



Contents lists available at ScienceDirect

Saudi Journal of Biological Sciences

journal homepage: www.sciencedirect.com

Original article

Screening of fluoride analysis as a biochemical parameter in the orthodontic treatment using fixed appliances

Nozha M. Sawan^a, Afnan A. Ben Gassem^b, Alhanoof Aldegheishem^c, Eman I. Alsagob^a, Abeer A. Alshami^{a,*}^a Preventive Dental Sciences Department, College of Dentistry, Princess Nourah Bint Abdulrahman University, Riyadh, Saudi Arabia^b Department of Pediatric Dentistry and Orthodontics, College of Dentistry, Taibah University, Al Madinah AlMunawwarah, Saudi Arabia^c Department of Dental Clinical Sciences, College of Dentistry, Princess Nourah Bint Abdulrahman University, Riyadh, Saudi Arabia

ARTICLE INFO

Article history:

Received 28 September 2021

Revised 24 October 2021

Accepted 25 October 2021

Available online 29 October 2021

Keywords:

Orthodontic treatment

Fixed appliances

Fluoride levels

Saliva

ABSTRACT

Saliva is a critical and useful biological fluid necessary for good health and for the appropriate execution of mouth activities. Orthodontic biomaterials have a complex relationship with many components, including the oral environment. Treatment with fixed orthodontic appliances may causes dental caries. As a result, it is necessary to comprehend how orthodontic therapy and various fluoride regimens affect the chances of developing dental cavities as well as individual risk factors. Usage of fluoride will tend to reduce the caries in the patients diagnosed with the fixed orthodontic treatment. The aim of this study was to screen the biochemical parameter of the fluoride levels in the patients undergone and completed the treatment of orthodontic fixed appliances. In this study, 35 patients have been visited on day 1 as well as day 35 and categorized as T₀ and T₁ groups. Saliva samples were collected and fluoride levels were measured between T₀ and T₁ groups. Using the fluoride kit with the spectrophotometer, fluoride levels were measured. The results confirmed similar fluoride levels between T₀ (26.11 ± 4.86) and T₁ (27.71 ± 4.40) groups. There was no significant association observed in this study (p = 0.56). Fluoride might have no role in the patients undergoing orthodontic treatment.

© 2021 Published by Elsevier B.V. on behalf of King Saud University. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

1. Introduction

One of the most undesirable side effects of fixed orthodontic treatment is enamel demineralization (Dai et al., 2019). Fixed orthodontic appliances can alter microbial colonization of the mouth cavity (Pellissari et al., 2021). Orthodontic brackets attached to the teeth could cause trouble in cleaning the teeth, biofilm formation and allowing new organisms to colonize (Alkhayyat and Alshammery, 2021). Orthodontic equipment can limit intraoral space, impair tongue movement, and hence distort certain specific sound frequencies (Melo et al., 2021). Orthodontic appliances dramatically affect the oral environment and make mechanical plaque removal difficult for orthodontic patients (Papadopoulou et al., 2021).

The secretory proteins present in saliva perform an indirect role in bone formation and resorption, which is related with periodontium remodeling for tooth movement as well as periodontal tissues (Baeshen, 2021). Therefore, saliva is crucial for sustaining oral hygiene. The formation of saliva varies from person to person, and there is no apparent correlation between saliva composition and the composition of blood. Studies suggest a link between lower levels of salivary buffering capacity, calcium, phosphate and tooth decay (Cunha-Cruz et al., 2013). A small amount of electrolytes and proteins are found in saliva, but they are critical for preserving oral health and maintaining the integrity of teeth (AlHudaithi and Alshammery, 2021). Depending on how frequently patients floss and brush their teeth, fixed orthodontic appliances may impede oral hygiene procedures and cause alterations in the oral microflora, which could decrease the acidity of the mouth and promote accumulation of plaque and increase the affinity to bacteria to metallic surfaces because of electrostatic reactions (Kouvelis et al., 2021). Orthodontic tooth mobility and fixed orthodontic treatment pain may be evaluated by examining salivary fluid (Bhat et al., 2021).

Fluoride ions help to prevent the development of white spot lesions in tooth enamel. In orthodontic treatment, many ways are used to administer fluoride to teeth, including topical fluorides

* Corresponding author.

E-mail address: aaalshami@pnu.edu.sa (A.A. Alshami).

Peer review under responsibility of King Saud University.



Production and hosting by Elsevier

and fluoride-releasing polymers (Benson et al., 2005). The constituents of the supplied fluoride source may also alter fluoride bioavailability in plaque. Various research have showed that after teeth brushing or rinsing with fluoridated mouthwash, salivary fluoride concentration increases rapidly, but returns to normal levels two hours after administering fluoride (Naumova et al., 2012). As an unstimulated, total-saliva sample, the fluoride concentration reflects the sum of fluoride that is present in ductal saliva and many other locations within the mouth where fluoride is retained (Talwar et al., 2016). Subsurface hypomineralization leads to the formation of dental fluorosis, which becomes more pronounced as it extends into dentine. The most likely connection here is that the hydrolysis and removal of proteins from the enamel is most likely to occur during the maturity of the tooth enamel (Stogiera et al., 2020). The incidence of dental caries in the Western world has fallen in the latter half of the 20th century thanks to the common usage of fluoride toothpaste. To put it simply, fluoridated goods exhibit a wide range of abilities when it comes to the amount and location of Fluoride in the oral cavity. It is possible to boost the fluoride level in saliva with rinsing solutions and toothpaste (Baeshen et al., 2010). Previous studies have shown the association and disassociation with the follow-up studies using many biochemical parameters including fluoride (Hallgren et al., 1990; Marini et al., 1999; Seyam et al., 2021). In this study, we aimed to investigate the fluoride levels in the follow-up study of patients undergone the orthodontic treatment for day 1 to day 35 in the Saudi population.

2. Materials and methods

2.1. Enrolment of the patients

In this follow-up study, 35 patients were enrolled after the completion of orthodontic treatment and also from other public hospitals of the capital city of the Kingdom. All patients who had surgery performed with a 0.022-in bracket system were treated with a bracket system that had a 0.022-in slot (3 M unitek, USA). In this study, we have opted the 14-men and remaining were women. All the participants were enrolled by signing the informed consent form. The minimum and maximum ages of the participants were between 15 and 35 years of age. All the participants who were involved in this study had undergone the treatment for at least 1 year were considered as inclusion criteria of this study. The exclusion criteria of this study were usage of antibiotics and smoking. The selection of participants based on the requirement criteria was obtained in the documented studies (AlHudaithi and Alshammery, 2021; Alkhayat and Alshammery, 2021).

2.2. Ethical issues

Ethical grant was obtained for this study and all the 35 participants involved in this study had signed the informed consent form to involve into the follow-up study.

2.3. Saliva collection

Thirty-five participants were selected based on the inclusion and exclusion criteria. From each participant, 2 ml of the saliva sample was collected twice *i.e.*, during initial visit and final visit. Initial visit indicates before debonding phase and final visit indicates five weeks after the debonding or retention period. Saliva samples were collected in an Oragene kits (Alharbi et al., 2020). Saliva collection was done during the early hours, between 8 a. m. and 11 a.m. To prevent contamination, participants were refrained from eating or drinking, brushing their teeth, or gargling

for 30–60 min before the saliva sample is collected. The saliva sample was obtained in the spitting method, using 2 ml (mL) as the collection volume. After consuming 2 oz of water, participants were instructed to rinse their mouths with distilled water for 1 min and then expectorate the water. Five minutes after the patients rinsed their mouths with the Oragene rinse, they were asked to spit into the tube. The dental chair position was suggested to them, where they should sit on the right side. To ensure saliva was flowing freely, the participants were asked to keep their heads lowered while they were speaking and to not speak until saliva had been flowing to the front of their mouths. Additionally, they were told not to cough up saliva because it is collected (AlHudaithi and Alshammery, 2021).

2.4. Fluoride test

Fluoride tests was performed with the saliva samples using the spectrophotometer with the colorimetric SPADNS method. 0.5 ml of saliva samples were mixed with 2 ml of SPADNS reagent and 8 ml of purified double distilled water. The experiment was performed with the spectrophotometer using the cuvettes. Fluoride ion concentration was measured at 570 nm using the Trisodium 2-(4-sulfophenylazo)-1, 8-dihydroxynaphthalene-3, 6-disulfonate (SPADNS) complex as a starting complex and the subsequent bleaching due to fluoride ion. Samples were repeated twice with different hours (Vincent and Thomas, 2019).

2.5. Statistical analysis

Fluoride levels were measured and tabulated between the first and second visits, and the results were compared using independent t-tests. The p-value of < 0.05 was considered significant and the SPSS software version 21.0 for windows for Windows (SPSS® Chicago, IL, USA) was used to conduct a statistical test (Khan et al., 2019).

3. Results

For this follow-up study, in which 35 individuals had previously undergone orthodontic treatment, 35 participants were found to be relevant. A treatment was classified into T_0 , which represents the debonding phase, and T_1 indicates thirty-five days after the treatment was defined as retention phase. Table 1 shows the information about the patients who participated in this study. In terms of treatment length, it took exactly 35 days for the orthodontic treatment to run its course. About 40% of the subjects were men, and 60% were women. The 35 participants had an average age of 21.5 ± 5.8 years. Women participants had an average age of 20.5 ± 6.2 , while male participants had an average age of 23.0 ± 4.91 . The duration of treatment was speckled based on the overall time of orthodontic treatment before de-bonding, which spans from 1 to 8 years. The male participants in this study ranged in age from 15 to 34 years old. In the case of women, the age range is 14–39 years. The mean treatment time was found to be 2.7 ± 1.6 ,

Table 1
Patient details of orthodontic treatment.

Details	Participants (n = 35)
Age (Years)	21.51 ± 5.8
Gender	M (14[40%]) & F (21[60%])
Retainer types	35 (100%)
Treatment duration	2.7 ± 1.6
Duration for male participants	2.9 ± 1.9
Duration for female participants	2.5 ± 1.4

Table 2
Biochemical analysis of the fluoride parameter.

Biochemical Test	T ₀	T ₁	Lower Limit	Upper Limit	Minimum variation	t	p
Fluoride	26.11 ± 4.86	27.71 ± 4.40	-3.81	0.61	-1.6	-1.44	0.56

Table 3
Descriptive statistics performed between fluoride levels in T₀ and T₁ phases.

Descriptive statistics	T ₀ (n = 35)	T ₁ (n = 35)
Total cases	35 (100%)	35 (100%)
Excluded cases	0 (0%)	0 (0%)
Number of Binned Values	35 (100%)	35 (100%)
Minimum	19	20
25% Percentile	22	24
Median	26	28
75% Percentile	29	30
Maximum	36	37
Mean	26.11	27.71
Std. Deviation	4.86	4.40
Std. Error of Mean	0.82	0.74
Lower 95% CI of mean	24.44	26.20
Upper 95% CI of mean	27.78	29.22

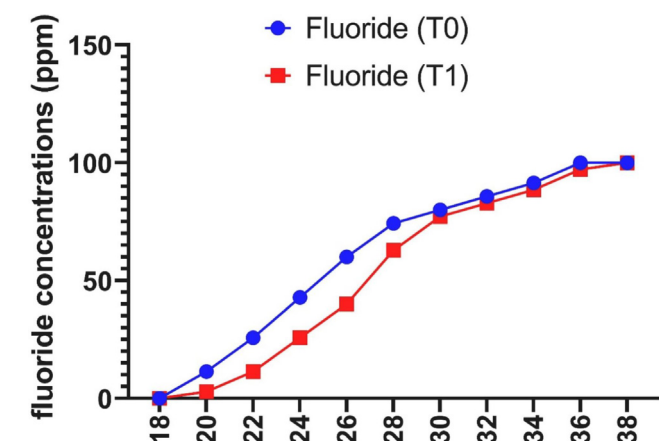


Fig. 1. Representation of fluoride levels in both T₀ and T₁ groups.

while the projected time for males was 2.9 ± 1.9 and 2.5 ± 1.4 for females.

Histogram represents (Fig. 1) the fluoride levels between T₀ and T₁ groups in the patients undergone the treatment. The mean age of the fluoride levels in T₀ and T₁ groups were found to be 26.11 ± 4.86 and 27.71 ± 4.40. When the student *t*-test was performed between both the groups, the study results were found to be almost all similar (p = 0.56). Both the higher and lower limits were in the range between 0.60 and -3.81. The minimum variation and *t*-stats were found to be -1.6 and -1.44 respectively Table 2.

Table 3 documents the descriptive statistics performed between the T₀ and T₁ groups. In both the groups, 35 participants were repeated within the gap between 1.25 months (5-weeks) and the minimum fluoride levels were found to be 19 and 20 in both the T₀ and T₁ groups. The fluoride levels were documented to be 22 and 24 as well as 36 and 37 in T₀ and T₁ groups. Both the 25% and 75% percentile were confirmed as 22 and 24, 29 and 30. The standard error of mean were 0.82 in T₀ and 0.74 in the T₁ groups. The lower (24.44 and 26.20) and upper (27.78 and 29.22) confidence intervals was observed in T₀ and T₁ groups. Fig. 2 presents the fluoride levels in both the T₀ and T₁ groups in the form of bar diagram.

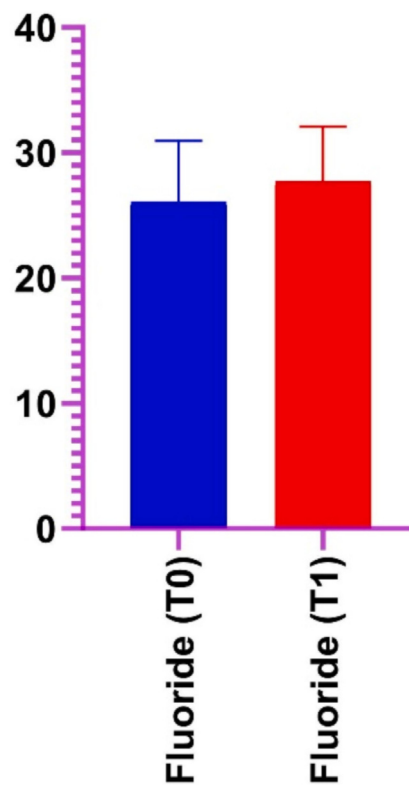


Fig. 2. Bar diagrams represents the fluoride levels between T₀ and T₁ groups.

4. Discussion

The aim of the present study was to investigate the role of fluoride levels among the patients who had undergone the fixed orthodontic treatment. The current study results confirmed the similar fluoride levels observed in both the groups (p = 0.56). A negative association of fluoride levels among the patients before and after the completion of the fixed orthodontic treatment was observed. One of the reasons for obtaining the negative results could be the lower sample size.

According to a study done by Peros, who states that use of 0.32% sodium fluoride-containing toothpaste 3 times daily is effective against mutans streptococci, however not to lactobacilli in the saliva of children with fixed orthodontic appliances (Peros et al., 2012). A wealth of studies has shown that topical fluoride can help with tooth decay by acting on dental plaque (Bowden, 1990; Johnstone et al., 2010; Zafar and Ahmed, 2015). The same findings have also been observed in other research, which have found that the administration and retention of topical fluoride in plaque results in microbial changes, leading to a less cariogenic environment. Fluoride toothpaste results showed that at one hour after use, the mean fluoride concentration in the users' saliva was above the baseline fluoride concentration (Emeke et al., 2019).

Global studies have confirmed both the positive and negative associations performed with the fluoride levels (Baeshen et al., 2010; Demito et al., 2011; Du et al., 2012; Enerbäck et al., 2019; Hallgren et al., 1990). Additionally, it has been a reliable treatment

measure to reduce caries potential when using traditional fluoride dentifrices (such as those that include fluoride, which is around 1000–1500 ppm). But for certain people, the traditional fluoride protocols have little to no effect on the caries rate (Vincent and Thomas, 2019). Of the prior trials, a 1,000-ppm fluoride toothpaste showed an 18% reduction in incremental caries rates as opposed to a toothpaste with greater fluoride levels (toothpastes) (Davies and Davies, 2008). Unfortunately, there are no meta-analysis studies carried out with the fluoride levels in the orthodontic treatment. However, fluoride toothpaste was shown to be an effective technique for avoiding dental cavities, as reported in a systematic review (Twetman et al., 2003). In previous investigations, the fluoride concentration in toothpastes has been shown to have a more or less linear relationship with the extent of caries reduction (Birkeland, 1972; Nordström and Birkhed, 2009; White and Nancollas, 1990). One of the previous study demonstrated that brushing with fluoride-free toothpaste is far less effective than brushing with fluoride-containing toothpaste in the prevention of tooth decay (Denes and Gabris, 1991).

One of the limitations of this study can be confirmed as only fluoride levels were measured that too only in a small number of patients. Other limitations of this study where we haven't repeated the fluoride tests with the toothpaste or miswak. The strength of this study can be confirmed as all the Saudi subjects were participated and the sample size was deemed to be adequate.

5. Conclusion

The present study confirms similar fluoride levels in both the groups. This study strongly recommends to carry-out the future studies with the additional groups as well as to repeat after quarterly, half-yearly and also for the annum with numerous time duration. This study also recommends to implement the meta-analysis studies with the fluoride levels of the group of patients undergone the orthodontic treatment with the fixed appliances and also fluoride level studies should be implemented in all the age group subjects with the large number of samples.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgment

This research project was funded by the Deanship of Scientific Research, Princess Nourah bint Abdulrahman University, Riyadh, Saudi Arabia through the Fast-Track Research funding program.

References

Alharbi, K.K., Al-Sheikh, Y.A., Alsaadi, M.M., Mani, B., Udayaraja, G.K., Kohailan, M., Ali Khan, I., 2020. Screening for obesity in the offspring of first-cousin consanguineous couples: A Phase-I study in Saudi Arabia. *Saudi J. Biol. Sci.* 27 (1), 242–246.

AlHudaihi, F.S., Alshammery, D.A., 2021. Screening of biochemical parameters in the orthodontic treatment with the fixed appliances: A follow-up study. *Saudi J. Biol. Sci.* <https://doi.org/10.1016/j.sjbs.2021.07.058>.

Alkhayat, D.H., Alshammery, D.A., 2021. Real time polymerase chain reaction analysis in the patients treated with fixed appliances after the orthodontic treatment: A follow-up study. *Saudi J. Biol. Sci.* 28 (11), 6266–6271.

Baeshen, H., Kjellberg, H., Birkhed, D., 2010. Oral fluoride retention in orthodontic patients with and without fixed appliances after using different fluoridated home-care products. *Acta Odontologica Scandinavica* 68 (4), 185–192.

Baeshen, H.A., 2021. Assessment of salivary pro inflammatory cytokines profile level in patients treated with labial and lingual fixed orthodontic appliances. *PLoS One* 16, e0249999.

Benson, P.E., Shah, A.A., Millett, D.T., Dyer, F., Parkin, N., Vine, R.S., 2005. Fluorides, orthodontics and demineralization: a systematic review. *J. Orthodontics* 32 (2), 102–114.

Bhat, S.S., Revankar, A., Patil, A., 2021. Biomarkers of orthodontic tooth movement and pain experienced during fixed orthodontic therapy in saliva: systematic review. *J. Dental Res. Practice*. 3–3.

Birkeland, J.M., 1972. Fluoride content of dental plaque after brushing with a fluoride dentifrice. *Eur. J. Oral Sci.* 80 (1), 80–81.

Bowden, G.H.W., 1990. Effects of fluoride on the microbial ecology of dental plaque. *J. Dent. Res.* 69 (2_suppl), 653–659.

Cunha-Cruz, J., Scott, J., Rothen, M., Mancl, L., Lawhorn, T., Brossel, K., et al., 2013. Salivary characteristics and dental caries: evidence from general dental practices. *J. Am. Dent. Assoc.* 144, e31–e40.

Dai, Z., Liu, M., Ma, Y., Cao, L., Xu, H.H., Zhang, K., et al., 2019. Effects of fluoride and calcium phosphate materials on remineralization of mild and severe white spot lesions. *BioMed Res. Int.*

Davies, R.M., Davies, G.M., 2008. High fluoride toothpastes: their potential role in a caries prevention programme. *Dental Update* 35 (5), 320–323.

Demito, C.F., Rodrigues, G.V., Ramos, A.L., Bowman, S.J., 2011. Efficacy of a fluoride varnish in preventing white-spot lesions as measured with laser fluorescence. *J. Clin. Orthod.* 45, 25–29.

Denes, J., Gábris, K., 1991. Results of a 3-year oral hygiene programme, including amine fluoride products, in patients treated with fixed orthodontic appliances. *Eur. J. Orthodontics* 13, 129–133.

Du, M., Cheng, N., Tai, B., Jiang, H., Li, J., Bian, Z., 2012. Randomized controlled trial on fluoride varnish application for treatment of white spot lesion after fixed orthodontic treatment. *Clin. Oral Invest.* 16 (2), 463–468.

Emeke, U., Obontu, T.J., Olushola, I., Akinyele, A., 2019. Salivary Fluoride Retention: A Comparative Analysis between Fluoride Containing Chewing Sticks and a Non Herbal Fluoridated Toothpaste. *J. Contemp. Dent. Pract.* 20, 370–376.

Enerbäck, H., Möller, M., Nylén, C., Ödman, Bresin C., Östman, Ros I., Westerlund, A., 2019. Effects of orthodontic treatment and different fluoride regimens on numbers of cariogenic bacteria and caries risk: a randomized controlled trial. *Eur. J. Orthodontics* 41, 59–66.

Hallgren, A., Oliveby, A., Twetman, S., 1990. Salivary fluoride concentrations in children with glass ionomer cemented orthodontic appliances. *Caries Res.* 24 (4), 239–241.

Johnstone, L., Spence, D., Koziol-McClain, J., 2010. Oral hygiene care in the pediatric intensive care unit: practice recommendations. *Pediatr Nurs* 36, 85–96.

Khan, I.A., Jahani, P., Hasan, Q., Rao, P., 2019. Genetic confirmation of T2DM meta-analysis variants studied in gestational diabetes mellitus in an Indian population. *DiabetesMetab Syndr.* 13 (1), 688–694. <https://doi.org/10.1016/j.dsx.2018.11.035>.

Kouvelis, G., Papadimitriou, A., Merakou, K., Doulis, I., Karapsias, S., Kloukos, D., 2021. A Prospective Cohort Study Assessing the Impact of Fixed Orthodontic Appliances on Saliva Properties and Oral Microbial Flora. *Oral Health Prev. Dentistry* 19, 67–76.

Marini, I., Pelliccioni, G., Vecchiet, F., Alessandri, B.G., Checchi, L., 1999. A retentive system for intra-oral fluoride release during orthodontic treatment. *Eur. J. Orthodontics* 21, 695–701.

Melo, P.E.D., Bocato, J.R., de Castro Ferreira Conti, A.C., de Souza K.R.S., Fernandes, T. M.F., de Almeida, M.R., et al., 2021. Effects of orthodontic treatment with aligners and fixed appliances on speech: A randomized clinical trial. *Angle Orthodontist*.

Naumova, Ella A., Kuehnl, Phillip, Hertenstein, Philipp, Markovic, Ljubisa, Jordan, Rainer A., Gaengler, Peter, Arnold, Wolfgang H., 2012. Fluoride bioavailability in saliva and plaque. *BMC Oral Health* 12 (1). <https://doi.org/10.1186/1472-6831-12-3>.

Nordström, A., Birkhed, D., 2009. Fluoride retention in proximal plaque and saliva using two NaF dentifrices containing 5,000 and 1,450 ppm F with and without water rinsing. *Caries Res.* 43 (1), 64–69.

Papadopoulou, C., Karamani, I., Gkoutrosogianni, S., Seremidi, K., Kloukos, D., 2021. A systematic review on the effectiveness of organic unprocessed products in controlling gingivitis in patients undergoing orthodontic treatment with fixed appliances. *Clin. Exp. Dent. Res.*

Pellissari, B.A., Sabino, G.S.P., de Souza Lima, R.N., Motta, R.H.L., Suzuki, S.S., Garcez, A.S., et al., 2021. Antimicrobial resistance of bacterial strains in patients undergoing orthodontic treatment with and without fixed appliances. *Angle Orthodontist*.

Peros, K., Mestrovic, S., Anic-Milosevic, S., Rosin-Grget, K., Slaj, M., 2012. Antimicrobial effect of different brushing frequencies with fluoride toothpaste on *Streptococcus mutans* and *Lactobacillus* species in children with fixed orthodontic appliances. *Kor. J. Orthodontics* 42, 263–269.

Seyam, Maha, Ibrahim, Samir, Gawish, Abeer, Sherif, Mohamed, 2021. Evaluation of the Effect of Fluoride Application on Periodontal Health during Orthodontic Treatment (A Clinical Study). *Al-Azhar Dental J. Girls* 8 (1), 157–167.

Stogiera, A., Barczak, K., Gutowska, I., Bosiacki, M., Chlubek, D., Szmidt, M., et al., 2020. Fluoride concentrations in saliva of chemical plant workers with respect to the general condition and microbial status of the oral cavity. *Fluoride* 53, 186–196.

Talwar, Manjit, Tewari, Amrit, Chawla, HS, Sachdev, Vinod, 2016. A comparative assessment of fluoride concentration available in saliva using daily prescribed topical fluoride agents. *Indian J. Dentistry* 7 (2), 76. <https://doi.org/10.4103/0975-962X.184647>.

Twetman, Svante, Axelsson, Susanna, Dahlgren, Helena, Holm, Anna-Karin, Källstäl, Carina, Lagerlöf, Folke, Lingström, Peter, Mejäre, Ingegerd,

- Nordenram, Norlund, Anders, Petersson, Lars G., Söder, Birgitta. . Caries-preventive effect of fluoride toothpaste: a systematic review. *Acta Odontologica Scandinavica* 61 (6), 347–355.
- Vincent, Sharon, Thomas, Abi M., 2019. (2019) Fluoride levels in saliva and plaque following the use of high fluoride and conventional dentifrices-a triple blinded randomised parallel group trial. *Sci. World J.* 2019, 1–7.
- White, D.J., Nancollas, G.H., 1990. Physical and chemical considerations of the role of firmly and loosely bound fluoride in caries prevention. *J. Dent. Res.* 69 (2_suppl), 587–594.
- Zafar, M.S., Ahmed, N., 2015. Therapeutic roles of fluoride released from restorative dental materials. *Fluoride* 48, 184.