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Research Report

The relationship between dental fear, anxiety and sociodemography in Jakarta, Indonesia

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ABSTRACT

Background: *The anxiety associated with dental visits is one of the obstacles preventing dentists from improving oral health and is also a significant predictor of dental visit evasion, which is frequently observed in Indonesia.* ***Purpose:*** *To identify the level of dental fear and anxiety in the population of Jakarta, Indonesia and establish the relationship with sociodemographic factors.* ***Methods:*** *A cross-sectional method was used with a sample size of 1811 respondents aged 17–65 years old who were asked to complete the validated Indonesian versions of modified dental anxiety scale (MDAS) and dental fear scale (DFS) questionnaires. The data obtained was then analysed using nonparametric and chi-square tests.* ***Results:*** *The prevalence of subjects with moderate to high dental anxiety and fear was 16.3% (295 respondents) and 36.1% (654 respondents), respectively. The primary sources of dental fear and anxiety were dental drilling and anaesthesia before tooth extraction. The results of the nonparametric and chi-square tests show that both are significantly related to gender, age, educational status, income level, insurance and history of dental visits ($p = < 0.05$).* ***Conclusion:*** *Several sociodemographic factors are associated with dental fears and anxiety among the participants in Jakarta, Indonesia.*

Keywords: *Dentist visit; dental fear and anxiety; sociodemography; Indonesia*

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INTRODUCTION

The fear of dental care is a major obstacle to preventing problems related to dental and oral health, which are known to interfere with daily activities. Dental fear is a challenge for dentists because it complicates medical procedures and leads to irregular dental visits (and ultimately poor oral health).[1–4] This type of fear, which can be defined as an emotional response to a threat or danger involving dental treatment, is a common phenomenon in dentistry.[5,6]

In Indonesia, a survey on dental fear and anxiety measured the prevalence of anxiety towards certain dental treatments: tooth extraction, dental fillings and oral hygiene; the results showed that around 20–30% of subjects felt fear and anxiety towards the treatments. There is limited data on the common causes of dental fear and anxiety in Indonesian society. However, research in other countries reports that the frequency of dental anxiety ranged from 5% to 20% and was higher in females.[7,8] Furthermore, the prevalence of dental anxiety among children ranged from 6% to 20%, and in adolescents, this increased to 11%.[6] The prevalence of dental anxiety varies from 4% to 30%.[9] The percentage of dental care utilisation in Jakarta province is only 16.4%;[10] therefore, it can be concluded that while Jakarta is the province with the fastest rate of progress and development in Indonesia, residents are less likely to participate in dental health care. The low number of dental visits or avoidance of dental treatment may both be a result of dental anxiety and fear.

Several instruments have been developed to measure anxiety and fear related to dental care. One of the most common instruments is the dental anxiety scale (DAS). The DAS is widely used and has been updated by Dailey et al.[11] to the modified dental anxiety scale (MDAS), which is more

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concise and is both valid and reliable.[6] It consists of five questions, with each question offering responses ranging from 1 to 5 ('not anxious' to 'very anxious', respectively). The minimum total score was 5 and the maximum was 25; 19 and above indicated high dental anxiety, which may require special attention from the dentist.[12]

The Kleinknecht's Dental Fear Scale is the second most frequently used instrument, and it focuses on specific situations and procedures.[13] The updated version contains 20 questions rated on a five-point scale where 1 is 'no fear' and five means 'extreme fear'; hence, the total scores ranged from 20 to 100.[14] The purpose of this study is to provide data on the prevalence of fear and anxiety levels associated with dental care in Indonesia – especially in the province of Jakarta – and identify the main causes of these and their relationship with sociodemography using the MDAS and DFS. Data on the prevalence of dental fear and anxiety is critical for Indonesian dentists and governments in order to see how much this will affect dental care and how to address this phenomenon.

MATERIALS AND METHODS

This study used an analytic cross-sectional design to determine the relationship between sociodemographic factors and the fear and anxiety levels towards dental care. The population included 17–65-year-old residents from the Thousand Islands and regions in the Jakarta province (Central, East, West, North and South Jakarta). The study was conducted from September to November 2017 following the review and approval of the research ethics protocol by the YARSI University Ethics Commission (certificate number 316/KEP-UY/BIA/XI/2017). The participants signed informed consent, and multistage cluster sampling was used: the sample was taken randomly up to the district level and in accordance with the proportion of the population of Jakarta with a total of 1811 respondents.

The procedure was conducted by requesting permission from the relevant agencies and testing the validity and reliability of the MDAS and DFS questionnaires (0.844 and 0.935, respectively) using Cronbach alpha and a p value of < 0.05. The calculated r value is greater than r table for all question items from the two questionnaires using the Pearson product moment correlation test. The DFS and MDAS scoring, which initially consisted of a five-point scale, was converted into two-point scale for analysis in logistic regression. The scores in MDAS are divided as follows: 0–5 for not anxious, 6–10 for somewhat anxious, 11–14 for moderately anxious, 15–18 for highly anxious and 19–25 for extremely anxious. For DFS, the scores are divided as š 60 for high dental fear, 34–59 for moderate fear, 21–33 for low fear and scores < 20 for no fear.

The surveys were first calibrated by six interviewers then administered by researchers and extended for approximately two months. Furthermore, each respondent was asked to provide sociodemographic data consisting of age, region, sex, education level, income, questions related to health insurance and history of dental visits. The Indonesian versions of the DFS and MDAS were made available. The subjects' ages were classified into 17–25, 26–35, 36–45, 46–55 and 56–65, and education level was divided into basic (elementary–junior high school), secondary (high school) and higher (higher education/university). Income level was categorised into non-income and income below and above the Jakarta UMR (regional minimum wage).[15] The data obtained was analysed using SPSS software with a nonparametric test to compare the medians because the data did not have a normal distribution or chi-square values for proportions and binary logistic regression.

RESULTS

Based on sociodemographic status, the frequency distribution of research respondents is shown in Table 1. The data in Table 1 shows a gender variable characterised by 1012 male respondents (55.9%) and 799 female respondents (44.1%). In the age variable, the largest proportion was in the group of 17–25-year-olds (818 respondents, 45.2%) while the smallest was 37 respondents (2%) in the 56–65 age group.

Table 1. Sociodemographic status of research subjects

Variable	N	%
Gender		
Male	1012	55.9
Female	799	44.1
Region		
North Jakarta	300	16.6
Central Jakarta	201	11.1
East Jakarta	414	22.9
West Jakarta	404	22.3
South Jakarta	382	21.1
Thousand Islands	110	6.1
Age of subject		
17–25 years old	818	45.2
26–35 years old	491	27.1
36–45 years old	273	15.1
46–55 years old	190	10.5
56–65 years old	37	2.0
Level of Education		
Higher Education	508	28.1
Secondary Education	921	50.9
Basic Education	381	21.0
Income		
No income	479	26.4
< Minimum wage	433	23.9
≥ Minimum wage	899	49.6
Insurance		
Yes	1251	69.1
No	560	30.9
Ever been to dentist		
Yes	1370	75.6
No	441	24.4
Total	1811	100.0

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Table 2. Frequency of sources of fear in DFS questionnaire items

Questions	No Fear	Some Fear	Moderate Fear	High Fear	Extreme Fear	Total
The source of fear during treatment						
Promise to visit the dentist	1137 (62.8%)	519 (28.7%)	110 (6.1%)	24 (1.3%)	21 (1.2%)	1811 (100%)
Approach the dentist's clinic	1310 (72.3%)	391 (21.6%)	82 (4.5%)	19 (1.0%)	9 (0.5%)	1811 (100%)
Sit in the dentist's waiting room	1140 (62.9%)	526 (29.0)	112 (6.2%)	21 (1.2%)	12 (0.7%)	1811 (100%)
Sitting in the dental care chair	898 (55.7%)	671 (37.1%)	184 (10.2%)	42 (2.3%)	16 (0.9%)	1811 (100%)
Smell of the dental clinic	1307 (72.2%)	365 (20.2%)	96 (5.3%)	31 (1.7%)	12 (0.7%)	1811 (100%)
Seeing the dentist enter the room	1133 (62.6%)	509 (28.1%)	132 (7.3%)	23 (1.3%)	14 (0.8%)	1811 (100%)
The sight of syringe for anaesthesia	579 (32.0%)	667 (36.8%)	372 (20.5%)	113 (6.2%)	80 (4.4%)	1811 (100%)
The feeling of injected syringe	554 (30.6%)	744 (41.1%)	309 (17.1%)	127 (7.0%)	77 (4.3%)	1811 (100%)
Seeing the dental drill	623 (34.4%)	655 (36.2%)	347 (19.2%)	100 (5.5%)	86 (4.7%)	1811 (100%)
The sound of the dental drill	636 (35.1%)	688 (38.0%)	321 (17.7%)	89 (4.9%)	77 (4.3%)	1811 (100%)
The vibration of the dental drill	565 (31.2%)	714 (39.4%)	347 (19.2%)	100 (5.5%)	85 (4.7%)	1811 (100%)
After the teeth cleaning process	1287 (71.1%)	381 (21%)	94 (5.2%)	32 (1.8%)	17 (0.9%)	1811 (100%)

Table 3. MDAS and DFS nonparametric test results based on sociodemographic status

Variable	MDAS Median (Mean±SD)	p value	DFS Median (Mean±SD)	p value
Gender				
Male	9.00(9.85±4.08)	0.0001*	30.00(33.13±12.06)	0.0001*
Female	10.00(10.86±4.12)		33.00(35.42±12.24)	
Region				
North Jakarta	9.00(10.02±3.77)	0.0001*	30.00(32.28±10.74)	0.0001*
Central Jakarta	9.00(9.55±4.03)		31.00(33.25±11.81)	
East Jakarta	10.00(10.92±4.13)		33.00(35.65±12.44)	
West Jakarta	10.00(10.49±4.54)		31.00(34.86±13.27)	
South Jakarta	9.00(9.83±3.88)		31.00(33.09±11.38)	
Thousand Islands	11.00(11.13±4.06)		33.50(36.04±13.08)	
Age of subject				
17–25 years old	10.00(10.51±4.09)	0.0001*	32.00(34.93±12.18)	0.0001*
26–35 years old	10.00(10.34±4.18)		32.00(34.47±12.07)	
36–45 years old	9.00(10.06±4.05)		30.00(32.98±11.83)	
46–55 years old	9.00(9.86±4.335)		29.50(32.40±12.86)	
56–65 years old	9.00(9.08±3.507)		25.00(30.00±11.19)	
Education				
Higher Education	9.00(9.68±4.06)	0.0001*	30.00(32.92±12.31)	0.0001*
Secondary Education	10.00(10.36±4.00)		31.00(34.51±12.13)	
Basic Education	10.00(10.98±4.41)		33.00(34.90±12.08)	
Income				
No income	10.00(10.74±3.99)	0.0001*	32.00(34.96±12.17)	0.0001*
< Minimum wage	10.00(11.11±4.66)		33.50(36.64±13.59)	
≥ Minimum wage	9.00(9.67±3.88)		30.00(32.50±11.21)	
Insurance				
Yes	9.00(9.77±3.98)	0.0001*	31.00(33.48±11.77)	0.0001*
No	10.00(10.98±4.37)		33.00(35.63±12.97)	
Ever been to dentist				
Yes	9.00(9.91±3.86)	0.0001*	30.00(33.07±11.17)	0.0001*
No	11.00(11.50±4.68)		34.00(37.48±14.43)	

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The sociodemographic data for the education variable showed that the highest proportion had a high school education (921 respondents, 50.9%), while 381 (21%) had a basic education. Furthermore, 899 respondents (49.6%) received income above the minimum wage (minimum wage in Jakarta is rupiah 3,648,035), and a majority (1251, 69.1%) also had either government (BPJS) or private insurance. A total of 1370 respondents (75.6%) have been to the dentist, while the remaining 441 (24.4%) have not.

In this study, the levels of dental anxiety in 1811 respondents were as follows: high-level anxiety in 216 respondents (11.9%), extreme anxiety in 79 respondents (4.4%), moderate anxiety in 461 respondents (25.5%), low anxiety in 842 respondents (46.5%) and no anxiety in 213 respondents (11.8%). Moreover, the DFS questionnaire (Table 2) attributed the main source of fear to the sight and sensation of dental drills and anaesthesia (questions 7–11). This was indicated by the Likert scale, with scores of 4 (high fear) and 5 (extreme fear). The percentages of dental fear

Table 4. The MDAS and DFS chi-square test results based on sociodemographic status

Variable	Dental Anxiety (%)						Dental Fear (%)			
	Not Anxious	Somewhat Anxious	Moderately Anxious	Highly Anxious	Extremely Anxious	p value	No Fear	Low Fear	Extreme Fear	p value
Gender										
Male	14.7	47.6	23.5	10.5	3.7	0.0001*	67.8	24.5	7.7	0.001
Female	8.0	45.1	27.9	13.8	5.3		58.9	31.0	10.0	
Region										
North Jakarta	10.7	49.0	27.7	10.35	2.3	0.0001*	69.0	24.0	7.0	0.088
Central Jakarta	16.9	48.8	20.4	10.4	3.5		67.7	25.9	6.5	
East Jakarta	6.8	44.9	30.0	13.0	5.3		58.7	31.6	9.7	
West Jakarta	15.1	43.8	21.0	13.6	6.4		62.9	26.0	11.1	
South Jakarta	13.9	49.2	22.8	11.3	2.9		64.9	28.3	6.8	
Thousand Islands	4.5	41.8	37.3	10.9	5.5		62.7	25.5	11.8	
Age of subject										
17–25 years old	8.8	46.7	28.2	11.5	4.8	0.0001*	60.3	30.6	9.2	0.024*
26–35 years old	11.6	46.4	25.3	11.6	5.1		64.0	28.3	7.7	
36–45 years old	15.0	46.2	22.0	13.9	2.9		67.4	23.1	9.5	
46–55 years old	18.4	45.3	20.5	12.1	3.7		71.6	19.5	8.9	
56–65 years old	21.6	51.4	18.9	8.1	0.0		78.4	16.2	5.4	
Education										
Higher	14.2	53.0	19.5	9.4	3.9	0.0001*	70.7	22.2	7.1	0.003*
Secondary	11.2	44.7	28.1	12.6	3.4		62.1	28.2	9.7	
Basic	10.0	42.3	27.0	13.4	7.3		59.3	32.0	8.7	
Income										
No income	7.5	45.1	29.4	13.4	4.6	0.0001*	60.3	29.4	10.2	0.0001*
< Minimum wage	11.5	38.6	27.0	15.7	7.2		55.0	33.0	12.0	
≥ Minimum wage	14.1	51.1	22.6	9.3	2.9		70.1	23.6	6.3	
Insurance										
Yes	10.5	41.3	24.3	18.0	5.9	0.0001*	57.3	31.6	11.1	0.0001*
No	12.3	48.8	26.0	9.2	3.7		66.8	25.5	7.7	
Ever been to dentist										
Yes	9.3	37.9	27.4	16.6	8.8	0.0001*	53. 3	33.1	13.6	0.0001*
No	12.6	49.3	24.8	10.4	2.9		67.3	25.5	7.2	

*p = < 0.05: significant

Table 5. Binary logistic regression analysis with DFS and MDAS

Variable	MDAS		DFS	
	p value	Odds ratio	p value	Odds ratio
Gender	0.05	0.631	0.0001*	0.660
Age	0.253	0.621	0.008*	0.646
Education	0.017*	0.543	0.169	0.843
Income	0.780	1.080	0.507	0.925
Insurance	0.138	1.428	0.001*	1.411
Ever been to dentist	0.0001*	2.854	0.0001*	1.756

*p = < 0.05: significant; Nagelkerke R2 DFS = 0.05; Nagelkerke R2 MDAS = 0.066

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in this study were as follows: high fear in 76 participants (4.2%), moderate fear in 693 participants (38.3%), low fear in 872 participants (48.2%) and no fear in 170 participants (9.4%). Bivariate analysis was performed to establish a relationship between gender, region, age, education, income, insurance and history of dental visits (independent variables) and MDAS and DFS (dependent variables). This analysis involved the use of nonparametric tests (Table 2) and chi-square tests (Tables 3 and 4).

Table 3 shows the nonparametric test results obtained using the Mann-Whitney/Kruskal-Wallis tests and indicates a significant difference in MDAS and DFS scores in terms of all the variables. MDAS and DFS scores were higher for females (10.00[10.86±4.12]; 33.00[35.42±12.24]) than males (9.00[9.85±4.08]; 30.00[33.13±12.06]), and the age group of 17–25-year-olds scored the highest (10.00[10.51±4.09]; 32.00[34.93±12.18]). The scores declined with the subsequent increase in age groups. Furthermore, the education variables show higher MDAS and DFS scores in participants with lower levels of education (10.00[10.98±4.41]; 33.00[34.90±12.08]) and in those with no or low income below the minimum wage (10.00[10.74±3.99]; 33.50[36.64±13.59]). Individuals without insurance and those who had never visited a dentist indicated a higher level of fear and anxiety (11.00[11.50±4.68]; 34.00[37.48±14.43]).

The chi-square test results in Table 4 showed significant differences in the gender variables: females had a higher proportion of moderate to extreme anxiety and fear towards dental care compared to males. This phenomenon is also higher among the age groups of 17–45-year-olds compared to individuals between 46 and 65 years old. This study also identified differences based on education level, income, insurance and history of dental visits.

DISCUSSION

Sociodemographic factors (gender, age, education, etc.) play a role in determining an individual's fear and anxiety towards dental care.[16] The gender variable in this study based on bivariate nonparametric and chi-square analysis of the MDAS and DFS scores had a p value of < 0.05, indicating the presence of statistically significant differences, with a higher mean score for females than males (Tables 4 and 5). This outcome was congruent with the studies conducted by Saatchi et al.[14] and Fayad et al.[7]. Furthermore, physiological conditions in the nature of phobias, panic, stress, depression and fear are also more common in females; hence, there is a possibility that dental anxiety is related to these.

Age is one of the factors commonly reported in various studies; although this study reported a significantly higher level of dental fear and anxiety in adolescents than in adults, this contradicts previous studies that found increased dental anxiety in adults 31–35 years of age and which decreased after 60+ years.[8] Do Nascimento et al.[17] found that the highest scores (found in the age group of 30–39-year-olds) were a result of the relationship between age, an individual's experiences and views and their maturity level.[18] This assumption is supported by the research conducted by Fayad et al.[7]

This study demonstrates that a person's educational status (primary, secondary or higher) affects the level of fear and anxiety because of the significant differences based on the nonparametric and chi-square tests; the results of this study indicate that the higher a person's education, the lower the level of dental fear and anxiety. These findings are consistent with other studies that associated a higher level of education to reduced anxiety related to dental care.[7,17] An individual with a higher level of education is more likely to maintain better oral health and visit the dentist more frequently.[8] This finding is contrary to other studies that reject the assumptions that stipulate the presence of a relationship.[7,14]

An assessment of the socioeconomic factors related to fear and anxiety towards dental care shows less fear among individuals with higher socioeconomic status. This is congruent with a study conducted by Armfield et al.[18] in Australia, although other studies showed different results.[17] The ability to pay for dental care or affiliated insurance premiums is directly related to an individuals' job status, income and wealth.[19] The results obtained from the bivariate analysis with nonparametric tests between MDAS and DFS scores with the coverage value showed a p value of < 0.05, which indicates a statistically significant difference between anxiety and fear between people who have insurance and those who do not; this may be because insurance can lower cost-related stress related to dental treatment. However, there are several types of insurance that have limited coverage, especially in terms of some dental procedures, so this can cause concern. There is a need for a broader level of insurance for someone with a higher level of fear, followed by a demand for more care needs. This study provides the same results as previous studies that reported a relationship between dental fear and individuals who have private insurance.[18]

The regularity of dental visits is considered another important contributor to fear and anxiety for dental care. In this study, the results of the bivariate analysis using nonparametric tests between MDAS and DFS scores showed a p value of < 0.05, which means that the difference is statistically significant between the MDAS and DFS scores of respondents who visited the dentist (MDAS: 9.00[9.91±3.86]; DFS: 30.00[33.07±11.17]) and those who never visited the dentist (MDAS: 11.00[11.50±4.68]; DFS: 34.00[37.48±14.43]). This was in line with the research conducted by Doganer et al.,[20] which showed higher anxiety in participants evading dental treatment than those attending regular appointments. Therefore, patients with fear tend to keep their dental appointments only when necessary (e.g. when they can no longer endure the pain); they also avoid routine dental visits.[20]

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The chi-square test results between MDAS and DFS scores in relation to respondents' visits to the dentist indicated the absence of statistically significant differences ($p = > 0.05$). Furthermore, the results show an absence of any possible influence on fear and anxiety towards dental care due to the higher proportion of respondents that regularly visit the dentist. This outcome was in contrast with the results of Svensson et al.,[8] which demonstrated a significant difference between dental anxiety and the rate of visits.

Based on the results and discussion, the percentages of dental fear and anxiety in the province of Jakarta were 4.2% (high fear), 38.3% (moderate fear), 4.4% (extremely anxious) and 25.5% (moderately anxious). In this study, several sociodemographic factors were confirmed to be related to dental fear and anxiety. In addition, nonparametric test results showed an association between gender, age and income level ($p < 0.05$), while the chi-square results confirmed a correlation between gender and dental anxiety ($p < 0.05$) as well as income level and dental fear ($p < 0.05$). The limitation of this study is that it cannot explain the relationship between sociodemographic factors and dental fear and anxiety, so further longitudinal research is required. However, there are several other factors that are expected to be analysed in future research: the relationship between dental fear and anxiety and respondent behaviours, dental treatment experiences, dentist attitudes and others.

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Research Report

Effect of different final irrigation solutions on push-out bond strength of root canal filling material

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ABSTRACT

Background: *The adhesion of root canal filling material to dentin is one of the crucial factors in determining the success of endodontic treatment. However, the smear layer that forms during instrumentation serves as an interface that impedes the bonding mechanism of the filling material. A proper irrigation solution is required to remove the smear layer and provide a dentin surface that supports the bonding mechanism of the filling material in establishing good adhesion.* ***Purpose:*** *This study aims to evaluate and compare the bond strength of filling material with different final irrigation solutions.* ***Methods:*** *Mandibular premolars were prepared by a crown down, pressure-less technique and divided into three final irrigation groups (2.5% NaOCl, 17% EDTA and 20% citric acid). The root canal of each tooth was obturated using epoxy sealer and gutta-percha. A two-millimetre-thick section of the apical third portion of each group was arranged for the push-out assessment using a universal testing machine in an apical to coronal direction at 1 mm/min crosshead speed.* ***Results:*** *A one-way ANOVA test indicated the difference in push-out bond strength among the groups ($p<0.05$). A post hoc Bonferroni test presented a statistically significant difference in the bond-strength value between the 2.5% NaOCl group compared with the 20% CA group ($p<0.05$).* ***Conclusion:*** *The push-out bond strength of root canal filling material is increased by applying a chelating agent as the final irrigation solution where 20% of CA presents the highest push-out bond strength.*

Keywords: *20% citric acid; 17% EDTA; 2.5% NaOCl; push-out bond strength*

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INTRODUCTION

The bonding ability to dentin is a crucial feature of the root canal filling material. The material, which is frequently used as a root canal filling, is gutta-percha. Nevertheless, gutta-percha must be combined with a root canal sealer since gutta-percha does not adhere to root canal dentin.[1] There are two main concerns regarding material adhesion to root canal dentin. In static situations, the adhesion would prevent fluid percolation between the filling material and the root canal dentin. In dynamic situations, it would prevent dislodgement of the filling material from the root canal during subsequent manipulations, thereby reducing the risk of contamination.[2]

The smear layer serves as an interface between the root canal filling material and the dentin.[3] The removal of the smear layer advances sealer adhesion and affects the bond strength of the AH Plus sealer.[4,5] Smear layer removal increases the contact area and the sealing ability of the sealer, so it produces better adaptation. Smear layer removal enables the sealer tags extension to the dentin tubules, which results in the formation of mechanical locking and efficient micro-retention.[5,6] The sealer contact to the dentin also becomes closer, so it optimises the adhesion due to the formation of chemical bonds.[7]

Ethylenediaminetetraacetic acid (EDTA) is suggested as an irrigation solution because of its nature as a chelating agent. This irrigation solution has the ability to eliminate the inorganic portions of the smear layer. However, EDTA that is used as a single irrigation solution is not effective to eliminate the smear layer entirely.[8] A proteolytic agent, one of which can be sodium hypochlorite (NaOCl), must

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be used to eliminate the organic portion of the smear layer. NaOCl and EDTA irrigation solutions can remove the inorganic portions of the smear layer and expose collagen fibres. Moreover, the collagen fibres serve as a substrate for sealer infiltration and hybrid layer formation.[8,9] A combination of NaOCl with a chelating agent or acidic material is required to eliminate both organic and inorganic portions of the smear layer. Consequently, it has been recommended to apply NaOCl along with EDTA or citric acid for irrigation procedures.[10]

Citric acid has been recommended as an alternative chelating agent. The effectiveness of 10%–50% citric acid in removing inorganic portions of the smear layer has been evaluated.[11] Olivieri et al.[11] reported that citric acid has a more effective smear layer removal effect in the apical and middle third root canals compared to EDTA 17%. Besides, Prado et al.[12] showed that both EDTA and citric acid are more effective in eliminating the smear layer at the apical third with a three-minute application.

The push-out test has been described as one of the most reliable, accurate, effective, and easy methods to measure the bond value between the sealer, dentin, and core material. Likewise, the push-out test can evaluate the bond strength to a low value at various depths of root canal dentin.[8,13] The current study was performed to analyse the push-out bond strength between gutta-percha and epoxy resin sealer to dentin with the final irrigation solution 17% EDTA and 20% citric acid.

MATERIALS AND METHODS

The ethics committee of the Dentistry Faculty, Universitas Lambung Mangkurat No. 023 / KEPKG-FKGULM / EC / I / 2020 approved this research and declared it to be clear from any ethical issues. This study used a post-test only with a control group design. The samples were 21 premolar teeth with the following inclusion criteria: mandibular premolar teeth extracted due to orthodontic treatment, straight and perfectly formed apex, and no root fractures. Teeth with caries, root morphological anomalies, and more than one root canal were excluded.

Root canal treatment was carried out on the selected teeth. The teeth were cut through the cemento-enamel junction with a double-sided, diamond disk (Suzhou Syndent Tools Co., Ltd, Suzhou, China) to leave a 14 mm root section with a working length of 13 mm.[13,14] Preparation of the root canal was performed by a crown down, pressure-less technique with ProTaper hand-use instruments (Dentsply Maillefer, Ballaigues, Switzerland). Preparation was initiated by K-file #10 (Dentsply Maillefer, Ballaigues, Switzerland) throughout 2/3 of the working length. Thereafter, the preparation was carried out with S1 and S2 files according to the working length for widening the 2/3 coronal portion. Furthermore, a 1/3 apical portion preparation was performed with F1, F2, and F3 files according to the working length (according to the manufacturer's instructions).

All of the root canals were irrigated with a 3 ml 2.5% NaOCl solution during instrumentation for each file size up to the F3 file (size 30, 0.09 taper). At the end of instrumentation, the root canals were randomly divided into three final irrigation groups, namely Group I (5 ml 2.5% NaOCl), Group II (5 ml 17% EDTA), and Group III (5 ml 20% citric acid). The irrigation was carried out using a 30 G, close-end, single side, vent needle (OneMed, Sidoarjo, Indonesia) for three minutes.[15,16] Irrigation was done by a manual, dynamic-agitation technique (hand-activated, well-fitting, #F2 gutta-percha (Dentsply Maillefer, Ballaigues, Switzerland)) with push-pull movement 100 times/30 seconds.[17] Thereafter, a paper point was inserted to dry up the root canal.[18]

The obturation was performed by manipulating the sealer (AH Plus, Dentsply, DeTrey GmbH, Konstanz, Germany) and ProTaper #F3 gutta-percha (Dentsply Maillefer, Ballaigues, Switzerland) and used a single-cone technique as stated by the manufacturer's instructions. Then, a plugger (Cerkamed Medical Company, Poland) was heated to cut off the remaining gutta-percha that exceeded the root canal. Furthermore, the obturation was covered with zinc phosphate cement (Elite Cement 100, GC Corporation Tokyo, Japan) and radiographed to ensure a hermetic obturation system. After that, the sample was conditioned in a plastic container that contained moist gauze for the incubation procedure of seven days at 37°C with 100% humidity.[14]

The sample was segmented in the transverse plane, perpendicular to the root canal's long axis using a circular diamond disk (Louyang Penghao Ceramic Technology Co., Ltd, Louyang, China). The apical third of the root was removed with a thickness of 2 mm. The procedure was continued to obtain an apical third sample that would be used in the test. Furthermore, the sectioning was carried out to a thickness of 2 mm and measured with an electronic digital calliper (Mitutoyo, Kawasaki, Japan). The coronal surface of each sample was marked and coded for each group.[13]

The sample was positioned on the surface of a custom-made, cylindrical, resin fixture (20 mm diameter x 7 mm height) with a hole in the middle (2 mm diameter), which would accommodate material dislodgement during the push-out test.[14] The push-out test was conducted with a universal testing machine (TN 20 MD, France) with a 0.53 mm stainless-steel plunger (custom made) that pushed the filling material at a crosshead speed of 1 mm/min in apical-coronal direction.[19] The bond strength was calculated by the following formula:[13]

$$PBS = F / A$$

Where: PBS = push-out bond strength (MPa), F = maximum load (N), A = bonding area of root canal filling (mm^2), calculated by the following formula:[20]

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$$A = \pi (r_1 + r_2) \sqrt{(r_1 - r_2)^2 + h^2}$$

Where: $\pi = 3.14$, r_1 = coronal radius, r_2 = apical radius, h = sample thickness.

The data were processed using IBM SPSS Statistics for Windows version 26.0 (IBM Corp., Armonk, NY, USA) for normality with the Shapiro–Wilk test and homogeneity was carried out with Levene's test. Statistical analysis was conducted with a one-way ANOVA test to compare each group. Furthermore, the data were further tested with a post hoc test using the Bonferroni method to determine the value of comparison between groups (significant level set as $p<0.05$).

RESULTS

The mean, standard deviation, and one-way ANOVA significant value of filling-material bond strength are described in Table 1. The one-way ANOVA test implied that the bond strength value of the filling material was statistically different among final irrigation solution groups ($p<0.05$). According to the summary of the Bonferroni test in Table 2, there was a difference that was statistically significant between the 2.5% NaOCl group compared to the 20% citric acid group ($p<0.05$). Otherwise, the differences between the final irrigation 2.5% NaOCl group compared to the 17% EDTA and between 17% EDTA group compared to 20% citric acid were not statistically significant.

Table 1. One-way ANOVA test result: The push-out bond strength of root canal filling material with final irrigating solution 2.5% NaOCl, 17% EDTA, and 20% citric acid

Final irrigation group	N	Mean ± Standard Deviation	Sig
2.5% NaOCl	7	2.05 ± 0.75 MPa	
17% EDTA	7	2.75 ± 0.60 MPa	0.041*
20% Citric Acid	7	2.98 ± 0.59 MPa	

N: Number of specimens, *: Value shows a significant difference at $p<0.05$.

Table 2. Post hoc test result using the Bonferroni methods: The push-out bond strength of root canal filling material with final irrigating solution 2.5% NaOCl, 17% EDTA, and 20% citric acid

Final irrigation group	2.5% NaOCl	17% EDTA	20% Citric Acid
2.5% NaOCl		0.182	0.048*
17% EDTA			1.000
20% Citric Acid			

*: Value shows a significant difference at $p<0.05$.

DISCUSSION

The push-out test provides information about the material bonding properties and material resistance, and it is intended to assess the degree of material bonding to the dentin. As the push-out bond-strength value of the filling material gets higher, the adhesion of the material also gets better. In endodontics, the push-out test is conducted to study filling material resistance, perforation improvement, post retention, and sealer bonding to dentin.[10,21] Moreover, the push-out test provides better outcomes in assessing the bond strength of intra-canal materials than the conventional shear test method. This is due to the dislodgment of material that occurs parallel to the dentin and thus is more useful in representing the clinical setting.[22]

The current study complies with the research of Alkhudhairy et al.[23] and Rocha et al.,[24] which reported a deleterious effect on the filling material bond strength when 2.5% NaOCl was used as the final irrigation solution. This research confirmed that NaOCl as a single irrigation solution does not effectively eliminate the smear layer. The physicochemical properties of NaOCl only work on the organic portions of the smear layer.[25] An NaOCl irrigation solution can degrade dentin collagen. Consequently, it affects sealer bond strength.[26] AH Plus sealer is chemically bonded with collagen.[27] Collagen is the main component of dentin, which plays a critical role in the bonding between the resin sealer and dentin.[28] The bonding mechanism of the epoxy resin sealer is the arrangement of covalent bonds from the open epoxide ring to the amino group of collagen dentin.[29] Thus, the removal of collagen fibrils from the dentin due to the use of an NaOCl irrigation solution leads to a decrease in the bond strength value of the adhesive system.[28]

The deproteinisation effect of NaOCl makes the amino group of the collagen become unstable and easily dissolve.[30] This produces a less receptive dentin surface, provides weak micromechanical bonds, and decreases the bond strength of the epoxy resin sealer.[27,30] The deproteinisation of dentin that is irrigated by NaOCl leads to hydrophilic surface properties that do not support the spreading of the hydrophobic AH Plus sealer.[31]

The removal of dentin organic matrix (fragmentation between carbon atom bonds and degradation of the primary structure of collagen) potentially restrains the hybrid layer formation. After breaking down long collagen chains, NaOCl also chlorinates protein terminal groups. The presence of chloramine protein results in premature termination of the polymer chain and incomplete resin polymerisation.[27] The release of oxygen from NaOCl may inhibit the polymerisation process and thereby reduce bond strength, especially in the apical third.[23,24] Besides, a negative correlation has been found between the exposure time of NaOCl and the material bond strength.[26]

A combination of NaOCl and the chelating agent has a positive effect on the push-out bond strength of

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the epoxy resin sealer.[6,32] The chelating agent that was used in this study, EDTA 17% (One Med) and 20% citric acid (Biochemistry Laboratory, Universitas Lambung Mangkurat), indicated similar bond-strength values. Filling material that previously was irrigated with 2.5% NaOCl - 17% EDTA and 2.5% NaOCl - 20% citric acid indicated higher bond strength in comparison with the group that was irrigated with 2.5% NaOCl as a single irrigation solution. The current study is in accordance with the research by Berástegui et al.[25] that showed that a higher bond strength was obtained when NaOCl was combined with a chelating agent. However, statistical analysis of the current study is in line with Farag et al.,[13] who conducted a push-out test of filling material with different irrigation solutions. The study reported that the difference of filling material bond strength between the irrigation group with 2.5% NaOCl compared to the irrigation group with 2.5% NaOCl-17% EDTA was not statistically significant.[13]

Alkhudhairy et al.[23] and Gündoğar et al.[33] explained that smear layer removal became more effective when the root canal was irrigated with EDTA solution and provided the higher bond-strength value than irrigation with an NaOCl solution. Irrigation with EDTA showed the higher bond-strength value was caused by its effectiveness in removing the smear layer, demineralising dentin, opening dentin tubules, and increasing dentin surface roughness.[23,34] Therefore, 17% EDTA, which was used as the final irrigation solution, facilitated collagen exposure, increased sealer spreading, and established a dentin substrate more conducive to AH Plus adhesion.[35]

Final irrigation with EDTA shows a higher bond-strength value because EDTA can significantly reduce dentin wetting, thereby resulting in a dentin substrate that has a favourable condition for hydrophobic properties of AH plus.[5,36] A comparative evaluation of the contact angle suggests that the contact angle of the sealer is reduced after irrigation with EDTA 17%.[37] The AH plus sealer shows better surface wetting in the application of EDTA and NaOCl irrigation solutions than using NaOCl irrigation solutions only. This is due to the intimate contact between the dentin surface and the sealer, possibly achieved by adequate smear layer removal, which enhances sealer infiltration into the dentinal tubules.[38]

The effect of EDTA on dentin is determined by its concentration and time of exposure.[39] In the present study, the final irrigation was intended for three minutes as mentioned by Mello et al.,[15] who suggested that root canal irrigation with 5 ml of EDTA for three minutes could eliminate the smear layer effectively. Besides, the dynamic manual agitation technique was also used during irrigation. This technique has been proven to be more capable of removing dentinal debris, the smear layer, and biofilm than the static irrigation technique.[17] Statistical analysis of the current study indicated that the bond strength of the final irrigation group EDTA 17% and the final irrigation group 20% were not significantly different. The current study is in line with Ravikumar et al.[36] who examined the bond strength of filling material with these chelating agents. The study showed that the difference of bond strength among the final irrigation group with EDTA and citric acid was not significant.[36]

Different concentrations of citric acid (1–50%) have been widely used in removing the smear layer.[7] Citric acid with a concentration of 20% was used in the current study because of its biocompatibility and capacity to demineralise the inorganic portion of the smear layer. This was conformable with the study that stated that concentrations of 1–40% citric acid were adequate in eliminating the smear layer, dissolving dentin debris, and demineralising intra-tubular dentin to expose the dentinal tubules. Besides, citric acid with a concentration of 20% does not have any detrimental effect on the dentin surface. Based on this reasoning, the current study was carried out using a concentration of 20% to increase its capacity as a chelating agent.[18] The highest bond strength of the final irrigation group with 20% citric acid can be attributed to the previous study, which showed that the root canals that were irrigated with citric acid showed more effectiveness than the 17% EDTA in eliminating the smear layer at the apical and middle third portion of the root canal. Besides, the application of citric acid with a concentration of 20% also increases the chelating effect.[12,25]

Several factors that determine the effect of the chelating agent are contact time, pH, concentration, and the volume of the solution. Research conducted by Berástegui et al.[25] showed a similar capacity for the smear layer removal between 20% citric acid and 17% EDTA. The concentration of citric acid 20% does not have any damaging effect on the surrounding tissue because it is not highly ionised. Another study that applied citric acid at a concentration of 20% showed that the chelating effect of citric acid became higher with increased concentrations. The application of 20% citric acid as a chelating agent results in an increase in the contact area and covalent bond, thereby resulting in a higher AH Plus sealer bond to the dentin.[25,40]

However, due to its ability to disinfect and dissolve organic tissue, NaOCl irrigation remains an option in contemporary endodontics, although the current study showed the lowest filling material bond strength compared to other groups.[13,41] NaOCl does not remove the smear layer that coats the dentin and occludes the orifice of the dentinal tubules, thereby restricting the sealer penetration into dentinal tubules.[13,41,42] Meanwhile, the use of EDTA or citric acid as final irrigation solution can remove the smear layer and open the dentinal tubules, which facilitates the collagen exposure in intratubular dentin, thereby providing a higher filling material bond strength as the adhesion mechanism of the epoxy resin sealer is an arrangement of covalent bonds by any exposed amino groups in dentin collagen to the open epoxide ring of AH Plus sealer.[29,41]

The bond strength of the epoxy resin sealer is also associated with creep capacity, low shrinkage levels during setting, flow-ability properties, low polymerisation shrinkage, sealer volumetric expansion, and long-term

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dimensional stability.[1,23] Several studies have found diversity on the sealer bond strength values when the samples were examined with different root canal depth-level sections.[13,19,23,29] Nonetheless, the current research used samples in the apical one-third that tended to show the lowest value of bond strength compared to the middle and coronal third.[3] The reduction in root canal diameter, anatomic variation, and vapour lock effect in the apical third interferes with the irrigation solution flow and makes removing the smear layer even more challenging.[12] The decrease in dentinal tubule density, sclerotic dentin, and inhomogeneous hybridisation of dentin in the apical third also reduces the level of material adhesion to dentin.[3,6,23,43] The results of the current study indicate that there are differences in the bond strength of root canal filling material with different final irrigation solutions. The push-out bond strength of root canal filling material is increased by applying a chelating agent as final irrigation. The final irrigation with 20% citric acid shows the highest bond strength value of filling material and implies a significant difference in bond strength compared to 2.5% NaOCl.

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Case Report

Burning mouth syndrome caused by xerostomia secondary to amlodipine

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ABSTRACT

Background: *Xerostomia, generally referred to as dry mouth, has been identified as a side effect of more than 1,800 drugs from more than 80 groups. This condition is frequently unrecognised and untreated but may affect patients' quality of life and cause problems with oral and medical health, including burning mouth syndrome (BMS).* ***Purpose:*** *The purpose of this case is to discuss how to manage a patient with BMS caused by xerostomia secondary to medication that has been taken by the patient.* ***Case:*** *We reported that a 45-year-old male military officer from the Royal Malaysian Air Force came to Kuching Armed Forces Dental Clinic with dry mouth and a burning sensation since he started taking 10 mg of amlodipine due to his hypertension. After a thorough physical and history examination, we made a diagnosis of burning mouth syndrome (BMS) caused by xerostomia secondary to amlodipine.* ***Case Management:*** *Oral hygiene instructions, diet advice and prescription of Oral7 mouthwash has been given to reduce the symptoms of BMS. The patient has been referred to the general practitioner to reduce his amlodipine dosage from 10 mg to 5 mg (OD) in order to prevent xerostomia, and oral hygiene instructions have been given. A review after two weeks showed significant changes in the oral cavity, and the patient was satisfied as he is no longer feeling the burning sensation and can enjoy his food without feeling difficulty in chewing and swallowing.* ***Conclusion:*** *Adverse drug events are normal in the oral cavity and may have a number of clinical presentations such as xerostomia. Xerostomia can cause many implications as saliva helps in maintaining oral mucosa and has a protective function. The signs of adverse drug incidents in the oral cavity should be identified to oral health care professionals.*

Keywords: *amlodipine; burning; dry mouth; military officer; xerostomia*

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INTRODUCTION

Burning mouth syndrome (BMS) is a benign disorder that presents as a burning sensation in the absence of any apparent findings in the mouth and in irregular blood tests. The underlying BMS aetiology remains unclear and is typically characterised by a feeling of burning, itching, or tingling, preferentially at the tip and sides of the tongue, lips, and anterior palate.[1] The manifestations of BMS are usually bilateral but in some cases may prove unilateral.[2] According to Cerchiari et al.,[3] BMS can be classified according to the associated risk factors: idiopathic, psychogenic, local, and systemic. Psychopathological processes such as anxiety, depression, and certain phobias are among the psychogenic risk factors. In essence, local causes include infectious processes, allergic reactions, and irritative phenomena, while the systemic aetiological factors include salivary secretion changes, endocrine disorders, neurological changes, dietary factors, and drug substance.[2]

It is estimated that more than 400 drugs affect the salivary gland function and contribute to hyposalivation.[4,5] Drugs with anticholinergic activity, including antihypertensive drugs, can cause hyposalivation by reducing the acetylcholine released by the parasympathetic nerves.[6,7] Amlodipine, which is an antihypertensive agent that acts as a calcium channel blocker, can cause xerostomia through the muscarinic M3 receptor, which results in reduced salivary flow.[8,9] Diagnosis is based on the history and chronology of oral adverse reactions that are usually identified within weeks or months of the drug being administered.

Xerostomia is a concomitant symptom in patients with burning mouth syndrome, with prevalence varying between

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34 and 39%.[8,9] Saliva offers a protective function as well as an antimicrobial, buffering, and lubricating feature to help cleanse and eliminate food debris within the mouth. Patients will begin to develop oral problems before experiencing dryness, such as a burning mouth sensation, when the protective environment created by saliva is altered. The purpose of this case is to discuss how to manage a patient with BMS caused by xerostomia secondary to medication that has been taken by the patient.

CASE

A 45-year-old male military officer from the Royal Malaysian Air Force came to Kuching Armed Forces Dental Clinic with complaints of a burning sensation and dryness throughout his mouth for the past two months. The patient claimed that he feels hot and sharp and has been on a cold food diet as he cannot swallow any spicy or hot food.

A clinical examination was performed to rule out any pre-existing pathology other than dry mouth. The patient is fit, conscious and has no other underlying medical diseases besides hypertension. The patient admitted feeling the changes in his mouth since taking anti-hypertensive medication, which was 10 mg of amlodipine per day given by the general practitioner in the last two months. Further inquiry into the patient's social history revealed no tobacco or alcohol use. The patient is happily married with three children, and after a deeper investigation, the patient claimed that he feels neither stress nor any psychological disturbances related to his daily life. However, he had not been able to eat well during the past two months due to the burning sensation he was having, and since then he had started to drink lots of cold water and apply lip balm on his cracked lips (Figure 1).

Figure 1. Photograph showing the patient's cracked and peeled lips.

An intraoral examination demonstrated that both the buccal and palatal sides of the mouth were reddish, dry, and inflamed, while the tongue appeared scalded and burnt (Figure 2). The patient is not wearing any dentures and has relatively good oral hygiene, with no teeth restored.

Subsequently, the patient was referred to a medical for a full blood count (FBC) with differential analysis to check for any possible relationship with diabetes or Sjögren's Syndrome and deficiencies of iron, folate, zinc, and group B vitamins. The results came out that the patient was fit and well without any abnormality or deficiencies. Besides, the swab test showed the absence of candidiasis infection, although there is a severe decrease in unstimulated salivary flow rate (0.1 mL per minute) and stimulated salivary flow rate (0.5 mL per minute) according to the spit test that was done. The Challacombe scale of clinical oral dryness shows a score of 6, which indicates moderate dryness of the patient's mouth. Based on the overall clinical and laboratory findings, the differential diagnosis of this patient is BMS secondary to xerostomia due to amlodipine.

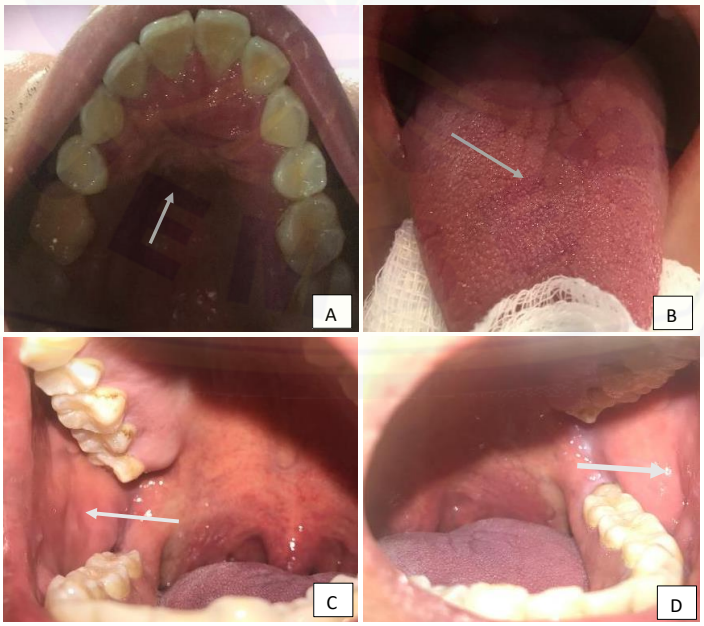

Figure 2. Pre-treatment photographs showing dry and inflamed palatal side (A), scalded tongue (B), and inflammation of both sides of the buccal region (C and D).

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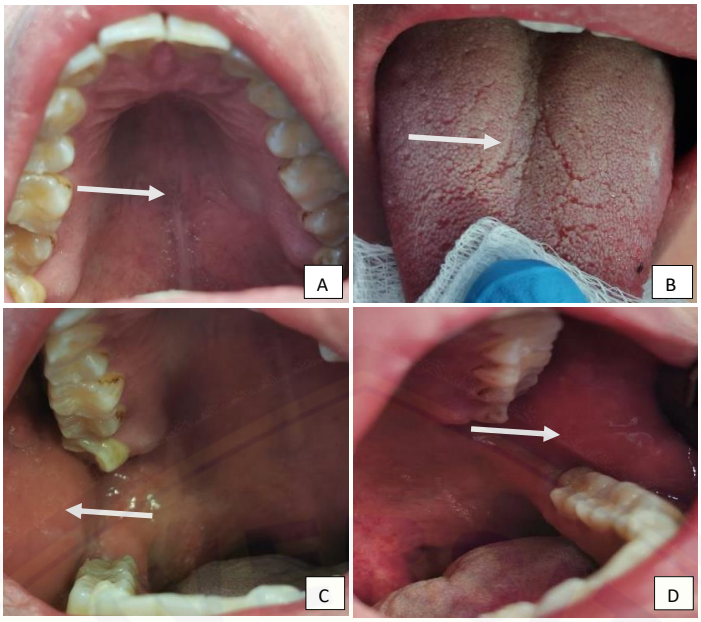

Figure 3. Two weeks later, follow-up treatment shows improved mucosa appearance, which is not dry or sticky (A, C, and D) and includes the non-scalded appearance of the tongue (B).

CASE MANAGEMENT

The patient was referred to the general practitioner for advice about lowering his dose of 10 mg of amlodipine or replacing it with other types of antihypertensive drug that can reduce xerostomia leading to burning mouth syndrome. Besides prescribing a hydrating mouthwash, oral hygiene advice has been given to the patient, including on how to relieve his burning and dry mouth symptoms, such as frequent sucking on ice cubes, increasing the intake of water and watery fruits, avoiding hot and spicy food, and chewing gum.

A follow-up appointment was given 14 days after the initial treatment. The patient claimed absence of burning sensation and dry mouth and said he can enjoy his food well without feeling difficulty in swallowing. Intraoral examination revealed an improved mucosa appearance without dry and sticky appearance or scalded appearance of the tongue (Figure 3). The dosage of 10 mg of amlodipine has been reduced to 5 mg, which has less effect in drying the mouth.

DISCUSSION

This clinical report described the BMS caused by xerostomia secondary to amlodipine. Initial management focused on eliminating the cause of xerostomia in order to treat the burning mouth syndrome. After establishing a diagnosis, a step-wise management approach should be implemented. This includes alleviating symptoms, the implementation of preventive measures, the treatment of oral diseases, the enhancement of salivary function, and the management of any underlying systemic condition.[10] Initial treatments of this case include changing or lowering the dose of 10 mg of amlodipine by referring the patient to the general practitioner. The dry mouth side effects of medications may be alleviated or reduced by substituting the problem medications with similar drugs that have lesser side effects.[10] In addition, alteration in the timing or dosing schedule of medication doses at night-time, when salivary flow is usually at its lowest, can maximise the dry mouth effects.

The diagnosis of burning mouth syndrome caused by dry mouth is based on clinical findings, with the characteristic of burning and itching sensation located bilaterally in the mouth, including the palate and tongue, with the absence of any oral mucosal pathology.[11] According to Millsop et al.,[12] patients with dry mouth have clinical manifestations of difficulty in swallowing, chewing, and/or speaking and present with burning mouth, halitosis, dry buccal mucosa, cracked and peeled lips, and oral candidiasis. This patient encountered burning mouth sensation together with dry mouth, difficulty in swallowing, and cracked lips, without any presence of other oral pathology, after taking an antihypertensive drug, and he was diagnosed with burning mouth syndrome caused by dry mouth secondary to amlodipine.

Persistent hyposalivation leading to dry mouth can leads to infections, such as candidiasis and dental caries, as well as bacterial sialadenitis.[13] Lubrication loss can also result in erythema and mucosal susceptibility to frictional damage to the teeth, causing the patient to feel discomfort.[14] The more commonly used agents for dry mouth can be categorised into chewing gums, salivary stimulants, and saliva substitutes.[12] Chewing gums or candies should be sugar-free to prevent dental caries. The patient has been prescribed with a hydrating mouthwash to minimise the dry

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mouth effect as well as to stimulate the production of saliva, which helps to eliminate the burning sensation. Saliva substitutes that commonly contain carboxymethylcellulose, xanthan gum, mucins, hydroxyethylcellulose, polyethylene oxide, or linseed oil may help to increase salivary viscosity by resembling natural saliva.[15]

Very clearly, amlodipine can cause dry mouth, leading to burning mouth syndrome. Antihypertensive agents are the drugs most often associated with the appearance of symptoms compatible with burning mouth syndrome because they can act upon the angiotensin-renin system.[2] Salivary secretion depends on parasympathetic and sympathetic signalling, and parasympathetic activation leads to increased Ca^{2+} release and water fluxing out of the cell.[16] Thus, taking a calcium channel blocker such as amlodipine, which acts as a Ca^{2+} antagonist, may cause dry mouth by inhibiting the voltage-dependent Ca^{2+} channels that are activated by depolarisation to cause resting salivation.[16] Of the worldwide cases of medication-induced BMS, 33% were known to be dose-dependent phenomena, as the burning sensation only occurred when the medication dose was increased in pursuit of therapeutic efficacy.[2]

BMS can be managed easily with adequate diagnosis and treatment planning, or it can give discomfort to the patient psychologically; in the worst case scenario, other oral diseases might arise, such as dental caries and fungal infections. The first phase is an acute treatment to prevent the progression of burning mouth syndrome and to provide pain relief. The second phase is the amelioration of pre-existing conditions such as dry mouth, where symptoms would be improved after the initial acute phase. This included intense oral hygiene instruction and control of systemic factors such as smoking, diet, and stress. The alteration of medication that causes the burning mouth syndrome comes in the third phase and is considered only if it is confirmed to be the cause of BMS. The fourth and final treatment phase proceeds through supportive therapy to maintain oral hygiene and control of systemic and local factors.[15]

The patient discussed in this report went through the first and second phases of treatment, where we managed to treat the acute phase, and the patient was compliant with the treatment. For the third phase, of altering the patient's medication, the general practitioner has been consulted, and the patient is under review every three months as part of oral condition maintenance. In this case, as discussed in this report, a proper diagnosis was followed by improvement of the patient's condition.

Xerostomia or dry mouth is the result of reduced or absent salivary flow. It may occur as a result of ongoing drug use and happen concurrently with BMS. There are over 500 widely used medications, including various antidepressants, antipsychotics, antihistamines, diuretics, and sedatives, which list xerostomia as a side effect. Temporary relief options include oral mouthwashes, gels and sprays, lozenges, and change of dietary habit; however, more prolonged treatment options will be medication such as pilocarpine or saliva substitutes with longer mucosal surface retention.

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Case Report

Orthodontic camouflage treatment using a passive self-ligating system in skeletal Class III malocclusion

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ABSTRACT

Background: *The treatment options for adults with skeletal Class III malocclusion can be dentoalveolar compensation, also known as orthodontic camouflage, or orthognathic surgery. Camouflage treatment can be carried out with teeth extractions, distalisation of the mandibular dentition, and use of Class III intermaxillary elastics. However, intermaxillary elastics as anchorage has its own risk–benefit.* ***Purpose:*** *To explain that camouflage treatment with teeth extractions can be performed in a mild to moderate skeletal Class III malocclusion using intermaxillary anchorage with elastics, while minimising the deleterious effects and achieving a satisfactory treatment outcome.* ***Case:*** *Our patient was a 25-year-old female who had a skeletal Class III pattern, with normal maxilla and a protruded mandible. She had a straight facial profile with a Class III canine and molar relationship on her right and left sides. Anterior crossbite was also present with crowding on both the maxilla and the mandible.* ***Case Management:*** *The treatment plan was carried out with dentoalveolar compensation by extracting teeth. Extraction of the lower first premolars was conducted to eliminate the crowding and correct the anterior crossbite. The mandibular incisors were retroclined and the maxillary incisors were proclined with dentoalveolar compensation. Passive self-ligating system was used with standard torque prescription, intermaxillary anchorage, and no additional appliances for anchorage control. Class I canine and incisor relationship were both achieved at the end of the treatment, while maintaining the Class III molar relationship.* ***Conclusion:*** *Orthodontic camouflage treatment in an adult patient using a passive self-ligating system and intermaxillary anchorage can improve facial profile and improve dental occlusion.*

Keywords: *Class III malocclusion; orthodontic camouflage treatment; passive self-ligating system*

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INTRODUCTION

Edward Angle described Class III malocclusion as a lower molar which is mesially positioned relative to an upper molar, with no specification of the line of occlusion. Nonetheless, Class III malocclusion can be constituted from skeletal and dental irregularities. Skeletal Class III malocclusion can be a result of maxilla deficiency, mandible excessiveness , or a combination of both. Dental features include retroclined mandibular incisors, proclined maxillary incisors, edge-to-edge incisor relationship and negative overjet.[1,2] Studies have showed that the prevalence of Class III malocclusion affects a great variety of different populations. It has been documented that there is a greater prevalence of it in Asian races compared to other races.[2–4]

The treatment of choice for skeletal Class III malocclusion in adult patients often requires a combination of orthodontic and surgical procedures. However, with camouflage treatment it is also possible to correct skeletal Class III malocclusion, depending on the level of severity. Adult patients who have a mild to moderate skeletal Class III malocclusion and a fairly good facial profile can be treated with camouflage treatment. Camouflage treatment can be conducted by extracting teeth, distalising the mandibular dentition, and using Class III intermaxillary elastics. Strategies in skeletal Class III malocclusion camouflage treatment are to procline the upper incisors and retrocline the lower incisors. Acceptable occlusion, function, and facial aesthetics with dentoalveolar compensation are the objectives from camouflage treatment.[5–8]

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Intermaxillary elastics have been used as intermaxillary anchorage and are available in many sizes and strengths.[9] However, Class III intermaxillary elastics can promote extrusion of upper molars, proclination of upper incisors, distal tipping of lower molars, and extrusion of lower incisors.[9,10] In some studies, a combination of skeletal anchorage and intermaxillary elastics were used to minimise the unwanted effects of intermaxillary elastics alone.[11,12] This case report demonstrates a camouflage treatment in an adult patient with skeletal Class III malocclusion by the extraction of lower first premolars with the use of intermaxillary elastics and no additional appliances.

CASE

The patient was a 25-year-old woman, who came to the orthodontic clinic at the Faculty of Dentistry in the Universitas Indonesia Dental and Oral Hospital. She was concerned about her crowded and crossbite of the anterior teeth; hence, she did not feel confident when smiling. The photographs taken before treatment showed a symmetric face and a dolichofacial appearance. Her facial profile was straight and her lips were competent (Figure 1). The intraoral examination showed anterior crossbite with –3 mm overjet, +4 mm overbite, single posterior crossbite of the upper right second premolar, and Class III canine and molar relationships. The degree of crowding on her maxilla was mild, while on the mandible it was moderate. There was no deviation on her maxillary dental midline with her facial axis, but there was a deviation in the mandibular dental midline by as much as 1mm to the right. There was premature contact on the upper right first incisor with lower right first incisor causing functional displacement to the anterior when closing the jaw. Her oral hygiene and periodontal tissues were good, and all teeth were present (Figure 2).

The lateral cephalometric analysis revealed a pattern of skeletal Class III malocclusion with normal maxilla and prognathic mandible, concave skeletal profile, proclined maxillary incisors, and a normal interincisal angle. A panoramic radiograph showed impacted maxillary third-molars and partially erupted mandibular third-molars (Figure 3).

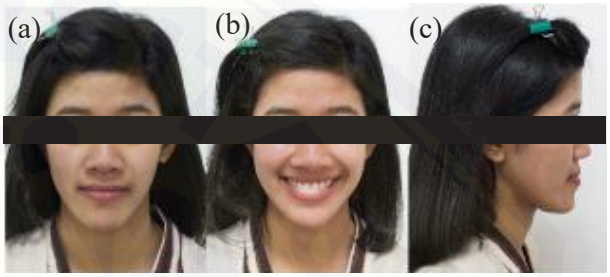

Figure 1. Pre-treatment extraoral photographs. Facial photos of (a) frontal view at rest, (b) during smiling, and (c) lateral view.

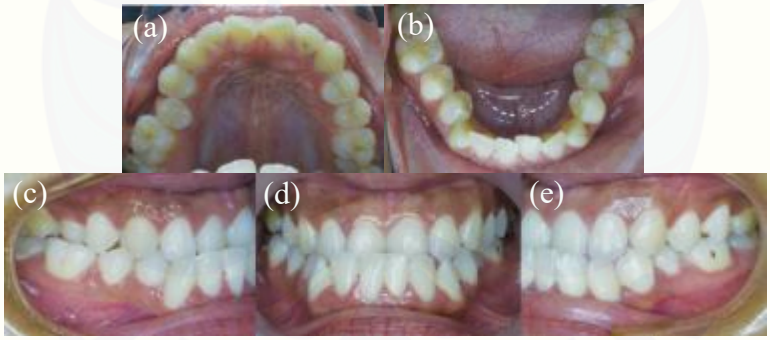

Figure 2. Pre-treatment intraoral photographs. Intraoral view of (a) upper occlusal, (b) lower occlusal, (c) right lateral, (d) frontal, and (e) left lateral.

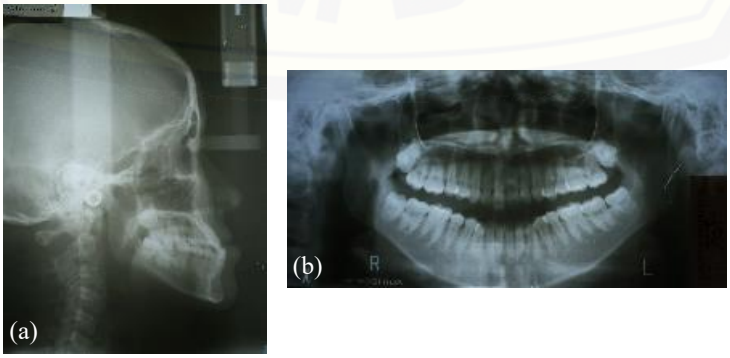

Figure 3. Initial (a) lateral cephalometric and (b) panoramic radiographs.

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According to the patient, she knew of no relatives in her family that had skeletal Class III malocclusion features. Meanwhile, the etiologic possibility for the single posterior crossbite of the upper right second premolar could be the retention of the upper right second deciduous molar.

The treatment objectives were to improve the occlusion, including correction of the anterior and posterior crossbites and to achieve ideal overjet and overbite. The ideal treatment for skeletal malocclusion was a combination of orthodontic and surgical procedures to improve the facial profile. However, as the patient refused to have surgery, she chose to have the camouflage treatment, involving the extraction of the lower first premolars with fixed orthodontic appliances.

CASE MANAGEMENT

Clinicians should be able to make a proper diagnosis and establish realistic treatment objectives with the patient, in order to prevent undesirable outcomes when performing a camouflage treatment in a mild to moderate skeletal Class III malocclusion. It has been suggested that changes in the three aspects, such as skeletal, dental, and soft tissue, can be successfully camouflaged without damaging the periodontal tissue.[7]

In this case, the camouflage treatment was conducted by extracting lower first premolars. The patient had a well-formed maxillary arch with mild crowding, while the mandibular arch was prognathic with moderate anterior crowding. By extracting the lower first premolars, the extraction space was used to relieve crowding and retract the lower incisors. The lower first premolars were extracted before bracket bonding. A Damon Q passive self-ligating system (0.022x0.028-inch slot; Ormco, Glendora, California) with standard torque prescription was bonded on the upper and lower teeth. Bite raisers were used on the mandibular posterior teeth and the patient was given an instruction to use the early Class III elastics (2 oz, 5/16-inch Ormco). Open coil springs were used between upper lateral incisors and canines to protract the upper incisors. Power chain and Class III elastics were simultaneously used for retracting the mandibular anterior teeth. After six months of treatment, the overjet became positive and crowding was resolved. As we progressed to 0.018x0.025-inch copper-nickel-titanium archwire, we inverted the brackets of the four upper incisors, so that the upper incisors with labial root torque were inclined labio lingually. Aligning and levelling with sequential copper-nickel-titanium archwires was achieved in 12 months. Then, 0.019x0.025-inch stainless steel archwires were put into the upper and lower arches and elastics were also constructed to be used for improving interdigitation and detailing occlusion. After 22 months of treatment, the brackets and molar tubes were debonded and vacuum-formed Essix retainers were used for stability on both upper and lower arches.

A straighter soft tissue profile and a pleasant smile were obtained at the end of the treatment (Figure 4). An ideal overjet and overbite were also attained with a Class I canine relationship, while the molars are maintained in Class III relationship. Crowding on both arches were relieved and the crossbite on the second upper premolar was also corrected (Figure 5).

After 20 months of treatment, a lateral cephalometric radiograph showed changes in skeletal, dental, and soft tissue parameters (Figure 6). Analysis from lateral cephalometric radiograph were shown in Table 1. The ANB angle showed improvement from –2° to 0° and the angle of convexity was also improved from –5° to 0°, while the lower facial height was maintained. Dental parameters

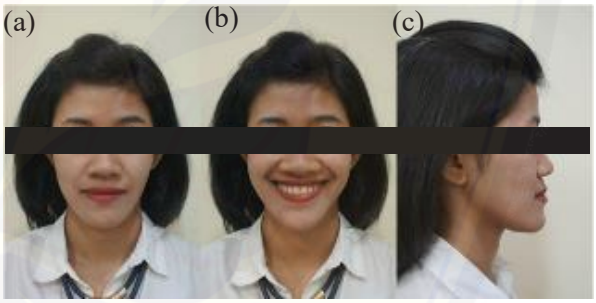

Figure 4. Post-treatment extraoral photographs. Facial photos of (a) frontal view at rest, (b) during smiling, and (c) lateral view.

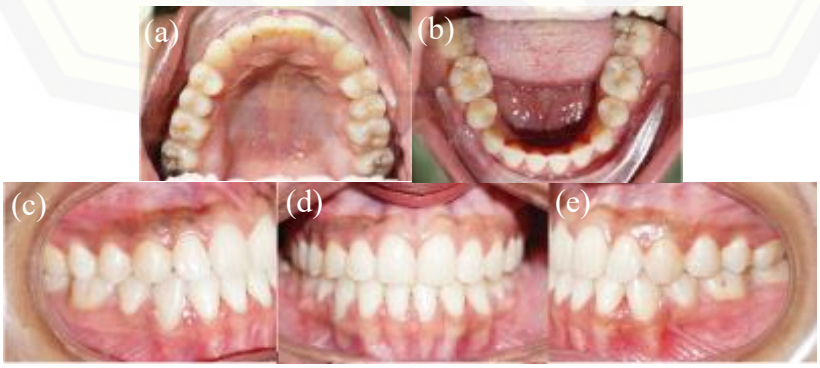

Figure 5. Post-treatment intraoral photographs. Intraoral view of (a) upper occlusal, (b) lower occlusal, (c) right lateral, (d) frontal, and (e) left lateral.

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showed that the upper incisor to maxillary plane angle and lower incisor to mandibular plane angle decreased from 120° to 115° and 86° to 81°, respectively. Soft tissue parameters showed that the positions of the upper and lower lip positions were also improved and confirmed in the lateral cephalometrics superimposition (Figure 7).

DISCUSSION

In this case, Class III molar relationship was maintained with a Class I canine relationship and an ideal overjet and overbite. This type of occlusion is also known as therapeutic Class III occlusion.[13] Previous study found that good occlusal stability and periodontal health were observed in patients with Class III molar relationship after 13–14 years of treatment.[14] The alternative treatment by extraction of maxillary second premolars and mesialisation of the first molars to achieve a Class I molar relationship could risk to depress the face, as the patient already had a fairly straight profile.[15]

The patient's facial profile was improved as there were several skeletal and dental changes that affected the position of the upper and lower lip. Increase in ANB angle and the angle of convexity might be attributed to the protraction of the upper incisors and also the retraction of the lower incisors (Table 1). The inclination of upper incisors were initially proclined as a common feature of dental compensation in skeletal Class III malocclusion, while the lower incisors have normal inclination. It has been suggested that using Class III elastics can cause some unwanted tooth movements. Therefore, we used and prescribed the patient with light-force elastics and a bigger wire in the maxillary arch, so that the whole maxillary arch became an anchorage for mandibular anterior teeth retraction and also to minimise any unwanted effects. Light force in Class III elastics was also used to prevent the maxillary posterior teeth from extruding, as this can cause

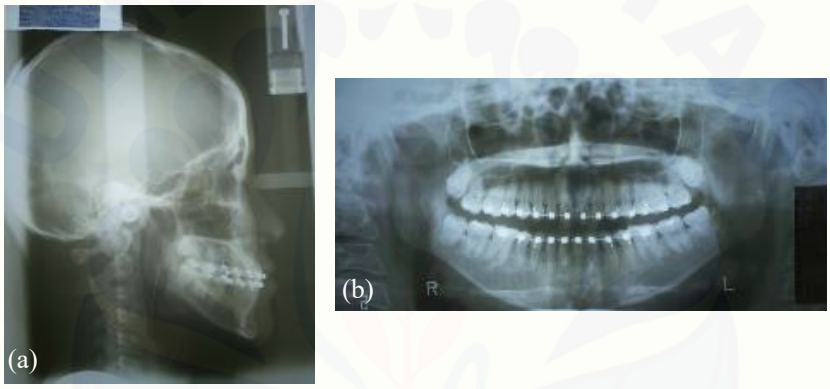

Figure 6. Pre-debonding (a) lateral cephalometric and (b) panoramic radiographs.

Table 1. Comparison of skeletal, dental, and soft tissue values of pre- and post-treatment lateral cephalometric radiographs

Measurement	Mean	SD	Pre-treatment	Post-treatment
Horizontal Skeletal				
SNA (°)	82	2	84	84
SNB (°)	80	2	86	84
ANB (°)	3	2	–2	0
The Wits (mm)	1	2	–12	–5
Angle of convexity (°)	0	10	–5	0
Vertical Skeletal				
Y-axis (°)	60	6	59	58
Go-angle (°)	123	7	134	134
SN-mandibular plane (°)	32	3	32	32
MMPA (°)	27	4	26	26
LAFH (%)	55	2	55	55
Anterior Dental				
Interincisal angle (°)	135	10	130	135
U1-palatal plane (°)	109	6	120	115
L1-mandibular plane (°)	90	4	86	84
Soft Tissue				
Upper lip –E Line (mm)	1	2	–6	–5
Lower lip – E Line (mm)	0	2	1	0

Figure 7. Superimposition of lateral cephalometrics on before (black) and after treatment (red). Note there was changes in maxillary and mandibular incisor angulation, and also in the lip position.

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the mandible to rotate backwards, therefore increasing the lower facial height.[16]

We also inverted the maxillary incisor bracket position to prevent more proclination on maxillary incisor teeth. The torque value for Damon Q brackets with standard prescription are +15° on the upper central incisors and +6° on the upper lateral incisors. When the brackets were inverted, the torque values were changed to –15° and –6° for the upper central and lateral incisors, respectively. Therefore, the upper incisors had a higher labial root torque placed on them. The same effects were also obtained in previous study with Damon 3 brackets by inverting the bracket position.[17]

The inclination of lower incisors was retroclined by the end of the treatment. A meta-analysis study found that self-ligating brackets could promote inclination of incisors become 1.5° less than the conventional brackets.[18] Another study also reported acceptable facial aesthetics and good dental occlusion when camouflaging skeletal Class III malocclusion with a passive self-ligating system without the use of auxiliary appliances.[19]

Retention is needed after an active phase of orthodontic treatment because there is tendency to relapse. A vacuum-formed retainer was used for this patient to maintain the tooth alignment and arch width stability. A previous study suggested that vacuum-formed retainers were more effective than the Hawley retainer at holding the incisors in alignment.[20] However, a recent systematic review also suggested that there are no differences between the Hawley retainer and the vacuum-formed retainer in terms of cost, time, maintaining the arch width, occlusal contacts, and patient satisfaction.[21] There is also limited evidence that suggests fixed retainers are better than vacuum-formed retainers. Further studies are needed to make some recommendations about retention after orthodontic treatment.[22] In conclusion, orthodontic camouflage treatment with passive self-ligating and intermaxillary anchorage can improve facial profile and dental occlusion.

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Research Report

Acceleration of post-tooth extraction socket healing after continuous aerobic and anaerobic physical exercise in Wistar rats (*Rattus norvegicus*)

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ABSTRACT

Background: *Physical exercise has been proven to accelerate wound healing. Physical training itself consists of aerobic (continuous training) and anaerobic (interval training) exercise. The effectiveness of continuous physical exercise on post-tooth extraction wound healing is the focus of this study.* ***Purpose:*** *This study aims to investigate the differences in post-tooth extraction wound healing in Wistar rats (Rattus norvegicus) after aerobic and anaerobic exercise based on the number of fibroblasts and neovascularisation.* ***Methods:*** *Wistar rats were divided into three groups: the control group (K1); K2 undertook continuous aerobic exercise, swimming at 50% maximum swimming capacity (MSC) with an additional 3% bodyweight load; K3 undertook anaerobic continuous exercise, swimming at 65% MSC with a 6% load. The rats swam three times per week for six weeks. The number of fibroblasts and neovascularisation were examined three days after tooth extraction. Data was analysed using the one-way analysis of variance (ANOVA) and Least Significant Difference (LSD) tests ($p<0.05$).* ***Results:*** *There was a significant difference in the number of fibroblasts between the K2 and K3 groups. There was no significant difference between K2 and K3 in the amount of neovascularisation.* ***Conclusion:*** *There were differences in the number of fibroblasts but not neovascularisation after tooth extraction in Wistar rats given aerobic and anaerobic continuous training.*

Keywords: *continuous aerobic physical exercise; continuous anaerobic physical exercise; fibroblasts; neovascularisation; post-tooth extraction wound healing*

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INTRODUCTION

Tooth extraction is an irreversible surgical procedure that sometimes causes complications if it is not handled properly.[1] Dental extraction complications can be divided into intraoperative complications, complications shortly after extraction and complications a long time after extraction. These occur because of microorganism infections, trauma, drugs or smoking. There have been efforts to reduce complications and accelerate wound healing using antibiotics, antifibrinolytics and topical drug applications.[2] However, the administration of drugs can cause further problems, such as allergies, resistance and systemic complications. Antibiotics can be used as life-saving drugs and treatments to prevent infection, but they are also a predisposing factor for superinfection due to resistance and toxicity.[3,4] The effect of antibiotic resistance is called the Eagle effect, which increases the minimum bactericidal concentration (MBC) of antibiotics so that the dose needed to kill the bacteria must be increased.[5]

Efforts to improve wound healing with drugs are considered to have shortcomings, so an alternative form of therapy with exercise has been studied, but this is still not the primary choice. Regular physical exercise produces positive health effects, such as reducing various cardiovascular diseases, disorders of metabolic syndrome and osteoporosis.[6] It can supply nutrients and oxygen, which can accelerate the wound healing process, and it can

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increase the secretion of healing factors to help the wound healing process.[7]

Previous studies have shown that physical exercise or sport is proven to heal wounds significantly in people who participate compared to those who do not.[8] One study explored the effect of continuous moderate-intensity physical exercise on the number of fibroblasts and neovascular disease in Wistar rat extract scars. The study found that participating in exercise increased the number of fibroblasts and neovascularisation in tooth extraction scars.[9] Physical exercise even accelerated wound healing in the inflammatory phase after tooth extraction. The study showed that physical exercise is one of the factors that can accelerate the wound healing process.[10] The proliferation phase occurs at the end of the inflammatory phase for up to 14–21 days after the injury. This phase's main objective is to repair injured tissue by fibroplasia, which includes wound closure, angiogenesis, re-epithelialisation and fibroplasia. Fibroblasts play a huge role in the repair process responsible for product preparation and protein structure that is used during the tissue reconstruction process.[11]

The response made by fibroblast cells in fibroplasia is proliferation, migration, formation of the extracellular matrix and wound contraction; angiogenesis or neovascularisation also occur in this stage. Angiogenesis is the process of forming new capillaries in a wound, and it is significant in the proliferation stage of the wound healing process because it generates new blood vessels to access nutrients, oxygen and other components in the wound healing process.[12]

At this stage, extracellular matrix biosynthesis occurs, which is the temporary matrix formed mainly from fibrin and fibronectin tissue and replaced by collagen matrix enriched in proteoglycans, glycosaminoglycans and non-collagen glycoproteins, which, in turn, causes the restoration of proper tissue structure and function. Cells that play an essential role in ECM biosynthesis are fibroblasts that secrete extracellular matrix products and components and the formation of granulation tissue. First, components and growth factors previously secreted by macrophages will stimulate the formation of a scaffold in the form of a granulation tissue matrix, such as collagenase, fibronectin and extracellular matrix components. The scaffold formed by these components will function as a facility for the collagen fibrogenesis process. Furthermore, within two weeks after injury, collagen increases and fibroblasts decrease due to apoptosis.[13] Based on the above explanation, this study aims to investigate the differences in the effectiveness of continuous aerobic and anaerobic exercise on the acceleration of wound healing after tooth extraction in Wistar rats (*Rattus norvegicus*) by observing the number of fibroblasts and neovascularisation.

MATERIALS AND METHODS

This research was conducted after obtaining an ethical eligibility certification issued by the research ethics commission under 190/HRECC.FODM/IV/2019. This study involves a post-test only control group with *in vivo* true experimental design. Thirty Wistar rats (*R. norvegicus*) were used with the criteria being 8–12 weeks-old, male and 250–300 grams in body weight. The animals were kept in a plastic cage during the day with enough air and light for acclimatisation. The cages were divided by groups and labelled to distinguish each cage. After the acclimatisation process, each rat's body weight was measured to determine the load given.

The rats were divided into three groups: the control group (K1) did not perform any physical activity; K2 participated in continuous aerobic exercise (swimming) with an additional load of 3% of the rats' body weight; K3 participated in continuous anaerobic exercise (swimming) with an additional load of 6%. The load was in the form of a paper clip tied to a rope one-third of the way from the base of the rat's tail. The control group was not given a load. After being given a load, the rats' maximum swimming capacity (MSC) was calculated to distinguish between the types of physical activity in each treatment group. This was obtained using the rats' weights and the maximum time they could swim for (until they started to sink, marked by the emergence of air bubbles) or when they stopped swimming. The MSC calculation was not carried out for the control group because the rats were placed in containers of water that came up to their feet so that the body temperatures of the control group and treatment groups were equalised. K2 swam at 50% MSC with a load of 3% body weight, and K3 swam at 65% MSC with an additional load of 6% body weight.[14,15]

All three groups were treated three times a week for six weeks. In the seventh week, each rat was anaesthetised with a ketamine injection to extract the mandibular left incisor. This was performed with modified pliers then irrigated with distilled water to remove debris and the remnants of the extraction. On the third day after the extraction, euthanasia was carried out on all rats so their mandibles could be removed. Histology tissue was taken to check the number of fibroblasts and neovascularisation in the post-tooth extraction socket.[10]

The tissue was extracted using a microtome with a thickness of 4–6 µm then it was attached to a glass object. Xylol was used for deparaffination and 90% alcohol for rehydration. Hematoxylin-eosin (HE) staining (Merck Chemical, Darmstadt, Germany) was used, which, in acidic conditions, will attract alkaline substances/solutions so they will turn blue, and the cytoplasm is alkaline, which will attract acidic substances/solutions so they turn red. To colour the nucleus and cytoplasm, 0.6% HCl was stained for differentiation using 0.5% lithium carbonate to give the nucleus a blue colour, and eosin stain was used to give the cytoplasm a red colour. The process ended with dehydration and mounting. Preparations were read by counting the number of fibroblast cells and neovascularisation in five fields using a light microscope with 400x magnification. There were three observers.[15]

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After histological readings to find the number of fibroblasts and neovascularisation, data was collected for statistical tests using Statistical Package of Social Science (SPSS) version 16.0 for Windows (IBM, New York, USA) with normality and homogeneity tests conducted in advance to determine the different tests performed. The normality test was conducted using the Shapiro-Wilk's test and Levene's test was used to test homogeneity ($p>0.05$); subsequently, a parametric comparison test was performed with one-way ANOVA along with a non-parametric comparison using the Kruskal-Wallis test ($p<0.05$). The LSD posthoc test was used to analyse the difference between groups after the ANOVA test was performed ($p<0.05$).

RESULTS

The observation of fibroblast cells in the mandibular incisor socket of the Wistar rat can be seen in Figure 1. The average results of the number of fibroblasts in the wound healing process after the extraction of the Wistar rat tooth and the results of normality and homogeneity tests are available in Table 1.

Table 1. The mean, normality test and homogeneity test of the number of fibroblasts in each group

Groups	Mean ± SD	Normality test	Homogeneity test
K1	15.00 ± 0.8165	0.144*	
K2	17.86 ± 1.9518	0.200*	0.101*
K3	20.71 ± 3.3523	0.119*	

*significant at p>0.05

The highest mean value of the number of fibroblasts was found in the anaerobic continuous training group (K3), while the lowest fibroblast mean values were found in the control group that did not swim (K1). In the normality test results using the Shapiro-Wilk's test, all groups had normal distribution values ($p> 0.05$), whereas the homogeneity test results using Levene's test had a significance of 0.101, which provides homogeneous data.

Table 2 displays the results from the ANOVA test, which obtained a significance value of 0.001 ($p<0.05$); this indicates a significant difference between the groups. The results of the LSD posthoc tests, in Table 3, shows significant differences between all three groups ($p<0.05$).

The observation of neovascularisation in the mandibular incisor socket of the Wistar rat can be seen in Figure 2.

Table 2. Overall difference test of fibroblast groups

	One-way ANOVA
p-value	0.001*
α	0.05

*significant at p<0.05

Table 3. Difference test between fibroblast groups using LSD posthoc test

Groups	K	K1	K2
K1			
K2	0.031*		
K3	0.000*	0.031*	

*significant at p<0.05

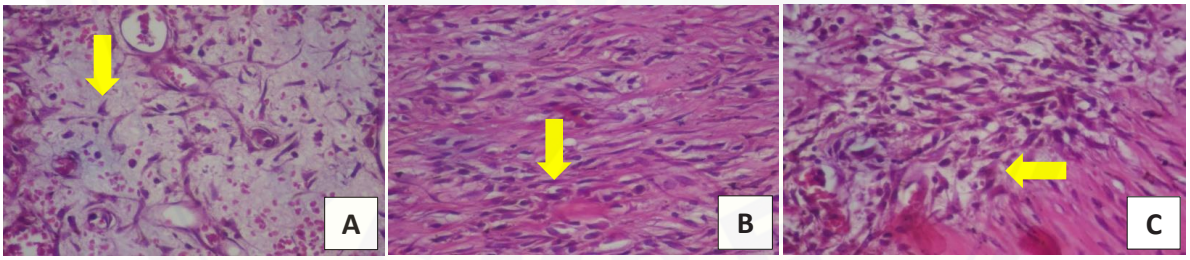

Figure 1. Fibroblast in the socket of a maxillary incisor tooth extraction with HE staining, indicated by the yellow arrows. Groups K1 (A), K2 (B) and K3 (C).

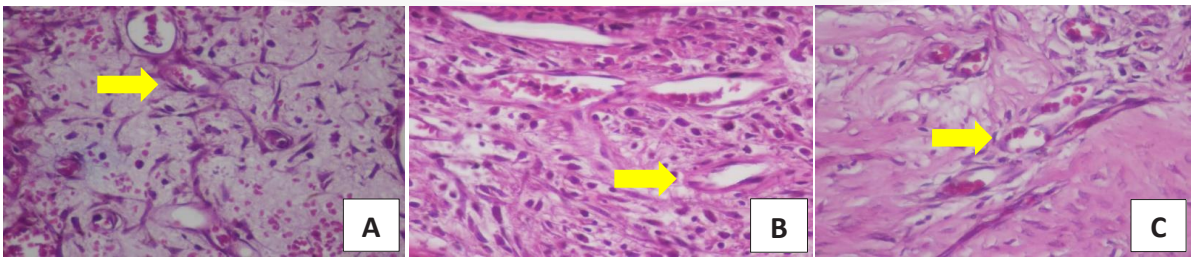

Figure 2. Neovascularisation in the socket of a mandibular incisor tooth extraction with HE staining, indicated by the yellow arrows. Groups K1 (A), K2 (B) and K3 (C).

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Table 4. The mean and standard deviation of neovascularisation amounts

Groups	Mean ± SD	Normality test	Homogeneity test
K1	8.57 ± 1.1339	0.262*	
K2	9.28 ± 1.7043	0.140*	0.335*
K3	10.71 ± 2.2887	0.518*	

*significant at p>0.05

Table 5. Overall difference test of the neovascularisation groups

One-way ANOVA	
p-value	0.098
α	0.05

p-value: One-way ANOVA significance value of neovascularisation variable; α: Significance value of the different tests.

Table 4 shows the neovascularisation mean values along with the results of the normality and homogeneity tests. In the table, the highest neovascularisation mean is in the continuous anaerobic exercise group (K3), and the lowest average is in the control group (K1). The normality test for each group shows that the distribution data is normal because p> 0.05, whereas the homogeneity test results show 0.335, which means homogeneous data continued with the one-way ANOVA parametric test. Table 5 shows that the different test results for the whole neovascularisation group gave a result of 0.098 (p>0.05), which means there were no significant differences between the groups on neovascularisation variables.

DISCUSSION

The wound healing process has several healing phases, including haemostasis, inflammation, proliferation and remodelling. As one of the factors that influences wound healing is oxygen, participating in physical exercise is expected to increase tissue oxygen consumption (VO_2 max) so that the wound healing process is faster due to the stimulation of cells. Cells that play a role in wound healing include PMN cells, macrophages, fibroblasts and neovascularisation.[16]

As experimental animals, Wistar rats have several advantages: they are cheap, easy to obtain, breed and keep, and they have a physiological body that is almost the same as humans. During the experiment, several rats became sick and died, so they had to be removed from the study. The swim test was used because the research tools and materials could be easily prepared and have been proven by other researchers to obtain reasonably good results.[17]

The groups for aerobic and anaerobic continuous exercise training were determined by calculating the results of the MSC and body weight of the experimental animals. Aerobic exercise intensity was 50% of the MSC and a weighting of 3% (medium intensity), while 65% of the MSC and a load of 6% were chosen as anaerobic physical exercise (high intensity). An obstacle in determining anaerobic physical exercise occurred because all of the literature suggests that methods of high-intensity anaerobic exercise involve interval training. However, the focus of this study was on continuous training. Therefore, as a preliminary study, we experimented with continuous anaerobic exercise at 65% MSC and a 7% load. However, several Wistar rats experienced fatigue and then death, so the researchers lowered the MSC and load without reducing the essence of the high-intensity physical exercise.[18,19]

Wound healing occurs in the proliferation phase, after haemostasis and inflammation but before remodelling. In the proliferation phase, several vital processes are related to studying the number of fibroblasts and neovascularisation. Macrophage cells found in the inflammation phase secrete matrices and growth factors to stimulate fibroplasia and angiogenesis. These include metalloproteinase, platelet-derived growth factor (PDGF), fibroblast growth factor (FGF), epidermal growth factor (EGF), transforming growth factor-beta and alpha (TGF-β/α) and vascular endothelial growth factor (VEGF). The process of angiogenesis and the proliferation of fibroblasts occur in tandem. When a wound occurs, branches of capillary blood vessels form around the edge resulting in bleeding for blood clot formation. The proliferation phase occurs after the fibroplasia process.[12]

Fibroplasia is the process of stimulating fibroblast cells to actively proliferate, migrate and form an extracellular matrix (ECM). The matrix will function as a scaffold for the next wound healing process. After fibroplasia comes angiogenesis, which is the process of forming neovascularisation. This process restores blood circulation to the injured area and prevents the development of necrotic tissue. The process is stimulated by factors and growth components, such as basic FGF, TGF-β, tumour necrosis factor-α (TNF-α), VEGF, angiogenin and angiotrofin. The cells that perform neovascularisation are stimulated and migrate to the temporary matrix (scaffold) that has been formed by fibroblast cells. Subsequently, cells develop and form a new network of blood vessels into tubular structures. The processes of fibroplasia and angiogenesis go hand-in-hand and synergise to form a layer of collagen and epithelium, which is a sign of the final phase of wound healing or remodelling.[20,21]

This statement supports the proven research results on fibroblasts in the treatment group rather than the control group. The process of fibroplasia in wound tissue after tooth extraction in Wistar rats is mediated by fibroblast cells stimulating the formation of the extracellular matrix so that fibroblasts can be seen in histological preparations. In this process, angiogenesis will be followed by neovascularisation. However, on the histopathological readings, the neovascularisation results were insignificant. This is likely to occur because the angiogenesis process occurs on the second and third day when the fibroplasia process has already taken place.[22]

Another factor affecting the results is the determination of the training intensity method, which has almost the

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same effect. In the continuous anaerobic exercise group, researchers have not used a standard method for determining the magnitude of the MSC and the load that is given, so the results can be biased if there is no standardisation. Additionally, it proves that interval-type training is better than continuous training because there is a resting phase that improves the effectiveness of the exercise.[20] Non-standardised tooth extraction techniques can also affect the results due to the occurrence of root fractures and bleeding during high extractions, which will disrupt the wound healing process.[15]

Theoretically, physical exercise will affect the wound healing process due to the appearance of free radicals in the body, which will disturb or delay wound healing. This happens if the period between the process of injury and participation in physical activity is close together, or even physical activity is only carried out after the injury. The healing process will be disrupted due to a lack of oxygen to the wound tissue and the build-up of lactic acid during sports activities. At a molecular level, the build-up of lactic acid causes reactive oxidative stress (ROS) and free radicals and inhibits scaffold formation in fibroplasia.[9]

In this study, physical exercise was performed before tooth extraction in Wistar rats. The training involved either continuous aerobic training or continuous anaerobic training. The results of this study indicated a higher number of fibroblasts and neovascularisation in the anaerobic training group (K3) because the heart and body adapt after 7–10 days after exercise by thickening the heart muscle and increasing lung capacity.[21] At the beginning of exercise (<7 days), lactic acid increases and VO_2 max decreases, which is proven to be significant, and this was demonstrated at the highest level in the anaerobic exercise group.[22]

Anaerobically, VO_2 max increases and the heart supplies blood to the body more efficiently and effectively due to the body's adaptation processes.[23] These conditions accelerate the wound healing process. Statements and research by Flora et al. and Shi et al. support the results of this study, which showed the number of fibroblasts in the continuous anaerobic exercise group (K3) was higher than the continuous aerobic exercise group (K2).[22,24] Based on the results of this study, we concluded that anaerobic exercise provides a wound-healing acceleration effect on increasing the number of fibroblasts compared to aerobic exercise.

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Research Report

Maxillary anterior root resorption in Class II/I malocclusion patients post fixed orthodontic treatment

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ABSTRACT

Background: *Previous studies on root resorption were reviewed by panoramic radiographs. Cone-beam computed tomography (CBCT) showed that 41.5% of teeth experienced resorption when panoramically examinated, while 68% of teeth experienced resorption when the examination method used was CBCT. Root resorption occurs in the maxillary central incisor (as much as 74%) and in the maxillary lateral incisor (as much as 82%). The maxillary canines have the most resorption, followed by the lateral maxillary incisors.* ***Purpose:*** *The aim of this study was to determine the differences of apical resorption in anterior maxillary teeth before and after orthodontic treatment in skeletal Class I/II cases of extraction.* ***Methods:*** *Samples from this study were the results of panoramic photographs of 50 patients treated by fixed orthodontic appliances at the Dental and Oral Hospital Airlangga University. These were selected according to the sample criteria. The evaluation method consists of measuring root and crown lengths with a digital application (RadiAnt DICOM Viewer). Subsequently, the measurements were evaluated using CBCT images.* ***Results:*** *The data were statistically analysed using normality tests with Shapiro–Wilk and Kolmogorov–Smirnov tests. Based on the results of paired sample tests, it was found that every treatment group had significant differences in the average length of the crowns and roots, with a result of p=0.000 (p<0.05).* ***Conclusion:*** *The use of CBCT is considered quite effective and accurate in evaluating root resorption compared to panoramic photographs.*

Keywords: *CBCT; Class II division I malocclusion; maxillary anterior; panoramic photographs; root resorption*

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INTRODUCTION

Malocclusion is a type of connection between the maxilla and mandible that deviates from the standard form, but is accepted as a normal form. Malocclusion can be caused by dentofacial balance. This dentofacial balance is not caused by one factor alone, but several factors that influence each other. These factors influence heredity, environment, growth and development, ethnicity, function and pathology.[1,2] Malocclusion can be treated using orthodontic appliances to obtain normal occlusion and a proportional facial profile.[2] The prevalence of malocclusion in Indonesia is still high (seen in around 80% of the population) and may increased dental and oral health problem[3]

Orthodontic treatment always uses mechanical force to move teeth. The mechanical force on the tooth that will be moved orthodontically will be transmitted to the entire tooth's supporting tissue, which starts a remodelling process to help the tooth move through the bone.[4]

Orthodontic treatment has a positive effect but can have undesirable secondary effects. During orthodontic treatment, the application of various procedures, tools and materials can cause side effects, both local and systemic. One of the side effects is root resorption, which is clinically difficult to identify when radiographs are made, especially in cases of orthodontic treatment.[5]

External Apical Root Resorption (EARR) is a state of permanent loss of the apex structure of the tooth. Cross-sectional studies show that EARR is a common iatrogenic consequence and minor problem for the average orthodontic patient, with the mean radiographic resorption being less than 2.5 mm.[6,7]

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One study found that the risk factors involved in root resorption were dental trauma, bone density, root morphology, age and length of treatment. Most of the research on root resorption has focused more on maxillary incisors because they are thought to be more prone to root resorption than other teeth. More specifically, root resorption often occurs in maxillary incisors and other teeth with an abnormal root shape; for example, those that are pipette-like, blunt or macerated. The maxillary lateral incisors were most frequently subjected to root resorption, followed by the maxillary central incisors.[4]

Root resorption occurs in the maxillary central incisor (up to 74%) and in the maxillary lateral incisor (as much as 82%). The maxillary canines have the most resorption, followed by the maxillary lateral incisors, which are measured using cone-beam computed tomography (CBCT).[8] Research shows that root resorption in maxillary first molars has a mean resorption of 53.3%-63.3%. This absorption occurs because the force applied to the molars is greater than that on the premolars. In addition, the resorption in the extraction case was 3.72 times greater than that in the non-extraction case.[7,8]

Several studies of root resorption and its relationship with orthodontic treatment have found that numerous factors influence root resorption: age, sex, nutrition, genetics, type of appliance, the amount of force used during treatment, extraction or non-extraction, length of treatment and distance of tooth movement. There is positive correlation between the strength of orthodontic style, length of treatment and increased resorption.[9,10]

Individuals with skeletal anterior open bite have a greater risk of resorption during orthodontic treatment compared to those with other types of malocclusion. Dental intrusions are four times more likely to cause EARR than extrusion movements.[4,11]

Previous studies on root resorption were reviewed by panoramic radiographs and CBCT. It was found that 41.5% of teeth experienced resorption when panoramically examined, while 68% of teeth experienced resorption when the examination method used was CBCT.[9] An advantage of CBCT is its accuracy in measuring root resorption, but its disadvantage is that radiation levels are 1.5 to 33 times higher than levels in panoramic photographs; thus, careful consideration is needed when using CBCT.[9]

In this study, panoramic radiographs show some degree of distortion, which was caused by the lack of a three-dimensional image. CBCT imaging allows the three-dimensional evaluation of teeth and adjacent anatomical structures, which provides a more detailed visualisation of the tooth and surrounding structures and can diagnose EARR with accuracy.

Based on the above, the authors were interested in researching the evaluation of maxillary anterior tooth root resorption that occurred before and after orthodontic treatment in Class I/II malocclusion by using panoramic radiographs and CBCT. The aim of this study was to determine the differences of apical resorption in anterior maxillary teeth before and after orthodontic treatment in skeletal Class I/II cases of extraction and to help the operator prevent the occurrence of more severe root resorption when finding it on radiographs.

MATERIALS AND METHODS

This study is an observational analytic research. The sample of this study was made up of patients who had been treated by fixed orthodontic appliances at Dental and Oral Hospital Airlangga University from 2014 to 2018. They were selected according to the sample criteria: patients needed to have Class II division 1 skeletal patterns, have had both first maxillary premolars extracted. Ethical clearance was obtained from the health research ethics commission of the Faculty of Dental Medicine, Universitas Airlangga (number: 614/HRECC.FODM/IV/2019). The minimum sample size needed was 35 to avoid drop out. It is necessary to over-sample, so this study also used 50 samples from the data obtained at the Dental and Oral Hospital Airlangga University. These samples qualified according to the Lameshow formula.[7]

Panoramic Measurements: Methodology developed by Fontana et al.[11] demonstrates periapical radiographic measurements of central incisors with root lengths (reference teeth) taken before treatment and after treatment. The evaluation method involves measuring the length of the roots and crowns using a digital application (RadiAnt DICOM Viewer) (Figure 1). The root apex, incisal edge and cemento enamel junction (CEJ) of each tooth were

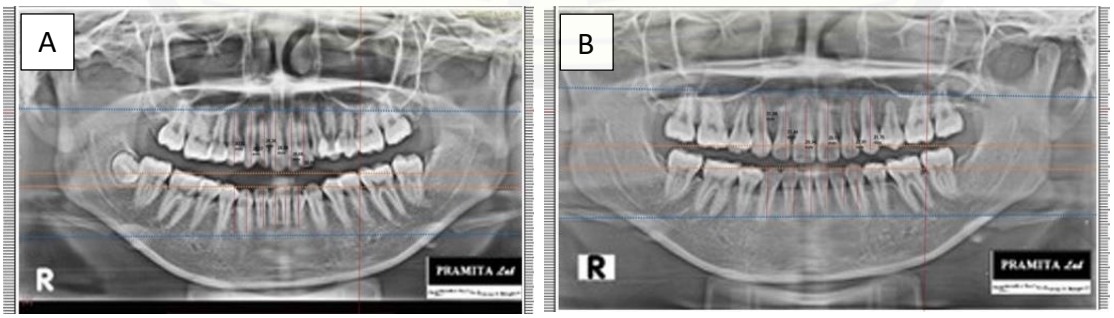

Figure 1. A) Before orthodontic treatment. B) After orthodontic treatment. An example of calculating tooth length before and after treatment using RadiAnt DICOM software.

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determined by making a point. The longitudinal axis of each tooth is projected from the tip point of the root to the edge of the incisal following the root canal. The perpendicular axis is then directed down the longitudinal axis from the mesial to the distal side of the CEJ. The value of the crown length is automatically calculated from the incisal edge to the CEJ projection and the root length from the CEJ projection to the apex of the root (Figure 2). The difference between the two measurements shows EARR.[11]

Measurements on CBCT: Then, the measurements were evaluated using the CBCT image. First, the CBCT image of the maxillary central incisor obtained after orthodontic treatment was aligned using the CEJ angle. Next, the amount of apical root resorption was calculated as the distance between the root apex before and after orthodontic treatment on the axis of the maxillary central incisor. The root resorption area was measured using a digital application (RadiAnt DICOM Viewer) and classified as labial and palatal after identifying tooth axes (Figure 3). The ratio of labial root resorption is defined as the ratio of the area of labial root resorption to the resorption area for all roots, and the ratio of palatal root resorption is defined as the ratio of the area of palatal area to the area of resorption for all roots.

The measurements of the lengths of the crowns and roots that were obtained were then tested using IBM SPSS 26 for Mac to determine the distribution of the data. The first data analysis that was performed focused on data normality. Analysing data is needed to determine the use of the next statistical test or whether parametric or non-parametric tests should be used next. If the data is normally distributed, parametric statistics can be used.[13] The normality tests used in the data analysis of this study were the Kolmogorov–Smirnov test and the Shapiro–Wilk test. These tests can be used on both research with small samples and research with large samples.[8]

RESULTS

Based on the measurements of the lengths of the crowns and roots using a digital application (RadiAnt DICOM Viewer), several results were obtained. The results of paired sample tests found that almost every treatment group had significant differences in their average lengths of crowns and roots, as p=0.000 (p<0.05) (Table 1). This significant difference indicates the presence of root resorption after orthodontic treatment.

As shown in this diagram (Figure 4), tooth 11 had an average root resorption of 0.98 mm, tooth 12 had an average of 1.17 mm, tooth 13 had an average of 0.86 mm, tooth 21 had an average of 0.93 mm, tooth 22 had an average of 1.13 mm and tooth 23 had an average of 0.87 mm. Based on the diagram above, the highest root resorption was in tooth 12 and the lowest was in tooth 13.

In this study, the authors included 10 participants who had completed orthodontic treatment and were then recalled

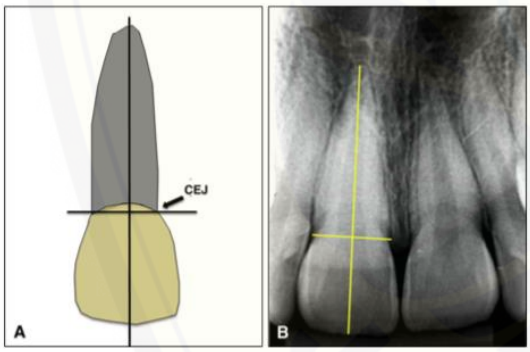

Figure 2. A) Anatomic landmarks for measuring EARR: cementoenamel junction (CEJ). B) A reference for measuring X-rays.[12]

Table 1. Mean and standard deviation of the calculations of crown and root lengths with panoramic measurements

Teeth	Mean ± SD		Delta (%)	p value
	Before treatment	After treatment		
11	29.34±3.48	28.46±3.25	-2.93	<0.0001
12	27.44±3.05	26.27±3.11	-3.11	
13	31.58±3.89	30.72±3.75	-2.83	
21	29.51±3.26	28.58±3.25	-2.98	
22	27.81±3.23	26.68±3.23	-3.08	
23	31.76±3.84	30.89±3.76	-2.89	

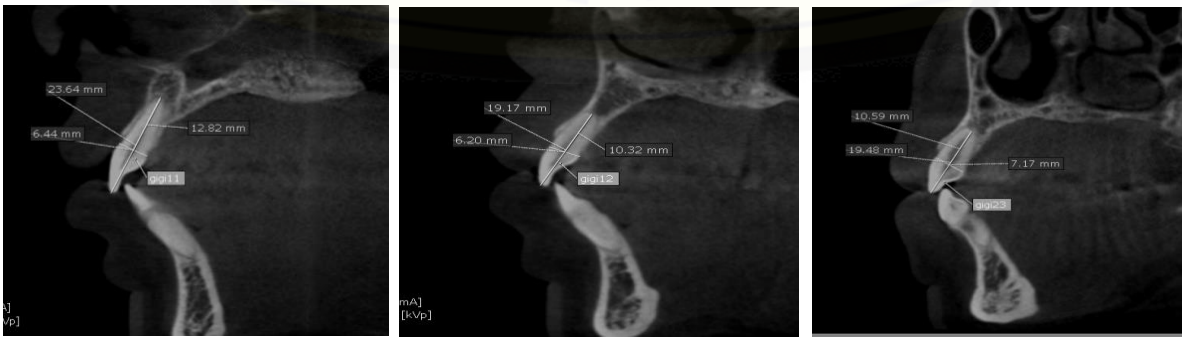

Figure 3. Measurement of the degree of absorption in CBCT with the use of RadiAnt DICOM software.

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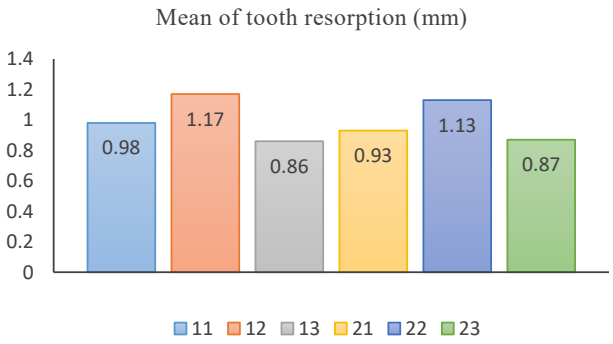

Figure 4. Diagram showing the mean of crown and root length calculations with panoramic measurements.

Table 2. Mean and standard deviation of the crown and root length calculations with CBCT

Teeth	Mean ± SD
11	29.82 ± 3.21
12	26.83 ± 3.13
13	34.02 ± 3.45
21	25.99 ± 3.11
22	25.01 ± 3.07
23	29.13 ± 3.14

for CBCT photos. The authors compared root resorption on panoramic radiographs after treatment with CBCT photographs (Table 2). The results obtained indicated that seven samples showed mild resorption by panoramic (an average of 1.2 mm) and three samples showed moderate resorption (around 2.5 mm). However, using CBCT revealed that eight samples showed moderate resorption and two samples showed mild resorption. This is consistent with Dudic's study in which 275 teeth were evaluated with panoramic radiographs and CBCT to measure apical root resorption.

DISCUSSION

In general, Class II malocclusion often occurs with tooth root resorption of mild to moderate severity.[14] Patients with Class II malocclusion have an increased frequency of tooth root resorption. Patients with Class I malocclusion had tooth root resorption with an average value of 1 mm, while patients with Class II malocclusion had an average of 2 mm. Maxillary central incisors had greater resorption values in Class II malocclusion patients.[15]

The classification of Class II malocclusion in this study was based on skeletal anteroposterior discrepancy. The antero-posterior relationship between the maxilla and mandible was evaluated through the A point, Nasion, B Point (ANB) angle, where the ANB size was significantly greater in skeletal Class II than in skeletal Class I.

In patients treated using bracketed MacLaughlin, Bennet and Trevisi (MBT), root resorption was 18.26%, while patients treated using edgewise brackets had a root resorption of 14.82%. Tooth root resorption in MBT patients was greater than in edgewise patients. This was due to the tooth root in MBT.

Torque with an increasing angle will affect the severity of tooth root resorption. The increase in torque angle and the duration of torque usage causes the apical tooth to have a hollow hyaline zone. The accumulation of this hollow hyaline zone will result in a short tooth root and will reduce the dimensional ratio between the root and crown of the tooth.[6]

Root absorption often occurs in the apical part of softer teeth and contains less of Sharpey's fibre. Another cause of this is the use of torque, as it presses the periodontal tissue at the apical part so that the tooth is more susceptible to root resorption.[6]

Significant differences were observed between the two methods and for all levels of resorption. One hundred and forty-five teeth evaluated panoramically showed no resorption, whereas, out of those evaluated by CBCT, only 80 teeth showed no resorption. Ninety-two teeth showed mild apical root resorption with panoramic evaluation, and this increased to 128 teeth with CBCT. Only 21 teeth had moderate panoramic resorption, but this increased to 48 teeth with CBCT. In addition, two teeth had severe resorption when assessed by CBCT.

CBCT imaging allows for a three-dimensional evaluation of teeth and their adjacent anatomical structures, resulting in a detailed visualisation of the tooth as well as its neighboring structures. Subsequently, the area of the tooth resorption can be detected easily. In contrast, the panoramic radiographic image is a two-dimensional radiographic image that experiences distortion. This results in difficult interpretation with minimal accuracy, which makes it less helpful in measuring root resorption.

In conclusion, this study has proven the existence of root resorption after orthodontic treatment in Class II/I malocclusion cases. The highest resorption (a value of more than 1 mm) was found in tooth 12 (1.13 mm) and the lowest was found in tooth 13 (0.86 mm). The use of CBCT is considered quite effective and accurate in evaluating root resorption compared to panoramic photos.

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Research Report

Electronic application for oral health school programme enhances the quality of the information in dental health data records

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ABSTRACT

Background: *Dental and oral health problems among elementary students can be resolved through an oral health programme in schools. The main factor that inhibited this scheme was that the recording and reporting still employed a manual system, making it less effective and efficient. The electronic application of this programme can help managers to complete both of these tasks.* ***Purpose:*** *This study's aim is to assess the effectiveness of the electronic application that is utilised in the oral health school programme to increase the quality of the information relating to the recording of dental health data in schools.* ***Methods:*** *This study used a pre-test and post-test one-group design, and the sample consisted of 37 oral health programme managers in schools who were chosen via simple random sampling. The data in this study was analysed using the Wilcoxon signed-rank test.* ***Results:*** *The results showed that the use of the electronic application as part of the oral health scheme influenced the quality of the information when details were recorded and reported. This can be seen in the rise in the standard of the information that was noted and disclosed when comparing data before and after using the electronic application; the average value of 1.54 (standard deviation=1.45) increased to an average value of 3.58 (standard deviation=2.84) with a significance level of 0.000 ($p<0.05$).* ***Conclusion:*** *The electronic application used in conjunction with the oral health programme was effective in raising the quality of dental health records in schools.*

Keywords: *electronic application; oral health school programme; quality information; recording and reporting*

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INTRODUCTION

Oral and dental health are a part of the overall well-being of the body, but they have often been neglected by some people. Keeping our mouths and teeth healthy is not only useful for chewing, talking and our appearance, it also supports our general welfare as living beings. World Health Organization (WHO) data for 2005–2015 showed that 90% of dental caries ware found in school-age children.[1] The Fit for School programme research conducted in Cambodia, Indonesia and Laos stated that 94.4% of children had caries in their primary teeth and 73.2% were shown to have odontogenic infections, which can cause disruptions to eating and sleeping, poor quality of life, school absence and growth retardation.[2] The results of the 2018 Basic Health Research, or Riset Kesehatan Dasar (RISKESDAS), disclosed that 57.6% of Indonesia's population had dental and oral problems and only 10.2% received dental medical treatment. Meanwhile, in West Java, 58% of the populace had dental and oral health issues and only 11.9% had received treatment.[3]

However, often neglected dental and oral health problems in children can be overcome through a full integration of oral and dental health into public health promotion strategies and school health programmes. Such schemes in places of education teach children to maintain their oral and dental health and to comprehensively and continuously care for their mouths and teeth; thus, improving their health status in this regard. Collaboration between schools and health workers was an important

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element in maximising this oral-health-based school programme. Nevertheless, there were obstacles in terms of accessing information, such as difficulties in providing information on the condition of children's oral health and the availability of details related to the programme with regards to schools and parents.[4]

The development of information and communication technology provided convenience when strengthening and developing health information systems. Currently, there is a need to utilise information and communication technology in the health information system to improve the management and implementation of the field's progression. These types of systems could be used as a tool to help oral health school programme managers provide data quickly and easily to schools, simplifying the process of monitoring performance and reporting activities online in a way that is both integrated and easily accessible by everyone.[5]

Recording and reporting were part of the health information system, which was an indicator of success when assessing a programme, and the details generated from the processed data could be used for planning subsequent schemes. Indeed, the information had an important role in the decision-making process. At the time, the recording and reporting of data in relation to the oral health school programme were still done manually, and the management team continued to use a book to record the results of the scheme's oral health activities in schools. Doing so was less effective and efficient because the person in charge of the scheme had to rewrite the results of said activities and had subsequent difficulties finding the relevant data; therefore, they took a relatively long time to compile the reports. Electronic recording systems in the health sector have emerged as a result of the development of information and communication technology, which has been utilised to improve the quality and efficiency of health services.[6] This kind of system could be easily accessed, reduce paper costs and diminish the number of problems when sharing information between patients and health workers. In addition, it could provide accurate and consistent care record details that are required for further maintenance,[7] which can lead to the sustainability and success of a programme.[8]

Furthermore, an electronic recording system could integrate data into one place that allows analysis to be done easily and quickly when making decisions. The use of electronic health records also leads to more comprehensive data input by patients, and can make examinations more accurate if a more detailed previous medical history has been given.[9] The electronic recording system makes it easy to find patient data and their histories, so it saves time and is more effective. Additionally, data pertaining to those being treated is stored more safely, meaning it is not easily lost and confusing patient data can be avoided. Android is an operating system for smartphones based on Linux, which is often used in the health sector as an innovation in the use of information technology. The advantage of Android compared to other smartphone operating systems is that it employs an open-source code, making it easier for developers to create and modify applications or features that do not already exist within the operating system according to their wishes.[10]

The West Java Province School's health programme advisory team has created a MEDISis website application, which is a student health management information system. Its purpose is to store a history of the health and physical growth of students obtained through several measurements and examinations that can be done as part of the institution's healthcare scheme. Moreover, pupils can see the state of their well-being by using the aforementioned Android-based application. The aim is to use it to collate medical histories after they have been entered through the MEDISis website by the teacher or the MEDISis operator. The Faculty of Dentistry at Airlangga University has also developed the simdagilut application, which is an online-based information system website and a tool for data management for dental and oral health surveys. Dental health records on the MEDISis website and simdagilut used an odontogram, which recorded the dental health condition of school children, but did not explain in detail their dental and oral hygiene or the condition of any dental caries. The follow-up plan and the treatment carried out were also not included in the MEDISis and simdagilut applications. The simdagilut version of this digital tool only displays the status of immediate care needs without presenting the type of treatment needed.

The results of a preliminary study conducted at public health centres, or Pusat pelayanan kesehatan masyarakat (PUSKESMAS), in Bandung City show that the recording and reporting of oral health school programmes still utilises a manual system that involves the assist book. Indeed, MEDISis and simdagilut applications have not been used at all by these places in the city, and manual notation and reporting make it difficult to find dental health data records; consequently, dental health statuses and the related care required for each child have not been included in the oral health programme report in schools. In addition, data from the scheme as reported to the Health Office is still being combined with information from visits to PUSKESMAS and details from the community-based dental health programme. While the report on the oral health school programme is very important for determining policies or resolving problems related to oral health in elementary school children, the aim of this study is to investigate the effectiveness of the scheme's electronic application in increasing the quality of the information held in dental health data records in schools.

MATERIALS AND METHODS

This research has received approval from the Research Ethics Commission of Padjadjaran University (1505/UN6.KEP/EC/2019) and has met the basic principles of ethics based on the Belmont Report, namely respect for

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persons (respect for human dignity), beneficence and non-maleficence (principle benefit and not harming anyone) and justice. This research was conducted in Bandung City from December 2019 to April 2020.

The sample used in this study consisted of the managers of oral health school programmes in elementary schools within the working boundaries of the public health centre in Bandung City, and they were chosen via a simple and randomised sampling method. The researchers compiled a list of the names of public health centres, and made a paper containing the number of notes that corresponded to this list. The paper was rolled up for the purpose of a draw to select a sample with a predetermined number, and the numbers on the paper that were picked were selected as the research sample.

The inclusion criteria for this study were managers of oral health school programmes in elementary schools within the working parameters of health centres in Bandung City; having oral health schemes in the elementary schools within the aforementioned area; the ability to use an Android-based mobile phone that supports the programme's electronic application; and the propensity to operate said application. Concurrently, the exclusion criteria for this study was oral school programme managers who were not present at the time of the research. Meanwhile, the conditions for dropping out were the programme managers who resigned in the middle of the research. This study did not use a control group, and the treatment group alone was given a pre-test and post-test. The sample formula used pairwise numerical analytic research (two-average hypothesis testing with a single sample) as follows:

$$n = \left[\frac{(Z_\alpha + Z_\beta)\, S}{X_1 - X_2}\right]^2$$

Where: n = minimum sample size, Z_α = standard deviation of *alpha* / Z value to a certain degree of significance, Z_β = standard deviate *beta* / Z value at a given power test, S = standard deviation of the difference in value between groups, X_1 - X_2 = the difference between the minimum mean which is considered significant.

This study also used mean and standard deviation values based on the research by Hoogerheide et al.[11] because it was considered to be similar in nature, with a mean difference of 8.84 and a standard deviation of 0.12. Estimation was done with a 5% degree of significance and 90% test strength. Through the calculation of the sample size, the minimum number of subjects was 33 respondents. Anticipating contributors dropping out, the number of subjects was increased by 10%, meaning the total number of respondents was 37.[11]

This research was conducted using a pre-test and post-test one-group design. Respondents were asked to do a pre-test by filling out a questionnaire on the quality of the recording and reporting of information that is currently used, which still employs a manual system that involves the use of a book. The subject was then given an explanation of the uses of the oral health school programme's electronic application. After that, the researcher asked scheme managers to enter existing dental and oral health screening data, specifically in relation to first graders based on last year's screenings. The decision behind choosing this information in relation to this group of pupils was based on the programme's guidelines that dental and oral health screening should be carried out on first-grade students. After 30 days, those in charge of the oral health schemes in schools were asked to fill out a questionnaire (the post-test) that quizzed them in the same manner as the pre-test to assess the quality of recording and reporting after using the scheme's Android-based electronic application. This was completed on the Google Form sent by the researchers. During the applicable trial period of 30 days, the scholars involved monitored the activities of the oral health school programme managers when collecting apposite data using the aforementioned application.

The instrument used in this study was a questionnaire for assessing the quality of information recording and reporting on the oral health programme in schools, which consisted of 22 questions divided into four dimensions, namely the intrinsic, contextual, accessibility and representation elements. The assessment was carried out by the researcher and was assisted by the dental nurse. The independent variable in this study is the use of the electronic application, and its dependent counterpart is the quality of the recording and reporting information as part of the same scheme. The quality of the information can be measured from a person's point of view by using a product and service that is in accordance with users' expectations, and it can be quantified via the quality dimension method, which is a determining factor in the degree to which the benefits it contains have been achieved. Wang and Strong[12] categorised the dimensions of information quality as intrinsic, contextual, representation and accessibility.

The intrinsic dimension is a basic assessment that appears in relation to individuals that are using a product or service. This includes evaluating the accuracy, objectivity, content validation and the reputation of the information's source. The contextual dimension scrutinises the content of an interrelated detail to make it easier for users to receive information. Meanwhile, the contextual dimension relates to the relevance, added value, timeliness, reliability and completeness of information, and the representation dimension is a form of conveying information to users. The parameters of the presentation include the ability to interpret, ease of understanding and the conciseness/ consistency of the way the data is presented. Lastly, the accessibility dimension assesses the ease of accessing information and the security system for guaranteed information or the existence of an arrangement for the sake of privacy.

The instrument used has been tested for its validity and reliability on 30 oral health school programme managers and was found to have a Cronbach's alpha value of 0.92 (very good), person reliability of 0.90 (good) and item

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reliability of 0.82 (good). The univariate analysis of the frequency distribution of general data, such as age, gender and education, can be utilised to describe the characteristics of each variable studied as well. In the meantime, bivariate analysis was used to determine the influence of the oral health programme's electronic application on the quality of recording and reporting in the scheme. The data was examined using IBM statistical analysis for social science (SPSS) software version 20 (IBM corporation, Illinois, Chicago, US), and the Shapiro-Wilk test was employed to assess its normality. Based on the Shapiro-Wilk test, it was found that the data was not normally distributed, so the next form of analysis used the Wilcoxon test and found a significant value of 0.000 ($p< 0.05$), which indicates a significant change.

RESULTS

In terms of the characteristics of the respondents in this study, 83.78% of them were female, 51.36% were aged 26–45 years, those with a DIII education accounted for 75.66% of contributors and 86.47% of the sample group worked as dental nurses. The quality of information recorded and reported during the activities completed by Android-based electronic application for oral health school programme were assessed based on users' perceptions regarding the standard of the data provided, and were assessed using the following scores: one (very poor), two (poor), three (sufficient), four (good) and five (very good).

Table 1 highlights the fact that the average value of the recording and reporting quality of the oral health school programme increased after using the associated electronic application; the overall improvement in the standard of notation and delineation after using the application went from an average value of 1.54 (standard deviation=1.45) to an average value of 3.58 (standard deviation=2.84) where a significant value of 0.000 (<0.05) indicates a noteworthy change. When viewed based on the dimensions of information quality, only the intrinsic, contextual and accessibility dimensions show significant changes with a significant value <0.05.

DISCUSSION

A high calibre of information was needed to support the success of the system's development. The results showed that the Android-based electronic application for oral health school programme gave influenced to the quality of electronic application for oral health school programme recording and reporting. This was in accordance with Salsabila's[13] research, which states that the use of an Android-based smartphone can be a ready-made means of shortening the time spent on data collection and automatic data processing. This android-based recording and reporting application can also minimise paper requirements; therefore, reducing the risk of lost and corrupted data and facilitating the creation of fast and accurate reports based on the data needed at the highest level.[13]

Android applications are mobile devices that provide a good platform to develop from, and they can be used as data collection systems. The relatively low cost, its portable nature and the ease of access to wireless networks where there is network range make these implements very attractive to users.[14] The results of Asgari's[15] research in Iran showed that electronic dental health records could provide benefits with regards to storage, processing and data utilisation, which were all major components in delivering effective and efficient service based on the available evidence. Digital versions of these records are more effective and efficient because they reduce paperwork, lead to the fast processing of data, facilitate ease of analysis and increase the accuracy, precision and quality of data. In addition, dental and oral health data records can be used in conjunction with programme planning and intervention.[15] In relation to this, Petersen et al.[16] found in their research that in constructive health-related decision-making and planning, the information system must cover aspects of risk factors associated with oral health and the quality of information related to it, services and interventions, administrative processes and the quality of clinical care outcomes as well as epidemiological details.

Using the school programme's Android-based application minimises errors when entering data, makes it easier to create reports and offers information about the status of oral

Table 1. Analysis of pre and post changes in the quality of electronic application for oral health school programme recording and reporting

Variable	N	Mean	SD	Min	Max	Significant value
Pre-all	37	1.54	1.45	-0.97	5.48	0.000
Post-all	37	3.58	2.84	-3.46	9.52	
Pre-intrinsic	37	1.15	2.30	-3.06	5.50	0.021
Post-intrinsic	37	-0.53	4.37	-8.50	8.29	
Pre-contextual	37	1.68	2.09	-2.50	6.73	0.044
Post-contextual	37	0.66	3.29	-7.08	6.90	
Pre-representation	37	1.74	2.05	-2.02	7.07	0.056
Post-representation	37	0.19	5.03	-9.65	9.64	
Pre-accessibility	37	1.33	2.64	-3.90	7.45	0.004
Post-accessibility	37	2.89	2.76	-3.14	8.19	

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health that can be used when making choices that bolster dental and oral health schemes. This was in keeping with what Detsomboonrat's et al.[17] discovered in 2019, which was that Android applications can be used to assist health care professionals in carrying out many important tasks, including time management, maintenance of and access to medical records, consultation, referrals, information gathering, clinical decision-making and educational health. The application was seen to be capable of reducing the number of errors and the time spent on data recording, and it could provide a good system for collecting information that can be used to assess the dental and oral health status of a population to determine policies and strategies that support dental and oral health programmes.[17]

The success of the information system was assessed by ascertaining the level of usefulness of the information when making reports and settling on related decisions. Its degree of accomplishment could also be scrutinised from the perspective of the information generated as its quality was indispensable when the validity of the system's development needed to be verified. The dimension of information quality was an approach used to measure the standard of data from the aforementioned electronic application based on users' perceptions as user perception has become the most important factor in determining the value of the information supplied because everyone's needs are different in this respect.[18]

In this study, measurements of the information in relation to the programme's recording and reporting were undertaken using Wang and Strong's theory,[12] which divides the data into four strata: intrinsic, contextual, presentation and accessibility. The results of the influence analysis when using the school scheme's electronic application to affect the quality of recording and reporting on oral health from each of these subdivisions showed that the ones that had a meaningful influence were the intrinsic, contextual and accessibility dimensions; meanwhile, the representation dimension was shown to have an insignificant bearing.

The intrinsic dimension assessed the accuracy, objectivity and validity of the informational content as well as the reputation of the source of the data. The electronic application used in conjunction with the oral health programme in schools provided details that were free from the errors that may usually occur in the implementation of data processing, because it can automatically provide the results of the calculation of the dental hygiene index. The presentation of accurate information was vital as what was provided from the source to the recipient can cause interruptions and mistakes that can damage the data collated from the information. The material gathered by the programme's application reflected the actual conditions and was in accordance with the requirements of the oral health manager in the specified schools. Furthermore, the application presented information in an objective, complete and reliable manner, and the users' trust in the details it offered showed that the information has a high level of credibility. This is supported by Hazen's et al.[19] who asserted that the quality of the information depends on the accuracy of the data in line with the actual situation.

The contextual dimension assessed the relevance, added value, timeliness, reliability and completeness of the information. The application linked to the aforementioned school programme provided details that were relevant to the oral health scheme, meaning they complete and useful for recording and reporting on it. Complete information was imperative for both the provider and the recipient of the information, so that it can be understood clearly. The electronic application also helped the programme managers to create reports on time as they no longer need to make repetitive notes. Information must be given in a timely manner so that its recipient is not too late to act, or to make decisions based on what has been brought to light. Moreover, the scheme's application provided added value, because it could automatically calculate the status of dental and oral health and generate reports that can be downloaded directly onto it. This was consistent with the findings of a study by Lee et al.[20], which stated that in the contextual dimension, users must make relevant, timely, complete and accepted considerations, which has added worth when providing changes to existing information, making it superior in terms of how it can be utilised.

The representation dimension assessed the ability of interpretation, ease of understanding and the conciseness and consistency of presentation. The electronic application that was employed during the oral health scheme provided information that was easy to understand, systemic, consistent, in accordance with the reported needs and that could facilitate the production of reports on time. The use of clear and succinct language was also an added value when assessing the quality of the information. Good recording and reporting produced statistics that did not conflict with previous information and did not vary. The results also underlined that the electronic application had no significant effect on the dimensions of the presentation. This was because the oral health program managers in schools had only just recently been introduced to it, meaning that they were not familiar with its employment. Consequently, those in charge of the above oral health schemes were confused by application, so they had to ask about how to use it once again. This is supported by research by McGill et al.[21], which proposes that the utilisation of developed information systems should refer to how often users employ them; the more often they use information systems, the more they learn about them.[21]

The accessibility dimension assessed the ease of accessing information as well as the standard of the security system for guaranteed information or the existence of a system for privacy. The aforementioned application can be accessed without difficulty to get the information needed when the programme leader wants to make important decisions, which can in turn increase the success of such a scheme. The quality of the information was determined by its availability; in other words, the extent to which it can be retrieved whenever and wherever it is necessary. Failure to

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access the school programme's electronic application was found to occur if the internet network was interrupted or if it was in the process of being maintained. The application was also equipped with a data security system, and the information that was presented had a privacy system, which meant that the data stored in the application could not be easily corrupted by means of duplication, dissemination or by being changed by irresponsible people.[22]

Information quality was often measured as a major component of user satisfaction. Indeed, the quality of the information was a determining factor in increasing the intention to use information technology. If users believed in the quality of the system they were utilising and felt that it was not difficult to use, they would then believe it would provide greater benefits and could optimise their performance. If the information generated by the system was more accurate, timely and exhibited a good degree of reliability, it will further increase user confidence in the system. Rai et al.[23] came to similar conclusions, stating that an increase in the trust of users of information systems was expected to further improve one's performance. Nevertheless, with regards to the limitations of this study, the electronic application for oral health school programme that was used is still a prototype, so suggestions are needed for further improvement and development. Additionally, the details entered into the application were secondary data due to circumstances that make it impossible to carry out direct dental health checks on elementary school students.

Based on the results of the research on the influence of the Android-based electronic oral health school programme application on the quality of recording/reporting in elementary schools around the working parameters of the public health centre in Bandung City, it can be concluded that the tool was effective in increasing the calibre of the information contained in dental health records. The above application was utilised in accordance with the needs of the scheme's managers and the existing conditions, so that it could be a solution to the problems associated with the manual recording and reporting system. It made these dual tasks effortless, prevented the necessity for repetitive work and could be accessed at any time. This research has contributed to enriching studies in the field of public dental health and producing new innovations regarding the electronic application for oral health school programme, which is used for noting down and delineating dental health in schools. It was hoped that the Android-based electronic application relating to the oral health school programme can be used as a medium for recording and reporting in order to monitor the dental and oral healthcare of elementary school students.

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Research Report

Combining 10% propolis with carbonated hydroxyapatite to observe the RANKL expression in a rabbit's alveolar bone

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ABSTRACT

Background: *Periodontitis causes an increased receptor activator level in the nuclear factor-κβ ligand (RANKL), which is one of the inflammatory mediators that plays a role in osteoclastogenesis. The open flap debridement (OFD) technique is the preferred treatment when accompanied by regenerative periodontal treatment using guided tissue regeneration (GTR) and guided bone regeneration (GBR). Carbonated hydroxyapatite is a GBR material that serves as a scaffold and has strong osteoconductive properties for bone regeneration. Propolis is natural product that can decrease osteoclastogenesis in periodontitis by decreasing the RANKL expression.* ***Purpose:*** *To investigate the RANKL expression after open flap debridement by applying carbonated hydroxyapatite to 10% propolis in the alveolar bone of rabbits.* ***Methods:*** *Nine induced-periodontitis rabbits (Oryctolagus cuniculus) were divided into three treatment groups of Group A OFD, Group B OFD followed by the application of carbonated hydroxyapatite, and Group C OFD followed by application of 10% propolis-carbonated hydroxyapatite. Each group was selected one to euthanised on the seventh, 14th and 28th day, respectively, and prepared using histology slides. The data was analysed using a two-way ANOVA followed by a post-hoc LSD test ($p<0.05$).* ***Results:*** *The RANKL expression in each group showed significant differences ($p=0.00$; $p<0.05$) on the seventh, 14th and 28th day. The post-hoc LSD test showed that the RANKL expression in the treatment group with carbonated hydroxyapatite-10% propolis had significant differences ($p<0.05$) in the intergroup analysis at different time points.* ***Conclusion:*** *Combining 10% propolis with carbonated hydroxyapatite in OFD treatment can decrease the RANKL expression in a rabbit's alveolar bone.*

Keywords: *10% propolis; carbonated hydroxyapatite; periodontitis; RANKL expression*

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INTRODUCTION

Periodontal disease is caused by *Porphyromonas gingivalis (P. gingivalis)* and increases inflammatory cell infiltration, i.e. T lymphocytes, B lymphocytes and neutrophils in the connective tissues of the periodontium.[1] These inflammatory cells may lead to an increase in inflammatory mediators, such as prostaglandin E2 (PGE2), interleukin-1 (IL-1) and the receptor activator of nuclear factor-κβ ligand (RANKL). RANKL is an inflammatory mediator that plays a role in osteoclastogenesis. T lymphocytes can activate RANKL during inflammation in periodontal tissues, which causes the RANKL expression to increase during periodontitis.[2]

Periodontal treatment using the open flap debridement (OFD) technique is the preferred treatment when it is accompanied by regenerative periodontal treatment using guided tissue regeneration (GTR) and guided bone regeneration (GBR).[3] Carbonated hydroxyapatite is a GBR material that serves as a scaffold. It has strong osteoconductive properties for bone regeneration, can be well-resorbed by osteoclasts in the body and has good solubility in weak acidic conditions, i.e. when osteoclasts resorb bone by releasing H+ ions.[4] The addition of 10% propolis in carbonated hydroxyapatite can stimulate the growth of fibroblast cells, and it is a good candidate for alveolar bone regeneration.[5] A study by Kusumawati

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et al.[6], which loaded the propolis into the carbonated hydroxyapatite using the immersion method, found that the 10% propolis solution had the strongest chemical bond with carbonated hydroxyapatite. This experiment is in line with the study by Devitaningtyas et al.[7], which found that 10% propolis with carbonated hydroxyapatite had a strong antibacterial inhibition against *P. gingivalis bacteria.*

The largest active compounds in propolis are flavonoids and phenols. The phenolic content in propolis is usually called caffeic acid phenethyl ester (CAPE).[8] The CAPE compound can increase osteoprotegerin (OPG) in tissues and prevent osteoclastogenesis, as OPG works by preventing RANKL from binding to the receptor activator of nuclear factor-κβ (RANK).[9] Bone regeneration processes can be observed from bone formation biomarkers, one of which is RANK.[10] Carbonated hydroxyapatite can be combined with 10% propolis, which has anti-inflammation, anti-tumour and immunomodulator properties, to increase the bone graft material ability for regeneration by decreasing the RANKL expression pathway. This study aimed to determine the effect of the application of carbonated hydroxyapatite-10% propolis in an open flap debridement on RANKL expression.

MATERIALS AND METHODS

This study was an experimental study with a randomised control group design. The carbonated hydroxyapatite that was used in this study was Gama-CHA (PT. Swayasa Prakarsa, Yogyakarta, Indonesia). To acquired carbonated hydroxyapatite-10% propolis, the Gama-CHA block was divided into 10mg and immersed in 1ml of 10% propolis solution for 24 hours at room temperature.[6] The experimental animals that were used were nine male rabbits (*Oryctolagus cunicullus*) aged 5–8 months that weighed 1500–2000g. Periodontitis was induced in the experimental animals in the mandibular incisors using the ligation method with silk 3-0 and an injection of 0.05ml LPS *P. gingivalis* using a tuberculin needle into the interdental area three times a week for six weeks.[11] Clinical signs of the induced periodontitis that were observed in the rabbits were tooth mobility, gingival recession and redness of the gingiva. The sampling method was stratified random sampling. The experimental animals were divided into three groups after ligation. Group A had open flap debridement treatment, Group B had open flap debridement treatment with the application of carbonated hydroxyapatite and Group C had open flap debridement treatment with the application of carbonated hydroxyapatite-10% propolis.

Open flap debridement was done under anaesthesia using ketamine 40mg/kgBW and xylazine 5mg/kg BW. A sulcular incision was performed using scalpel no. 15 in the buccal sulcus of the mandibular central incisors, and the flap incision was then reflected using a small raspatorium. Debridement was performed on both soft tissues and hard tissues. Once OFD was done, irrigation with distilled water and flap repositioning was performed, followed by a suture using 4-0 nylon thread.[12] Groups B and C were given the same treatment; however, carbonated hydroxyapatite material was added to Group B and carbonated hydroxyapatite-10% propolis was added to Group C. After the treatment, the laboratory animals were administered soft food for 24 hours, tramadol at dose of 0.2–0.5mg/kgBW and one interflox antibiotic at a dose of 0.1 mg/kgBW after the treatment through intramuscular injection. One rabbit from each group was randomly selected to be decapitated on the seventh day after surgery, and the remaining rabbits were taken on the 14th day and 28th day after surgery. They were then euthanised using an intermuscular injection of an overdose of sodium pentobarbital, i.e. 120mg/kgBW. Mandibular decapitation was carried out and fixed with formalin before the mandible was cut to obtain mandibular incisor specimens and placed onto four microscopic slides. The immunohistochemistry examination was conducted using antibody polyclonal RANKL from Bioss USA to measure the RANKL expressions in the alveolar bone, which were viewed using a light microscope in 400x magnification on three different fields of view by two observers. Each field of view showed both positive and negative cells. The calculation used the following formula:[13]

$$\text{RANKL Expression} = \frac{\text{Number of Positive Cells}}{\text{Total Cells}} \times 100$$

The data was analysed using the software SPSS version 21 for Windows (IBM, Chicago, USA). The data analysis was performed using the normality test with the *Shapiro–Wilk* test. A homogeneous variation test was conducted to discover the data variation in the groups with Levene's test ($p>0.05$) with a two way-ANOVA and multiple comparison LSD test ($p<0.05$).

RESULTS

Figure 1 shows the alveolar bone of *Oryctolagus cuniculus* with a magnification of 400x at the seventh, 14th and 28th day in each group. Osteoblasts were found in the sides of the bone. Osteoblasts that positively expressed RANKL were marked by dark brown cytoplasm, whereas those that negatively expressed RANKL were marked by bright blue cytoplasm. The results from this experiment are shown in Table 1. The carbonated hydroxyapatite-10% propolis group had the lowest expression of RANKL.

The normality test showed that the RANKL expression in each treatment group and on each decapitation day had a significance greater than 0.05 ($p>0.05$). The homogeneity test also showed a significance greater than 0.05 ($p>0.05$). Based on the normality and homogeneity tests results, it could be concluded that the RANKL expression data was normally distributed and homogeneous. Therefore, a statistical test using the parametric test – two-way ANOVA – was carried out. The results of the two-way ANOVA

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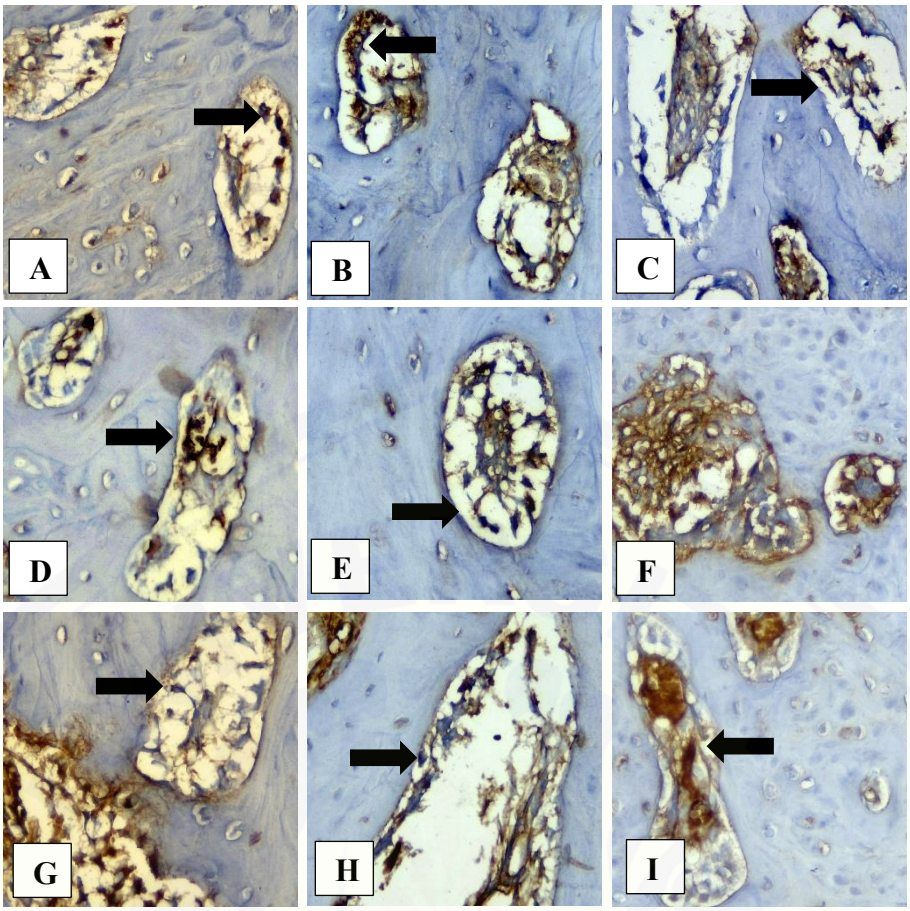

Figure 1. The expression of RANKL in the alveolar bone in each group on the 7th, 14th and 28th day of examination shows the following using a black arrow (osteoblasts which positively expressed RANKL were marked by dark brown cytoplasms): A. Group OFD 7th day; B. Group OFD 14th day; C. Group OFD 28th day; D. Group OFDCHA 7th day; E. Group OFDCHA 14th day; F. Group OFDCHA 28th day; G. Group OFDCHA-10% propolis 7th day; H. Group OFDCHA-10% propolis 14th day; I. Group OFDCHA-10% propolis 28th day.

Table 1. Mean and standard deviation of the RANKL expression

Groups	*X*±*SD*			P
	7th day	14th day	28th day	
OFD	43.21±8.72	30.79±2.76	46.77±6.35	
OFDCHA	42.90±4.52	26.08±4.66	37.49±6.81	0.00*
OFDCHA-10% propolis	22.60±6.77	15.670±6.42	14.42±2.97	

*significant (p<0.005)

Table 2. LSD test of the RANKL expression

Time Point	Group Treatment	OFD	OFDCHA	OFDCHA-10% Propolis
7th day	OFD		0.952	0.002*
	OFDCHA			0.002*
	OFDCHA-10% propolis			
14th day	OFD		0.203	0.002*
	OFDCHA			0.002*
	OFDCHA-10% propolis			
28th day	OFD		0.045*	0.000*
	OFDCHA			0.000*
	OFDCHA-10% propolis			

* significant (p<0.005)

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showed that there were significant differences ($p<0.05$) (Table 1). The result of the post-hoc LSD test showed that the OFD followed by application of 10% propolis-carbonated hydroxyapatite (OFDCHA-10% propolis) group had a significant value ($p<0.05$) compared to the OFD and OFD followed by application of carbonated hydroxyapatite (OFDCHA) groups at all time points (Table 2), as well as the OFDCHA to the OFD group at the 28th day ($p<0.05$). The OFDCHA group compared to the OFD group at the seventh and 14th day was not significant (Table 2).

DISCUSSION

RANKL is a cytokine that regulates bone remodelling and is expressed by osteoblast cells.[2] The RANKL expression is stimulated by cytokines that bind to gp130 signal transducers, such as IL-6, IL-1 and TNF-α. With periodontitis, increasing IL-6, IL-1 and TNF-α will stimulate *activators of transcription* (STAT) dan *mitogen-activated protein kinase* (MAPK) in osteoblast cells, so that the RANKL expression will increase and stimulate ostoclastogenesis.[14,15] Carbonated hydroxyapatite is a strong drug delivery system because it has a uniform pore size, high pore volume, mesoporous (2–5nm) and a large surface area. The –OH group in carbonated hydroxyapatite is an active compound that binds with the bioactive molecule on propolis and creates a hydrogen bond. These hydrogen bonds make the carbonated hydroxyapatite easier to load and release the propolis molecules.[16]

The results of the study showed that the RANKL expression in the group that was treated with OFD and had the application of carbonated hydroxyapatite-10% propolis was the lowest compared to the OFD and OFDCHA groups at all observation time points on day seven, 14 and 28. This indicates that propolis that is incorporated into carbonated hydroxyapatite decreases the RANKL expression in the alveolar bone of rabbits until day 28. The results of this study are in line with the study by Andrade et al.[17], which showed that propolis-incorporated alloplastic bone graft material has good porosity and is able to release active substances until the 30th day. The addition of propolis to a carbonated hydroxyapatite graft material aims to boost the performance of the graft material by reducing the inflammatory response and providing osteoinductivity. Propolis has antibacterial, antiviral, antifungal, anti-tumour and immunomodulatory properties.[18] Propolis is able to reduce the RANKL expression by activating Wnt signalling. Activation of canonical Wnt signalling leads to β-catenin over-expression in cytoplasms, which is translocated to the nucleus of osteoblasts. An increase in the β-catetnin expression suppresses the RANKL expression.[19] Furthermore, propolis is anti-inflammatory as it decreases pro-inflammatory cytokines, such as IL-1β, IL-1, IL-8 and TNF-α. Cytokine cause periodontal destruction by increasing the RANKL expression and inducing osteclastogenesis.[20]

The RANKL expression of the OFDCHA-10% propolis group decreased from day seven to day 28. This indicates that propolis addition can reduce the RANKL expression from the seventh day, whereas the reduction in the other groups began to take place on the 14th day. Day seven is the end of the inflammatory phase and the start of the proliferation phase. At the end of the inflammatory phase, the RANKL expression declines because the osteoblasts are preparing to secrete bone matrix. This is in line with research by Steen et al.[21], which showed that propolis could suppress the RANKL expression at the beginning of inflammatory phase between days three to six. This is in line with the study by Tang et al.[22], which showed that propolis has the ability to suppress and regulate RANKL in three phases of osteoblast development. Although there was an increase during the proliferation phase, this was not significantly different to the mineralisation and maturation phases. This condition allows osteoblasts to secrete more bone formation matrices, which optimises tissue regeneration. In addition, the caffeic acid phenethyl ester (CAPE) in propolis triggers the osteoclastogenesis process by inhibiting osteoclastogenesis at the early stage of differentiation by suppressing RANKL-induced NF-kB activation.[23] The limitations of this research are that there were no baseline conditions and the duration of the experiment was limited. The conclusion from the study was that combining 10% propolis with carbonated hydroxyapatite in OFD treatment can decrease the RANKL expression in a rabbit's alveolar bone. It is necessary to conduct further research on the effect of the application of carbonate hydroxyapatite-10% propolis in open flap debridement with a longer period of observation.

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Research Report

Oral health profile of the elderly people in the Pandalungan community

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ABSTRACT

Background: *The Pandalungan community is a unique community established through the assimilation of two dominant cultures: the Javanese and Madurese. Both of these communities created a community with a new culture called the Pandalungan community culture. The people of this community live in coastal, rural and urban areas. Generally, research on the uniqueness in the oral health behaviour of the Pandalungan community has not been conducted since the oral health practices of the Pandalungan community are considered to be the same as that of the Javanese community.* ***Purpose:*** *In order to develop programmes for oral health prevention, this research aims at comparing the oral health profiles of the elderly (classified as per age) living in the rural and urban areas in the Jember Regency.* ***Methods:*** *The research employs a cross-sectional approach. The subjects of the research were selected on the basis of the total number of elderly people who attended the monthly meetings of the Karang Werda (those not willing to participate in the study were excluded). The study was conducted by organising extensive interviews, performing observations and intraoral examinations. Each group was classified into three subgroups on the basis of age: pre-elderly, elderly and high-risk elderly. The intraoral examination conducted included the oral hygiene index-simplified (OHI-S), the number of teeth missing, the depth of the pocket and the number of all functional tooth units (all-FTU).* ***Results:*** *The oral health profile of people in the rural community was poor when compared to the oral health profile of people living in the urban community (by accounting for nearly all the variables in the examination).* ***Conclusion:*** *The oral health profile of the elderly people in the Pandalungan community was poor. Adequate prevention and care are essential to maintain the oral health of people in the Pandalungan community.*

Keywords: *elderly; Javanese; Madurese; oral health; Pandalungan*

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INTRODUCTION

The Pandalungan community was established with the combination of two dominant cultures: Javanese and Madurese cultures.[1] Both cultures have different characteristics. The Javanese community is widely known as a community in which people speak softly and politely and live in harmony.[2,3] The Madurese community is known for being more religious and tough on defending dignity, even resorting to violence to resolve problems.[4] Both of these communities mingled and established a new culture called the Pandalungan community culture. Administratively, the Pandalungan community lives in the Eastern part of the East Java province, which includes districts and cities, such as Pasuruan, Probolinggo, Lumajang, Jember, Bondowoso, Situbondo and North and South Banyuwangi.[1] Some people live in coastal, urban and rural areas. Many people from this community lack education and have inadequate financial assistance. In general, the elderly in the rural areas work as farm workers, gardeners and fishermen,[5] while those who live in the urban areas work in many sectors.[1,6,7]

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Cultural behaviour has important implications for human health. The role of culture in a social system is shared among individuals and groups by sharing knowledge, beliefs and/or different practices between group members.[8] Thus, occasionally, a new behaviour is formed. Several aspects can affect the speed and change the way a new behaviour is formed, such as socioeconomic status, gender, religion and moral values. These factors play a role in changing dynamically the behavioural patterns pertaining to health among community members.[9–12]

Culture plays a crucial role in promoting health among the elderly. Foster and Anderson[13] state that culture might influence the health of an individual through many ways, including (1) influence through traditions (2) ethnocentric attitude (an attitude that regards one's own culture as the best), (3) values and norms in the community that influence and set out what is considered as the best behaviour, (4) pride in the group's status, (5) the influence of values that are inherited by the members in a community as part of socialisation, (6) fatalistic attitude (an attitude where members do not seek immediate help or treatment and instead give up) and (7) the consequences of innovation on healthy behaviour.[13] Changing the dental and oral health practices of a community is difficult. Additionally, what makes the change even more difficult is that these behaviours are tied to a culture that has existed for a long time.

Annually, there has been an increase in the number of elderly people in Indonesia. The percentage of elderly in the Jember District in 2010 was 10.85%. In 2020, this percentage reached 14.30%.[14] If a large number of elderly people are not taken care of properly, it will have an impact through an increase in the morbidity rate and an increase in the cost of health care services (individual, family and government funds). On the basis of this reasoning, the elderly would burden those who are young.[15,16] Although age restrictions are placed in research and health planning, there is no general agreement about age limits.[17] Generalised age thresholds are used as an indicator for deciding old-age thresholds and also making biological age assumptions. However, until now, there has been no definite agreement in any country to mark an old-age threshold. This is, presumably, because the development of old age is not always proportional to biological age.[18] Therefore, in this study, we have employed some modifications to decide on the age limit among the elderly in Indonesia.[17,18] The elderly were classified into the young elderly (middle age, ages between 45 and 59 years), elderly (ages between 60 and 74 years), old elderly (ages between 75 and 90 years) and the very old elderly (over 90 years of age).[19,20]

Dental problems in the elderly are different from those found in other ages.[21] Usually, elderly people have poor oral health status. Oral health cannot be separated from the overall health of the body. Poor oral health may cause difficulty during chewing and lead to nutritional disturbances (which leads to diseases all over the body).[22–24]

This research focused on the study of oral health status among the elderly in the Pandalungan community. The examination was conducted using the modified oral hygiene index-simplified (OHI-S),[25] the depth of the pocket,[26,27] the number of teeth missing[17] and the number of all-FTU.[13] These important indicators influence the function of mastication.[28] In order to develop programmes for oral health prevention and care, this research aims at comparing the oral health profiles of the elderly (classified as per age) who live in the rural and urban areas of the Jember Regency. The findings from the study will provide basic data for providing optimal oral healthcare (based on the special needs of the elderly community).

MATERIALS AND METHODS

The research employs a cross-sectional descriptive approach to verify the oral health profile and treatment needed for elderly people in the rural and urban areas of the Pandalungan community. The area where the elderly people lived was randomly assigned to reduce bias. The research subjects arrived at on the basis of the total number of elderly people who attended the monthly meetings of the Karang Werda (a platform to support the welfare needs and accommodate the activities of the elderly). Those who were not willing to participate in the study were excluded. The study was conducted by organising extensive interviews and performing observations and intraoral examinations. The elderly in the Pandalungan community were classified based on the location where they lived: rural or urban areas in the Jember District. Each group was further classified into three subgroups on the basis of age: pre-elderly, elderly and high-risk elderly.[16] The rural areas comprised the Puger-Grenden village and the Sucopangepok village (n = 90). The urban areas comprised the Kaliwates sub-district and Sumbersari (n = 78). The elderly respondents in each area were arrived at by using a total sampling technique in which all of the elderly who attended the Karang Werda meetings willingly took part in the research and were categorised into three sub-groups based on their ages: (1) pre-elderly, ages between 45 and 59 years old (n = 66); (2) elderly, ages between 60 and 74 years old (n = 87); and (3) elderly at high risk ≥ 75 years old (n = 15). All of the respondents completed the informed consent form. Ethical clearance was carried out by the Ethics Commission of the Faculty of Dentistry at the University of Jember (number 924/UN25.8/KEPK/DL/2019).

The respondents for this research were interviewed by employing the interview guidelines provided by the Center of Environment, Ageing and Health 2018.[21] The interviews were conducted to examine oral hygiene habits, including tooth brushing frequency and methods as well as other procedures related to the risk of tooth loss. Oral health practices (guided by oral health surveys from the World Health Organization, 2013[16]) were examined, such as dental and oral hygiene using OHI-S based on the

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Greene–Vermillion index,[25] pocket depth,[26,27] the number of teeth lost[17] and the number of all-FTU.[28] Teeth in the elderly were measured by examining 28 teeth in their mouth cavities and by removing the wisdom molar teeth. The measurement of OHI-S was obtained by the addition of index debris and calculus covering the tooth's surface (with some modifications, such as tooth loss, remaining tooth, edentulous and extruded tooth). Considering the criteria for OHI-S, a good score of oral hygiene ranged between 0.0–1.2, a moderate score of oral hygiene ranged between 1.3–3.0, and a poor score of oral hygiene ranged between 3.0–6.0.[15] The criteria for a healthy pocket depth was ≤3 mm, medium criteria was 4–5 mm and heavy criteria was ≥6 mm.[28]

All-FTU estimations were based on the total number of functional tooth units (FTU), defined as a similar, natural tooth pair and/or the opposite of a replaced tooth (anterior and posterior) that could be supported by an implant, dental bridge pontics or removable prosthetics.[28] The total FTU was divided into six categories: natural to natural teeth (nt-FTU), natural teeth to fixed prosthetic (nf-FTU), natural teeth to removable prosthetic (nr-FTU), fixed prosthetic to fixed prosthetic (ff-FTU), fixed prosthetic to removable prosthetic (fr-FTU) and removable prosthetic to removable prosthetic (rr-FTU). This estimation did not include the third molar teeth, a tooth with wide coronal destruction, tooth loss and a tooth that had contact with a non-similar tooth (the latter three aforementioned categories of tooth were categorised as non-functional). The molar tooth was considered as two units, and, thus, right–left was eight units. The premolar and anterior teeth were considered as one unit. The premolar right–left was considered as four units. Therefore, the total number of FTU in the mouth cavity was 18 units/intact dentition.[28]

Data analysis was conducted using a statistical product and service solutions (SPSS, version 22) (IBM, New York, USA). The data was tabulated and tested for homogeneity using the Levene test, followed by a t-test to determine differences in oral health profiles between the elderly people (based on age group) residing in the urban and rural areas of the Pandalungan community.

RESULTS

The data presented in Figure 1 pertains to respondents who willingly joined the research. The percentage of female and male respondents was 83.93% and 16.07%, respectively. Based on this data, it can be observed that the Karang Werda monthly meetings were predominantly attended by elderly women.

Additional, the elderly in rural areas had a poor OHI-S score (Table 1), pocket depth (Table 2), tooth loss (Table 3) and all-FTU (Table 4) when compared to the elderly in urban areas (considering all ages). The result of the t-tests revealed a significant difference, especially in the pocket depth and all-FTU among the elderly (ages between 60 and 74 years) in urban and rural areas ($p < 0.05$).

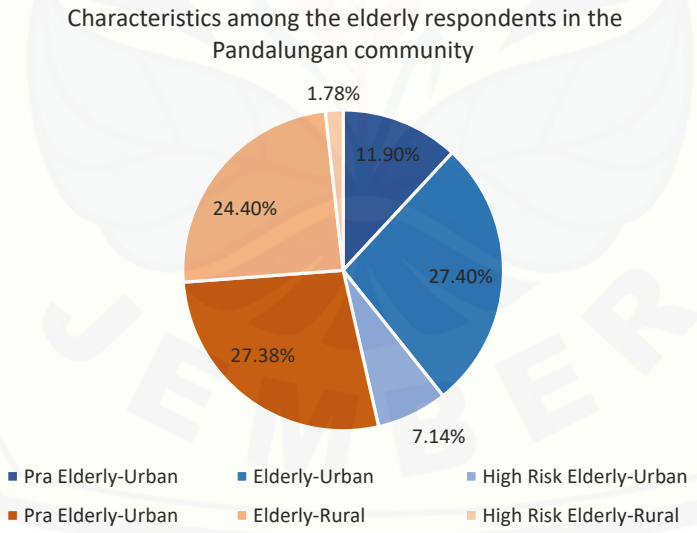

Figure 1. Characteristics among the elderly respondents in the Pandalungan community.

Table 1. Average OHI-S among the elderly in the rural and urban areas in the Pandalungan community.

Elderly Groups	Urban		Rural		p-*value*
	n	X ± SD	n	X ± SD	
Pre-Elderly	20	1.56 ± 1.45	46	1.70 ± 1.30	0.720
Elderly	46	0.94 ± 5.15	41	2.67 ± 1.75	0.189
High-Risk Elderly	12	1.28 ± 0.93	3	1.67 ± 1.53	0.900
Total	78		90		

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DISCUSSION

The elderly women in the Pandalungan community (rural and urban areas) were more active in joining the Karang Werda monthly meetings when compared to the elderly men. This is because the number of elderly women was higher than the number of elderly men.[29] Mamai-Homata et al.[30] stated that women are more concerned about their health than men. Hamzah et al.[31] also declared that a person who is concerned about their health adopts healthy behaviour. Elderly men were more inclined to passively partake in activities conducted during the Karang Werda monthly meetings; the reason for this could be due to a smaller percentage of elderly men attending the Karang Werda monthly meetings.

The elderly people in the rural areas had a higher OHI-S score, indicating that their oral hygiene was poor when compared to the elderly from the urban areas (considering all ages). However, no significant difference was observed in the OHI-S scores among the elderly in the rural and urban areas. The reason for this could be due to the improved dental facilities and better dental information provided in the urban areas when compared to the rural areas. Based on the interviews, pre-elderly people in rural areas brushed their teeth twice a day and used mouthwash; however, there was no improvement in their oral hygiene. An assumption that could be made from this finding is that their toothbrushing technique was not effective. In addition, some elderly and high-risk elderly people mentioned that they did not brush their teeth, as they encountered pain while brushing (because of the high number of tooth loss). These concerns of the elderly and high-risk elderly are supported by a previous study that found that the elderly people who had a high number of missing teeth had difficulties in cleaning the remaining tooth root.[32] Furthermore, on the basis of the findings from the interview, these two groups (elderly and high-risk elderly) said that they only rinsed their mouth when performing wudu (an Islamic procedure for washing parts of the body prior to salah) and had been following instructions handed over to them by their parents for years. From these findings, it is essential to devise a suitable and novel method for cleaning the oral cavities in the elderly who have a lot of missing teeth.

With the passage of time, the culture in the Pandalungan community is undergoing both social and cultural changes in families and societies due to improvements and changes in the educational, economic and health sectors, as evidenced by the practices followed by the present generation of family members in both rural and urban areas. The social and cultural values that have been maintained in the Pandalungan community are also undergoing more positive changes,[33] particularly in the urban areas. These findings are indicated in the t-test results of oral hygiene among the elderly people (between 60 and 74 years of age in the urban areas).

Table 2. Average pocket depth among the elderly in the rural and urban areas in the Pandalungan community.

Elderly Groups	Urban		Rural		p-*value*
	n	X ± SD	n	X ± SD	
Pre-Elderly	20	0.80 ± 0.89	46	0.91 ± 0.83	0.900
Elderly	46	0.59 ± 0.65	41	1.02 ± 0.69	0.007*
High-Risk Elderly	12	0.55 ± 0.69	3	0.33 ± 0.58	0.660
Total	78		90		

* significant different

Table 3. Average of tooth loss among the elderly in the rural and urban areas in the Pandalungan community

Elderly Groups	Urban		Rural		*p-value*
	n	X ± SD	n	X ± SD	
Pre-Elderly	20	3.50 ± 2.50	46	4.37 ± 5.17	0.536
Elderly	46	4.67 ± 4.11	41	6.07 ± 6.38	0.216
High-Risk Elderly	12	9.36 ± 7.67	3	15.67 ± 7.77	0.005*
Total	78		90		

* significant different

Table 4. Average of all-FTU among the elderly in the rural and urban areas in the Pandalungan community

Elderly Groups	Urban		Rural		*p-value*
	n	X ± SD	n	X ± SD	
Pre-Elderly	20	8.74 ± 3.77	46	8.00 ± 5.73	0.558
Elderly	46	7.59 ± 5.15	41	5.12 ± 4.38	0.023*
High-Risk Elderly	12	4.91 ± 5.65	3	2.00 ± 2.89	0.320
Total	78		90		

* significant different

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As per the findings in the periodontal pocket depth examination (Table 2), the elderly people in the rural areas had a significantly deeper pocket depth compared to those in the urban areas. Razak et al.[32] explained that an increase in age has a bearing on the duration of the periodontal tissue that is exposed to the dentogingival bacterial plaque (which indicates a history of individual oral cumulative). Accumulation of bacterial plaque can cause mild to moderate alveolar bone resorption, resulting in deeper periodontal pockets, tooth mobility and can eventually lead to tooth loss.[34]

Table 3 indicates that tooth loss is directly proportional to age among elderly people. A higher incidence of tooth loss is observed in rural areas than in urban areas. These findings may be related to the data shown in Tables 1 and 2, which indicates poor oral hygiene and deeper periodontal pockets. The results of the t-test indicate there is no significant difference in the pre-elderly and elderly groups in both rural and urban areas. The average number of tooth loss in rural areas is significantly more (twice than the average number of tooth loss in urban areas). A higher number of tooth loss leads to progressive changes in the structure and function of the oral cavity, including masticatory efficiency, and can thus affect the general health.[21–23,32,33]

With regards to tooth loss, elderly people can undergo a reduction in all-FTU, which plays an important role in mastication. Based on the measurements carried out on all-FTU, the number of all-FTU among the elderly is smaller in rural areas than in urban areas (considering all ages) (Table 4). The findings reveal that the number of teeth used for mastication decrease, which leads to a decline in masticatory efficiency. These findings correspond with a study conducted by Shinsho[35], who revealed that a minimum of 20 healthy, natural teeth are required for avoiding masticatory difficulty among the elderly people.

Table 4 indicates that there is no significant difference in measurement of all-FTU in the pre-elderly and high-risk elderly in both rural and urban areas, while there is a significant difference in the elderly people. This difference could be attributed to a high number of the crown, or tooth loss or changes in tooth position. To obtain a higher number of all-FTU, dental filling and tooth replacement with a dental fix or a removable denture is required.[32] Elderly people who live in rural areas, usually, prefer to opt for the services of an illegal dental practice, offering a direct, partial or a full denture and a door to door service (despite the quality of the treatment being poor and not in accordance with health standards) than visit a licensed dentist. The elderly also do not need to stand in queues to obtain treatment, which is more convenient.

Based on the above results, it appears that the dental and oral health of the elderly in the Pandalungan community is still poor. Findings from researches conducted in several countries indicate that the dental health services among the elderly are still inadequate, especially the elderly who lack education and are facing socio-economic limitations.[36] The elderly, usually, possess physical disabilities that make it difficult for them to brush their teeth thoroughly and effectively.[32,37] The elderly also face difficulties in grasping the handle of a conventional toothbrush.

The findings from this study present a big challenge for dental care among the elderly, especially the elderly, in the Pandalungan community. The elderly need support to practise oral health procedures that take into account the actual oral health of the elderly. Self-care related to oral hygiene for the elderly is challenging and includes multiple factors, including cultural values that have long influenced the health and behaviour of the elderly. Therefore, the data pertaining to the oral health profile of an elderly person must be accessed in order to develop future plans for dental and oral health services among the elderly. This study has not identified all the factors that could affect the ability of the elderly in the Pandalungan community to carry out comprehensive oral care.

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Research Report

The difference between Begg and Straightwire appliances on molar position, occlusal plane angle, and anterior and posterior facial height changes

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ABSTRACT

Background: *Bimaxillary and bidental protrusion Class I Angle malocclusions have a characteristic convex facial profile and protrusion lips due to the labial inclination of the anterior teeth. Extraction of the first four premolars is the most common choice for orthodontic treatment of these cases when all the permanent teeth are complete and in good condition. Orthodontic treatment can be performed using the Begg or Straightwire techniques.* ***Purpose:*** *This study aims to investigate the difference in the effect of orthodontic treatment with Begg and Straightwire appliances on molar position, occlusal plane, and anterior and posterior facial height.* ***Methods:*** *Sixty pairs of lateral cephalograms before and after the treatment of patients with bimaxillary and bidental protrusive Angle malocclusion Class I, aged 18–35 years old, who underwent orthodontic treatment using the Begg and Straightwire techniques with the extraction of all first premolars that met the inclusion criteria. Data analysis was performed using two-way repeated analysis of variance ($p<0.05$) and Pearson correlation ($p<0.05$).* ***Results:*** *Molar position, occlusal plane angle, and anterior and posterior facial heights increased significantly after the Begg technique treatment and decreased significantly after the Straightwire technique treatment ($p<0.05$), but there were no significant differences between the four variables in the two techniques ($p>0.05$). Medium correlation was found between variables in both the Begg and Straightwire techniques.* ***Conclusion:*** *Molars were extruded and mesialized and the occlusal plane angle and height of the anterior and posterior faces increased after the Begg appliances treatment. The molars moved mesially and occlusally and there was a decrease in the occlusal plane angle, as well as the height of the anterior and posterior faces, after treatment with the Straightwire appliances. However, there was no difference between the two techniques.*

Keywords: *Bimaxillary and bidental protrusion; fixed orthodontic treatment; molar position; occlusal plane; facial vertical height*

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INTRODUCTION

Malocclusion is a condition deviating from the normal occlusion that occurs due to a discrepancy between the dental arch and the jaw arch.[1] This situation can occur in the upper and lower jaw and results in disturbances in chewing, phonation and aesthetics.[1–3] The prevalence of Class I Angle malocclusion in the Indonesian Deutero-Malay population is 48.8%.[4,5] Class I bimaxillary protrusion malocclusion has a convex profile.[2] The orthodontic treatment objective is the correction of the malrelation and malposition of teeth to achieve stable occlusion function and pleasant facial aesthetics.[2,6]

The vertical dimension of the face of the patient undergoing orthodontic treatment is an important aspect to consider because it determines facial aesthetics.[7] The height of the vertical dimension of the face is influenced by the angle of the occlusal plane, the height of the anterior face, the height of the posterior face and the movement of the molar in the horizontal and vertical directions.[8–10]

The Begg technique is a fixed orthodontic treatment technique that has long been used.[11] Round section

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archwires provide the free tipping motion of crowns.[11,12] The function of the anchorage bend is to open the anterior bite and control anchorage, thereby preventing the tipping of the mesial anchoring molars.[11,12] Correction of a malocclusion Angle Class I bimaxillary protrusion uses Z elastic (intramaxillary elastic combined with intermaxillary elastic Class II) from the first stage of treatment.[11–13] The anchorage bend creates a force vector that acts on the anterior mandibular teeth down and front, whereas on the mandibular molars it is down and back.[10,11,14] Intermaxillary elasticity causes forward and upward force vectors on the mandibular molars as well as down and backward on the anterior mandibular teeth.[10,11,14] An extrusion of the molars will result in a rotation of the mandible backwards and downwards so that the occlusal plane angle increases and the facial height increases.[10,11,14–16]

The Straightwire technique is one of the orthodontic treatment techniques.[2] Orthodontic tooth movement uses sliding mechanics.[1,2] Stainless steel bow wire measuring 0.016 x 0.022 inches is used at the anterior retraction stage so that there is bodily tooth movement, and maximum anchorage is required.[1–3] Maximum anchorage is obtained by bonding the buccal tube to the first and second molars.[2,3] A gable bend is used during retraction functions to increase anchorage control in the molars.[2,3,17] The vector of forces acting on the mandibular molars is forward and downward, whereas in the anterior mandibular teeth it is backward and upward due to archwire deflection.[17] A gable bend on the mesial buccal tube will create a forward and upward force vector of the mandibular molar, whereas in the anterior teeth the force vector is forward and down.[17] Anchorage loss causes the molars to move mesially and there is a forward and upward rotation of the mandible resulting in smaller mandibular plane angles and a shortening of facial height.[17–19]

Alkumru et al. disclosed that the vertical dimension of the face is not affected by the movement of the molar to the mesial.[20] The research results of Tarvade et al. are contrary to the popular opinion that the Begg technique causes greater vertical dimensional height increases than the preadjusted appliances technique (Edgewise and MBT).[15] Based on the data that has been described, it is necessary to conduct a study to investigate the differences between the orthodontic treatments using the Begg and Straightwire techniques on molar position, angle of the occlusal plane, and anterior and posterior facial height.

MATERIALS AND METHODS

An ethics permit was obtained from the Research Ethics Commission of the Faculty of Dentistry, Universitas Gadjah Mada with the number 00435 / KKEP / FKG-UGM / EC / 2020. The research object was secondary data, in the form of an initial 120 lateral cephalograms followed by fixed orthodontic treatments using the Begg or Straightwire techniques, which are appropriate standard requirements by the Faculty of Dental Surgery, The Royal College of Surgeons of England, which provide a clear contrast and sharpness of the image.[21] Lateral cephalograms were calibrated using Corel Draw X5 (Corel Corp., Ottawa, Canada). Inclusion criteria: a. 18–35 years old; b. Angle Class I malocclusion is bimaxillary and bidental protrusion; c. ANB angle 0°–4°; d. upper and lower lips in front of the S line; e. index of orthodontic treatment need/IOTN (Dental health component/DHC) scores 1–3; f. complete number of teeth except third molars; g. network periodontal healthy; h. do not have systemic diseases; i. treatment plan the first four premolars were removed. Exclusion criteria: a. anodontia; b. there are edentulous; c. badly crowded teeth; d. impacted other than the third molars.

Determination of the position of the left mandibular first molar in the horizontal direction was calculated using the Pancherz parameter, namely the linear distance from the mesiobuccal molar cusp to the vertical mandible (Figure 1a). The position of the left mandibular first molar in the vertical direction was calculated using the Pancherz parameter, namely the linear distance of the mesiobuccal molar cusp to the horizontal mandible (Figure 1b). The position of the left maxillary first molar in the horizontal direction was calculated using the Pancherz parameter, which is the linear distance between the mesiobuccal molar cusp to the maxillary vertical (Figure 2a). The position of the first molar of the left maxilla in the vertical direction was calculated using the Pancherz parameter, namely the linear distance of the mesiobuccal molar cusp to the horizontal maxillary (Figure 2b). The occlusal plane angle was calculated using the Steiner parameter (Figure 3a), which is the angle formed from the occlusal plane (overlapping lines of the first molar and premolar) and sella-nasion. The anterior face height was calculated using the Gebeck parameter (Figure 3b), which is the distance between the palatal plane (ANS-PNS) perpendicular to the menton. The posterior facial height was calculated using the Gebeck parameter (Figure 3c), which is the distance between the articular and the mandibular plane (gonion-menton).

The data obtained in this study were tabulated and tested for normality and homogeneity, then analysed using

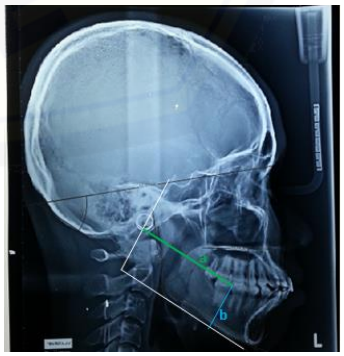

Figure 1. Measurement of the horizontal mandibular molar position (a) and vertical mandibular molar position (b) using Corel Draw X5.

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Figure 2. Measurement of the horizontal maxillary molar position (a) and vertical maxillary molar position (b) using Corel Draw X5.

Figure 3. Measurement of the angle of the occlusal plane (a), and height of the anterior face (b) and posterior face (c) using Corel Draw X5.

the parametric test. The change in molar position, angle of the occlusal plane, anterior facial height and posterior facial height before and after orthodontic treatment were analysed by means of a two-way repeated analysis of variance (ANOVA). The relationship between changes in molar position, angle of the occlusal plane, and height of the anterior face as well posterior facial height after orthodontic treatment were analysed using Pearson's parametric correlation and regression. The level of trust that was used in the study was 95% ($\alpha = 0.05$). Analysis was carried out using the Statistical Package for Social Science (SPSS) (IBM, Illinois, US) version 23.

RESULTS

The results showed an increase in vertical maxillary molars, vertical mandibular molars, occlusal plane angle, and anterior and posterior facial height after the Begg technique orthodontic treatment. Decreased horizontal maxillary molars, horizontal mandibular molars, occlusal plane angle, and anterior and posterior facial height were found after fixed orthodontic treatment with the Straightwire technique (Table 1).

There were significant differences between molar positions, occlusal plane angles, and anterior and posterior facial heights after treatments using the Begg and Straightwire techniques ($p = 0.000$) (Table 2). There was no significant difference ($p > 0.05$) in molar position, occlusal plane angle, and anterior and posterior facial height after the Begg and Straightwire orthodontic treatments (Table 3).

Table 1. Mean (x) and standard deviation (SD) values of molar positions, angle of the occlusal plane, and height of the anterior and posterior faces of subjects with orthodontic treatment using the Begg and Staightwire techniques

Variable	Mean ± Standard deviation (SD)			
	Begg		Straightwire	
	Before	After	Before	After
Horizontal maxillary molar (mm)	66.02±6.60	64.94±5.72	67.30±9.34	66.43±7.07
Vertical maxillary molar (mm)	22.25±2.10	22.92±2.41	22.52±2.93	22.88±2.79
Horizontal mandibular molar (mm)	68.35±6.78	67.37±5.54	70.13±9.58	69.66±8.60
Vertical mandibular molar (mm)	27.80±3.48	28.24±3.89	27.54±4.49	28.65±3.76
Occlusal plane angle (°)	22.27± 6.62	23.75±5.29	23.74±5.63	22.61±4.44
Anterior facial height (mm)	64.78±5.26	66.43±5.44	68.16±8.06	66.55±6.46
Posterior facial height (mm)	41.94±5.50	43.17±7.29	41.62±4.96	40.81±4.94

Table 2. Two-way repeated ANOVA test results, molar position, occlusal plane angle, and anterior and posterior facial height of subjects with orthodontic treatment using the Begg and Straightwire techniques

Group	F	Sig.
Treatment stage	73.829	0.000*
Treatment stage * type of treatment	3.296	0.075
Treatment effect	1327.033	0.000*
Effect of treatment * type of treatment	1.417	0.207
Treatment stage * treatment effect	1275.541	0.000*

* significant difference $p < 0.05$

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Table 3. The results of the two-way repeated ANOVA test for molar position, occlusal plane angle, and anterior and posterior facial height between the Begg and Straightwire techniques

Variable	df	F	Sig.
Type of treatment	1	0.742	0.392

* significant difference p <0.05

Table 4. Pearson correlation of fixed orthodontic treatment using the Begg and Straightwire techniques

	Begg		Straightwire	
	Correlation coefficient	Sig.	Correlation coefficient	Sig.
MMxH-MMxV	0.442	0.020	0.442	0.020
MMxH-MMdH	0.442	0.020	0.412	0.020
MMxH-MMdV	0.442	0.020	0.442	0.020
MMxH-SBO	0.415	0.018	0.401	0.020
MMxH-TWA	0.456	0.010	0.455	0.010
MMxH-TWP	0.373	0.023	0.363	0.023
MMxV-MMdH	0.443	0.020	0.423	0.020
MMxV-MMdV	0.443	0.020	0.443	0.020
MMxV-SBO	0.411	0.024	0.411	0.024
MMxV-TWA	0.491	0.006	0.481	0.006
MMxV-TWP	0.414	0.021	0.373	0.023
MMdH-MMdV	0.420	0.020	0.420	0.020
MMdH-SBO	0.391	0.022	0.391	0.022
MMdH-TWA	0.605	0.000	0.565	0.000
MMdH-TWP	0.365	0.022	0.363	0.023
MMdV-SBO	0.420	0.017	0.389	0.022
MMdV-TWA	0.620	0.000	0.590	0.000
MMdV-TWP	0.425	0.014	0.373	0.023
SBO-TWA	0.420	0.017	0.412	0.019
SBO-TWP	0.397	0.021	0.389	0.024
TWA-TWP	0.404	0.019	0.414	0.019

Information:
MMxH: Horizontal maxillary molars MMxV: Vertical maxillary molar SBO: occlusal plane angle
MMdV: Vertical mandibular molar MMdH: Horizontal mandibular molar SWA: Straightwire
TWA: anterior face height TWP: posterior face height

Table 5. Regression of fixed orthodontic treatment using the Begg and Straightwire techniques

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate
Begg	0.784	0.581	0.359	1.23654
Straightwire	0.759	0.529	0.098	1.67787

Table 6. The results of regression analysis of molar position, angle of the occlusal plane, and height of the anterior and posterior faces on the Begg and Straightwire appliances

	Begg			Straightwire		
	B	t	Sig.	B	t	Sig.
MMxH-MMxV	0.223	0.232	0.019	0.196	0.557	0.023
MMxH-MMdH	0.199	0.764	0.020	0.344	1.170	0.000
MMxH-MMdV	0.158	0.272	0.028	0.383	1.677	0.017
MMxH-SBO	0.106	0.350	0.010	0.211	0.800	0.032
MMxH-TWA	0.194	0.805	0.019	0.239	1.031	0.013
MMxH-TWP	0.356	0.556	0.013	0.281	0.233	0.011
MMxV-MMdH	0.258	0.138	0.008	0.383	0.557	0.023
MMxV-MMdV	0.116	0.189	0.010	0.212	1.170	0.000
MMxV-SBO	0.159	0.033	0.015	0.196	0.715	0.022
MMxV-TWA	0.181	0.318	0.025	0.265	0.752	0.020
MMxV-TWP	0.123	0.055	0.019	0.274	0.112	0.011
MMdH-MMdV	0.139	0.182	0.048	0.383	1.677	0.007
MMdH-SBO	0.139	0.329	0.045	0.101	0.800	0.032
MMdH-TWA	0.450	0.044	0.043	0.281	1.031	0.013
MMdH-TWP	0.379	0.605	0.021	0.239	0.455	0.033
MMdV-SBO	0.233	0.049	0.023	0.129	1.871	0.044
MMdV-TWA	0.638	0.422	0.006	0.426.	1.871	0.044
MMdV-TWP	0.379	0.264	0.011	0.426	0.126	0.039
SBO-TWA	0.214	0.025	0.013	0.204	0.273	0.048
SBO-TWP	0.146	0.030	0.019	0.124.	0.032	0.025
TWA-TWP	0.233	0.542	0.033	0.462	0.780	0.011

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The fixed orthodontic treatment using the Begg and Straightwire techniques showed a correlation in direction and moderate closeness. The effect of vertical mandibular molars on the angle of the occlusal plane has the greatest value when compared to other molars in the Begg technique. Horizontal maxillary molars have the greatest value when compared to other molars in the Straightwire technique (Table 4).

The contribution of molar position, anterior face height and posterior face height is 58.1% on the angle of the occlusal plane in the Begg technique and 52.9% in the Straightwire technique (Table 5). There was significant influence between the four variables in both the Begg and Straightwire techniques (p> 0.05). Each 1 mm increase in horizontal maxillary molars, vertical maxillary molars, horizontal mandibular molars and vertical mandibular molars caused the occlusal plane angles to increase by 0.106°, 0.159°, 0.139° and 0.233°. An increase in the angle of the occlusal plane by 1° cause the anterior and posterior facial heights to increase by 0.214 mm and 0.146 mm. The results of the regression analysis on the Straightwire technique showed that every 1 mm increase in the horizontal maxillary molar caused the occlusal plane angle to increase by 0.211° (Table 6).

DISCUSSION

This study found that there were changes in molar position, occlusal plane angle, and anterior and posterior facial height after orthodontic treatments with the Begg and Straightwire techniques. The after-treatment effect of the Begg technique causes the maxillary molars to extrude and move mesially, and the angle of the occlusal plane and the anterior and posterior facial height to increase, while the treatment effect of the Straightwire technique causes the maxillary and mandibular molars to move mesially and extrude, and the angle of the occlusal plane and the anterior and posterior facial height to decrease, which shows a statistically significant difference.

Molar extrusion and mesialization in the Begg technique are probably due to the use of intramaxillary elastic. Maxillary molars receive orthodontic force from the use of intramaxillary elastic for retraction and the use of an anchorage bend is intended to prevent anchorage loss as well as to avoid a deep bite due to anterior retraction.[10,11] Use of an anchorage bend to the mesial buccal tube causes the molar to tip distally. This force can be neutralised by using intramaxillary elastic as the molars will receive an anterior force from the intramaxillary elastic. The anchorage bend angle used in the treatment of the subject was 30–45° and dynamic because it was adjusted to the subject's overbite each time the control is carried out. Intramaxillary strength was ¼ light to ¼ medium or 2.5–4.5 oz. The maxillary molars remain in the initial position, but if the anchorage bend angle is too small or the use of the intramaxillary elastic is too strong, the maxillary molars can extrude and move mesially as in the results of this study.

The retraction in the Straightwire technique consists of two stages, namely canine retraction followed by anterior/incisor retraction, which can cause the molars to move mesially. Efforts to prevent molar mesialization include using a gable bend that serves as anchorage preparation. The decrease in the occlusal plane angle and facial height are due to molar mesialization. Maxillary and mandibular molars receive orthodontic force through the use of a powerchain and gable bend that are fixed during orthodontic treatment. The molars tip distally due to the use of a gable bend.[17] It is intended that the molars remain in their position when the retraction stage starts using the powerchain because the molars act as anchorage.[2,11] Not all orthodontists use a gable bend as this can make it easier for molars to move mesially. The use of force for retraction is 100–250 grams, however, the use of this force is less certain because a tension gauge is not used. A force that is too large is also one of the causes for a molar to move mesially.[11] The shorter height of the anterior face despite molar extrusion could be due to the vertical movement of the molar being smaller than the horizontal movement, or the molar extrusion being smaller than the mesial to the molar shift. The movement of molar to mesial can also be caused by the principle of bodily motion in the Straightwire technique so that the molar as anchorage moves mesial.[2,11] During the finishing stage of this technique, both box elastic and intermaxillary Class II elastic were used.[11,18] The use of elastics can cause the extrusion of molars. Other factors that lead to molar extrusion include placing the buccal tube deeply into the gingival.[2,11]

The changes that occurred after orthodontic treatment using both the Begg and the Straightwire techniques were not statistically significant. This could be due to the difference in the values of the two techniques that are not very dissimilar. The results of this study are the same as those of Tarvade et al., who stated that there was no significant difference in facial height increase between the Begg and preadjusted (MBT) technique treatment groups.[15] The results of the correlation test for fixed orthodontic treatment using the Begg and Straightwire techniques show that there was a moderate correlation between the angle of the occlusal plane, molar position, and anterior and posterior facial height. In the Begg technique, vertical mandibular molars have the greatest correlation with the angle of the occlusal plane compared to other molar positions, indicating that the tooth has the greatest influence on the angle of the occlusal plane. For each increase in vertical mandibular molars by 1 mm, there is an increase in the angle of the occlusal plane by 0.233°. This could be due to the use of intramaxillary elastic, Class II intermaxillary elastic and the use of an anchorage bend to open the anterior bite. Bratu et al. stated that when intermaxillary elastic is used throughout the day, the effect of the vertical component is much greater than that of the horizontal component.[22]

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In the Straightwire technique, horizontal maxillary molars have the greatest value compared to other molar positions. For each increase in horizontal maxillary molar by 1 mm, there will be an increase in the angle of the occlusal plane by 0.196°. A study by Chandra et al. reported that mandibular molars moved mesially by 2.26 mm.[23] This could be due to the application of excessive force and the eruption of the third molars.[23,24] Some of the study subjects had impacted third molars and some of the third molars had erupted. According to Nanda, a maximum anchorage with two molars as anchors will still have a 25% chance of moving the posterior teeth mesially.[17] This study used different cephalogram instruments. Efforts to overcome this, namely by calibration of the lateral cephalogram and a validity test of the head length (glabella-occipital), were carried out between the patient and lateral cephalogram. Suggestions for further research need to be carried out in prospective studies with the same cephalogram instrument.

Based on the research results, it can be concluded that the molars extruded and mesialized, and the occlusal plane angle and height of the anterior and posterior faces increased after the Begg technique treatment. The molars moved mesially and occlusally and there was a decrease in the occlusal plane angle and the heights of the anterior and posterior faces after treatment with the Straightwire technique. However, there was no difference between the two techniques.

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Research Report

Lemuru fish oil gel as host modulation therapy in periodontal ligaments induced with *Porphyromonas gingivalis*

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ABSTRACT

Background: *Periodontitis affects approximately 20%–50% of the global population and is caused by gram-negative bacteria, such as Porphyromonas gingivalis (P. gingivalis). Host modulation therapy (HMT) is part of a periodontal therapy that is used as an adjunct to conventional periodontal treatment to reduce tissue damage. Lemuru fish oil containing eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) can reduce the formation of matrix metalloproteinase species (MMPs) and will further increase the number of fibroblasts thereby stimulating collagen formation.* ***Purpose:*** *To determine the effect of lemuru fish oil gel on the collagen density and width of the periodontal tissue induced by P. gingivalis and the correlation between these parameters.* ***Methods:*** *Thirty male Wistar rats were divided into five groups. Induction of P. gingivalis was carried out first, then lemuru fish oil gel was applied to the gingival sulcus for 14 days, according to collagen scores in histological preparations using Masson's trichrome (MT). The width of the periodontal ligament was measured with an image raster program in µm. The data were analysed using statistics to test hypotheses using statistical product and service solutions (SPSS) version 24.* ***Results:*** *Significant differences in the results of the collagen density were observed between groups K- and K+ and groups K+ and P2. Meanwhile, no significant difference was observed between groups K- and P2, P3, P2 and P3 and K+ and P1. The mean values of the periodontal ligament widths were 299.61 ± 51.82µm (K-), 425.85 ± 61.54µm (K+), 346.93 ± 33.53µm (P1), 370.15 ± 49.42µm (P2) and 379.6 ± 49.26µm (P3).* ***Conclusion:*** *Lemuru fish oil can affect the width of the ligament and the collagen density with an optimal concentration of 20%. The correlation between the collagen density and the periodontal ligament width was negative and not significant.*

Keywords: *Collagen density; lemuru fish oil; periodontitis-width of the periodontal ligament*

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INTRODUCTION

Periodontal disease is a common issue, as seen in the Basic Health Research data published in 2018, which recorded the prevalence of periodontal disease in Indonesia reaching 73.1%–75%. Chronic periodontitis has been found to be one of the most widespread diseases among Indonesians.[1] The main factor causing periodontitis is anaerobic negative plaque bacteria. One of the most dominant bacteria found in chronic periodontitis is *Porphyromonas gingivalis* (*P. gingivalis*).[2]

P. gingivalis bacteria secrete endotoxin lipopolysaccharides (LPS), which can stimulate the activation of lymphocyte B cells to produce antibodies and stimulate the excretion of mediators by macrophages, including the tumour necrosis factor alpha (TNF-α), interleukin 1 (IL-1), interleukin 6 (IL-6), prostaglandin E2 (PGE2) and matrix metalloproteinase species (MMPs).[3] In pathological conditions such as inflammation, TNF-α prevents macrophages from excreting intermediates such as TNF-α, IL-1, IL-6, PGE2, and MMPs.[4] In pathological conditions such as inflammation, TNF-α

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inhibits macrophage activity. The inhibition of collagen synthesis and the presence of MMPs increase collagen destruction. Collagen is absorbed continuously and replaced by inflammatory cells so that damage occurs in the periodontal tissue and the collagen density decreases.[2,5] Untreated periodontitis can result in tooth loss. The goal of the treatment in periodontitis is to control bacteria as a local factor to minimise the systemic effects as a form of non-surgical treatment for periodontal disease.[6,7] Host modulation therapy (HMT) is part of a periodontal therapy that is used as an adjunct to conventional periodontal treatments such as scaling and root planning, and aims to reduce damage and regenerate the periodontal tissue by reducing the destructive aspects of the host response.[2,8]

The HMT properties can be found naturally in lemuru fish oil. Lemuru fish oil contains n-3 polyunsaturated fatty acids (PUFA), namely eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). This has the potential as an anti-inflammatory that works by degrading potent eicosanoids in the form of PGE2 and leukotriene B_4, so that the production of proinflammatory cytokines is inhibited. Lemuru fish oil will also affect growth factors by increasing the fibroblast growth factor (FGF), which plays a role in the proliferation of fibroblasts by stimulating collagen formation.[9,10] Research on the topical application of catfish oil with content similar to EPA and DHA to tooth extraction sockets, in concentrations of 5% and 10%, showed an increase in the inactivation and amount of the bone morphogenetic protein-2 (BMP-2), which was most effective at a concentration of 10%. However, in the available research on lemuru fish oil with topical administration, there are no references proving which concentration is effective in curing periodontitis. Available research investigated the effect of applying lemuru fish oil with modified concentrations of 10%, 20% and 40%.[11] Given these limitations in the current research, the aim of this paper is to investigate the effect of lemuru fish oil gel on collagen density and on the width of the ligament in the periodontal tissue of Wistar rats induced by *P. gingivalis*.

MATERIALS AND METHODS

This research received the approval of the Ethics Commission of the Faculty of Dentistry, Hang Tuah University, with the number S.ket / 068 / KEPK-FKGUHT / XII / 2019. The type of research was a true experimental laboratory investigation with a post-test only design. The samples used in this study were male Wistar rats (n = 30) aged 3–4 months, weighing 250–300g and divided into five groups, namely normal (K-) group, *P. gingivalis* bacterial induction without therapy (K+) group, *P. gingivalis* bacterial induction with 10% lemuru fish oil gel therapy (P1) group, *P. gingivalis* induction with 20% lemuru fish oil gel treatment (P2) group and *P. gingivalis* induction with 40% lemuru fish oil gel treatment (P3) group.[11]

Periodontitis was obtained by bacterial induction using *P. gingivalis* (Pg) American Type Culture Cell (ATCC) 33277. The application of 2 ml of a 1 10^9 CFU / ml solution of *P. gingivalis* bacteria, with 1.5 ml of a 1 10^9 CFU / ml solution of live bacteria in phosphate buffered saline (PBS) with 2% of carboxymethylcellulose was carried out orally. In addition, 0.5 ml of bacteria was smeared using a cotton swab along the gingival groove on all teeth and anus in the colorectal area. The frequency of the *P. gingivalis* induction was three times in four days (0h, 48h and 96h). Periodontal tissue damage takes as long as four weeks counting from when the first bacterial induction is given.[12] Rats with periodontitis presented bleeding, redder than normal gingiva and a decrease in bone height, according to criteria described in a previous study.[4]

Lemuru fish oil was obtained from a caning waste factory from Banyuwangi, East Java (CV. Biji Sesawi, Banyuwangi, Indonesia). The induction of *P. gingivalis* was carried out first, then lemuru fish oil gel with concentrations of 10%, 20% and 40% was applied topically using a micro brush, once a day for 14 days. Approximately 1 ml/day of lemuru fish oil gel was applied topically each day on the lower jaw gingival sulcus of the Wistar rats using a micro brush (Cotisen, China). Therapy was carried out for 14 days in order to follow the period of the angiogenesis process, the process of osteoblast formation and to overcome the operator error process.[4]

The rats were euthanised one day after administration of the therapy using ketamine (Sigma, Germany) for termination at a dose of 100 mg/kg body weight (BW) intraperitoneally. Diazepam at a dose of 5 mg/kg BW was administered by inhalation. The rat was put into a container containing ether, and once it was in a sedative state, its neck was dislocated. Then the whole mandible was taken, and the rat was buried. The mandible was transferred to disposable polypropylene tubes (GP, China) containing 10% formalin liquid. Then the mandible was decalcified using 10% formic acid and cut sagittally between the mandibular left molar and the jawbone in the interdental area of the posterior mandible of the *Rattus norvegicus*, which was then stained using Mayer's haematoxylin (Orsatech, Germany).

The histometric analysis was carried out using an Olympus CX-22 (Olympus, Germany) and the Optilab program (Miconos, Indonesia), with 400x magnification. Histometric was performed with modification methods from the Damaiyanti and Mulawarmanti experiment, where the sample slides were divided into five fields of view and scored as +0 (no collagen fibres found in the wound area), +1 (density of collagen fibres in the low wound area (25%)), +2 (density of collagen fibres in moderate wound area (50%)), +3 (density of collagen fibres in tightly wound areas (75%)) and +4 (the density of collagen in the wound area is very tight (100%)).[13,14]

The periodontal ligament width was measured in the cross section using a 400x magnification microscope, the Olympus CX22. The measurements were carried out using a raster image program at three locations in one field of

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view with a micrometre unit. The reading was carried out by two observers.[15]

The data obtained were analysed using descriptive statistics to obtain a description of the data distribution and ranking in relation to the dependent variable (collagen density and periodontal ligament width), before proceeding with the analysis test. The Kruskal–Wallis test was used to compare the collagen density data between groups and the one-way analysis of variance (ANOVA) parametric statistical test was performed to analyse the periodontal ligament width. All tests were carried out using statistical product and service solutions (SPSS) version 24 (IBM, Armonk, US).

RESULTS

The data obtained from the results of the study were tabulated and analysed using statistics to test hypotheses using SPSS version 24. Because the collagen density data were measured as interval scores, nonparametric analysis was used. The mode of collagen density is shown in Table 1.

Based on Table 1 and Figure 1, the lowest collagen density mode was observed for the K+ and P1 groups and the highest for K- and P2. A nonparametric test was performed using the Kruskal–Wallis test with a significance level of $p < 0.05$. The result of the significance between groups was $p = 0.001$ ($p < 0.05$). This shows that there are significant differences in the negative group, positive group and treatment group. Subsequently, the Mann–Whitney analysis was carried out. Significantly different results were obtained between groups, except between K- and P2, P3, P2 and P3, and K+ and P1, where no significant results were obtained, with $p > 0.05$ (Table 2).

Based on the results of the histological examination and the statistical calculations, the results of the width of the periodontal ligament obtained between groups are shown in Table 3 and Figure 2. The histopathology of the periodontal ligament width shown in Figure 2 was measured in three areas, with every field of view slide stained with haematoxylin eosin. Table 3 shows that the highest periodontal ligament width measured from bone and tooth was observed in the control positive group (K+ = 425.85µm) and the narrowest periodontal ligament width was observed in the control negative group (K- = 299.87µm).

Table 1. Collagen density score mode value in the experiments

Group	Number of Samples	Mode
K-	6	4
K+	6	1
P1	6	1
P2	6	4
P3	6	3

Table 2. Significance test between groups using the *Mann–Whitney test*

Groups	K+	P1	P2	P3
K-	.002*	0.004*	0.589	0.132
K+		0.589	0.004*	0.004*
P1			0.015*	0.026*
P2				0.485

*significant ($p < 0.05$)

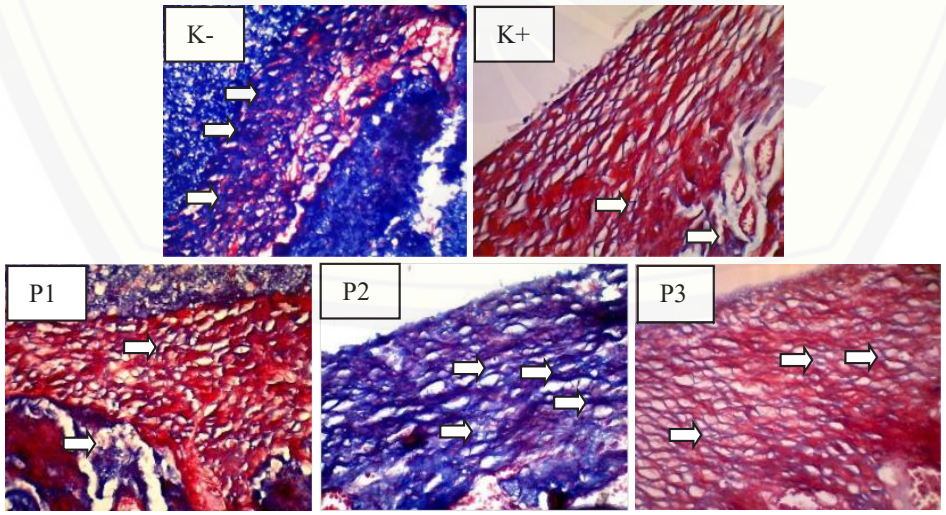

Figure 1. Histopathology of collagen density with MT staining at 400x magnification (collagen = blue colour) for the groups control negative (K-), control positive (K+), lemuru fish oil gel 10% (P1), lemuru fish oil gel 20% (P2) and lemuru fish oil gel 40% (P3).

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The data collected on the periodontal ligament width were analysed using the Shapiro–Wilk normality test and resulted in a normal distribution because of $p > 0.05$. The one-way ANOVA parametric statistical test resulted in a significance of $p = 0.004$ ($p < 0.05$), which means that there is a significant difference in the periodontal ligament width. Subsequently, the post hoc Least Significant Differences (LSD) test was performed, as shown in Table 4, to determine the value of the difference between groups. The results of this test show that there are significant differences between the groups K- and K+, K- and P2, K- and P3, and K+ and P1.

The Spearman correlation analysis was used to determine the correlation between the parametric data, namely the width of the periodontal ligament, and the nonparametric data on collagen density scores. The correlation value obtained was -0.292, which indicates an opposite relationship, i.e., the higher the collagen score, the smaller the periodontal ligament width. This shows that the higher the density value, the smaller the periodontal ligament widening, but the significance result obtained was $p = 0.11$, which means that the relationship between the collagen density and the periodontal ligament width is not significant ($p > 0.05$) as shown in Table 5.

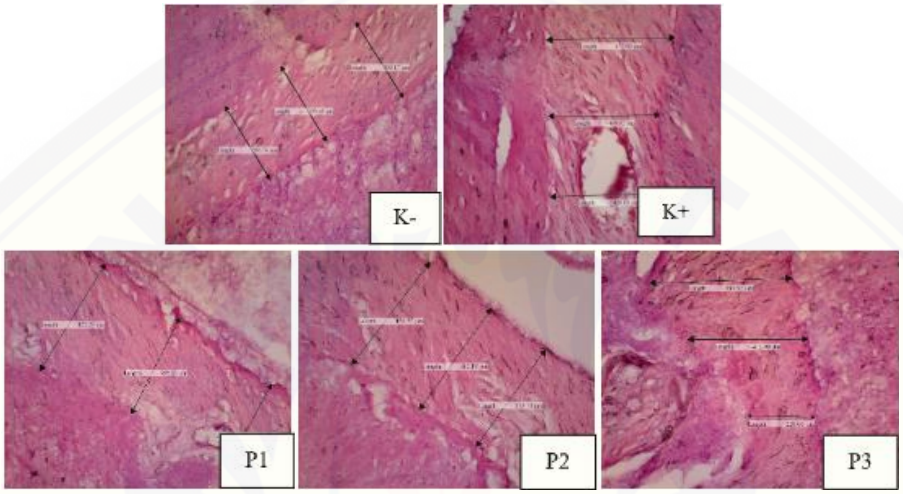

Figure 2. Image of the histopathological preparations showing the periodontal ligament width with haematoxylin eosin (HE) staining and 400x magnification (arrow between bone and tooth).

Table 3. Mean and standard deviations of the estimation of the periodontal ligament width at each group experiment

Groups	Replication	Average (μm)	Standard Deviation
K-	6	299.612	51.82
K+	6	425.850	61.54
P1	6	346.932	33.53
P2	6	370.159	49.42
P3	6	379.671	49.26

Table 4. Significance test of the periodontal ligament width

	K+	P1	P2	P3
K-	.000*	0.113	0.022*	0.01*
K+		0.011*	0.065	0.122
P1			0.428	0.267
P2				0.744

Table 5. Correlation between collagen density and periodontal ligament width

Correlations Spearman's rho		
		Collagen density score
Periodontal ligament width	Correlation coefficient	-0.292750482
	Sig. (2-tailed)	0.116427602
	N	30

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DISCUSSION

In this study, the induction of *P. gingivalis* bacteria causes an inflammatory response in the periodontal tissue because they secrete biologically active endotoxins or LPS and cause the activation of macrophages. This plays an important role in the synthesis of pro-inflammatory cytokines such as IL-1 and TNF-alpha, PGE2 and hydrolytic enzymes.[4,16]

Secretion of inflammatory mediators such as cytokines and prostaglandins responds to produce MMPs, proteolytic enzymes that affect the degradation of extracellular matrix macromolecules, namely collagen. In pathological conditions such as inflammation, TNF-α inhibits the activity of fibroblasts thereby inhibiting collagen synthesis as well as the presence of MMPs, which triggers collagen destruction.[5,17] The induction of *P. gingivalis* bacteria was shown to cause periodontal ligament damage characterised by an increase of the periodontal ligament width, which is more visible in the positive control group compared to negative control group. According to the periodontal regeneration model conducted by Montevecchi et al.,[18] a sign of periodontal ligament repair is a shorter width of the periodontal ligament compared to when the periodontal tissue damage occurred. This is because the formation of a good alveolar bone during the healing process causes the periodontal width to decrease.[18]

The groups induced by *P. gingivalis* bacteria and the group treated with 20% lemuru fish oil gel therapy had a significant difference in collagen density. This shows the positive effect of 20% lemuru fish oil gel on the increase of the collagen density. Eicosapentaenoic acid and DHA act as anti-inflammatory agents because of their ability to bind eicosanoids. They are contained in lemuru fish and compete with arachidonic acid, which can reduce the formation of MMPs and stimulating fibroblast cell regeneration. Fibroblasts, active inflammatory cells such as macrophages and neutrophils, epithelial cells and vascular endothelial cells stimulate the formation of MMPs. With the help of MMPs, fibroblasts digest the fibrin matrix and replace it with glycosaminoglycan. Over time, this extracellular matrix is replaced by type III collagen, which is also produced by fibroblasts. Furthermore, type III collagen is subsequently replaced by type I collagen during the maturation phase.

Lemuru fish oil also affect growth factors, namely FGF, which plays a role in the proliferation of fibroblasts so that it stimulates collagen formation. When extracellular matrix deposition occurs, collagen synthesis is augmented by growth factors, such as platelet-derived growth factor (PDGF), FGF and transforming growth factor beta (TGF-β), so the tissue remodelling process modulates the synthesis and the activation of metalloproteinases, an enzyme that functions to degrade the extracellular matrix. The result of the synthesis and degradation of the extracellular matrix is the remodelling of the connective tissue framework. This structure is the main feature of tissue healing in chronic inflammation.[4,19]

The sample group with the induction of *P. gingivalis* bacteria and 10% lemuru fish oil gel did not result in a significant difference from the induced groups that did not receive therapy. This shows that lemuru fish oil gel with a concentration of 10% did not have a significant effect on collagen density and periontal ligament-width. This is because the low doses were unable to stimulate growth factors such as FGF2. This mediator is needed in the healing process to trigger cell healing and differentiation and to initiate the recovery of damaged tissue.[20,21]

Compared to the smaller concentration, the 20% concentration of lemuru fish oil gel showed better repair of the collagen density and the periodontal ligament. However, there was no significant difference in the results compared to the 40% concentration. This shows that if the gel preparation has a high concentration or excessive molecular weight, it will produce a thick gel layer when applied. The penetration of the gel through the hypodermic layer becomes less effective, thus making the therapeutic effect last longer.[15,22]

The results showed that the negative control group did not have a significant difference in collagen density when compared with the study group treated with lemuru fish oil gel therapy at a concentration of 20%. Omega-3 fatty acids, especially EPA, have been shown to increase the number of fibroblasts and stimulate the formation of collagen. Eicosapentaenoic acid plays a role in increasing the amount of IL-6 cytokines, which consequently increases collagen production by fibroblasts and stimulates endothelial cells to form neovascular tissue through the process of angiogenesis and lead to the healing process.[9]

Lemuru fish oil contains unsaturated fatty acids consisting of omega-3 and omega-6. Omega-3 has been shown to regulate various proteins in periodontal tissue, such as MMP-8, MMP-13, MMP-14 and tissue inhibitor of metalloproteinases (TIMP). Omega-6 can affect the production of eicosanoids, PGE2, leukotriene and lipoxin. The ability of lemuru fish oil to influence eicosanoid metabolism is related to the long-chain structure of EPA and DHA, which has similarities to the long-chain structure of Arachidonic Acid (AA). Because of this, EPA and DHA can become AA competitor substrates to blend with the phospholipid membrane and directly inhibit the enzymes cyclooxygenase-2 (COX-2) and lipoxygenase. Inhibition of the cyclooxygenase pathway results in inhibited prostaglandins so that there is no monocyte activation mechanism to produce TNF-α and interleukin 1β, which can inhibit collagen synthesis.[9,23] The limitation in this study was the difficulty in ensuring that the whole 1ml of lemuru fish oil gel was fully absorbed in the sulcus of the periodontal ligament. Therefore, further research on the absorption capacity of lemuru oil is required, to ensure the effect of lemuru as a topical drug in the treatment of periodontitis.

Based on this research, lemuru fish oil gel influences the increase of collagen density in the periodontal tissue of Wistar rats induced by *P. gingivalis*. The concentration

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of lemuru fish oil gel that had the most profound effect on the increase of the collagen density and the width of the ligament of the periodontal tissue of Wistar rats induced by *P. gingivalis* was 20%. The correlation between collagen density and the periodontal ligament width was negative and not significant.

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