However, the statistics declined shockingly by the end of the first year (<25%) due to higher relapse rate.[30] According to Ramseier et al., the relapse rate is 50% to 60% among the quitters within the next year.[31]

From this study, several barriers of achieving smoking abstinence among our patients were found: stress and boredom. According to Asif et al., one of the reasons the university students of Bahawalpur Pakistan start smoking is stress (33%).[32] Another study has also found that smoking can help in coping with stress among adolescents.[33] Health practitioners can plan strategies to assist smokers to overcome the precipitating factors. They should discuss with patients any stressful events that can cause relapse and suggest alternative means to cope with the problems.[34]

A limitation of this study is sample size. We only collected data in one tobacco cessation centre. Furthermore, some students did not fill in the tobacco cessation registry in detail. We saw a few blank entries while gathering the inputs needed for the research. The negligent actions made us face difficulties while compiling the data. We were unable to achieve our initial sample size of 196 patients.

The TCC registry in the Oral Health Suite consists of the carbon monoxide level, number of cigarettes and FTND for three consecutive visits. Another column for nicotine replacement therapy (NRT) acquisition should be added. A lot of studies advocate that NRT contributes to a near bi-fold success of cessation rates procured by non-pharmacological intervention, irrespective of the level of the intervention.[35] The long term effectiveness and health benefits of nicotine replacement therapy combined with non-pharmacological approaches have been evidently achieved.[35-36] Victoroff et al. reported that 87% of the dental students agreed to the fact that educating patients on the negative impacts of tobacco to systemic health is the dentist's obligation.[37] This shows that with effective training modules and incorporating tobacco cessation in the dental curricula, dental students in the university can assist smokers to quit smoking and may continue when they practice as dental practitioners in primary care.[38]

Conclusions

Tobacco cessation counselling delivered by the students to patients attending Oral Health Suite in UiTM has shown favourable outcomes and can be improved in facilitating the patients to quit smoking. Greater effort should be taken to promote and assist tobacco cessation to patients, tighten enforcement of smoke-free environments and in training effective tobacco cessation techniques.

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Original Article

Qualitative Analysis of Apacider® Mangostin Adhesive Pastes on Acid Resistance of Dental Enamel Around Orthodontic Brackets: An *In vitro* Study

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Abstract

Objectives: This *in vitro* study was aimed to compare the resistance of dental enamel to acid around orthodontic brackets. Fluoride varnish (FV), Apacider® Mangostin Adhesive Pastes (AMAP), resin-modified glass ionomer cement bonding (RMGIC), casein phosphopeptide–amorphous calcium phosphate (CPP-ACP), and a negative control were compared. **Methods**: The buccal surfaces of teeth extracted from patients undergoing orthodontic treatment were divided into five groups: Group 1, FV; Group 2, AMAP; Group 3, the specimens were bonded with RMGIC (Fuji Ortho LC, GC America); Group 4, CPP-ACP (GC Tooth Mousse, RECALDENT™); Group 5, no treatment. The topical agents were placed around the bracket margins, and the specimens were immersed in a demineralizing solution for 96 hours with the periodic application of AMAP and CPP-ACP to second and fourth group every four hours. The lesions were examined using scanning electron microscopy (SEM) and polarized light microscopy (PLM) to evaluate the surface characterization and obtain the depth of the enamel demineralization. **Results**: ehT surface characteristics revealed by the SEM images showed homogenous smooth enamel surface in the AMAP group. Slight porosity and some roughness were found on the FV-treated surface. The CPP-ACP-treated surface showed greater porosity and surface roughness compared with the AMAP and FV applications. The highest porosity and surface roughness were found on the untreated and the RMGIC-treated group. The PLM images of the surface treatments showed a greater reduction in the depth of enamel demineralization compared with the control group. AMAP showed the greatest protection of the enamel surface in the reduction of lesion depth, followed by FV, CPP-ACP, and RMGIC, in ascending order of depth. **Conclusion**: Despite the limitations of this *in vitro* study, the results suggest that applications of AMAP, FV, and CPP-ACP have the potential to enhance the resistance of dental enamel to acid around orthodontic brackets. However, further clinical investigations are needed to support the results of this study.

Key words: demineralization, remineralization, acid resistance, orthodontic bracket, Apacider® mangostin adhesive paste, qualitative analysis

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Introduction

The prevention of demineralization or white spot lesion (WSL) during orthodontic treatment is one of the greatest challenges experienced by pediatric dentists and orthodontists despite the many modern advances in caries prevention.[1] However, calcium phosphate-based technologies show potential efficacy in the prevention of WSLs.[1,2]

Apacider® Mangostin Adhesive Pastes (AMAP) are newly developed materials comprised of Apacider®-AW (Sangi Co. Ltd., Tokyo, Japan). In AMAP, calcium phosphate and antibacterial agents promote remineralizing activity. Moreover, alpha mangostin has strong antibacterial activity against cariogenic *S. mutans*. This material is mixed in an adhesive paste that is applied to the tooth surface. AMAP is an alternative agent for the prevention and management of WSLs in patients. Because of the adhesive properties of AMAP, it can hold on to the tooth surface longer than other remineralizing agents can. In addition, because the product is white in color, unlike fluoride varnish, it does not cause a yellow stain on the tooth surface[2-4]

Materials and Methods

This *in vitro* study was conducted in the Faculty of Dentistry at Khon Kaen University. The study protocol was exempted from review by the Khon Kaen University Ethics Committee in Human Research (HE602321).

Tooth specimen preparation

Twelve human premolars that had been extracted for the purpose of orthodontic treatment were collected. The tooth specimens were stored in a 0.1% thymol solution prior to the experiment. Calculus and soft tissue debris were removed using a Gracey curette and then randomly separated into five groups. Specimens with carious lesions, cracks, abrasions, enamel hypoplasia, fluorosis, tetracycline stains, or restorative material, which were visible to the naked eye, were excluded from this study. The specimens were mounted in plastic blocks and then coated with acid-resistant nail vanish (Revlon, New York, USA) except a 4x5 mm^2 window area in the middle of the buccal surface (Fig. 1). The specimens were allocated randomly into five groups. The specimens in four groups were treated with Transbond Plus Self-Etching Primer before the brackets were adhered by a light-cured resin composite cement (Transbond XT, 3M Unitek). Any visible excess cement was removed before light curing was performed. The only remaining group of specimens was treated with GC Ortho Conditioner before the brackets were set in place using RMGIC (Fuji Ortho LC, GC America).

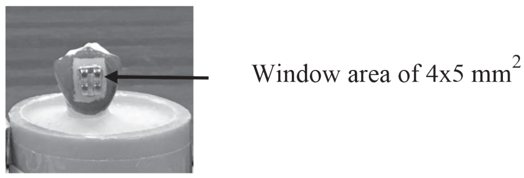

Fig. 1 Sample of tooth specimen

Sample allocation and treatment protocol

The specimens were allocated randomly into the following five groups:

Group 1: Fluoride varnish (Duraphat®) was applied on the first day as recommended by the manufacturer.

Group 2: AMAP was applied for three minutes every four hours.

Group 3: The specimens were bonded using RMGIC bonding.

Group 4: CPP-ACP (GC Tooth Mousse, RECALDENT™) was applied for three minutes every four hours as recommended by the manufacturer.

Group 5: No treatment was applied.

Acid challenge to simulate the caries process

The enamel samples were then stored individually in 10 mL of demineralizing solution consisting of 2.20 mmol/L calcium, 2.20 mmol/L phosphate, and 0.05 mol/L acetic acid at pH 4.4[5] for 96 hours at 37 °C. Every four hours, the teeth were removed from the solution and rinsed with deionized water. AMAP (Group 2) and CPP-ACP (Group 4) were reapplied every four hours, whereas only a single application of fluoride varnish (Group 1) was performed during the study period. The immersion of the untreated enamel specimens in the demineralization solution for 96 hours was expected to result in the creation of deep enamel lesions, which would represent approximately three months of real time. In Groups 2 and 4, applications every four hours corresponded to approximately two weekly applications. After 96 hours, all the specimens were removed from the solutions. Then all brackets were removed and thoroughly rinsed with deionized water.

Scanning electron microscopy (SEM) preparation and imaging

The tooth crowns in each group were randomly selected for the evaluation of structural changes using SEM (Hitachi S3000N, Osaka, Japan). All specimens were separated from the root at the cementoenamel junction, and buccal-lingual sections were created using a tooth-cutting machine (Mecatome T081, Brié-et-Angonnes, France) with a diamond-coated blade under water cooling and then coated with a layer of gold palladium in a sputter coater under vacuum (K500X Emitech, Ashford, England). The outer surface of the enamel surrounded by bonding was photographed by SEM at magnifications of 500x, 1000x, and 2000x.

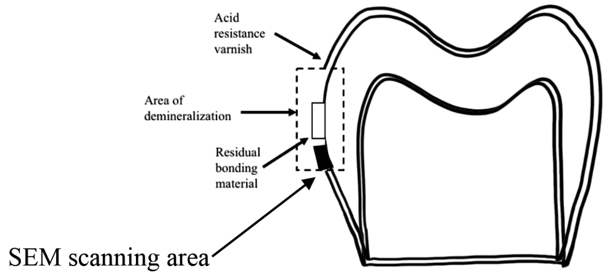

Fig. 2 Scanning the study area by scanning electron microscope (SEM)

Polarized light microscope (PLM) preparation and imaging

The specimens were sectioned through the center of the lesions using a diamond-coated blade under water cooling to obtain sections that were 100 ± 20 μm thick. The sections were examined under a polarized light microscope (PLM) (Nikon Eclipse80i, Kanagawa, Japan) at magnifications of 5x and 10x. A photograph was taken to facilitate the calibration of the demineralized depth at the cervical area of enamel below the attachment of the orthodontic bracket. The depth of the lesion was measured from the border of the tooth surface to the demineralized lesion at the deepest area and marked on the photographs.

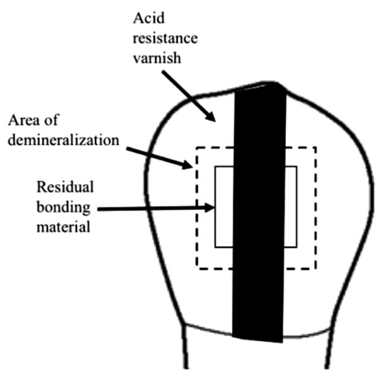

Fig. 3 Polarized Light Microscope (PLM) section

Results

Scanning electron microscope (SEM) analysis

Qualitative analyses were conducted on the surfaces of the sound teeth (Fig. 4A, 4B) in the experimental groups. The SEM results revealed that after demineralization for 96 hours, the specimens in the non-treated group (Fig. 4C, 4D) had greater porosity and surface roughness than the specimens in the RMGIC bonding group did (group 3) (Fig. 4E, 4F). These results indicated that remineralization was not fully compensated by treated agents. In contrast, a smoother surface was shown in the tooth specimens that were treated by AMAP (group 2) , FV (group 1), and CPP-ACP (group 4). The AMAP-treated enamel surfaces (Fig. 4G, 4H) were homogenous and smooth. The FV-treated surfaces (Fig. 4I, 4J) showed some porosity and slight roughness. The CPP-ACP-treated surfaces (Fig. 4K, 4L) showed greater porosity and surface roughness compared with the AMAP and FV applications. These results indicated that acid resistance was enhanced in the AMAP, FV, and CPP-ACP groups. In addition, the surfaces of the tooth specimens treated with FV and CPP-ACP had greater roughness than those in the AMAP-treated group did.

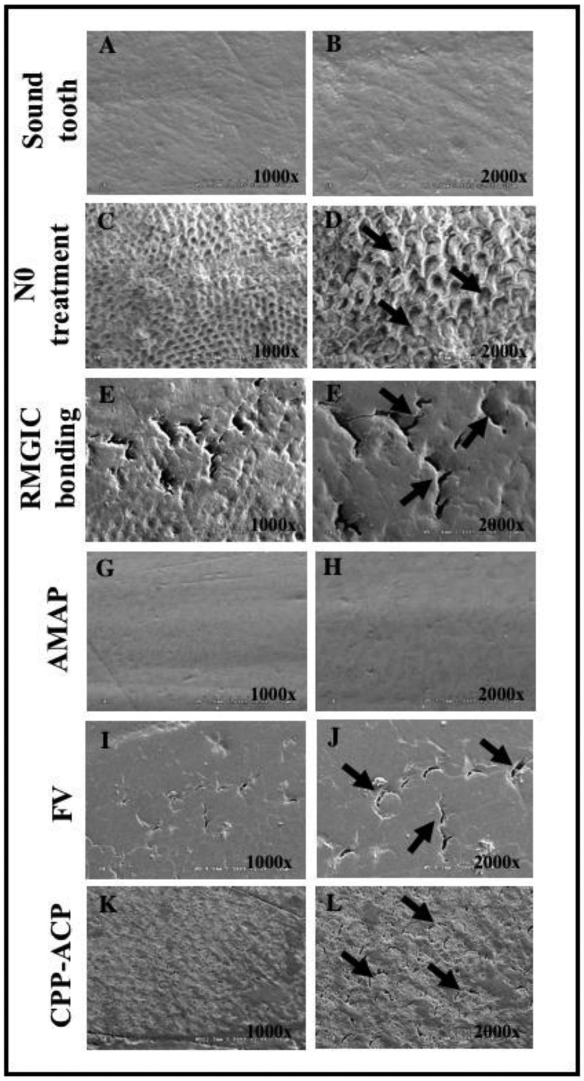

Fig. 4. Characterization of surfaces of the enamel specimens by SEM at magnifications of 1000x and 2000x of a sound tooth (Fig. 4A, 4B) after 96 hours of acid exposure in the non-treated group (Fig. 4C, 4D), the RMGIC bonding group (Fig. 4E, 4F), the AMAP group (Fig. 4G, 4H), the FV group (Fig. 4I, 4J), and the CPP-ACP group (Fig. 4K, 4L). The arrows point to porosity caused by demineralization, which occurred over the entire labial surface.

Polarized Light Microscope (PLM) analysis

The qualitative analysis of lesion depth showed that the enamel surfaces of the sound teeth did not evidence a radiolucency area in the demineralized lesions (Fig. 5A). In contrast, radiolucency areas in carious lesions were found on the artificial enamel carious sections (Fig. 5B). All three surface-treated sections showed a reduction in the depth of enamel demineralization compared with the non-treated group (249.49 μm). AMAP (116.41 μm) (Fig. 5C) provided the greatest protection of the enamel surface in terms of the reduction of lesion depth, followed by FV (143.11 μm) (Fig. 5D), CPP-ACP (179.41 μm) (Fig. 5E), and RMGIC (225.66 μm) (Fig. 5F).

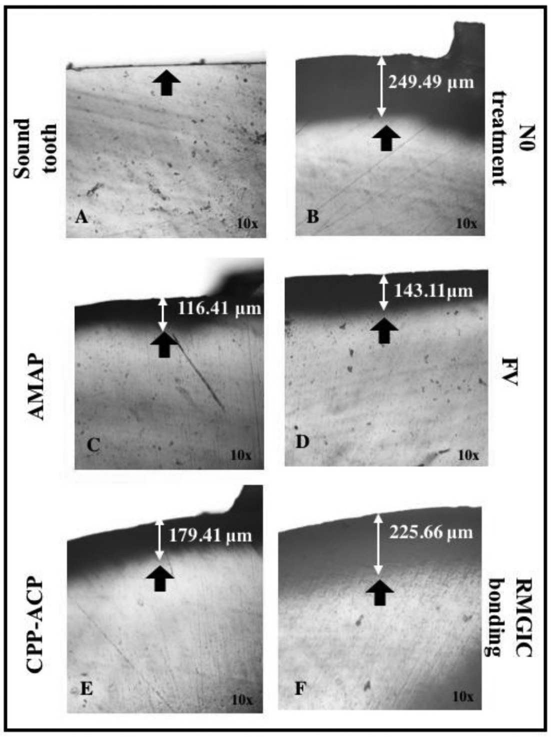

Fig. 5. Polarized light photomicrographs of enamel surfaces of sound teeth at magnifications of 10x (Fig. 5A) after acid resistance 96 hours in the non-treated group (Fig. 5B), AMAP group (Fig. 5C), FV group (Fig. 5D), CPP-ACP group (Fig. 5E), and RMGIC bonding group (Fig. 5F).

Discussion

The application of remineralizing agents in various forms has been the most commonly used caries prevention protocol in orthodontic treatment. Calcium phosphate-based remineralization systems have been introduced in recent years as supplements or substitutes for conventional fluoride-based systems.[6-8] Recently, there has been increased interest and technological development in the calcium phosphate-based remineralization of dental caries. However, no published studies have reported the effects of these products on the resistance of dental enamel to acid around orthodontic brackets.[9-11] The aim of this study was to compare the effectiveness of the resistance of dental enamel to acid around orthodontic brackets using AMAP, fluoride varnish, RMGIC bonding, casein phosphopeptide–amorphous calcium phosphate (CPP-ACP), and no treatment. The effectiveness of each material and no treatment was determined by the analysis of SEM and PLM images.

The results of this study showed that the greatest amount of enamel demineralization occurred in the non-treated group, which was subjected to the demineralizing solution without the application of any agents. The demineralizing solution of pH 4.5 and the study period of 96 hours were selected to limit the demineralization to a white spot lesion, which was then accurately measured.[6,10] Mild organic acids and acid buffers, such as lactic acid and acetate acid, are used to create demineralized lesions that mimic natural caries.[12,13]

The qualitative assessment was carried out using SEM analysis to evaluate the structural changes caused by the acid.[7,14] The results demonstrated that each agent used in the study offered some enamel protection compared with the untreated control group. The SEM images of the teeth specimens in the non-treated and RMGIC bonding groups showed greater porosity and surface roughness caused by the demineralizing solution. These effects were caused by the process in which the inorganic content of the enamel structure was lost, leading to occurrence of WSL. Soares et al. reported that SEM images of the sound enamel showed that the enamel crystals were homogeneously arranged and were clearly outlined.[13] In contrast, the demineralized enamel was disorganized and showed the loss of structural characteristics. All the treated groups showed either amorphous crystals or particles scattered on the surface along the prismatic borders.[14-16]

The AMAP-treated teeth presented homogenous smooth enamel surfaces and no porosity under magnification at x2000. These results are consistent with Suksupeaw,[16] who reported the effects of Apacider® varnish on the remineralization of dental enamel. The results of the present study confirmed that the enamel surfaces treated with Apacider® varnish presented more homogenous, smooth surfaces compared with the FV-treated group.[17] In addition, in 2015, Sodata showed that the surface morphology of sound tooth specimens after acid attacks in AMAP and FV groups presented homogenous smooth enamel compared with the untreated group.[2] In addition, Puisuwan reported that AMAP-treated teeth presented more homogenous and smoother enamel surfaces than the FV-treated group did.[18] These results could be explained as follows: partially demineralized crystal surfaces within the lesion acted as “nucleators,” and new surfaces grew on the crystals. These processes constitute remineralization, which is the replacement of minerals in the partially demineralized regions of the carious lesion of enamel.[19] AMAP is composed of Apacider®-AW in which calcium phosphate is the remineralizing and antibacterial agent, and alpha mangostin is the antibacterial agent. This material is mixed in an adhesive paste preparation that can be applied to the tooth surface. The proposed preventive anti-cariogenic mechanism of AMAP incorporates nanocomplexes on the tooth surface and acts as a reservoir of calcium and phosphate could have been absorbed into deeper enamel porosity, and significantly larger amounts of mineral were deposited in the subsurface lesions. In contrast, the fluoride varnish promoted the formation of intraoral fluoride reservoirs due to the formation of calcium fluoride (CaF2). Additionally, the remineralization potential of saliva was also limited by calcium and phosphate, which resulted in the incomplete formation of fluoapatite and remineralization.[2-4,18,20]

In the present study, the FV-treated surfaces presented slight porosity and some roughness. The ability of fluoride varnish to significantly inhibit enamel demineralization has already been documented.[6] Fluoride varnishes promote the formation of intraoral fluoride reservoirs due to the formation of calcium fluoride (CaF2). However, the formation of these reservoirs is limited by the availability of calcium ions and fluoride ions.[21,22] Additionally, the remineralization potential of saliva is also inhibited by insufficient calcium. Therefore, additional calcium and phosphate ions from sources such as varnish may lead to the improved remineralization of early lesions.[20-22]

In the present study, the CPP-ACP treated surfaces showed greater porosity and surface roughness compared with the AMAP- and FV-treated surfaces. Suksupeaw reported that the CPP-ACP treated tooth did not present an enamel surface that was as homogenous and smooth as the enamel surfaces in the Apacider® varnish and untreated groups.[17] These results are consistent with the proposed mechanism of anti-cariogenicity by the application of CPP-ACP on the tooth surface, which includes buffering plaque pH, depressing enamel demineralization, and enhancing remineralization. CPP-ACP is recognized for the importance of the neutral ion species in gaining access to subsurface lesions through a porous enamel surface.[23,24]

The PLM analysis software used in the study enabled the accurate and direct measurement of the depths of demineralization so that the effect of different agents could be compared. This accuracy is important for the realistic assessment of any therapeutic intervention because a product may not completely prevent demineralization, but it could reduce the area of the tooth surface affected as well as the amount of mineral loss and lesion depth.[5,25]

In the present study, the analysis of the PLM images demonstrated that after 96 hours, the acid resistance in the AMAP-treated group provided the greatest reduction in the depth of enamel demineralization compared with the other groups. These results are consistent with Suksupeaw, in which PLM images showed that the Apacider® varnish group presented depths of enamel demineralization less than in CPP-ACP and FV groups.[16] In addition, Sodata and Puisuwan both reported that in their studies, the depths of lesions in the enamel of teeth in the AMAP group were lower than in the FV and control groups.[2,17] Fluoride varnishes have been found to remain in contact with enamel for several days because of their viscous nature, which has been noted to create a durable physical barrier between the enamel and the cariogenic solution. Bichu et al. reported that teeth treated with GC Tooth Mousse, although the reduction in demineralization was less than in the enamel surfaces treated with fluoride varnish, but the measured depths were still significantly lower compared with the control. This finding was in line with other studies that demonstrated significant reductions in enamel demineralization using CPP-ACP *in vitro*.[5,26]

Calcium phosphate-based remineralization systems, or AMAP, should be applied as supplements or substitutes of fluoride to prevent WSL during orthodontic treatment. Systems such as AMAP depress enamel demineralization and enhance remineralization from their reservoir of calcium and phosphate ions, which promote remineralization during a cariogenic attack. Adhesive pastes can adhere to the tooth surface longer than other systems can.[2,17] They may also be a treatment alternative for patients who are allergic to fluoride varnish.[26,27]

Conclusions

Despite the limitations of this *in vitro* study, the results suggest that the applications of AMAP, FV, and CPP-ACP have the potential to enhance the acid resistance of dental enamel around orthodontic brackets. However, further clinical investigations need to be performed to support the results of this study.

Acknowledgements

The authors would like to acknowledge the guidance provided by Dr. Patteera Sodata and her support in preparing the testing agents. Financial support for this study was provided by the Faculty of Dentistry at Khon Kean University.

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The International Journal of Oral Health 14:22-31, 2018

Original Article

Ultrastructures of Enamel, Dentin, and Cementum associated with Hypocalcified Amelogenesis Imperfecta

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Abstract

Objectives: Amelogenesis imperfecta (AI) is a group of hereditary disorder of tooth development that affects the structure and appearance of tooth enamel. AI has been categorized based on clinical and radiographic appearances. However, understandings of the defects in ultrastructure and mineral composition defects of AI are very limited. This study aimed to analyze the ultrastructure, mineral density, and chemical compositions of enamel, dentin, and cementum in two teeth affected by autosomal dominant hypocalcified type of AI (ADHCAI). This study will expand knowledge of abnormalities in dental hard tissues associated with ADHCAI. **Methods**: Two Thai patients from different families affected with ADHCAI participated in this study. The history, clinical, and radiographic examinations were performed. An extracted tooth obtained from each patient was subjected for studies. The mineral density was examined by micro-CT, mineral composition by dispersive x-ray spectrometry (EDX), and ultrastructure by scanning electron microscopy (SEM). **Results**: The first patient exhibited yellowish brown discoloration with pitted enamel. The second patient showed brown-black colored crown with smooth surface. In both patients, the radiopacity of enamel was reduced. The disease was inherited through families suggesting autosomal dominant mode of inheritance. These suggest ADHCAI in both patients. SEM revealed that AI enamel showed atypical enamel rods containing several porosities and irregular dentinoenamel junction. Dentin exhibited partially obliterated dentinal tubules while cementum appeared unremarkable. The mineral density of AI enamel was decreased. The levels of calcium and phosphorus in affected enamel, dentin, and cementum were significantly reduced compared to the controls. These show alterations in the ultrastructure, mineral density, and compositions of the enamel, dentin, and cementum associated with ADHCAI. **Conclusions**: Our study demonstrates that ADHCAI could influence clinical and radiographic characteristics, ultrastructure, mineral density, and inorganic elements in the enamel, dentin, and cementum.

Key words: Amelogenesis Imperfecta, ultrastructure, teeth, EDX, SEM

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Introduction

Amelogenesis imperfecta (AI) is a hereditary disorder causing defects in dental enamel. It affects both primary and permanent dentitions. The prevalence of AI varies from 1:14,000 to 1:700 depending on population studied,[1,2] AI has been divided into four types including hypoplastic (type I), hypomaturation (type II), hypocalcification (type III) and hypomaturation-hypoplastic with taurodontism (type IV)[3] depending on the stage of enamel formation affected by genetic defects.

Hypoplastic type is caused by the reduced secretion of extracellular matrix. The quantity of enamel is decreased, which may be expressed clinically as thin or missing enamel such as pits and grooves. Hypomaturation type caused by anomalies at the maturation stage of enamel formation, presents clinically as opaque, mottled, discolored, and porous enamel but normal thickness. Hypocalcified type is due to defects in mineralization, characterized by discolored, rough, and soft enamel which is rapidly lost by attrition. Hypomaturation-hypoplastic with taurodontism is characterized by thin, mottled or spotted, and discolored and taurodont molars.[4,5] Radiographic features of AI reveal a variety of defects, depending on type of AI. Hypoplastic type shows reduced thickness of enamel and normal to slightly reduced radiopacity. The enamel contrast of hypomatured type is similar to or higher than that of the dentin while that of hypocalcified type is similar to or lower than dentin. The hypomaturation-hypoplastic with taurodontism is characterized by altered enamel contrast and large pulp chambers. The genetic inheritance patterns of AI can be autosomal dominant, autosomal recessive, or X-linked trait. The autosomal dominant hypocalcified type of AI (ADHCAI) is one of the most common forms of AI.

The development of dental hard tissues including enamel, dentin, and cementum is a complex process controlled by reciprocal interactions of genes and signaling. The abnormalities of enamel could affect the formation of dentin which influences that of the cementum. Majorities of previous ADHCAI studies have reported the structural defects of tooth enamel but showed highly variable data. These compromise the understandings of not only enamel anomalies but changes in dentin and cementum associated with ADHCAI. This study, identified two Thai patients from different families having different phenotypes of ADHCAI, therefore aimed to investigate ultrastructure, mineral density, and chemical compositions of two affected teeth using micro-computerized tomography, scanning electron microscopy, and energy dispersive x-ray spectrometry. The findings would expand the ultrastructural knowledge of affected dental hard tissues including enamel, dentin, and cementum and benefit treatment approach of ADHCAI in the future.

Materials and Methods

Subjects

Two Thai patients from different families were recruited for the study. They were diagnosed with ADHCAI according to the criteria by Witkop[3] at the Faculty of Dentistry, Chulalongkorn University based on clinical and radiographic examinations. The written informed consents were obtained from all individuals who participated in the study. The mandibular right first premolar was obtained from the first patient (called AI1 sample in this study) and the maxillary right lateral incisor was from the second patient (called AI2 sample) which were extracted according to their orthodontic treatment plans and poor prognoses, respectively. The research protocol was approved by the research ethics committee at Faculty of Dentistry, Chulalongkorn University. The patients' teeth were subjected for ultrastructural analyses compared with two age- and tooth type-matched sound teeth obtained from healthy *individuals.*

Micro-Computerized Tomography (μCT) analysis

The teeth were rinsed with phosphate-buffered saline and stored in 10% formalin solution. The samples were scanned with specimen μCT 35 (SCANCO Medical, Brüttisellen, Switzerland) and processed using the Image Processing Language (IPL, Scanco Medical AG). Thirty spots in the enamel and thirty spots in the dentin of the patients' teeth were selected to evaluate its mineral density compared with same areas in the controls via cross sections of micro CT. Subject to the availability of remaining tooth structure, the enamel and dentin were examined in AI1.

Energy-Dispersive X-ray (EDX) evaluation

The teeth were mounted on acrylic resin blocks with sticky wax and cut longitudinally along buccal-lingual direction using the slow-speed precision saw (Isomet 1000 Precision Saw, Buehler, Lake Bluff, IL, USA) with diamond disc at a speed of 450 rpm under constant water. They were then grinded with Grit#1200 silicon-carbide paper and polished with alumina powder on polishing pad (10-inch MICROPAD, Pace technologies). The samples were dehydrated with ethanol series and exposed to a critical point drying process using critical point dryer (Emitech K850, Emitech Ltd, Kent, England). Dried samples were coated with gold powder in argon-cathode atomization with fine coater media (JFC 1200, Tokyo, Japan) for 10 seconds. Three spots in the enamel, dentin, and cementum between AI and control teeth were selected to calculate the elemental levels (%) of carbon (C), oxygen (O), phosphorus (P), and calcium (Ca) under the EDX (Jeol, JSM-6610 LV Scanning Electron Microscopy, Japan) by a single operator who was not aware of the samples' preferences.

Scanning Electron Microscopy (SEM) evaluation

The specimens were etched with 37 percent phosphoric acid, rinsed with water, dehydrated, dried, and covered with gold powder for 110 seconds. For qualitative evaluation of the hard tissue surface, scanning electron microscopy (Quanta Feg 250, FEI Company, Oregon, USA) was performed on the affected samples and compared with the controls.

Statistical analysis

The data were analyzed by GraphPad Prism 5 software package (GraphPad Prism Software Inc., San Diego, CA, USA). Independent T-test was performed to determine significant differences of percent weight of each element between affected and control group ($p < 0.05$).

Results

Manifestation of AI

The first patient (AI1) is a 13 year-old Thai boy who presented for dental treatment at the Faculty of Dentistry, Chulalongkorn University with the complaint of yellowish teeth. Oral examination exhibited yellowish to brownish discolored, rough, and soft enamel with severe wear. Dental caries was found on his upper first molars. Generalized heavy plaque deposition and gingival inflammation were presented (Fig. 1A-B). Periapical radiograph revealed minimal enamel thickness with radiopacity slightly brighter than that of dentin. The unerupted third molar showed normal enamel thickness (Fig. 1C). Extractions of mandibular first premolars were performed based on orthodontic treatment plan. The mandibular premolar was collected for further evaluation (Fig 1D). Enamel defects were also observed in his mother, younger sister, and other mother's relatives.

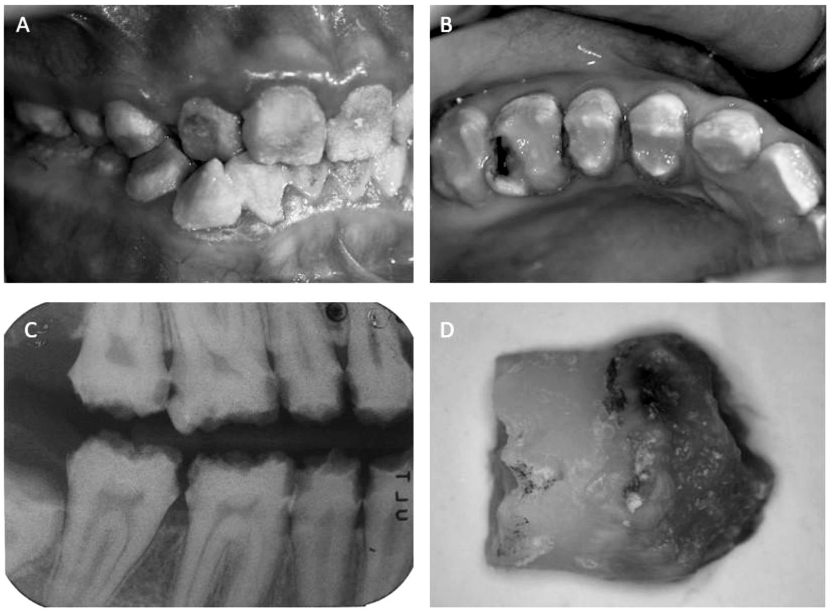

Fig. 1 Oro-dental manifestations of AI1 patient. A. The teeth were yellowish, rough, crowded, and deposited with heavy plaque and calculus. B. Oral photograph of maxillary right teeth showed loss of enamel and proximal contact. Large cavity was observed on maxillary right first molar. C. Dental radiograph showed irregular thin enamel and loss of tooth contact. D. Photograph of extracted mandibular right first premolar showed yellowish brown enamel with multiple pits.

The second patient (AI2) is a 42 year-old Thai male who presented for dental treatment at the Faculty of Dentistry, Chulalongkorn University due to his discolored and worn teeth. Oral examination revealed smooth, shiny, brownish-black teeth with severe attrition and caries leaving multiple retained roots. Several teeth were clinically absent (Fig. 2A). Radiologically, the enamel and dentin showed similar radiopacity. Pulp obliteration was presented. Multiple teeth had pulp necrosis with asymptomatic apical periodontitis (Fig. 2B) which were later extracted (Fig. 2C). His daughter was also affected with enamel abnormalities.

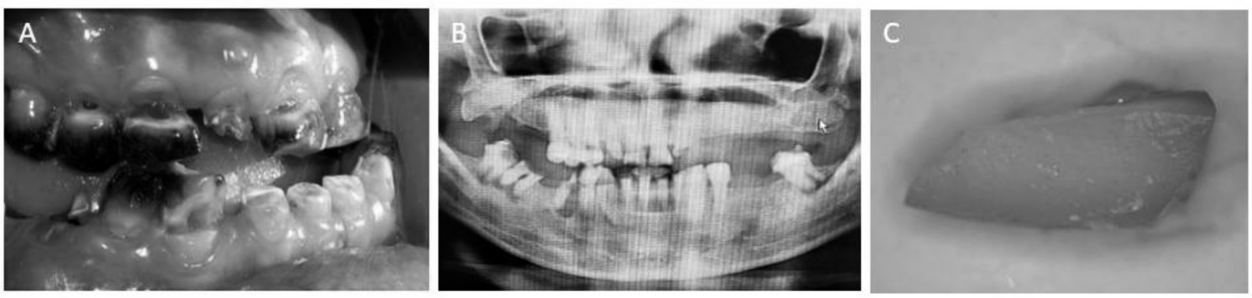

Fig. 2 Oro-dental manifestations of AI2 patient. A. Oral photograph of the teeth showed brownish-black, smooth, shiny teeth with severe attrition and caries leaving multiple retained roots. B. Panoramic radiograph showed similar radiopacity between enamel and dentin. Multiple teeth were broken and developed periapical lesions. C. Photograph of the extracted root of the maxillary right lateral incisor showed shiny white to yellowish color and irregular surface.

Micro CT analysis

In AI1, the mineral density of the enamel was 1,581.891 mgHA/cm^2, compared to the controls (2,137.418 and 2,091.817 mgHA/cm^2) while that of the dentin was 1,133.752 mgHA/cm^2, compared to the controls (1,234.914 and 1,173.842 mgHA/cm^2). These show that the AI tooth had substantially reduced mineral density in the enamel while slightly reduced density was observed in the dentin (Fig 3).

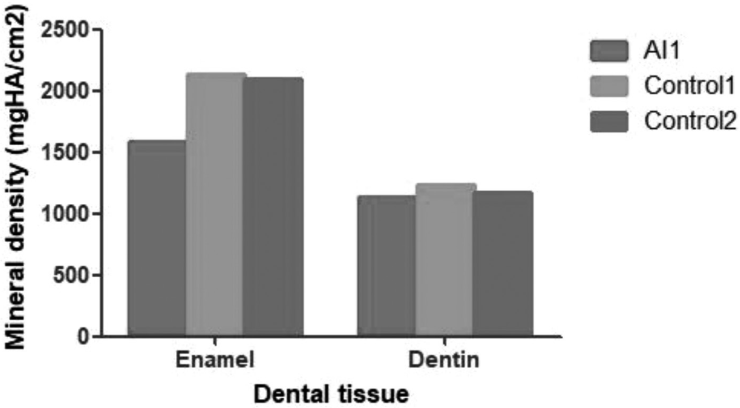

Fig. 3 Mineral density of enamel and dentin in AI1 sample and the controls analyzed by micro-CT. The mineral density of the AI1 enamel was apparently decreased while that of the dentin was similar to the controls.

Energy-Dispersive X-ray (EDX) analysis

Elemental analyses of the enamel revealed that AI1 had significantly lower levels of calcium (Ca, 31.73%) compared to the controls (33.57% and 33.06%). Its phosphate (P, 16.51%) and oxygen (O, 41.54%) contents were also significantly decreased (control; P, 17.63% and 17.57%; O, 42.25% and 42.81%) while the carbon (C, 10.21%) content and the mean C/O ratio (0.25) were significantly higher compared to the controls (C, 6.56%; C/O, 0.15). The calcium/phosphate (Ca/P) ratio in AI1 enamel (ratio 1.92) was significantly higher than controlAI1-2 (1.88) (*p*=0.0132) but not significantly different to controlAI1-1 (1.91) (Fig. 4A-B).

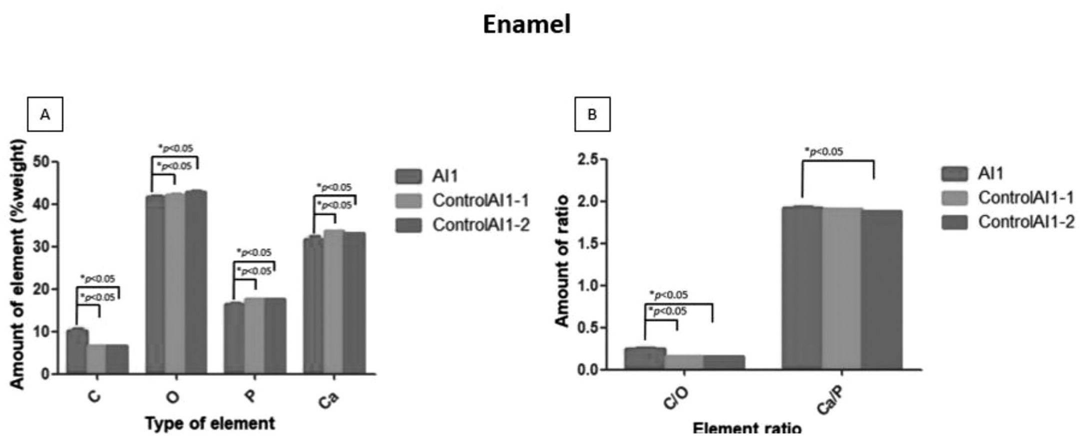

Fig. 4 Graphs of elemental quantities and ratios in the enamel. A. The AI1 enamel showed significantly higher C content but lower O, P, and Ca levels than the controls ($p<0.05$). B. The C/O and Ca/P ratios AI1 was significantly increased compared to the controls ($p<0.05$).

In the dentin, elemental analysis of AI1 exhibited that the C content and C/O ratio were statistically significantly higher in the AI1dentin compared to the controls. On the other hand, the P and Ca contents were statistically significantly lower in the AI1 dentin compared to the controls. The O content in AI1 dentin was statistically significantly lower in the AI1 compared to the controlAI1-2. The Ca/P ratio in AI1 dentin was statistically significantly higher in the AI1 compared to the controlAI1-2 but was not statistically significantly different when compared with controlAI1-1 (Fig. 5A-B).

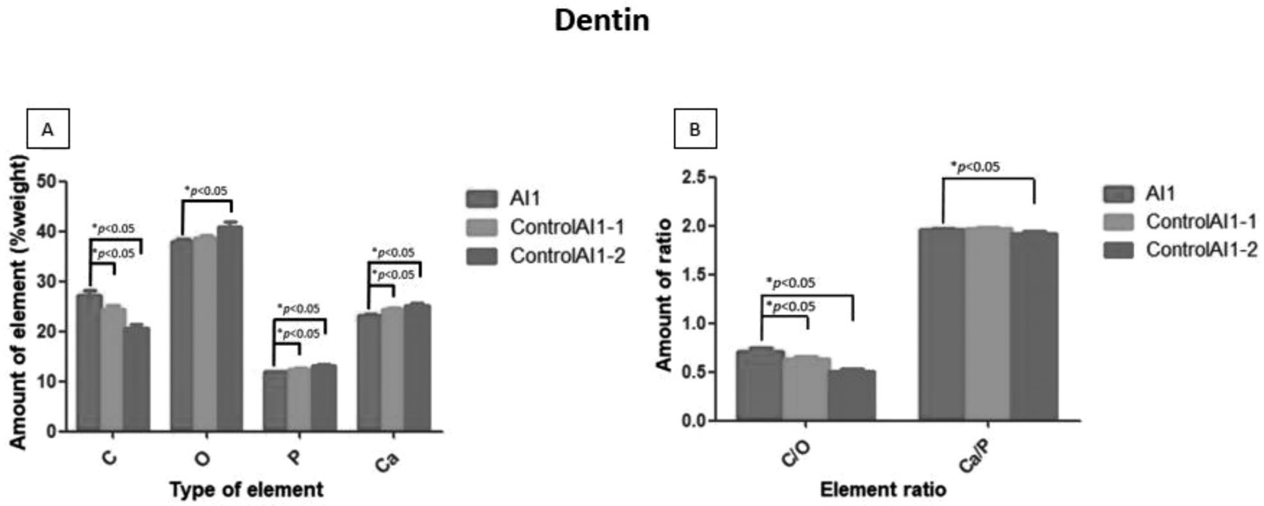

Fig. 5 Graphs of elemental quantities and ratios in the dentin. A. The Ca, O, and P contents of AI1 dentin were significantly lower than the controls. In contrast, the C content of AI1 was significantly increased ($p<0.05$). B. The C/O and Ca/P ratios of AI1' dentin was significantly increased compared to the controls ($p<0.05$).

In the cementum. AI2 showed significantly increased C and O contents and C/O and Ca/P ratios but decreased P and Ca levels, compared to the controls (Fig. 6A-B).

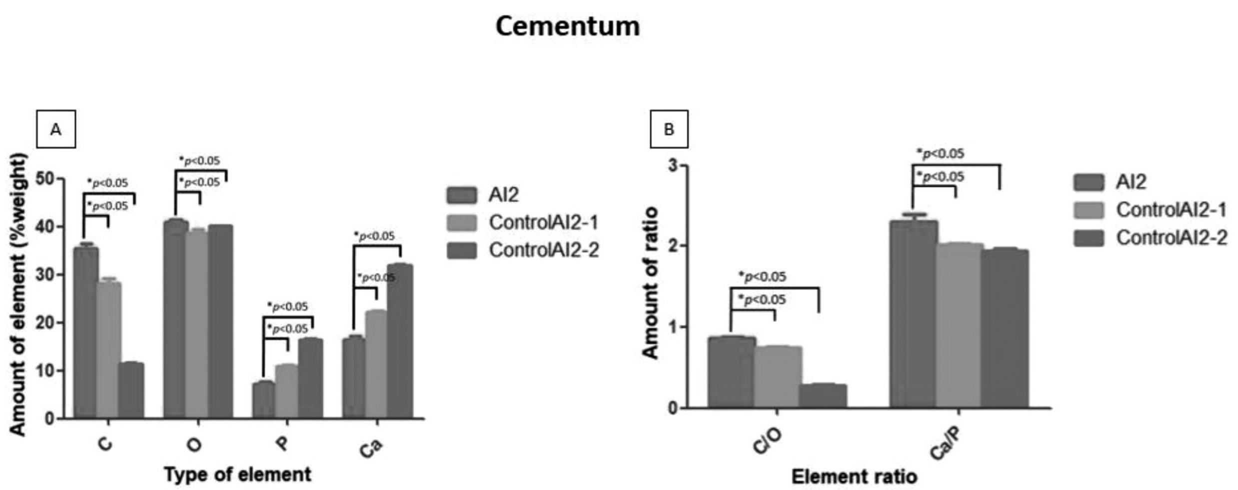

Fig. 6 Graphs of elemental quantities and ratios in the cementum. A. AI2 showed significant decreased Ca and P contents but increased C and O contents compared to the controls ($p<0.05$). B. AI2 cementum showed increased Ca/P and C/O ratios ($p<0.05$).

Scanning Electron Microscopy (SEM) Analysis

The AI1 enamel revealed porosities and disorganized and collapsed enamel rods. The interspaces between affected enamel rods were wider compared to those of the controls (Fig. 7A, B). The dentinoenamel junction of AI tooth was irregular (Fig. 7C, D). The dentinal tubules were present in AI dentin but partially obliterated (Fig. 7E, F). No obvious difference was observed between the cementum of AI2 and the controls (Fig. 7G, H).

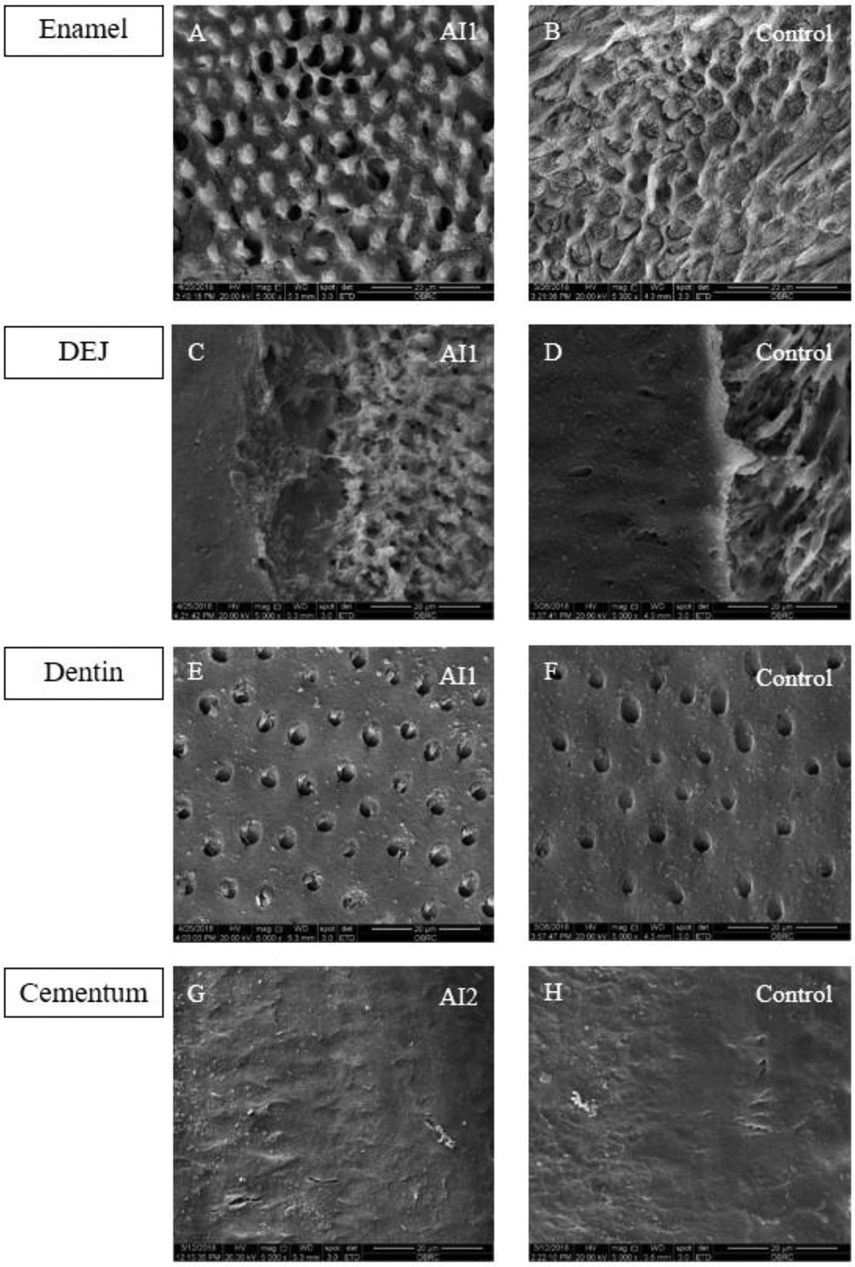

Fig. 7 SEM images at 5000x magnification of the enamel, DEJ, dentin, and cementum. A, B. AI1 showed irregular enamel rods with multiple pores. C, D. The DEJ of AI1 showed poorly organized orientation while the control showed typical scallop pattern. E, F. The dentin of the AI1 presented partially obliterated dentinal tubules compared to control. G, H. The cementum structure of AI2 was similar to the control.

Discussion

The ADHCAI is characterized by hypocalcified soft enamel with yellowish-brown in color affecting the whole crown. The disease is inherited as an autosomal dominance. In this study, we identified two Thai AI patients from different families exhibiting diverse dental manifestations. The features of the AI1 were consistent with ADHCAI categorized by Witkop[3] and other previous reports.[6-7] The AI1 exhibited rough and soft enamel with yellowish to brownish discoloration. Radiographically, the enamel thickness of erupted teeth was thin while that of unerupted teeth was normal. This phenotype corresponds to the developmental defects associated with ADHCAI. It was shown that the ameloblasts secreted normal quantities of enamel matrix proteins resulting in normal enamel thickness in ADHCAI, but the mineralization process was disturbed.[8-9] Correspondingly, our patient's teeth were weak, subject to wear and fracture soon after eruption and exposure to mastication forces. In AI1 family, his mother, younger sister, and other mother's relatives also presented enamel anomalies, suggesting that ADHCAI is inherited in this family.

In AI2 family, the patient and his daughter were affected with AI, indicating autosomal dominant mode of inheritance. Interestingly, the phenotype of AI2 was dissimilar to that of AI1 and to the typical rough enamel of ADHCAI. The clinical appearance of AI2 enamel was a brown-black discoloration with smooth and shiny surfaces. Consistently, Song *et al.*[10] also observed smooth and shiny enamel surface related to ADHCAI. These show that the ADHCAI patients could present a wide range of enamel features from yellow, brown, and black smooth to rough surface. These could be due to different ages of patients and/or genetic backgrounds.

In this study, only enamel and dentin of AI1 sample were available for analyses because its root was discarded during the operation. For AI2 sample, only the root portion was obtained. The formation of root dentin has unique control mechanisms involving Hertwig's epithelial root sheath and integration of the root with alveolar bone, blood supply, and nerve innervations which are different from that of crown dentin.[11-12] The root dentin was thus not included in this study.

The ultrastructure of AI1 enamel was porous containing irregular enamel rods and multiple crack lines. These were consistent with previous studies showing non-crystalline amorphous materials, crack lines, crevices, and porosity in the enamel of ADHCAI.[13-14] The enamel rods were irregularly associated with inappropriate retention of organic matrix.[7,15] These indicate that ADHCAI has enamel malformations. Quevado *et al.* (2004) showed that the dentin in ADHCAI had partial obliteration of the dentinal tubules.[16] Moreover, Zhang *et al.*[15] reported that ADHCAI had abnormal dentinal tubules with irregular, narrow, or partially obliterated lumens. These are consistent with partially obstructed dentinal tubules observed in our AI teeth. To our knowledge, there are no previous studies on ultrastructure of the cementum in ADHCAI. Kammoun et al. in 2017 reported the cementum ultrastructure of hypoplastic AI. The thickening of cellular cementum was observed, but was not consistent in their study.[17] In addition, the pathogenesis of hypoplastic AI is different from that of ADHCAI. The comparison between AI2 findings and previous studies cannot therefore be concluded. We show here that the structure of enamel is severely affected, the dentin is mildly altered, and the cementum is not likely to be influenced by ADHCAI. Wright *et al.*[18] found that the mineral density of the enamel in ADHCAI was decreased (45 - 80 % mineral per volume) compared to the normal range (70 - 98 %). This indicates that the mineral content of the enamel affected with ADHCAI could be widely varied from forty percent reduction to nearly normal level. Consistently, we observed that the mineral density of AI1 enamel was approximately twenty-five percent reduced compared to the controls.

Calcium and phosphorus are the main inorganic compounds involved in crystal formation and influence the physical and chemical properties of the enamel. The EDX analyses revealed that the Ca and P values in the enamel, dentin, and cementum of AI teeth were significantly decreased compared to those of the controls. It was shown the ADHCAI had marked reduction in the inorganic component of enamel, compared to other AI types including hypoplastic and hypomaturation.[19] These imply that the teeth affected with ADHCAI had reduced inorganic, but increased organic compositions causing fragile enamel. El-Sayed *et al.* (2010) reported that the Ca/P ratio in the ADHCAI enamel was not significantly different from that of the controls.[6] However, we found the inconsistent difference in Ca/P ratio between our AI sample and each control. More samples should be investigated to clarify this aspect in future studies. Interestingly, the C content and C/O ratio in the enamel, dentin, and cementum of AI teeth were significantly increased. Similarly, it was reported that the enamel in ADHCAI had higher C value and C/O ratio.[15-16] These demonstrate a wide range of alterations in inorganic contents of dental hard tissues in ADHCAI. The etiology and mechanisms of the abnormal AI features still require further studies.

Our study observed deviations in clinical and radiographic manifestations, ultrastructure, mineral density, and inorganic components of dental hard tissues in ADHCAI. These changes not only affect the appearance but also physical and mechanical properties of AI teeth. The teeth are prone to deteriorate upon mastication. The applications of dental adhesives, tooth-colored filling materials, and adhesive resins on AI teeth could be jeopardized. Knowledge about AI tooth defects would therefore assist dental practitioners to provide the suitable treatment for AI patients improving their quality of life.

Conclusions

Our study demonstrated that ADHCAI could present a wide clinical spectrum ranging from pitted, rough to smooth discolored enamel. The ultrastructural changes in enamel prisms and crystallite organization, and the reduction in mineral density and composition in enamel were the main ADHCAI features. The DEJ, dentin, and cementum in ADHCAI were also altered. Therefore, the weakness of AI teeth might not effect only enamel anomalies, but changes in the structure and compositions of other parts of the teeth as well. Our study demonstrated comprehensive characteristics of ADHCAI in Thai patients. These findings lead to a better understanding and proper management of AI in the future.

Acknowledgements

The study was supported by the Thailand Research Fund (TRF) and Office of Higher Education Commission (OHEC) Thailand (MRG6080001), the Chulalongkorn Academic Advancement Into Its 2nd Century Project, and the 90th Anniversary of Chulalongkorn University Fund (Ratchadaphiseksomphot Endowment Fund). We thank Anucharte Srijunbarl for laboratory assistance.

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The International Journal of Oral Health 14:32-41, 2018

Original Article

Ultrastructural Characteristics of Dental Hard Tissues Associated with Osteogenesis Imperfecta

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Abstract

Objectives: The aim of this study was to investigate the physical properties including surface roughness, hardness, elastic modulus, and ultrastructural appearance of dental hard tissues including the enamel, coronal dentin, and root dentin associated with syndromic dentinogenesis imperfecta (DGI). **Methods**: Patient included in this study was diagnosed as affected by osteogenesis imperfecta (OI) and DGI (syndromic DGI). Clinical and radiographic oral examinations were performed. Characteristics of the tooth affected with DGI (DGI tooth) including surface roughness, hardness, elastic modulus, and ultrastructural appearance were studied compared to the controls. **Results**: The patient was diagnosed with OI type IV and DGI. Physical examinations indicated classic signs of OI including bone deformities, curved extremities, short stature, and DGI. Oro-dental phenotypes of the patient showed brownish opalescent teeth with bulbous crown, cervical constriction, pulp obliteration, tooth deterioration, and malocclusion. Analyses of DGI tooth showed that the hardness of the enamel, coronal dentin, and root dentin was significantly reduced. The elastic modulus of DGI's enamel and root dentin was significantly decreased compared to that of the controls. The coronal dentin of DGI tooth displayed a markedly rough surface with the reduction in number and irregular appearance of dentinal tubules. The root dentin was disorganized with scattered of ectopic mineralization, irregular pattern and variable diameter of dentinal tubules. The dentinoenamel junction was irregular. The surface roughness of DGI's enamel was not decreased. **Conclusions**: The syndromic DGI tooth demonstrated significant changes in the hardness, elastic modulus, and formation of dentin and DEJ. These indicate that the tooth is malformed and weak which could negatively affect tooth integrity and adhesion property of dental restorations. Our study expands the understanding of pathologic changes of dental hard tissues associated with syndromic DGI and raises awareness of dental practitioner for the management of DGI patients.

Key words: Osteogenesis imperfecta, skeletal dysplasia, surface roughness, nanohardness, scanning electron microscopy

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Introduction

Osteogenesis imperfecta (OI), also called "bristle bone disease", is a heterogeneous group of genetic disorders affecting connective tissue which is mainly characterized by increased susceptibility to fractures. The prevalence of OI ranges from 1 in 5,000 - 20,000 live births.[1] The primary cause of OI is associated with heterozygous mutations in the *COL1A1* and *COL1A2* genes which encode the alpha 1 and alpha 2 chains of collagen type I.[2] The recessive mutations associated with genes encoding proteins involved in collagen type I biogenesis, other than *COL1A1* and *COL1A2,* are shown to cause more severe OI.[3]

The classification of OI is traditionally defined into four common types (type I-IV) based on clinical findings and inheritance pattern by Sillence in 1979.[4] Each type of OI shares common clinical characteristics such as bone fragility, skeletal deformities, and joint hyperlaxity which may differ in severity. The most common and mildest form is OI type I with specifically presence of blue sclerae and hearing impairment which can be observed over a half of patients. OI type II is the most severe form characterized by multiple skeletal fractures and severe deformities which mostly result in perinatal lethality. Type III usually involves a newborn presenting with severe progressive skeletal deformities, short stature and high mortality rate in early life. Type IV can be differentiated from the other aforementioned types by the absence of blue sclerae. The patient with this type of OI often has recurrent fractures and variable degrees of deformity of long bones and spine. The OI type V with moderate to severe bone fragility, characterized by presence of progressive calcification of the inter-osseous membranes in the forearms and legs, was suggested to be an additional type of OI classification.[5,6] Clinically, OI can be categorized into mild, moderate, severe, and extremely severe type based on its pre- and postnatal severity. These emphasize the importance of phenotypic characterization for diagnosis, classification, and severity assessment of the disease.[3]

The clinical manifestations of OI range from rarely recognized symptoms to perinatal mortality. Apart from bone deformities, other presentations of OI include blue sclerae, skeletal deformities, hearing loss, and DGI.[3] Oral manifestations in OI patients have been reported to include class III malocclusion, posterior crossbite, and generalized severe attrition.[7] The common radiographic features observed in OI include osteopenia, multiple bone fractures, and bone deformities. The skull shows multiple wormian bones in the sutures.[8]

DGI is one of the associated clinical features frequently found in OI which can affect both primary and permanent dentitions. The presence of DGI in OI patients has been suggested to be particularly related to severe physical manifestations.[3] The incidence of DGI is 1 in 8,000.[9] Clinically, the crown presents severe attrition of enamel with dentin exposed and change in color varies from yellow to opalescent gray or brown. Since collagen type I is the main component in dentin, the weak underlying dentin renders the teeth prone to rapid wear and fracture. Radiographically, the DGI teeth have bulbous crowns with constricted short roots in both dentitions. Pulp chambers may be abnormally wide but become obliterated over a period of time. Histologically, the dentinoenamel junction is malformed. The dentinal tubules are coarse, branched, and reduced in number.[10] DGI is widely classified into three types (type I - III). DGI type I is associated with OI, called syndromic DGI, and caused by mutation in *COL1A1* and *COL1A2* genes.[11] Type II is phenotypically similar to type I but without OI wherein mutations in the dentin sialophosphoprotein gene (*DSPP*) is responsible for the pathology.[12,13] DGI type

III is rare and only found in the triracial Brandywine population of Maryland, USA.

The characteristics of OI and DGI are extensively diverse and have not been fully investigated especially tooth defects associated with syndromic DGI. Majority of previous reports mainly focused on dentin defects via scanning electron microscope (SEM) which showed highly variable features.[14-18] In addition, the evidence showed that enamel was possibly affected by underlying dentin anomalies.[16] The measurement of surface roughness of the crown, a parameter used to quantify the micro-structure of enamel surface,[19] will be useful to represent consequences of dentin alteration. Furthermore, altered physical properties of the teeth including hardness and elastic modulus have been shown to affect adhesion of dental restorations.[20-22] To date, the information on physical properties of both enamel and dentin of DGI teeth is very limited.[23] Therefore, the present study aimed to investigate the physical properties including surface roughness, hardness, elastic modulus, and ultrastructural appearance of dental hard tissues including the enamel, coronal dentin, and root dentin associated with syndromic DGI. Our findings will expand the understanding of DGI which will be of assistance for clinicians to deliver appropriate dental management in affected patients.

Materials and Methods

Subject enrollment

A patient included in this study was diagnosed as affected by OI and DGI at the Genetic Clinics, Chulalongkorn Hospital and referred to the Faculty of Dentistry, Chulalongkorn University for oro-dental management. The diagnoses of the syndromes were based on clinical, radiographic, and laboratory findings. Extraoral and intraoral photographs were taken. Clinical and radiographic oral examinations were performed at the Faculty of Dentistry, Chulalongkorn University. The study was approved by the research ethics committee at Faculty of Dentistry, Chulalongkorn University. Informed written consent was obtained from the patient. The patient's maxillary left second molar (called DGI tooth in this study) was extracted due to poor prognosis. Two second molars were used as controls. They were collected from healthy individuals who had similar ages, and did not have any systemic diseases or medications affecting tooth and bone structures. After extraction, the teeth were placed in 10% formalin solution and stored in saline solution.

Surface roughness measurement

The surface roughness was studied on the lingual surface using a surface Profilometer (Talyscan 150, Taylor Hobson Ltd., UK). Each specimen was measured 30 times randomly at every 600 micrometer distance on Y-axis at the stylus speed of 1000 micrometer/second. Tracing area was 2 x 2 mm and cut-off length was 0.025 mm. The calculation of the surface topography parameters was carried out with the TalyMap Universal program. The same areas of the DGI and control samples were selected.

Tooth sectioning

Samples were embedded in acrylic block with sticky wax and divided along bucco-lingual plane parallel to the tooth axis using the slow-speed precision saw (Isomet 1,000 Precision Saw, Buehler, Lake Bluff, IL, USA) with diamond disc at a speed of 450 rpm under constant water cooling. Then, a cut surface was grinded with Grit#1000 silicon-carbide paper under water irrigation and polished with alumina powder on polishing pad (10-inch MICROPAD, Pace technologies) until the surface was flat and parallel. Once the samples were prepared, micrographs were obtained by stereomicroscopy (Olympus SZ61, Tokyo, Japan).

Measurement of nanohardness and elastic modulus

The indentation experiment was performed using nano-base indentation system (Ultra Micro-Indentation System, UMIS II, CSIRO, Australia). The finished specimens were mounted on metal base with thin double side tape. The test was done under dry condition. Elastic modulus and hardness of specimens were measured with the calibrated diamond Berkovich (three-sided pyramid) indenter. All samples were indented at loading forces from 1 to 200 mN on 30 locations randomly. In all loading-unloading cycles, more than 20 indentations were performed at each load. The IBIS software was used to calculate values for elastic modulus and hardness as a function of penetration depth, hardness testing (ht), for each indentation. The DGI teeth and controls were analyzed at the same areas.

Scanning Electron Microscopy (SEM)

Samples were etched with 37% phosphoric acid, rinsed with water, dehydrated, dried, and coated with gold powder for 110 seconds. The scanning electron microscopy (QuantaFeg 250, FEI Company, Oregon, USA) was employed to examine the dental tissues of DGI teeth compared with those of the controls.

Statistical Analysis

The statistical analysis was performed with the GraphPad Prism 5 Software Package (GraphPad Software, Inc., San Diego, CA, USA). All data was analyzed by Mann Whitney U test and Independent T-Test (significance level at 0.05).

Results

A 43-year-old Thai male was referred to the Faculty of Dentistry, Chulalongkorn University for extraction of carious tooth. He was diagnosed with osteogenesis imperfecta at the King Chulalongkorn Memorial Hospital, Thailand. The patient had experienced multiple episodes of bone fractures. He spontaneously broke his ribs and clavicles at the age of 3 months and his right femur at age 7 years. At 25 years of age, he had a car accident causing the fracture of both his femurs. Physical examinations revealed short stature (156 cm; 25th percentile), marked limb deformities, and widened antero-posterior body axis (Fig. 1A, B). The deformed limbs were not tender or swollen. The patient did not have blue sclerae.

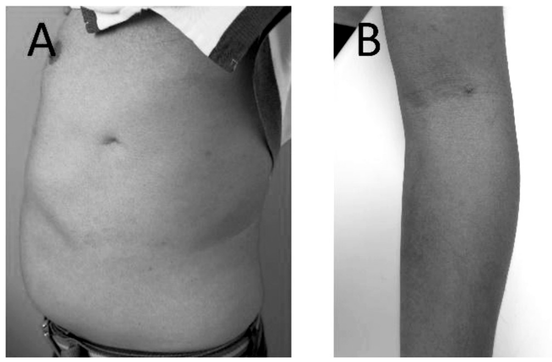

Fig. 1 Clinical features of the patient. A. The antero-posterior body axis was wide. B. The left arm showed marked deformities.

Radiographic examination revealed generalized osteopenia. His humerus and tibia were thin (Fig. 2A, B). The bone densitometry results concordantly showed reduced bone mineral density of lumbar spine (0.534 g/cm^2; z-score -3.0). The patient had never received bisphosphonate treatment.

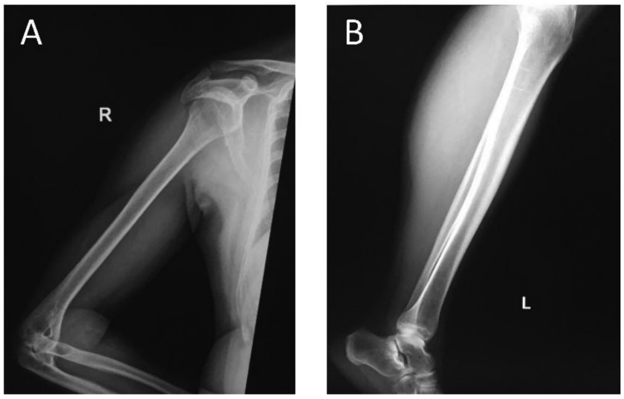

Fig. 2 Skeletal radiographs of the patient. The extremities were narrow with reduced radiopacity. A. Right humerus B. Left tibia

Intraoral examination of the patient revealed yellowish brown and opalescent teeth, severe attrition, and malocclusion. The oral soft tissue was unremarkable (Fig. 3A). The maxillary left second and third molars were extracted due to carious reasons. The teeth had bulbous crown, amalgam filling, and curved root (Fig. 3B). Dental radiograph showed marked cervical constriction and obliteration of coronal and radicular pulp cavities (Fig. 3C). The maxillary left second molar had large cavity. The root of maxillary left third molar showed periapical radiolucency.

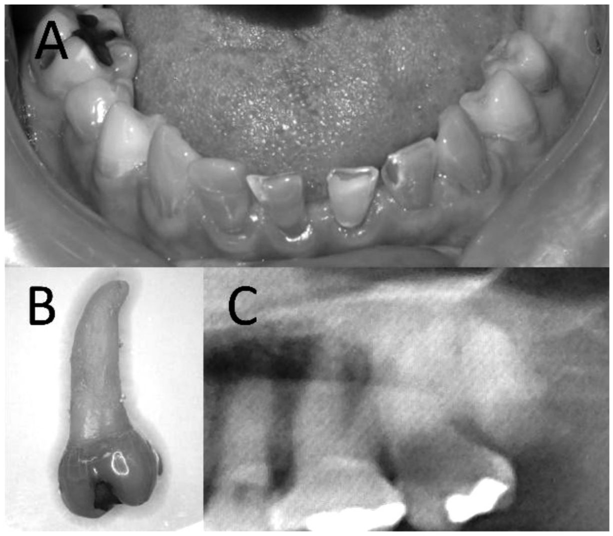

Fig. 3 Oro-dental manifestations of the patient. A. The mandibular teeth showed brown opalescent color with partial fracture. B. The maxillary left second molar showed bulbous crown with marked cervical constriction. C. Radiographically, the pulp cavities were obliterated and the roots were curved. Large cavity was observed on the maxillary left second molar.

Surface roughness measurement

In the enamel, the surface roughness analyses showed that the DGI tooth demonstrated a significantly lower roughness average (Ra) value than the controls ($p<0.0001$) (Fig. 4).

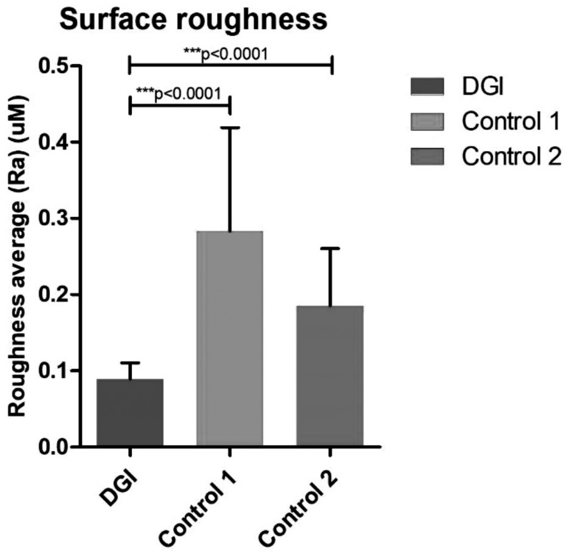

Fig.4 Bar graphs showed roughness average (Ra) values of DGI tooth compared to control 1 and 2. DGI tooth demonstrated the lowest value of surface roughness which was significantly different from the other controls.

Nanohardness and elastic modulus

The nanohardness of enamel, coronal dentin, and root dentin of DGI tooth was significantly lower than that of the controls (Fig. 5A). The difference in elastic modulus between DGI and controls showed similar pattern to the hardness values except the coronal dentin which did not show a statistical significance (Fig. 5B).

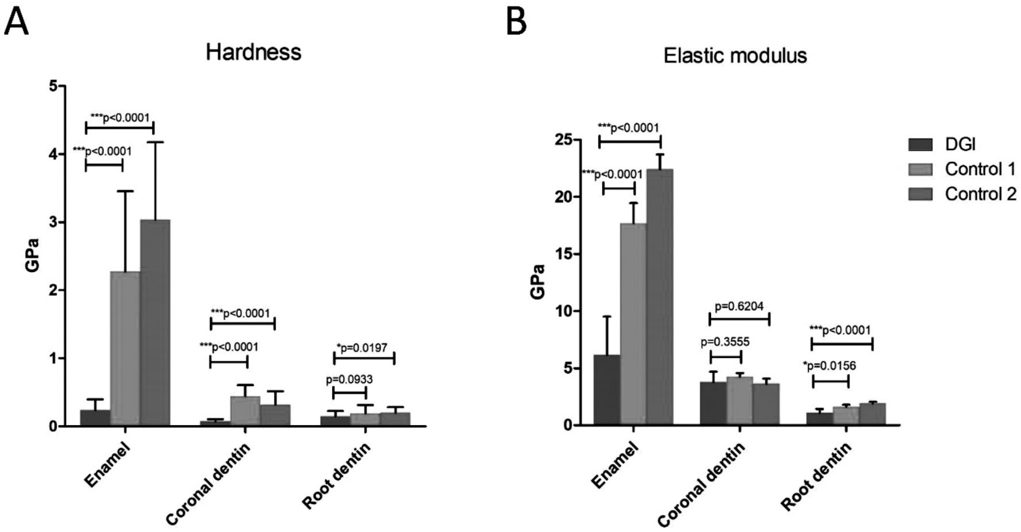

Fig.5 Bar graphs demonstrated the hardness (A) and elastic modulus (B) values of DGI tooth and controls. A. DGI tooth showed a significantly lower level of hardness in all three aspects, except at root dentin which was not significantly different from control 1's. B. The significant difference was noted at aspects of enamel and root dentin, whereas the difference at coronal dentin did not reach statistical significance.

Scanning Electron Microscopy (SEM)

In coronal dentin, DGI tooth displayed a markedly rough surface, reduced number of dentinal tubules, irregularly spaced tubules, and variable tubular diameter while the controls showed a typical pattern of regular dentinal tubules (Fig. 6A, B). At the dentinoenamel junction, the DGI tooth demonstrated a notably rough surface of dentin with appearance of scattered mineral deposits (Fig. 6C, D). The dentin surface at the DGI root was disorganized containing scattered ectopic mineralization and dispersed dentinal tubules with variable diameters (Fig. 6E-H).

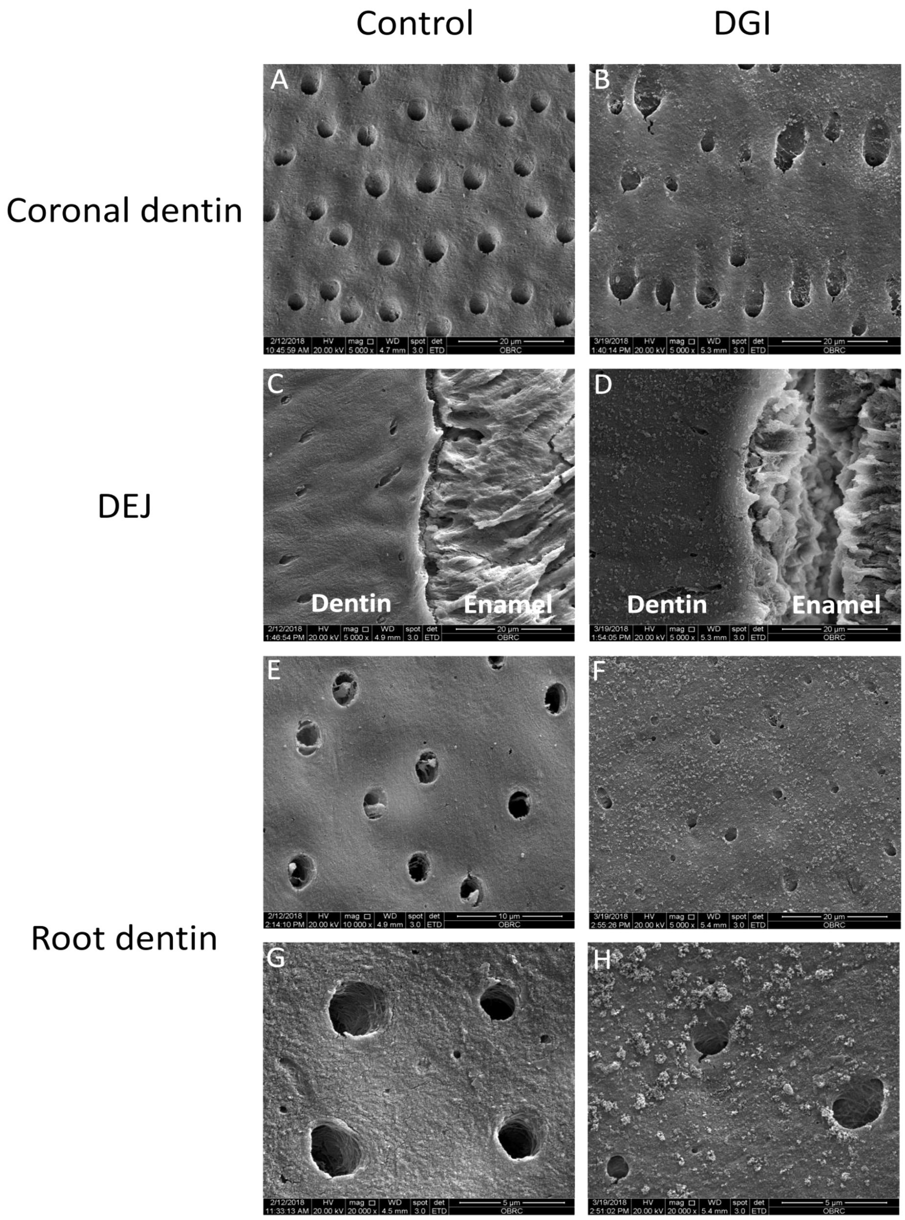

Fig. 6 Scanning electron microscopic findings of the DGI tooth compared to the controls. A, B. The coronal dentin of DGI tooth showed decreased and dispersed dentinal tubules compared to the typical pattern of tubules with uniform diameter. C, D. The DGI tooth showed rough DEJ. E-H. The root dentin of DGI tooth appeared rough containing ectopic mineralization and irregularly spaced tubules. (SEM original magnification x5,000 (E, F) and x20,000 (G, H))

Discussion

The present study demonstrates clinical and radiographic characteristics, ultrastructure, and physical properties of syndromic DGI. Based on the patient's physical features including mild skeletal severity without the presence of hearing loss and blue sclerae, the diagnosis of OI type IV was given.[4] The majority of OI has been associated with mutations in *COL1A1* and *COL1A2* genes which encode polypeptide chains of type I collagen. Pathogenic variants in those genes have been shown to cause abnormalities in collagenous tissue including bone, skin, sclerae, and dentin.[11] Consistently, our patient obviously showed bone deformity and abnormally yellowish-gray and opalescent tooth, bulbous crowns, and pulp obliteration, suggesting DGI associated with OI.

The enamel of our DGI tooth showed smooth surface as its roughness value was less than the controls. However, this finding cannot be compared with previous studies since surface roughness of DGI teeth has never been reported.

The ultrastructures of coronal and root dentin of our syndromic DGI including rough surface with deviated presence of dentinal tubules tooth was consistent with previous reports. Lygidakis *et al.*[14] reported that the main characteristics of DGI dentin were reduced in number, irregular pattern, and diameter of dentinal tubules, and rough surface of interdentinal space. We also observed scattered ectopic mineralization on the dentin surface of our DGI patient. This feature was first reported by Kantaputra *et al.*[24] They suggested that it was caused by copolymerization of normal and abnormal collagens and distortion of normal fibril morphology which could be responsible for tooth integrity.[24]

In addition, our SEM showed that the DEJ of DGI tooth was irregular compared to the controls. The DEJ of DGI teeth mostly demonstrated an irregular scalloped appearance interrupted with some areas of straight DEJ.[25,26] Previous study showed that the rough DEJ could contribute to the detachment of enamel from underneath abnormal dentin.[27] These altered structures of dentin, DEJ, and enamel of DGI teeth indicate the high susceptibility of the affected teeth to deterioration.

We observed that the hardness of enamel, coronal dentin, and root dentin of DGI tooth were significantly lower than that of the controls and the elastic modulus of DGI enamel and root dentin were significantly reduced. Consistently, Wieczorek *et al.* [23] reported that the hardness of DGI teeth was seven times decreased and Young's modulus was six times reduced, compared to those of sound teeth.

Based on several altered characteristics of DGI tooth including ultrastructure and physical properties of the dentin, DEJ, and enamel, the teeth could be prone to rapid deterioration and develop dental pathology. These are consequences of malformed collagen and dentin mineralization.[28] Studies have shown that penetration of dental adhesives to create a hybrid layer and resin tags are important factors for successful restorations of tooth-colored filling materials.[22] The defects in dentinal tubules could jeopardize the bonding of dental adhesive agents. Regular monitoring of dental restorations in DGI patient should therefore be implemented. Genetic analyses of the patient are currently being performed to elucidate disease-causing mutations. Future studies covering a large number of tooth samples obtained from DGI patients with identified pathogenic variants will provide solid data on physical, mechanical, and chemical phenotype correlated with genotype in DGI. These will expand understandings of the disease.

Conclusions

Our study demonstrates that the syndromic DGI teeth had defects in both ultrastructure and physical properties of dental hard tissues including the enamel, coronal dentin, and root dentin. These changes negatively affect tooth integrity and adhesion property of restorations. Importantly, the syndromic DGI patients suffer from both medical and oro-dental problems. Comprehensive management of the patient by medical and dental professionals should be properly arranged.

Acknowledgements

The study was supported by the Thailand Research Fund (TRF) (DPG6180001) and Office of Higher Education Commission (OHEC) Thailand (MRG6080001), the Chulalongkorn Academic Advancement into Its 2nd Century Project, and the 90TH Anniversary of Chulalongkorn University Fund (Ratchadaphiseksomphot Endowment Fund).

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The International Journal of Oral Health 14:42-51, 2018

Original Article

Antibiotic Prescribing Practices of Government Dentists in the Philippines

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Abstract

Objectives: Dentists prescribe antibiotics for odontogenic infections. However, there are reports of inappropriate prescribing and patients tend to self-medicate. These factors contribute to antimicrobial resistance, now a global public health problem. Other countries have antimicrobial stewardship strategies for dentistry, but none in the Philippines. This study aimed to describe antibiotic prescribing practices of government dentists to be part of basis for future antimicrobial stewardship policy for dentistry in the Philippines. **Methods**: The study utilized descriptive study design using self-administered questionnaire (SAQ) and focus group discussion (FGD). The questionnaire was pilot-tested and validated. Approval was sought from Emilio Aguinaldo College Ethics Review Committee. The SAQs with informed consents were distributed during the 53rd Annual Convention of Department of Health National Association of Dentists (DHNAD). Seventy-three (73) participants answered the questionnaires, while 20 participated in FGD. Data were analysed using descriptive statistics. **Results**: Amoxicillin was the first choice of antibiotics (82.19%). Clindamycin was the second choice (54.79%), then cephalexin (31.51%). Most of the respondents followed the indications for antibiotic therapy. However, some answered they would prescribe antibiotics for conditions without indications like chronic apical abscess (45%), periodontal abscess (50%), pericoronitis (23%), dry socket (22%), acute gingivitis (20.55%), acute dental pain (23.29%), pulpitis (26.03%) and odontogenic cysts (15.07%), and some would prescribe for usual procedures like uncomplicated extraction (26.03%), routine endodontics (6.85%), periodontal treatment without surgery (6.85%), and if anesthesia failure is expected (8.22%). Results also revealed inappropriate prescribing for prophylactic antibiotics for medical conditions. Most respondents were either “somewhat familiar” or “not so familiar” with antimicrobial stewardship. **Conclusion**: There is inappropriate antibiotic prescribing of participants on certain dental diseases, procedures, and medical conditions. The researchers recommend continuing education on antibiotics and creating antimicrobial stewardship policy for dentists to combat antimicrobial resistance.

Key words: antibiotics, antimicrobial stewardship, dentists, prescribing practices

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Introduction

Antibiotics are prescribed by dentists for management of dental infections. There are reports that dentists inappropriately prescribe antibiotics and patients tend to self-medicate with antibiotics. Misuse of antibiotics may lead to antimicrobial resistance (AMR), now a global public health problem. The US Centers for Disease Control and Prevention developed the Antibiotic Resistance (AR) Solutions Initiative to improve antibiotic use through antibiotic stewardship. The World Health Organization (WHO) policy package to combat antimicrobial resistance was developed to bring international attention to AMR. The United Kingdom developed the UK Five Year Antimicrobial Resistance Strategy to slow the development and spread of antimicrobial resistance, while Japan developed the National Action Plan on Antimicrobial Resistance. In the Philippines, the Interagency Committee on Antimicrobial Resistance was created and launched the Win the WAR against AMR. The Department of Health also published the Antimicrobial Stewardship Program in Hospitals.

Antimicrobial resistance (AMR) is the ability of a microorganism (like bacteria, viruses, and some parasites) to stop an antimicrobial (such as antibiotics, antivirals and antimalarials) from working against it, making standard antimicrobial therapy ineffective (World Health Organization), and stated that "both overuse and misuse of antimicrobial medicines accelerate the emergence of resistant microorganisms. Misuse can be due to poor prescribing practice, incorrect choice or dosage; self-medication; failure to finish a course of antimicrobial medicines or t`aking them for too long; lack of regulations or standards for health care workers".[1]

A study in the United States revealed that general dentists and dental specialists were responsible for more than 2.9 million antibiotic prescriptions with prolonged antibiotic treatment duration and commonly included broad-spectrum agents.[2] In the United Kingdom, a study revealed that over half of antibiotics (65.6%) were prescribed in situations where there was no evidence of spreading infection, 70.6% were used without operative intervention, and only 19.0% of antibiotics were prescribed in indicated situations. The study concluded that there is a high level of inappropriate antibiotic prescribing amongst the general dental practitioners (GDPs).[3] This is consistent with another study in 2001 which concluded that "there is a lack of knowledge of the use of antibiotics in practice and that GDPs need clear advice on when and what to prescribe, for how long and in what dosage".[4] In Australia, a study concluded that "there is a demonstrable lack of knowledge in some key areas including the incidence of adverse reactions, the development of multiresistant strains and difficulties in the appropriate use of prophylactic antibiotics.[5] While a different study revealed "heavy reliance on moderate and broad-spectrum antibiotics, with many being prescribed for conditions for which they are contraindicated".[6] In Belgium, a study concluded that antibiotics are often prescribed in the absence of fever and without any local treatment.[7] Different studies in Iran, Saudi Arabia, India, Yemen, and Indonesia also reported inappropriate antibiotic prescribing.[8-12]

This study aimed to describe antibiotic prescribing practices of government dentists in the Philippines as a basis for policy on antimicrobial stewardship. Specifically, to identify the first and second choice of antibiotics, duration of prescription, prescribing practices for selected dental conditions and procedures, medical conditions prior to dental surgery, non-clinical factors affecting prescribing practices, and familiarity with antimicrobial stewardship.

Materials and Methods

This research utilized descriptive study design. Self-administered questionnaire (SAQ) with questions taken from published studies was developed to answer the objectives. The questionnaires were pilot-tested and validated by experts. Ethics approval was sought from Emilio Aguinaldo College Ethics Review Committee. The SAQs with informed consents were distributed during the 53rd Department of Health National Association of Dentists (DHNAD) Annual Convention attended by 550 government dentists. All participants were invited but only 73 answered the questionnaires (13.27%). Twenty dentists who occupied supervisory positions in hospitals or rural health centers and who had worked in the government for at least three years participated in focus group discussion (FGD). Data were tabulated using Microsoft Excel and analyzed using descriptive statistics.

Results

Amoxicillin was the first choice for 82.19% of the respondents while only 2 dentists chose penicillin (2.74%) (Table 1). This was followed by Cephalexin (6.85%), clindamycin (4.11%), then amoxicillin with clavulanic acid (2.74%). One dentist (1.37%) answered "others" and stated that the choice was dependent on the severity of the infection. This was also corroborated by dentists during focus group discussion since amoxicillin is the antibiotic commonly supplied by the government.

Clindamycin was the second choice of antibiotics of most respondents (54.79%), then cephalexin (31.51%). Amoxicillin with clavulanic acid, cefuroxime, erythromycin equally got 4.11%, and cefuroxime (1.37%). Most ware rarely available, as stated in FGD. In these cases, it was up to the patient to buy their own medicines.

Table 1 Choice of Antibiotics

Antibiotic	First Choice		Second Choice	
	n	%	n	%
Amoxicillin	60	82.19	0	0
Amoxicillin with clavulanic acid	2	2.74	3	4.11
Cefuroxime	0	0	3	4.11
Cephalexin	5	6.85	23	31.51
Clindamycin	3	4.11	40	54.79
Doxycycline	0	0	1	1.37
Erythromycin	0	0	3	4.11
Penicillin	2	2.74	0	0
Others	1	1.37	0	0
Total	73	100	73	100

Most respondents (86.30%) prescribed antibiotics for 7 days, while 4.11% for less than 3 days, 5.48% for 3-5 days, and 4.11% more than 7 days (Table 2). FGD revealed that the initial dose was supplied, and it was up to the patient to buy the remaining prescription, but claimed that patients did not buy the remaining medicine due to financial reasons, or they stopped taking when they felt better.

Table 2 Duration of antibiotic prescription

Duration	Frequency	%
Less than 3 days	3	4.11
3 to 6 days	4	5.48
7 days	63	86.30
More than 7 days	3	4.11
Total	73	100

For dental infections, most respondents would prescribe antibiotics for infections with affected general conditions (68.49%), cellulitis (46.58%), and acute apical abscess (69.86%) (Table 3). However, most respondents would prescribe antibiotics for conditions not indicated. Twenty-seven percent would still prescribe for infections without affected general conditions and 45% for chronic apical abscess. Half of the respondents would prescribe for periodontal abscess, 23% for pericoronitis, and 22% for dry socket. Antibiotics will also be prescribed for other conditions such as acute gingivitis (20.55%), acute dental pain (23.29%), pulpitis (26.03%), and odontogenic cysts (15.07%). Participants of the FGD claimed that most dentists would just prescribe antibiotics if they were not sure with the diagnosis or the "just in case" scenario. This also applied to other dental procedures and medical conditions.

Table 3 Antibiotic prescribing for selected dental conditions (n=73)

Dental disease	Yes	%	No	%
Acute gingivitis	15	20.55	58	79.45
Stomatitis	20	27.40	53	72.60
Abscess with affected general conditions	50	68.49	23	31.51
Abscess with unaffected general conditions	20	27.40	53	72.60
Acute periapical abscess	51	69.86	22	30.14
Chronic periapical abscess	33	45.20	40	54.80
Pulpitis	19	26.03	54	73.97
Periodontal abscess	37	50.68	36	49.32
Acute dental pain	17	23.29	37	76.71
Pericoronitis	17	23.29	37	76.71
Dry socket	16	21.92	57	78.08
Cellulitis	34	46.58	39	53.42
Odontogenic cyst	11	15.07	62	84.93

Most dentists answered that they would prescribe for complicated extraction (75.34%), third molar surgery (71.23%), extraction of a tooth with chronic infection (69.86%), periodontal treatment with surgery (35.62), and endodontic surgery (16.44%). However, the respondents also answered that they would prescribe antibiotics on common procedures like uncomplicated extraction (26.03%), routine endodontics (6.85%), periodontal treatment without surgery (6.85%), and if anesthesia failure was expected (8.22%) (Table 4).

Table 4 Antibiotic prescribing for selected dental procedures (n=73)

Dental procedure	Yes	%	No	%
Periodontal treatment with surgery	26	35.62	47	64.38
Periodontal treatment without surgery	5	6.85	68	93.15
Routine endodontics	5	6.85	68	93.15
Endodontic surgery	12	16.44	61	83.56
Anesthesia failure is expected	6	8.22	67	91.78
Uncomplicated extraction	19	26.03	54	73.97
Complicated extraction	55	75.34	18	24.66
Extraction of tooth with chronic infection	51	69.86	22	30.14
Third molar surgery	52	71.23	21	28.77

For non-clinical factors, effectiveness in previous case (46.58%) tops the list, then cost (39.73%), knowledge gained from undergraduate/graduate education (28.77%), patient preference (26.03%), and availability of the nearest pharmacy (24.66%). However, several dentists answered they would prescribe when in doubt in diagnosis (17.81%), or recommended by a colleague (10.98%), while a small percentage (2.74%) would prescribe when under time pressure (Table 5).

Table 5 Non-clinical factors that affect antibiotic prescribing (n=73)

Factors	Yes	%	No	%
Patient preference	19	26.03	54	73.97
Reading of scientific materials	9	12.33	64	87.67
Knowledge gained from undergraduate/ graduate training	21	28.77	52	71.23
Availability of the nearest pharmacy	18	24.66	55	75.34
Cost of antibiotics	29	39.73	44	60.27
Recommended by a colleague	8	10.96	65	89.04
Effectiveness in previous case	34	46.58	39	53.42
When in doubt in diagnosis	13	17.81	60	82.19
When under time pressure	2	2.74	71	97.26
International guidelines	5	6.85	68	93.15

For medical conditions, 47.95% of the participants would prescribe antibiotics for heart conditions such as rheumatic heart disease (47.95%), infective endocarditis (31.51%), ventricular septal defect (31.51%), patients with pacemaker (13.70%), history of coronary bypass surgery (12.33%), and cardiac valve prosthesis (10.96%). For diabetes mellitus (32.88%) of the respondents answered they would prescribe but only 5.48% would prescribe for patients with decreased immune response due autoimmune disease, immunosuppressives, and renal transplant patients. For Hodgkin's disease, only 2.74% of the respondents answered that they would prescribe, and 8.22% for AIDS (Table 6).

Table 6 Antibiotic prescribing for certain medical conditions prior to dental surgery (n=73)

Factors	Yes	%	No	%
Diabetes mellitus	24	32.88	49	67.12
AIDS	6	8.22	67	91.78
Patient with pacemaker	10	13.70	63	86.3
Autoimmune diseases	4	5.48	69	94.52
Patients on immunosuppressive	4	5.48	69	94.52
Renal transplant patients	4	5.48	69	94.52
Rheumatic heart disease	35	47.95	38	52.05
Hodgkin's disease	2	2.74	71	97.26
History of coronary bypass surgery	9	12.33	64	87.67
Infective endocarditis	23	31.51	50	68.49
Cardiac valve prosthesis	8	10.96	65	89.04
Ventricular septal defect	23	31.51	50	68.49

Ten respondents (13.70%) answered very familiar with antimicrobial stewardship, while 3 (4.11) are "not at all familiar, while 6 (8.22%) did not provide answers. Twenty-seven (36.99%) respondents described themselves to be somewhat familiar and 26 (35.62%) not so familiar (35.62%), and only one dentist (1.37%) answered "extremely familiar" (Table 7).

Table 7 Familiarity with Antimicrobial Stewardship (n=73)

Familiarity	Frequency	%
Extremely familiar	1	1.37
Very familiar	10	13.70
Somewhat familiar	27	36.99
Not so familiar	26	35.62
Not at all familiar	3	4.11
No response	6	8.22
Total	73	100

Discussion

In this study, Amoxicillin was the first choice of the respondents, just like in the studies reviewed. However, in a study in Norway, **phenoxymethylpenicillin** was the most prescribed antibiotics, followed by Metronidazole, and concluded that, "reliance of dentists in Norway on phenoxymethylpenicillin as their first choice indicates low prevalence of antibiotic resistance among oral bacteria in Norway and shows the conservative antibiotic practice of dentists".[13] This is consistent with a 1992 study which concluded that "Norwegian dentists are somewhat restrictive in their prescription of antibiotics".[14]

Clindamycin was the second choice of antibiotics, followed by cephalexin. There is growing concern for *Clostridium difficile* infection (CDI) caused by disruption of indigenous intestinal microflora, associated with Clindamycin and third-generation cephalosporin. Restricted use of clindamycin and third-generation cephalosporins have been associated with reductions in CDI.[15-17]

Majority of the respondents prescribed antibiotics for 7 days, while others less than 7 days. Some literatures were challenging the standard 7 to 14 days of antibiotic therapy in medicine. Some studies concluded that shorter duration of antibiotic therapy was as effective as the standard.[18] In dentistry, antibiotics are prescribed for 7 days, however some studies suggest that shorter periods of 2 to 3 days can be used as management of dentoalveolar abscess provided that drainage has been established.[19]

Most respondents would prescribe antibiotics for infections with affected general conditions. A systematic review on use of antibiotics in odontogenic infections concluded that "antibiotics were prescribed only in situations of regional and/or systemic body manifestations", and recommended that "when the real need for antibiotic therapy is detected, antibiotics should be used for the shortest time possible until the patient's clinical cure is achieved".[20] However, some respondents answered that they would prescribe antibiotics for routine procedures. Some authors did not agree in giving routine prophylactic antibiotics for third molar surgery,[19, 21, 22] but a systematic review by the Cochrane Collaboration on the use of antibiotics for infection prophylaxis following tooth extraction found that antibiotics reduced the risk of infection.[23] The American Association of Endodontists recommends that "when using antibiotics in addition to adequate debridement and surgical drainage, such as in cases with spreading infections, the practitioner should use the shortest effective course of antibiotics, minimize the use of broad-spectrum antibiotics and monitor the patient closely". Supplemental antibiotics following adequate debridement and drainage in cases of localized endodontic infections is ineffective. Some evidence suggest that shorter courses of 2-3 days may be successfully used as adjuvant therapies.[24] The use of antibiotics in endodontics is limited and can be used as adjunct in treatment of acute apical abscess with systemic involvement. Antibiotics can be considered for patients with medical conditions with compromised immunity since they are more susceptible to complications arising from endodontic infection.[25]

Antibiotic prescribing is also influenced by non-clinical factors including shared attitudes and beliefs and diagnostic uncertainty which is related to the risk of suspected infection versus the risk of prescribing antibiotics[26] and the perception of clinicians that antimicrobial resistance is a public health problem and insignificant in clinical setting.[27] This was also mentioned during FGD especially when unsure about the immunity of the patient ("just in case" scenario).

Not all participants answered that they would prescribe antibiotics for indicated heart conditions and medical problems with decreased immune response prior to dental surgery. The American Heart Association simplified its recommendation for prophylactic antibiotics prior to dental procedures and only includes patients with high risk of infective endocarditis.[28] The European Society of Cardiology recommends that "antibiotic prophylaxis should only be considered for dental procedures requiring manipulation of the gingival or periapical region of the teeth or perforation of the oral mucosa, antibiotic prophylaxis is not recommended for local anesthetic injections in non-infected tissues, treatment of superficial caries, removal of sutures, dental x-rays, placement or adjustment of removable prosthodontic or orthodontic appliances or braces or following the shedding of deciduous teeth or trauma to the lips and oral mucosa.[29] Immunocompromised patients are more prone to bacteremia, which may lead to septicemia. These patients may benefit from antibiotics when receiving invasive dental procedures like extraction, deep periodontal scaling, and apical surgery.[30]

Only a small percentage of the respondents are very familiar with antimicrobial stewardship justifying the need for continuing education on antimicrobial resistance.

Conclusions

Results of this study suggest that there is inappropriate prescribing of antibiotics by dentists on certain dental diseases, dental procedures, and medical conditions.

Recommendations

- Further research with more participants to determine antibiotic prescribing practices of the general population of Filipino dentists
- Call for an expert panel consisting of oral surgeons, endodontists, periodontists, dental public health practitioners, general practitioners to create a national policy on Antimicrobial Stewardship for Dentists

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The International Journal of Oral Health 14:52-63, 2018

Original Article

Parent–Child Satisfaction and Safety of Silver Diamine Fluoride and Fluoride Varnish Treatment

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Abstract

Besides a bitter taste, black discoloration of dentinal caries is one of the shortcomings of silver diamine fluoride (SDF) application in children and could affect parental cooperation and satisfaction with the outcome. This study aimed to evaluate parent–child satisfaction with outcomes after three different types of fluoride applications and to investigate parent-reported signs and symptoms after two weeks. Subjects were 6–7 years old and had at least an active dentinal caries in their primary canines or molars. Three-hundred children were randomly assigned to Group 1: 38% SDF on dentinal carious lesion; Group 2: 5% NaF varnish on all tooth surfaces; or Group 3: a combination of SDF and NaF varnish. Immediately after application, children were interviewed regarding their satisfaction. Parental satisfaction at two weeks post-application was determined using a self-administered questionnaire. Most children were satisfied with the time spent, comfort level, taste and smell, and tooth color. There was no significant difference among groups for both children's and parents' satisfaction. Postoperative signs and symptoms as perceived by parents in Groups 1, 2, and 3, respectively, were as follows: pain, 15%, 11%, 10% ($p=0.53$); gingival swelling, 9%, 8%, 9% ($p=0.98$); gingival irritation, 10%, 15%, 17% ($p=0.36$); bad odor, 25%, 16%, 25% ($p=0.22$); and nausea or vomiting, 2%, 5%, 13% ($p=0.006$). Three methods were well accepted by children and parents. Tooth discoloration after SDF and NaF varnish application did not affect parent–child satisfaction or their perception of the effectiveness of the treatments.

Key words: silver diamine fluoride, fluoride varnish, patient satisfaction, dental caries, randomized controlled trial

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Introduction

Dental caries is the most common dental disease in children. According to the 7th National Oral Health Survey of Thailand in 2012, 75.9% of children aged five had untreated dental caries.[1] A traditional restorative approach requires powered dental equipment and well-trained dental professionals. An alternative method for effective caries management is crucial, particularly in communities where access to health care services is limited. Fluoride treatment is an easy-to-apply noninvasive method which requires minimal specialist training.[2, 3] Importantly, topical fluoride application by professionals has been shown to be one of the most cost-effective preventive methods.

Several clinical studies have shown that silver diamine fluoride (SDF) is effective in arresting dentine caries. A meta-analysis[4] reported the use of 38% SDF to arrest dentine caries where the overall proportion of arrested dentine was 65.9%. However, the permanent discoloration which occurs shortly after SDF application may discourage children and their parents from appreciating the effectiveness of the treatment. On the other hand, fluoride varnish (FV), the most common topical fluoride used by professionals, was reported to have a preventive fraction of 37% in primary teeth. It was shown to arrest 63.6% of the initial enamel caries[4] but was not effective in arresting dentine caries.[5] But FV does not stain the teeth permanently. It can be speculated that if used in combination, SDF might enhance FV in arresting and preventing dental caries in children. A few recent studies that evaluated the acceptance of SDF treatment were conducted in the USA[6,7] and Hong Kong.[8] However, the results may not be generalizable to the Thai population, with its different culture and values.

The objective of this study was to evaluate children's satisfaction, after applying three different topical fluoride treatments, with the procedures and with tooth color. Parental satisfaction at two weeks after treatment was also investigated together with side effects, based on parent-reported postoperative signs and symptoms.

Materials and Methods

This randomized controlled clinical trial was conducted in 18 primary schools in Khon Kaen, Thailand. The project was approved by the Khon Kean University Ethics Committee for Human Research, based on the Declaration of Helsinki and the ICH Good Clinical Practice Guidelines. Parents were asked for permission for their children to participate, and children were asked for their assent. The children who returned the consent form signed by the parents were screened at their school by one examiner to evaluate their eligibility, and they then received oral hygiene instructions.

Before recruiting, an eight-minute animation was shown to all schoolchildren. The story was about a panda who, instead of having tooth drilling, received SDF application, which turned the decayed teeth a black color. Together with the animation, a small exhibition on causes of caries and on caries prevention and treatment was organized at the participating schools.

Subjects were children between six and seven years of age at the time of enrollment who had at least one or more active dentin caries lesions in a primary canine or molar. We excluded the esthetic region to enhance parental participation and to compare our findings with previous studies where anterior teeth were included.[9] Exclusion criteria included receiving topical fluoride less than three months prior to enrollment, a history of allergic reaction to materials such as fluoride varnish, adhesive, or silver compound, and being uncooperative. Children whose primary caregivers were unable to respond to the questionnaire due to inadequate literacy were also excluded. Sample size was calculated based on the primary clinical outcome (arrested caries rate) of a previous study.[9] Three hundred children were recruited to achieve a power of 80% at a significance level of 5%.

After screening, the children were randomized into three groups using a block randomization method (https:www.sealedenvelope.com). Allocation was concealed in sealed opaque envelopes that were opened by the operator before treatment.

The subjects were examined at baseline, and then the fluoride treatment was applied. Children's satisfaction with the procedure was collected immediately after fluoride treatment, while parental satisfaction was collected at two weeks after treatment. Parents were instructed to contact the researchers via telephone if any concerns arose. Children who needed emergency care were referred to the nearest hospital or to the Khon Kaen University Faculty of Dentistry.

Fluoride application

All children were asked to brush their teeth to remove plaque before treatment. One dentist performed all treatments as follows.

Group I – application of a 38% SDF solution (Saforide; Toyo Seiyaku Kasei Co. Ltd. Japan)

SDF was applied directly to a dentinal carious lesion on a primary molar and/or canine without caries removal. Cavities in anterior teeth were given an application only when parental consent was obtained. The SDF solution was slowly rubbed into the cavitated caries using a microbrush, and excess solution was wiped off with dried gauze. Children were instructed not to rinse, spit, eat, or drink for 30 minutes after the application.

Group II – application of a 5% NaF varnish (Duraphat, Inpharma GmbH, Cologne, Germany)

The varnish was applied on all surfaces of every tooth. Children were instructed not to eat a hard diet for at least two hours after application.

Group III – application of a 38% SDF solution followed by 5% NaF varnish

SDF was applied in cavitated caries, followed by application of FV on all surfaces of every tooth. Children were instructed not to rinse or spit, with no eating or drinking for 30 minutes, and not to eat a hard diet for at least two hours after the application.

Child satisfaction

After the fluoride application, child satisfaction was immediately evaluated using a binary response questionnaire (satisfied/unsatisfied) regarding aspects encountered during the treatment. These included time spent for treatment, the taste and smell, discomfort, the color of teeth after treatment, overall satisfaction, and the willingness to receive the same treatment again in six months.

Parental satisfaction

Two weeks after the treatment, a self-administered questionnaire was sent to the parents for data collection. The questionnaire contained four parts.

I. General information

- The respondents were primary caregivers (Yes/No)
- The relationship to children (parents/grandparents/others)

II. Postoperative signs & symptoms

- Child had pain after fluoride treatment (Yes/No)
- Child had gingival swelling after fluoride treatment (Yes/No)
- Child had gingival irritation (whiter gingiva) (Yes/No)
- Child had bad mouth odor more than normal after fluoride treatment (Yes/No)

■ Child had nausea or vomiting after fluoride treatment (Yes/No)

III. Parental satisfaction with the fluoride treatment

■ Do you think that fluoride treatment is effective for dental caries treatment? (Yes/No)

■ Are you satisfied with the fluoride treatment given to your child? (Yes/No)

IV. Parental feeling toward tooth discoloration

This was collected using a five-point scale with the range of extremely unsatisfied, unsatisfied, somewhat satisfied, satisfied, and extremely satisfied.

Statistical analysis

Data were analyzed using SPSS for Windows version 19. Descriptive data were reported as frequency, percentage, mean, and standard deviation (SD). To evaluate the differences among groups, a chi-square test was used for categorical data and a Kruskal-Wallis test for continuous data. For multiple comparison, p-values were adjusted using the Bonferroni method. The significance level was set at 5%.

Results

A total of 300 school children, aged between six and seven, were recruited at baseline with 1,953 active dentine caries surfaces in primary teeth. A flow chart of subjects over the study period is shown in Figure 1. The distribution of children in all groups was similar in gender and age (Table 1).

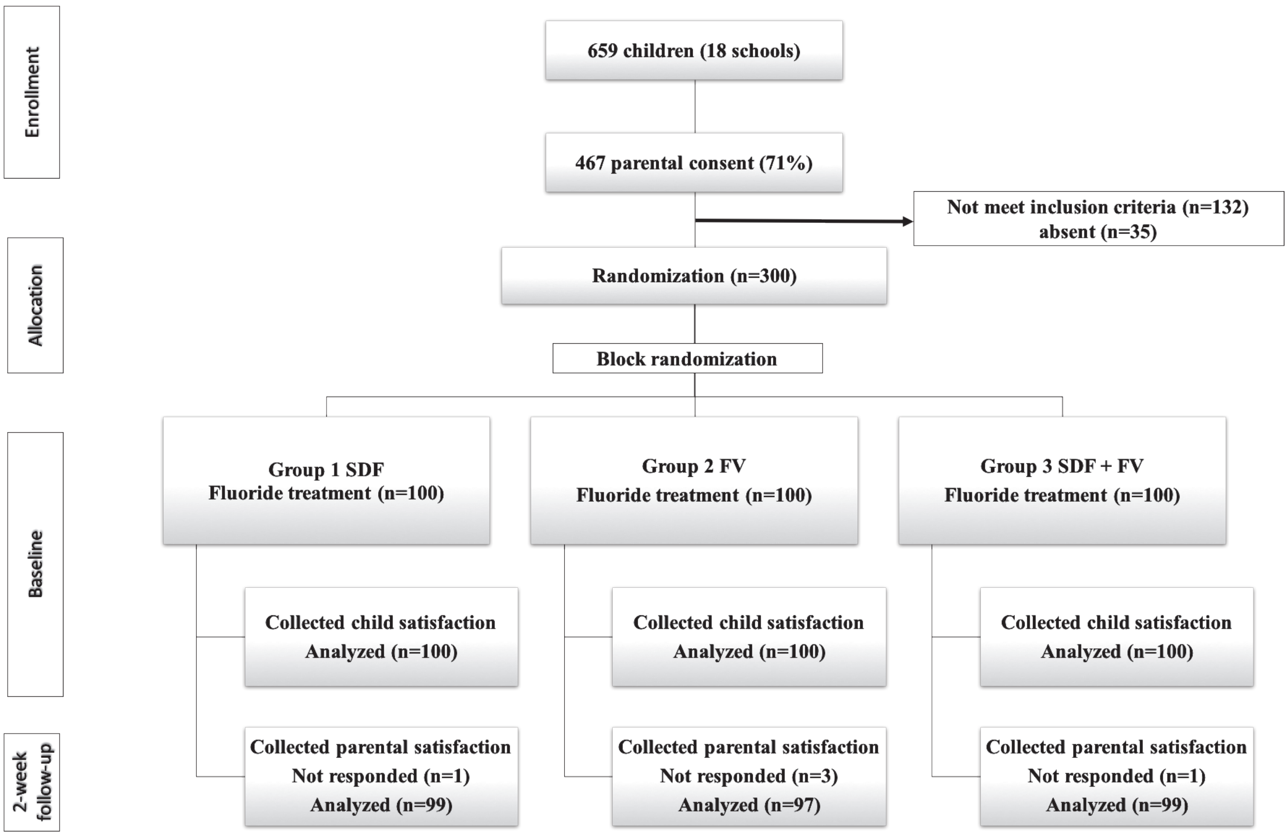

Fig. 1 Flow diagram of participants over the study period

Table 1 Characteristics of study participants at baseline examination

		Group 1 SDF (n=100)	Group 2 FV (n=100)	Group 3 SDF + FV (n=100)	Total n (%)
Age (year)	Median (Q1–Q3)	7 (6–7)	7 (6–7)	7 (6–7)	
	Mean (SD)	6.7 (0.5)	6.6 (0.5)	6.7 (0.4)	
Gender					
Boy		50	51	58	159 (53)
Girl		50	49	42	141 (47)
Questionnaire respondents					
Primary caregiver		98	93	96	287 (97)
Not primary caregiver		1	4	3	8 (3)
Relationship with child					
Parent		71	64	67	202 (69)
Grandparent		24	27	25	76 (26)
Other		4	4	7	15 (5)
Consent for application on anterior teeth					
Consent		90	88	87	265 (88)
No consent		10	12	13	35 (12)

The three groups were similar with respect to baseline caries experience (Table 2). The mean dmfs scores of the groups were as follows: 1) the SDF group, 23.9; 2) the FV group, 24.0; and 3) the SDF + FV group, 24.9. The distribution of active caries in the anterior and posterior teeth was also comparable among groups, with almost 40% of total active caries surfaces being found in primary incisors.

Table 2 Caries experience at baseline examination

		Group 1 SDF (n=100)	Group 2 FV (n=100)	Group 3 SDF + FV (n=100)	*p*-value
dmfs score	Median (Q1–Q3)	20.5 (9.3–34.0)	22.0 (12.0–34)	21.5 (13.0–35.0)	0.56[a]
	Mean (SD)	23.9 (1.7)	24.0 (1.6)	24.9 (1.5)	-
Active dentine caries surfaces	Median (Q1-Q3)	5.5 (3.0–8.0)	6.0 (4.0–9.0)	6.0 (3.0–D10.0)	0.20[a]
	Mean (SD)	6.07 (4.1)	6.88 (4.0)	6.59 (3.7)	-
Position of active dentine caries		**tooth surface n (%)**	**tooth surface n (%)**	**tooth surface n (%)**	***p*-value**
anterior teeth		241 (39.7)	263 (38.2)	251 (38.1)	1.00[b]
posterior teeth		366 (60.3)	425 (61.8)	407 (61.9)	1.00[b]

[a] Kruskal-Wallis test; [b] Chi-square test

Information on child satisfaction with six aspects of the fluoride treatment is shown in Figure 2. For the time spent, the "satisfied" responses for the three groups were 1) SDF = 100%; 2) FV = 98%; and 3) SDF + FV = 96%. For "whether the treatment is comfortable or not," all children in the SDF group felt comfortable, whereas 3% of children in Group FV and 2% in SDF + FV did not like the feeling of the cotton roll inside their mouths. The "satisfied assessment for taste and smell" constituted 95% of children in Group 1, 96% in Group 2, and 97% in Group 3. For color of teeth immediately after fluoride application, 98% in Group 1 were satisfied, 99% in Group 2, and 98% in Group 3. Assessment for overall satisfaction showed that 99% of children in Group 1 were satisfied, 98% in Group 2, and 99% in Group 3. Lastly, 98% of children in the SDF group, 95% in the FV group, and 99% in the SDF + FV group agreed to repeat the same treatment in six months.

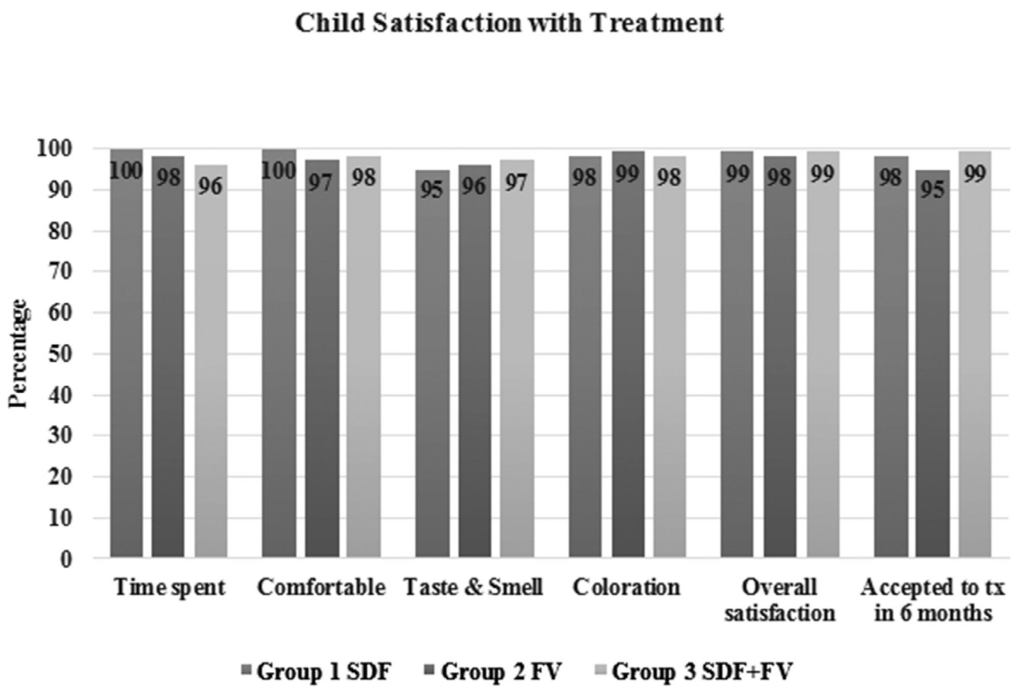

Fig. 2 Children's satisfaction with six aspects related to fluoride treatments

Parental reports of the postoperative signs and symptoms in children are shown in Table 3. Parents reported pain in 15%, 11%, and 10% of the children in Groups 1, 2, and 3, respectively. However, there was no further information in the comment sections regarding the source of the pain. About 9% of parents in all groups claimed that children had gingival swelling, and 14% reported gingival irritation. About 22% of parents reported that the child had bad breath. There were no statistical differences among the three groups with regard to pain, gingival swelling and irritation, and mouth odor. However, 13% of children in the SDF + FV group reported nausea, which was significantly higher than that reported in the SDF group (2%, p=0.009) but not different from that in the FV group (5%, p=0.16).

Table 3 Percentage of postoperative signs & symptoms at two weeks after fluoride treatment

	Group 1 SDF (n=99)	Group 2 FV (n=97)	Group 3 SDF + FV (n=99)	*p*-value
Pain	15	11	10	0.53
Gingival swelling	9	8	9	0.98
Nausea	2	5	13	0.006*
Gingival irritation	10	15	17	0.36
Bad mouth odor	25	16	25	0.22

Chi-square test

*For nausea symptoms: Group 3 SDF + FV > Group 1 SDF (Fisher's Exact test with Bonferroni adjustment, p=0.009)

Regarding tooth discoloration, on a five-scale evaluation, a majority of parents (Group 1 = 75%, Group 2 = 79%, Group 3 = 83%) responded as "satisfied" or "extremely satisfied." Only two parents in the SDF group, who consented their children to receive SDF on incisors, reported as "extremely unsatisfied" due to the darkened teeth of the child (Figure 3). This accounted for 1.6% of 125 children who received SDF on anterior teeth.

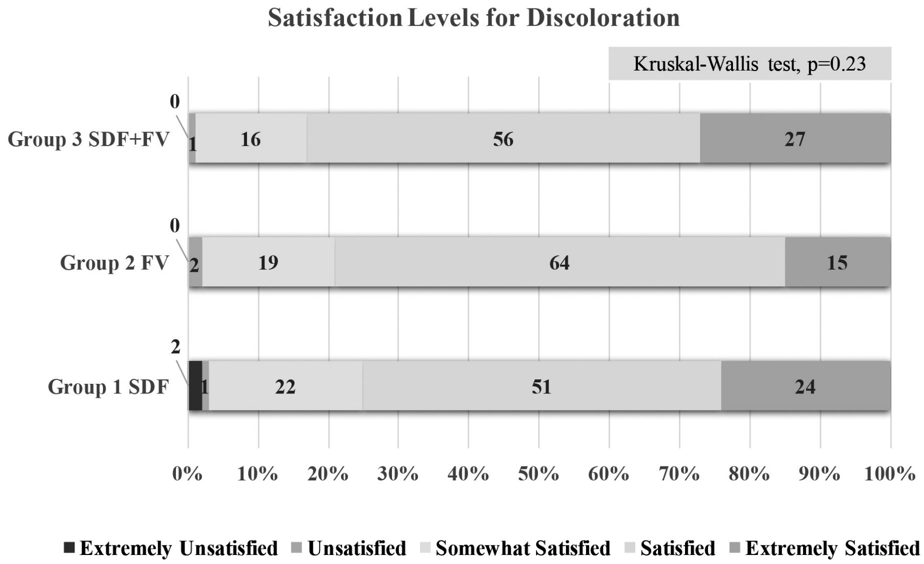

Fig. 3 Percentage of parental satisfaction with tooth discoloration after fluoride application

The analysis for parental satisfaction showed most parents (Group 1 = 86%, Group 2 = 93%, Group 3 = 87%; X^2, p=0.28) responded that the treatment received by their children was effective, and more than 90% (Group 1 = 96%, Group 2 = 94%, Group 3 = 94%; X^2, p=0.77) were satisfied with the overall treatment (Figure 4).

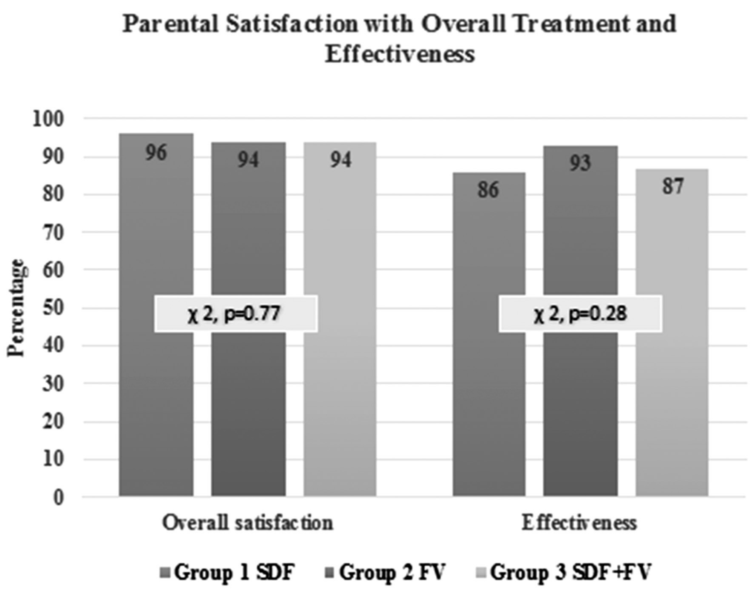

Fig. 4 Parental satisfaction with overall treatment and effectiveness

Discussion

This study was a randomized controlled clinical trial using a parallel group design. Each participant received one of the fluoride application methods. This study was conducted in a high-caries-risk population. Characteristics of the participants at baseline, such as caries experience, socioeconomic status, and oral care practice, were comparable among the three groups. In this preliminary report, we compared children's and parents' satisfaction with the three methods and investigated postoperative signs and symptoms after the fluoride treatment.

Patients' satisfaction is one of the outcomes of medical care and affects their decisions regarding health care.[10] For children, parental satisfaction plays an important role in decision-making. SDF usage for caries control is a considerably new protocol. Previous clinical studies[79] were mostly conducted in children of a younger age, whose satisfaction was difficult to assess directly. In this study, a binary response questionnaire was selected because of its advantages when responded to by children. Studies show that it is simple to score, reliable, preferred by respondents, and easy to administer.[11-13] Due to limitations of the children's literacy, they were interviewed by the examiner immediately after the fluoride treatment to reduce recall bias.

Topical fluoride application is a noninvasive method requiring minimal compliance.[2,3] Our results support this. Analysis of data from the questionnaire showed that children in all groups were satisfied with the treatment methods. The questionnaire was designed to evaluate children's perception of the treatment and their overall satisfaction. The SDF solution, at 38% concentration, manifests a metallic smell and taste, which may cause children to have a transient bad odor and may stimulate nausea and vomiting. In this study, the taste and smell of the fluoride reagents as well as the appearance of the teeth immediately after treatment were acceptable to almost all participants. However, a few children who received varnish as part of the treatment reported discomfort after treatment, whereas all participants who were administered only SDF appeared to be comfortable with the treatment. It is possible that children may not like the sticky texture of FV. A literature review[2] claimed that when SDF was carefully applied, response to its use with respect to taste and texture was more favorable than the response to FV. Regarding the time spent, it took 1–3 minutes per child for a single fluoride application and slightly longer to apply both reagents in Group 3. Most children were satisfied with the time spent for the treatments. As compared with other treatment methods without caries removal, such as resin infiltration, the simplicity of SDF application might have contributed to its higher acceptability.[14] Given that fluoride reagents had an unpleasant taste, care was especially taken to isolate the teeth when the reagents were applied. The children were satisfied with the fluoride treatment, confirmed by the result that more than 95% would be happy to repeat the same treatment in the next six months.

For parental satisfaction, we collected data using a self-administrated questionnaire at two weeks postoperatively, to ensure that the teeth had completely discolored. The comprehensive questions were also based on previously reported postoperative signs and symptoms, including pain, gingival irritation (white lesion), bad mouth odor, and nausea/vomiting. The mildly painful white lesion on the mucosa described in other studies could spontaneously disappear within two days.[8,15] The transient white lesion also observed in this study seemed to be a result of plasmolysis rather than a chemical burn or an ulcer.

In a previous study,[8] the incidence of adverse effects after SDF application, such as pain and a bad smell, were found to be lower than that reported in this study. This may be due to the fact that child participants in this study had more severe dental caries. As the data, as perceived by parents, were collected indirectly through a self-administered questionnaire, it would be difficult to explain the causes of the signs and symptoms, that is, whether they were due to the effects of fluoride application or to existing dental caries. The postoperative signs and symptoms in the two groups receiving SDF were no different from those manifested in the FV group. In this study, any deep dental caries whose pulpal involvement was uncertain were excluded. Reports of the children's oral health status and treatment needs were sent to their parents together with the questionnaire. Parents were also advised to seek appropriate treatment.

Regarding the nausea symptoms, parents of children in groups receiving either FV or varnish plus SDF reported a higher incidence than those who were given only the SDF application. This is possibly due to the sticky nature of FV, with or without SDF, associated with nausea, and when it was used in combination with SDF, the bitter taste could have made the children feel indigestion. We ruled out the possibility of acute toxicity when the two fluorides were used in combination. In children weighing 20 kg who received a combination of SDF and FV, the total amount of fluoride received was 11.28 mgF (2.24 mgF from SDF and 9.04 mgF from FV).[16,17] This amount was nine times lower than the probable toxic dose (PTD). We also observed that one girl vomited within five minutes after the FV treatment. A thorough investigation suggested that she was too excited by her participation in the study, although she cooperated well. Based on her body weight, the amount of fluoride received was 20 times lower than the PTD. It is also possible that she disliked the sticky nature of the varnish, which corresponded with her parents' report. During the study, parents were encouraged to contact the researchers via telephone if any concerns arose. Other serious adverse effects were not found in this study.

The discoloration of the teeth was generally well accepted by both children and parents of all groups. No statistically significant difference was found in the satisfaction with respect to tooth discoloration between groups receiving SDF and the group receiving varnish only. Only two parents in the SDF group who consented to their children receiving SDF on the anterior teeth reported being extremely unsatisfied with the discoloration. Although consent to the application of fluoride reagents on the anterior teeth was separate from the consent to participate in the study, parents might not have been fully aware of the actual effects. It has been shown that acceptance of SDF staining was greater for posterior teeth than for anterior teeth.[6] But if children have a major behavioral barrier, their parents prefer SDF treatment rather than advanced behavioral techniques,[6] such as anesthesia and general anesthesia, for traditional restoration. Parents' satisfaction level with respect to the effectiveness of SDF and the easy, pain-free process also outweighed the disadvantages with respect to discoloration and taste.[7]

Dentists have considered FV a standard for caries prevention in children,[3,18] whereas SDF has not been widely accepted despite its proven effectiveness,[19-21] for example, in Thailand, where children have high caries experience with a high burden of untreated caries. This lack of acceptance may partly be due to dentists' own lack of confidence in the safety of the treatment as well as some adverse effects and lack of child–parent acceptance. But the findings from this study strengthen the fact that postoperative signs and symptoms are slight and temporary, which should be reassurance for both the children and parents. Tooth discoloration may also be well accepted by both the children and their parents, provided that adequate and suitable information has been provided to them.

Only a few studies have investigated the efficiency of fluoride effectiveness with respect to the preferences of patients or parents[6,8] and have shown that SDF can control the progression of dental caries and may reduce pain and infection. These studies could lead to the improvement of children's quality of life.[22] Further long-term study should investigate oral health-related quality of life after SDF and FV treatments.

Conclusions

Treatment with SDF with FV together was well accepted by children and parents. Postoperative signs and symptoms perceived by a few parents included pain, gingival swelling or irritation, and bad mouth odor, which were less likely to relate to fluoride application. Black staining after SDF treatment, which had been perceived as the major disadvantage of this treatment, did not affect parental satisfaction to the degree that had been feared. As well as exercising stringent case-selection criteria, dental professionals should provide appropriate and adequate information prior to the treatment, both to the family and, where possible, to the child and the community.

Acknowledgements

This research was funded by the Biofilm Research Group and Graduate School, Khon Kaen University. We thank the New York University College of Dentistry for travel fund to present a part of this research at the 96th General Session of the International Association for Dental Research in London, United Kingdom.

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International Journal of Oral Health **Volume 14, November 2018**

The Official Journal of the Asian Academy of Preventive Dentistry