



Research article

Correlation between plaque maturity and white spot lesions on teeth during orthodontic fixed appliances treatment

Nita Hardiana, Siti Bahirrah*, Aditya Rachmawati, Muslim Yusuf, Mimi Marina Lubis

Department of Orthodontic, Faculty of Dentistry, Universitas Sumatera Utara, Medan, Indonesia

Corresponding author: SitiBahirrah, ✉ siti.bahirrah@usu.ac.id **Orcid Id:** <https://orcid.org/0000-0003-4458-2922>

Department of Orthodontic, Faculty of Dentistry, Universitas Sumatera Utara, Medan, Indonesia

© The author(s). This is an open access article distributed under the terms of the Creative Commons Attribution License (<https://creativecommons.org/licenses/by-nc/4.0/>). See <https://jmpas.com/reprints-and-permissions> for full terms and conditions.

Received - 15-11-2023, Revised - 03-12-2023, Accepted - 24-12-2023 (DD-MM-YYYY)

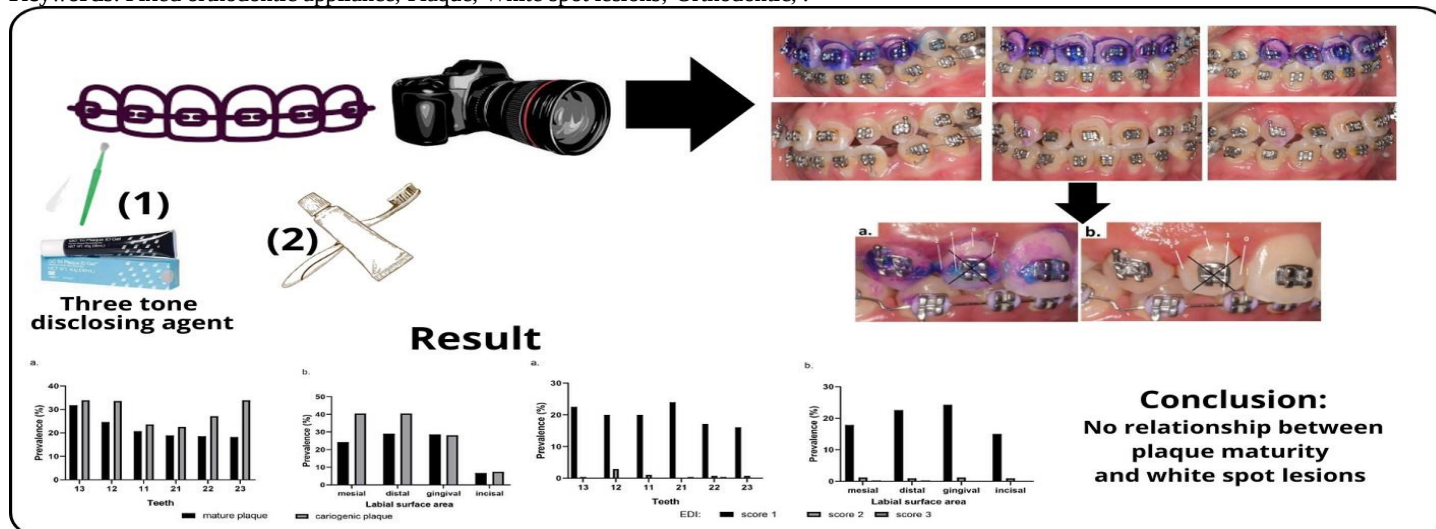
Refer This Article

Nita Hardiana, SitiBahirrah, Aditya Rachmawati, Muslim Yusuf, Mimi Marina Lubis, 2023. Correlation between plaque maturity and white spot lesions on teeth during orthodontic fixed appliances treatment. Journal of medical pharmaceutical and allied sciences, V 12 - I 6, Pages- 6267 – 6273. Doi: <https://doi.org/10.55522/jmpas.V12I6.5898>.

ABSTRACT

The main factor in the occurrence of white spot lesions on fixed orthodontic appliances is the difficulty in performing good oral hygiene. The pH of the dental biofilm becomes low due to the presence of fermentable carbohydrates, thereby accelerating the accumulation and maturation of dental plaque. This study aimto determine the relationship between plaque maturity and white spot lesions on teeth with fixed orthodontic appliances. The research design was observational analytic using digital photographs of the six upper anterior teeth of 70 patients undergoing orthodontic treatment at Teeth and Mouth Teaching Hospital Universitas Sumatera Utara. The six upper anterior teeth were given the three-tone disclosing agent to check the level of plaque maturation. The staining of the plaque is cleaned to examine the level of the white spot lesion. The examination was carried out from the intraoral photograph of the patient by dividing the four areas of the tooth surface. The Kruskal Wallis test to see the difference in the level of maturity of the six upper teeth has a significant difference ($p = 0.026$). Statistically, there is no relationship between plaque maturity and white spot lesions on the six upper anterior teeth ($p = 0.162$). In this study, the central incisor teeth have a greater chance of developing white spot lesions than other teeth (23.93%). White spot lesions on the use of fixed orthodontic appliances can form, one of which is due to plaque, but there was no significant relationship in the study.

Keywords: Fixed orthodontic appliance, Plaque, White spot lesions, Orthodontic, .



INTRODUCTION

White spot lesions are one of the side effects seen clinically in the use of fixed orthodontic appliances as an early manifestation of the caries process, thereby leading to poor esthetics at a prevalence of 2–96%^[1-3]. The main factor in the occurrence of these lesions is caused by difficulties in maintaining good oral hygiene. Plaque quickly accumulates and is difficult to clean due to fixed orthodontic appliance components adhering to the tooth surface, limiting the self-cleansing ability of saliva and oral muscles^[4].

Dental plaque contains *Streptococcus mutans* (S.mutans) and *Lactobacillus* microorganisms, which are aciduric and acidogenic due to caries^[4]. A study by Mummolo et al. stated that S.mutans and lactobacilli in patients' saliva treated with fixed orthodontic appliances increased progressively. Statistically significant differences were seen from baseline to three months of treatment and from baseline to six months of orthodontic treatment^[5]. The results of a meta-analysis conducted by Sundararaj et al. showed that the incidence of white spot lesions was 45.8%, and the prevalence was 68.4% of patients undergoing orthodontic treatment^[6].

Lucchese and Gherlone's study regarding the prevalence of white spot lesions formation before and during fixed orthodontic treatment found 40% in the 6-month-treated group and 43% in the 12-month-treated group^[7]. Mizrahi examined the spread of enamel surface lesions after orthodontic treatment, showing an increased incidence and severity of white spot lesions on the vestibular and lingual surfaces of the teeth. Significant increase in the cervical and middle third of the crown of the maxillary and mandibular first molars, maxillary lateral incisors and mandibular canines, especially on the vestibular surface^[8].

Operator factors that can trigger the formation of white spot lesions and mature plaques are the process of etching and bonding the brackets to the tooth surface. Yagci et al. stated that total etching of the entire labial surface of the tooth results in significant white spot lesions rather than partial etching^[9]. According to research by Sukontapatipark et al., mature plaque was present on the composite with excess after 2 and 3 weeks of bonding. Plaque accumulation was due to the advantages of the composite around the base of the bracket having a rough surface so that plaque accumulation is easy to form^[10]. In addition, a smooth adhesive surface with minimal excess adhesive around the bracket also risks the formation of marginal gaps with bacterial colonization, potentially triggering carious lesions^[11].

Diagnosis and clinical assessment of white spot lesions and mature plaques is a major challenge for orthodontists. Conventional caries detection methods can be carried out by visual-tactile sensation and radiographic examinations, but these two methods have side effects and are highly dependent on the dentist's experience^[12].

Another method for examining white spot lesions is photography^[13]. Richter et al. observed the surface of the teeth before and after fixed orthodontic treatment through photography. The results found that the incidence of new white spot lesions in patients treated with comprehensive orthodontics was significantly high at 72.9% and new cavity lesions at 2.3%^[14]. Furthermore, Khalaf said the photographic results revealed that the highest incidence of white spot lesions as recorded in the upper jaw on the canines and lateral incisors and the lower jaw on the premolars and first molars in patients with fixed orthodontic treatment^[15].

Dental clinical photography is indispensable in orthodontic treatment. In today's digital era, many clinicians download digital images of their treatment results on social media. Apart from being a record of treatment, digital images from photography can be used to trace areas of white spot lesions around orthodontic brackets. Therefore, the authors are interested in studying white spot lesions in fixed orthodontic treatment with dental photography.

MATERIALS AND METHODS

This study was an analytical research with a cross-sectional design. The study protocol was approved by the Health Research Ethics Commission of the Universitas Sumatera Utara [No.43/FEPK/USU/2023; Approval Date: 05/01/2023]. The samples of this study were the six upper anterior teeth of patients who were undergoing fixed orthodontic treatment at Teeth and Mouth Teaching Hospital, Universitas Sumatera Utara. The six upper anterior teeth consist of the upper right canine (13), upper right lateral incisor (12), upper right central incisor (11), upper left central incisor (21), upper left lateral incisor (22), and upper left canine (23). The sample selection method used purposive sampling that met the inclusion and exclusion criteria. The inclusion criteria were the six upper anterior teeth fully erupted, good general health, an absence of systemic disease determined by a medical history review, and existing fixed orthodontic appliances for at least three months. Exclusion criteria were current or recent use of antibiotics or an antibacterial/anti-plaque mouth rinse, allergy to benzoate preservatives, pregnancy, restored facial surfaces of the six upper anterior teeth, and any condition that impairs consenting. A total of 70 patients with 420 upper anterior teeth were included in this study.

The selected subjects were explained about the purpose, benefits, and research procedures to be carried out. If the subjects were willing to participate, subjects were asked to sign an informed consent sheet. All patient data is kept confidential. Retrieval data on plaque maturation and white spot lesions was carried out at the same time for each patient.

Retrieval data on maturation plaque

The patient sat in the dental chair, and then it was set at an

angle of 20°. The wire and ligature attached to the bracket are removed first. The patient was put on a cheek retractor, and then the teeth were drained with a three-way syringe. Apply the three-tone disclosing agent (GC Tri Plaque ID Gel™) with a micro brush to the labial surface of the six upper anterior teeth around the brackets. Leave it for 2 minutes, and the tooth surface will change color. The tooth surface is rinsed gently with a three-way syringe for 10 seconds while suctioning. The teeth were dried again with air from a three way syringe^[16]. Camera single lens (Nikon D3500, macro lens NIKKOR 60 mm f2.8G ED) settings: shutter speed 1/125, aperture F 25, and ISO 100. Ring flash tube settings A and B are the same, namely 1/128. Photos were taken approximately 30 cm from the ring flash to the labial with a ruler measuring 30 cm. Photographs were taken perpendicular to the point of contact between 13 and 12, 11 and 21, and 22 and 23^[17].

Retrieval data of white spot lesion

The cheek retractor is removed after taking pictures of plaque maturation on the teeth. The six upper anterior teeth with the disclosing agent were cleaned by asking the patient to brush their teeth. If staining was left, the operator helped clean it while educating the patient on brushing their teeth properly. The patient was put on the cheek retractor again, and then the teeth were dried with a spray of wind from a three-way syringe. The teeth were photographed the same way as the plaque maturity photograph.

Photo analysis

The results of digital photos of the maturity of the plaques and white spot lesions were transferred to a laptop, and then the images were analyzed using 3D paint computer application (Microsoft Corporation, Windows 10 Version 6.2310.24037.0). The

image of the labial surface of the tooth with and without the disclosing agent was divided into four areas: mesial, distal, gingival, and incisal (Figure 1). Maturation plaque analysis is carried out by observing plaque mature staining on each area of the labial surface of the tooth. Plaques stained with purple and light blue were noted as plaque mature staining. Each area of the labial surface of the tooth was recorded whether there was mature plaque staining; if there was no color, it was given a code of 0, and if there was color, it was given a code of 1^[18,19]. White spot lesions are analyzed by observing opaque lesions on each area of the labial surface of the tooth. White spot lesion per area of the tooth is scored EDI (Enamel Decalcification Index) by Banks and Richmond^[20]. Cutting the image per tooth element is transferred to Ms.Power Point, named the patient. Sectional images for each tooth element are arranged in plaque maturation and white spot lesions per the patient's name.

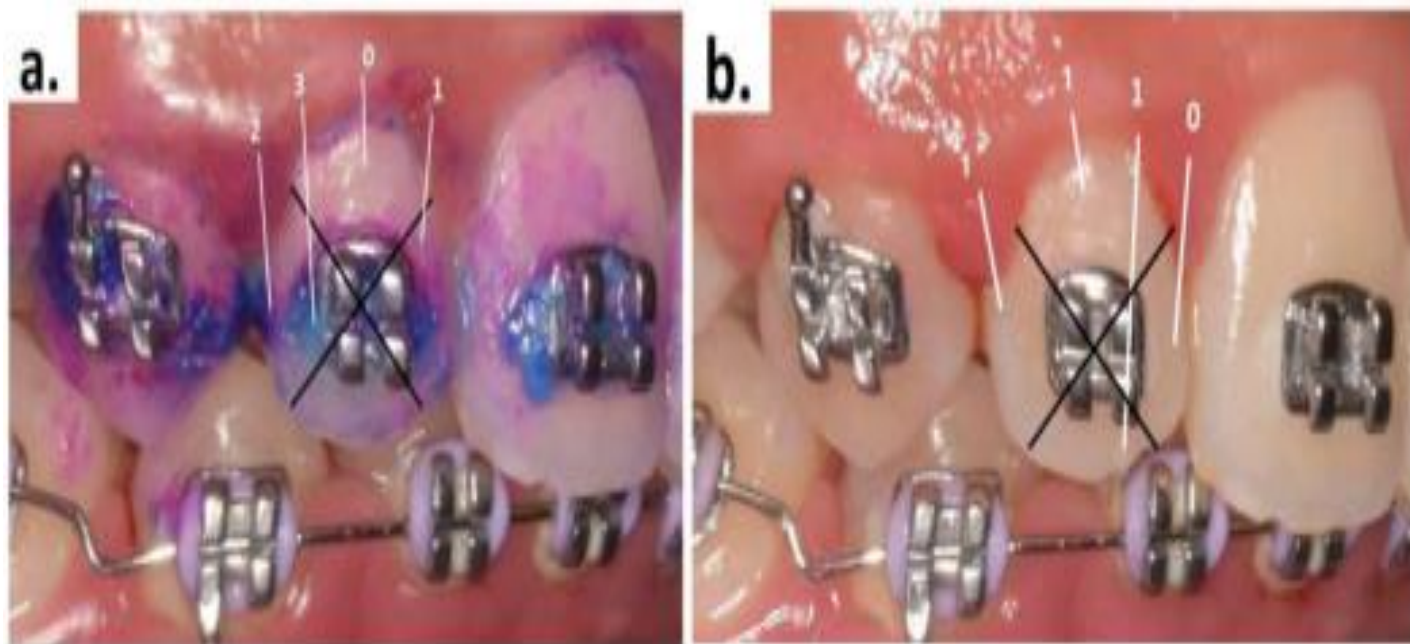
Statistical analysis

The statistical test used was the Kruskal-Wallis test to see differences in the level of maturation plaque and white spot lesions on the six upper anterior teeth. Pearson test used to see the relationship between plaque maturity level and white spot lesions.

RESULTS AND DISCUSSION

White spot lesions are the beginning of the enamel demineralization that occurs below the surface. Orthodontic fixed appliances attach to the surface of the tooth, an easy place for plaque retention because it is hard to clean compare to without a device. The accumulation of plaque that is not cleaned for a long time and the presence of acidogenic bacteria played a role in the acidogenicity of dental biofilm. Ecology interferes with the balance that causes caries, namely a sugar-rich diet, low pH, and salivary flow power^[21].

Figure 1: a. Multi-color three-tone disclosing agent of dental plaque: (0): no detectable plaque present, (1) New plaque (pink), (2) mature plaque >48 hours (blue/purple), (3) cariogenic plaque (light blue). b. Image analysis of white spot lesions, (0) No white spot lesions observed, (1) white spot lesion.



Maturation Plaque

Dental plaque can easily be seen and analyzed with the help of plaque coloring (disclosing agents). The three-tone disclosing agent (GC Tri Plaque ID Gel™) contains red pigments, blue, and sucrose. New plaque is colored pink/red, mature plaque (> 48 hours) is colored purple, and cariogenic plaque is colored light blue (Figure 1). Jayanthi et al.'s study cultured plaque samples colored by the three-tone disclosing agent for microbiological assessments. The results of his research showed that the light blue-colored plaque had a high level of *S. Mutans*^[22].

Figure 2: Diagram of the prevalence of maturati on and cariogenic plaque; a. each tooth of the six upper anterior teeth, b. based on the labial surface of the six upper anterior teeth.

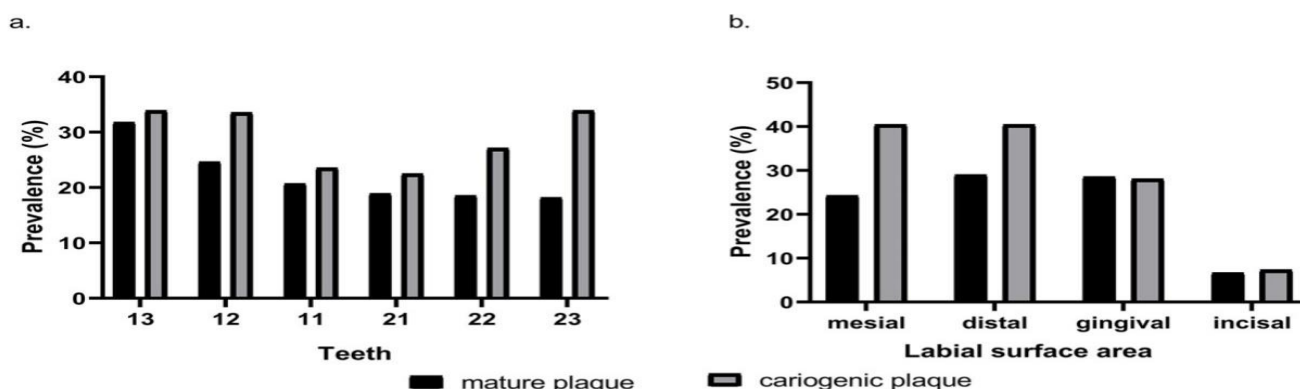
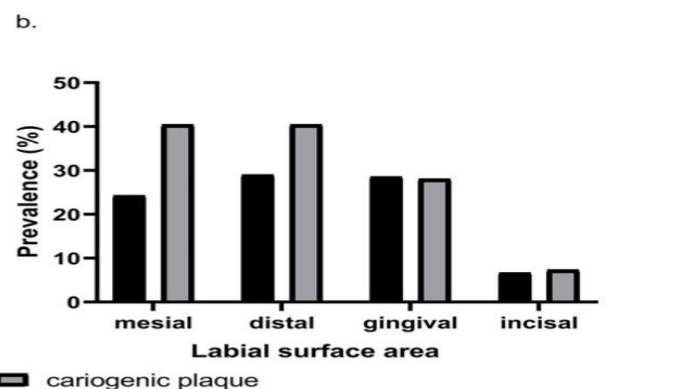


Figure 2b shows the maturation plaque of the six upper anterior teeth in the labial surface area of the tooth with the accumulation of mature plaque in the distal area of 29.05%. The accumulation of the most cariogenic plaque is seen in the mesial and distal areas, with an exact percentage figure of 40.48%. The least accumulations of plaque are seen in the incisal of the tooth; they are mature plaque by 6.67% and plaque cryogenic by 7.38%. In line with two previous studies, Furuichi et al. and Al Aneizi et al., the accumulations of plaque on orthodontic fixed appliances dominated the proximal area behind the wire path. It is often missed when brushing teeth^[24, 25].

The method of brushing teeth is the first line in cleaning debris and accumulation of plaque around the bracket. The method of brushing teeth most widely used by orthodontic patients is the Ramford method. Modification of the Stillman and Bass method is the most effective method in reducing plaque index in orthodontic patients. Anuwongnukroh et al.' research showed only 36.2% of patients routinely use interdental brushes, 51.4% sometimes, and 12.4% never. The use of tools such as an interdental brush other than the correct brushing method can also reduce the accumulation of plaque in the proximal tooth, especially behind the wire^[26].

Table 1 shows the differences in the level of plaque maturity between maturation and cariogenic plaque in the six upper anterior

teeth, which is a significant difference in anterior teeth where the mature plaque obtained $P = 0.031 < 0.05$. Hypothesis stating that there are differences in the level of plaque maturity with the dental element received. These results indicate that each anterior tooth has a different mature plaque, and it becomes a note in the control of the patient's plaque at home, especially in the upper right canines that have a large percentage of mature plaque. Plaque accumulation occurs due to inappropriate plaque control. Mature plaque is a plaque that has settled in teeth for more than two days. The most common types of brushing strokes used by the patient were horizontal and circling movements, which were most often observed by up to 88% of the total brushing duration^[27]. The natural technique of the patient is likely to cause teeth canines, which are located in the arch area that is often missed. White Spot Lesions



teeth, which is a significant difference in anterior teeth where the mature plaque obtained $P = 0.031 < 0.05$. Hypothesis stating that there are differences in the level of plaque maturity with the dental element received. These results indicate that each anterior tooth has a different mature plaque, and it becomes a note in the control of the patient's plaque at home, especially in the upper right canines that have a large percentage of mature plaque. Plaque accumulation occurs due to inappropriate plaque control. Mature plaque is a plaque that has settled in teeth for more than two days. The most common types of brushing strokes used by the patient were horizontal and circling movements, which were most often observed by up to 88% of the total brushing duration^[27]. The natural technique of the patient is likely to cause teeth canines, which are located in the arch area that is often missed. White Spot Lesions

Figure 3a, about white spot lesions of the six upper anterior teeth, shows the teeth that are most visible white spot lesions in tooth 21 by 23.93%. White spot lesions are at least visible in tooth 23 by 16.07%. Zachrisson's research reported the prevalence of white spot lesions on the maximum anterior teeth in central incisors^[28]. Gorelick reported that the teeth most often found white spot lesions were upper lateral incisors when treated by the orthodontic devices^[29]. Determination of certain teeth most often found in white spot lesions is not specific. According to Kawsar et al., there was no significant

difference in the incidence of white spot lesions between the dental elements ^[30].

Table 1: The difference in the level of maturation plaque in the six upper anterior teeth

Maturity level	Dental elements	Mean	Standard deviation	P
Maturation plaque	13	0.986	0.120	p = 0.026*
	12	0.900	0.302	
	11	0.871	0.337	
	21	0.800	0.403	
	22	0.843	0.367	
	23	0.871	0.337	
Cariogenic plaque	13	0.686	0.468	p = 0.158
	12	0.700	0.462	
	11	0.586	0.496	
	21	0.514	0.503	
	22	0.686	0.468	
	23	0.643	0.483	

Description: p<0,05*= Significant

Figure 3: Diagram of the prevalence of white spot lesions represented by EDI scores: a. each tooth of the six upper anterior teeth, b. based on the labial surface of the six upper anterior teeth

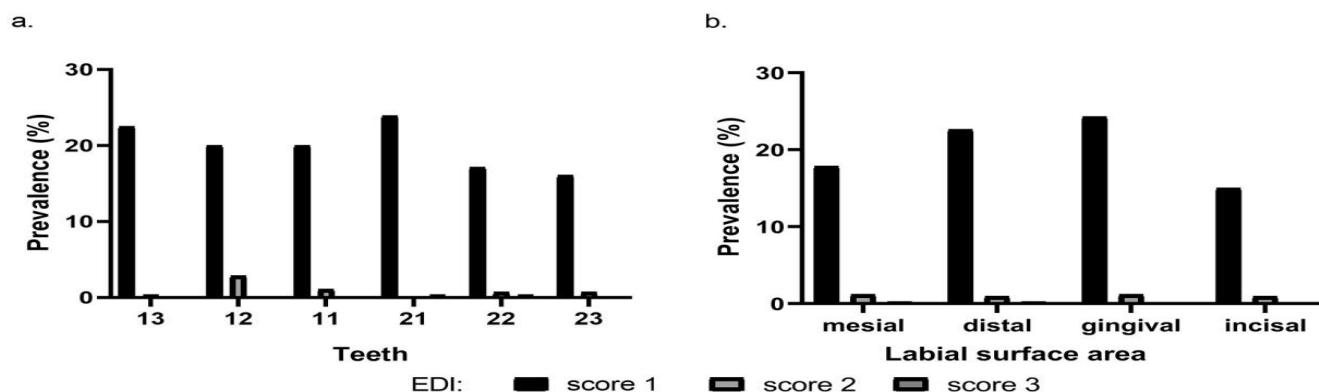


Figure 3b shows the white spot lesions on the six upper anterior teeth based on the area of the teeth surface. The gingival area is seen to have an EDI 1 score (white spot lesion <50%), which is high at 24.29%. The lowest EDI 1 score seen in the incisal area is 15%. In line with Khalaf et al.'s research, the location of white spot lesions on the tooth's surface is mostly found in the gingiva. The gingival area is one-third of the cervical tooth, which is the place where the teeth and the gingival meet and become a place for the growth of bacteria so that white spot lesions are often seen ^[31].

Table 2 shows the differences in white spot lesions on the six upper anterior teeth, showing that the highest average EDI score occurs in tooth 12 with a mean of 0.257, while the lowest average is in tooth 23 with a mean of 0.175. Kruskal-Wallis test results obtained p value = 0.162 > 0.05, then it was concluded that there was no significant EDI difference between the six teeth. The hypothesis that states there are differences in white spot lesions in the six upper anterior teeth is rejected.

Table 2: The difference between white spots lesions in six anterior teeth upper

Dental element	Mean EDI Score	Standard deviation	p
13	0.232	0.234	p = 0.162
12	0.257	0.289	
11	0.221	0.274	
21	0.250	0.229	
22	0.196	0.272	
23	0.175	0.227	

Description: p<0,05*= Significant

The relationship between the level of maturation plaque and white spot lesions in the six upper anterior teeth

Analysis of statistical tests to see the relationship between the level of maturation plaque and white spot lesions on the six upper anterior teeth is using the Pearson correlation test. Table 3 shows no relationship between the level of maturation plaque and white spot lesions on the six upper anterior teeth because of the value of P > 0.05. The hypothesis statement that there is a relationship between the level

of maturation plaque and white spot lesions on the six upper anterior teeth is rejected. Thanetchaloempong et al. examined half of the virulence gene on biofilm carcinogenicity. They used the three-tone disclosing agent in determining biofilm carcinogenicity and then collecting dental biofilm to determine the expression of genes with real-time PCR before and after the treatment of close orthodontics. Cariogenic genes were represented by GTFB, BRPA, SPAP, LUXS,

and GBPB in *S. mutans*. Statistical tests showed no significant relationship between the plaque maturity level and the cariogenic gene

expression [16].

Table 3: The relationship between the level of maturation plaque and white spot lesions in the six upper anterior teeth

Dental elements	The relationship between plaque maturity and white spot lesions			
	Mature plaque		Cariogenic plaque	
	r	p	r	p
13	-0.009	0.939	0.014	0.907
12	0.050	0.682	0.125	0.302
11	-0.040	0.741	-0.035	0.774
21	0.039	0.747	0.000	1.000
22	-0.013	0.915	0.094	0.441
23	-0.175	0.146	0.083	0.495
Description: p<0,05 *= Significant				

The six upper anterior teeth that were chosen in this study participated in Sardana et al.'s study, which saw white spot lesions from intraoral photos [17]. Several studies by Khalaf, Julien et al., and Enaia et al. indicated that white spot lesions are more common in the maxillary teeth than the mandibular, especially in the anterior teeth. [30, 32, 33] The anterior teeth are more susceptible to demineralization due to being located far from the salivary glands, especially the major, parotid, submandibular, and sublingual.

Saliva has the ability to remineralize enamel because it has the capacity of buffers, proteins, enzymes, fluorine ions, calcium ions, and phosphate saliva. [34] Salivary buffer capacity is essential in maintaining the pH level of saliva and plaque and helps neutralize the effects of acid exposure. Three buffer systems exist in saliva: carbonic acid, phosphoric, and protein. Salivary proteins such as proline, Catherine, and histatins have a binding power on the surface of the enamel to help remineralization. The lysozyme enzymes in saliva help dialysis bacterial cells, especially effective against gram-positive bacteria. Fluor in saliva has three roles: prevention of demineralization, encouraging remineralization, and disturbing bacterial growth. Phosphate and calcium ions are needed in fluorine to promote natural enamel remineralization. [35] Lindawati et al.'s study stated that the treatment of the fixed orthodontic increases the flow rate, pH, buffer capacity, and calcium saliva. [36]

CONCLUSIONS

Plaque accumulation, both mature and cariogenic plaques, is mostly found in the canines, followed by the lateral incisors, especially the mesial and distal parts. Statistically, there is a relationship between mature plaques on the six upper anterior teeth using orthodontic fixed appliances. The most common white spot lesions were found on the central incisors, but no significant difference existed between the six upper anterior teeth. It means that white spot lesions have the same chance on the six upper anterior teeth. Neither mature nor cariogenic plaques have a significant relationship with the incidence of white spot lesions on the labial surface of the six upper anterior teeth.

ACKNOWLEDGMENTS

The authors of the present study would like to thank all the participants who enrolled in this study.

Financial support and sponsorship: Nil

REFERENCES

1. Leeper DK, Noureldin A, Julien K, et al., 2019. Risk assessments in orthodontic patients developing white spot lesions. *J Investig Clin Dent*, 10(4), Pages- 1–7. DOI: 10.1111/jicd.12470.
2. Srivastava K, Tikku T, Khanna R, et al., 2013. Risk factors and management of white spot lesions in orthodontics. *J Ortho d Sci*, 2(2), Pages- 43–49. DOI: 10.4103/2278-0203.115081.
3. Kavvadia K, Seremidi K, Reppa C, et al., 2018. Validation of fluorescence devices for evaluation of white spot lesions in orthodontic patients. *Eur Arch PaediatrDent*19(2), Pages- 83–89. DOI: 10.1007/s40368-018-0327-y.
4. Metin-Gursoy G, Uzuner FD, 2018. The Relationship between orthodontic treatment and dental caries. In: Akarslan Z, editor. *IntechOpen Book. BoD - Books on Demand*, London Pages 55–77. DOI: 10.5772/intechopen.76470.
5. Mummolo S, Tieri M, Nota A, et al., 2020. Salivary concentrations of *Streptococcus mutans* and *Lactobacilli* during an orthodontic treatment. An observational study comparing fixed and removable orthodontic appliances. *ClinExp Dent Res*6 (2), Pages- 181–187. DOI: 10.1002/cre2.261.
6. Sundararaj D, Venkatachalapathy S, Tandon A, et al., 2015. Critical evaluation of incidence and prevalence of white spot lesions during fixed orthodontic appliance treatment: A meta-analysis. *J IntSocPrev Community Dent*5 (6), Pages- 433–439. DOI: 10.4103/2231-0762.167719.
7. Lucchese A, Gherlone E, 2013. Prevalence of white-spot lesions before and during orthodontic treatment with fixed appliances. *Eur J Orthod*35 (5), Pages- 664–668. DOI: 10.1093/ejo/cjs070.
8. Mizrahi E, 1983. Surface distribution of enamel opacities. *Am J Orthod*84 (4), Pages- 323–331. DOI: 10.1016/s0002-9416(83)90348-2.
9. Yagci A, Seker ED, Demirsoy KK, et al., 2019. Do total or partial etching procedures effect the rate of white spot lesion formation? A single-center, randomized, controlled clinical trial. *Angle Orthod*89 (1), Pages- 16–24. DOI: 10.2319/013018-84.1.
10. Sukontapatipark W, El-agroudi MA, Selliseth NJ, et al., 2001. Bacterial colonization associated with fixed orthodontic appliances a scanning electron microscopy study. *Eur J Orthod*23 (5), Pages- 475–484. DOI: 10.1093/ejo/23.5.475.

11. Hennig CL, Blochberger B, Symmank J, et al., 2023. Effects of reducing excess dental adhesive on bacterial adhesion in the bracket periphery. *Clin Oral Investig* 27 (5), Pages- 1993–2001. DOI: 10.1007/s00784-023-04924-4.
12. Duong DL, Duy Q, Nguyen N, et al., 2021. Proof-of-concept study on an automatic computational System in detecting and classifying occlusal caries lesions from smartphone color images of unrestored extracted teeth. *Diagnostics* 11(7), Pages- 1–14. DOI: doi.org/10.3390/diagnostics11071136.
13. Hassan IT, Allam GG, 2017. A standardized in vivo photographic technique to assess the remineralization of white spot lesions after orthodontic treatment. *Egypt Dent J* 63 (1), Pages- 121–128. DOI: 10.21608/edj.2017.74379.
14. Richter AE, Arruda AO, Peters MC, et al., 2011. Incidence of caries lesions among patients treated with comprehensive orthodontics. *Am J Orthod Dentofacial Orthop* 139 (5), Pages- 657–664. DOI: 10.1016/j.ajodo.2009.06.037.
15. Khalaf K, 2014. Factors affecting the formation, severity and location of white spot lesions during orthodontic treatment with fixed appliances. *J Oral Maxillofac Res* 5 (1), Pages- 1–10. DOI: 10.5037/jomr.2014.5104.
16. Thanetchaloempong W, Koontongkaew S, Utispan K, 2022. Fixed orthodontic treatment increases cariogenicity and virulence gene expression in dental biofilm. *J Clin Med* 11 (19), Pages- 1–12. DOI: 10.3390/jcm11195860.
17. Sardana D, Li KY, Ekambaram M, et al., 2022. Validation of clinical photography and a laser fluorescence device for assessment of enamel demineralization during multi-bracketed fixed orthodontic treatment. *Photo diagnosis Photodyn Ther* 38:102828, Pages- 1–8. DOI: 10.1016/j.pdpdt.2022.102828.
18. Widhianingsih D, Koontongkaew S, 2021. Enhancement of cariogenic virulence properties of dental plaque in asthmatics. *J Asthma* 58 (8), Pages- 1051–1057. DOI: 10.1080/02770903.2020.1753211.
19. Luengthamchat N, Koontongkaew S, 2022. Bitter taste perception and dental biofilm cariogenicity in orthodontics. *Int Dent J*. Pages- 1–6. DOI: 10.1016/j.identj.2022.07.003.
20. Banks PA, Richmond S, 1994. Enamel sealants: A clinical evaluation of their value during fixed appliance therapy. *Eur J Orthod* 16 (1), Pages- 19–25. DOI: 10.1093/ejo/16.1.19.
21. Marsh PD, 2006. Dental plaque as a biofilm and a microbial community Implications for health and disease. *BMC Oral Health* 6(SUPPL. 1), Pages- 1–7. DOI: 10.1186/1472-6831-6-S1-S14.
22. Jayanthi M, Shilpapiya M, Reddy V, et al., 2015. Efficacy of three-tone disclosing agent as an adjunct in caries risk assessment. *Contemp Clin Dent* 6(3), Pages- 358–363. DOI: 10.4103/0976-237X.161887.
23. Kadkhodazadeh M, Khodadustan A, Amid R, et al., 2012. Plaque removal ability in left-and right-handed patients in different parts of the oral cavity. *J Adv Periodontol Implant Dent* 4 (1), Pages- 24–28. DOI: 10.5681/jpid.2012.006.
24. Furuichi Y, Lindhe J, Ramberg P, et al., 1992. Patterns of de novo plaque formation in the human dentition. *J Clin Periodontol* 19(6), Pages- 423–433. DOI: 10.1111/j.1600-051X.1992.tb00673.x.
25. Al-anezi SA, Harradine NWT, 2012. Quantifying plaque during orthodontic treatment : A systematic review. *Int J Dent Hyg* 82 (4), Pages- 748–753. DOI: 10.2319/050111-312.1.
26. Anuwongnukroh N, Dechkunakorn S, Kanpiputana R, 2017. Oral hygiene behavior during fixed orthodontic treatment. *Dentistry* 7 (10), Pages- 1–5. DOI: 10.4172/2161-1122.1000457.
27. Ganss, C., Duran, R., Winterfeld, T., et al., 2018. Tooth brushing motion patterns with manual and powered toothbrushes-a randomised video observation study. *Clin Oral Investig* 22(2), Pages- 715–720. DOI: 10.1007/s00784-017-2146-7
28. Zachrisson BU, 1976. Cause and prevention of injuries to teeth and supporting structures during orthodontic treatment. *Am J Orthod* 69 (3), Pages- 285–300. DOI: 10.1016/0002-9416(76)90077-4.
29. Gorelick L, Geiger AM, John A, 1982. Incidence of white spot formation after bonding and banding. *Am J Orthod* 81 Pages- 93–98. DOI: 10.1016/0002-9416(82)90032-x.
30. Kawsar MA, Islam MN, Sen M, et al., 2019. Prevalence of white spot lesion during orthodontic treatment with fixed appliance. *Update Dent Coll J* 9(2), Pages- 40–42. DOI: 10.3329/updcj.v9i2.43739.
31. Julien KC, Buschang PH, Campbell PM, 2013. Prevalence of white spot lesion formation during orthodontic treatment. *Angle Orthod* 83 (4), Pages- 641–647. DOI: 10.2319/071712-584.1.
32. Enaia M, Bock N, Ruf S, 2011. White-spot lesions during multibracket appliance treatment: A challenge for clinical excellence. *Am J Orthod Dentofacial Orthop* 140 (1), Pages- e17–24. DOI: 10.1016/j.ajodo.2010.12.016.
33. Eltayeb MK, Ibrahim YE, El Karim IA, et al., 2017. Distribution of white spot lesions among orthodontic patients attending teaching institutes in Khartoum. *BMC oral health* 17(1), Pages- 1–6. DOI: 10.1186/s12903-017-0380-7.
34. Farooq I, Bugshan A, 2021. The role of salivary contents and modern technologies in the remineralization of dental enamel: A narrative review. *F1000Res* 9:171, Pages- 1–14. DOI: 10.12688/f1000research.22499.3.
35. Lindawati Y, Sufarnap E, Munawarah W, 2019. The effect of fixed orthodontic treatment on salivary component. *Dentika Dent J* 22(2), Pages- 30–33. DOI: 10.32734/dentika.v22i2.1073.