



Investigating the Effect of Orthodontic Treatment on Salivary IgA Levels

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ARTICLE INFO

Article Type:
Original Article

Received: 10 April 2024

Revised: 15 May 2024

Accepted: 19 June 2024

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ABSTRACT

Introduction: One of the most important and complex functions of saliva is its defensive role, which is mainly performed by immunoglobulins, especially secretory IgA. This study investigated the effect of orthodontic treatment on changes in the salivary levels of IgA.

Materials and Methods: Forty patients undergoing orthodontic treatment were examined in two fixed and removable groups. Saliva collection was done in three stages: before orthodontic treatment and three and six months after orthodontic treatment. Salivary IgA level was measured using the ELISA method. First, the salivary samples were centrifuged, and then the amount of IgA in the upper part of the solution was determined using the ELISA method.

Results: In the fixed and removable orthodontic treatment groups, the amount of salivary IgA before treatment, three months after treatment, and six months after treatment, according to ANOVA, showed a significant difference in the mean of salivary IgA levels.

Conclusion: With the start of orthodontic treatment, one can witness an increase in the defense mechanisms of saliva, as evidenced by an increase in the amount of IgA, with differences between fixed and removable orthodontic treatments.

Keywords: Orthodontic treatment; Saliva; IgA.

Please cite this Article as:

Arefi AH, Khorshidi O, Hosseinabadi A. Investigating the Effect of Orthodontic Treatment on Salivary IgA Levels. J Craniofac Res 2024; 11(3): 168-174. DOI:



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Introduction

Orthodontic appliances in the oral cavity can create a different environment in terms of physical and chemical statuses and also serve as an extra location for food impaction and surfaces for the adhesion of normal oral flora in addition to tipping the balance of the microbial ecosystem of the oral cavity [1]. The oral environment is well protected by salivary immunoglobulins [2]. One of the most important and complex functions of saliva is its defensive role, which is mainly performed by immunoglobulins, especially secretory IgA [3]. This defense system acts against all oral microflora in different areas. Secretory IgA (S-IgA) also acts as the main axis of specific immune defense in saliva and plays an important role in oral microflora homeostasis [4].

Oral surfaces maintain their integrity through this salivary antibody. These surfaces include tooth enamel and mucous membranes that act as the first line of defense. IgA also plays an important role in antigen and antibody reactions, preventing the penetration of bacterial toxins such as lipopolysaccharides into deeper tissues [4–6]. Serum immunoglobulins are commonly measured by special immunological methods such as ELISA [7]. The desirable features of ELISA include high sensitivity and no need for radioisotopes [8].

IgA antibody is produced at a higher rate than other antibodies during the day. This antibody is the second most prevalent serum antibody and the most dominant antibody in mucous secretions, which has two A1 and A2 subtypes, which are almost equally present in the serum [9]. Antibodies of this type participate in the preservation and integrity of the oral surfaces (enamel and mucous membranes) by preventing the adhesion of microorganisms [10]. S-IgA antibodies independently or in complex participate in antigen-antibody reactions in the mucous membrane (to some extent in tooth enamel) and limit the penetration of bacteria and toxins [11]. The highest amount (90%) of S-IgA is produced by parotid and submandibular salivary glands [6]. The amount of immunoglobulins in saliva and serum is different. In some pathological processes, these relationship changes are meaningful and can have diagnostic value. A decrease in S-IgA results from changes in the immunity of the oral cavity and is the cause of some pathological processes in the oral cavity [12,13]. Studies have shown that the average amount of S-IgA in the saliva in both groups under fixed and removable orthodontic treatment after treatment is significantly higher than before treatment, and

the amount of salivary IgA in the fixed orthodontic group is significantly higher than that in the removable group [14]. In addition, the level of salivary IgA in children with gingivitis was significantly higher than that of healthy children [15]. A study examining the relationship between gingivitis and salivary immunoglobulins in patients with thalassemia major concluded that salivary immunoglobulins do not increase in response to gingivitis [16]. Considering the few studies on the changes in the levels of salivary IgA due to orthodontic treatments, we investigated the effect of orthodontic treatment on the levels of salivary IgA in this study.

Materials and Methods

This cross-sectional/analytical study was conducted in the Zahedan Faculty of Dentistry on 40 patients, including two groups of patients using fixed and removable orthodontic appliances, with 20 in each group, including 10 girls and 10 boys referring to the Specialty Clinic of the Faculty of Dentistry, Zahedan University of Medical Sciences, who met the inclusion criteria. Inclusion criteria include not having genetic, systemic diseases, no drug treatment, no history of chemotherapy and radiotherapy, no oral pathology in the examination, no dry mouth, no active caries, good oral hygiene, age of 8–15 years, patient consent to participate, and no smoking. Salivary samples were collected in three stages: before orthodontic treatment and three and six months after orthodontic treatment. The unstimulated spitting method was used to collect salivary samples. The logic behind collecting unstimulated saliva was to obtain S-IgA in sufficient concentration, while stimulated saliva increases salivary flow and further decreases S-IgA concentration. The patients were advised to avoid eating and drinking (except water) and chewing gum for an hour before saliva collection. This ensures the minimization of possible food residues or any salivary flow stimulation. It is established that the circadian rhythm also affects the salivary flow and concentration, so the samples were collected between 9:00 and 12:00 AM. The participants were asked to rinse their mouths, and after 5 minutes, their unstimulated saliva was collected in sterile tubes. The participants were asked to collect saliva on the floor of their mouth and then pour it into a previously labeled sterile tube. Then, 1.5mL of saliva was withdrawn using a dropper and transferred into a test tube. Salivary samples were stored in dry ice, immediately transferred to the laboratory, and frozen at a normal temperature of -20°C. Salivary IgA level was measured using the ELISA method according to the manufacturer's instructions. First, the salivary samples were centrifuged, and then

the concentration of IgA in the supernatant solution was determined by ELISA using the EASTBIOPHARM Company kit (Xihu District, Hangzhou City, Zhejiang Province, China). Data were analyzed using frequencies and frequency percentages in each group using SPSS 20. If the findings were normal, ANOVA and post hoc Tukey tests were used to compare IgA levels at the three time intervals. The independent samples t-test was used to compare the amount of IgA in two types of orthodontic treatment.

Results

In this research, 20 patients were using fixed orthodontic appliances. Table 1 presents the salivary IgA levels before and 3 and 6 months after orthodontic treatment in the groups. Table 1 shows that in the fixed orthodontics group, salivary IgA levels were 215.7 ± 96.37 , 310.3 ± 140.07 , and 356.7 ± 152.56 $\mu\text{g/mL}$ before treatment, three months after treatment, and six months after treatment, respectively. ANOVA showed significant differences in the mean salivary IgA levels between the investigated time intervals. Tukey tests for the two-by-two comparisons of time intervals showed that salivary IgA levels before treatment and three months after treatment and salivary IgA levels before treatment and six months after treatment were significantly different, but salivary IgA levels three months and six months after treatment were not significant-

ly different. In this study, 20 patients were using removable orthodontic appliances. Table 3 presents the salivary IgA levels before orthodontic treatment and 3 and 6 months after orthodontic treatment in this group. The results of Table 3 show that in the removable orthodontic treatment group, the levels of salivary IgA before treatment, three months after treatment, and six months after treatment were 204.58 ± 70.09 , 268.94 ± 151.98 , and 314.9 ± 170.16 $\mu\text{g/mL}$, respectively. ANOVA showed a significant difference in the means of salivary IgA between the three time intervals. Tukey test for two-by-two comparison of time intervals showed that the salivary IgA levels before treatment and three months after treatment and salivary IgA levels three months after treatment and six months after treatment did not show any significant difference. However, the salivary IgA levels showed a significant difference before treatment and six months after treatment. In this research, 40 patients were present in two groups of 20, and the salivary IgA levels in patients with fixed and removable appliances before and 3 and 6 months after orthodontic treatment are presented in Table 5 and Figure 1. Table 5 shows that the salivary IgA level is similar between fixed and removable orthodontic patients. Three and six months after treatment, there was no significant difference between the salivary IgA levels between fixed and removable orthodontic patients.

Table 1. Comparison of salivary IgA levels in the fixed orthodontics group at the three intervals.

Interval	No.	Mean ($\mu\text{g/mL}$)	SD	Min	Max
Before treatment	20	215.70	96.37	105.5	370.06
After 3 months	20	310.3	140.07	170.1	521.3
After 6 months	20	356.7	152.56	180.04	702.1
P-value			0.002		

Table 2. Pairwise comparisons of time intervals in terms of salivary IgA levels in fixed orthodontic treatment.

Time	(J)	Mean difference (I-J)	P-value	95% confidence interval	
				Lower bound	Upper bound
Before treatment	After 3 months	-94.60*	.049	-194.96	5.75
After 3 months	After 6 months	-141.035*	.004	-241.39	-40.67
After 6 months	After 6 months	-46.430	.510	-146.79	53.93

Table 3. Comparison of salivary IgA in removable orthodontic patients in the three investigated intervals.

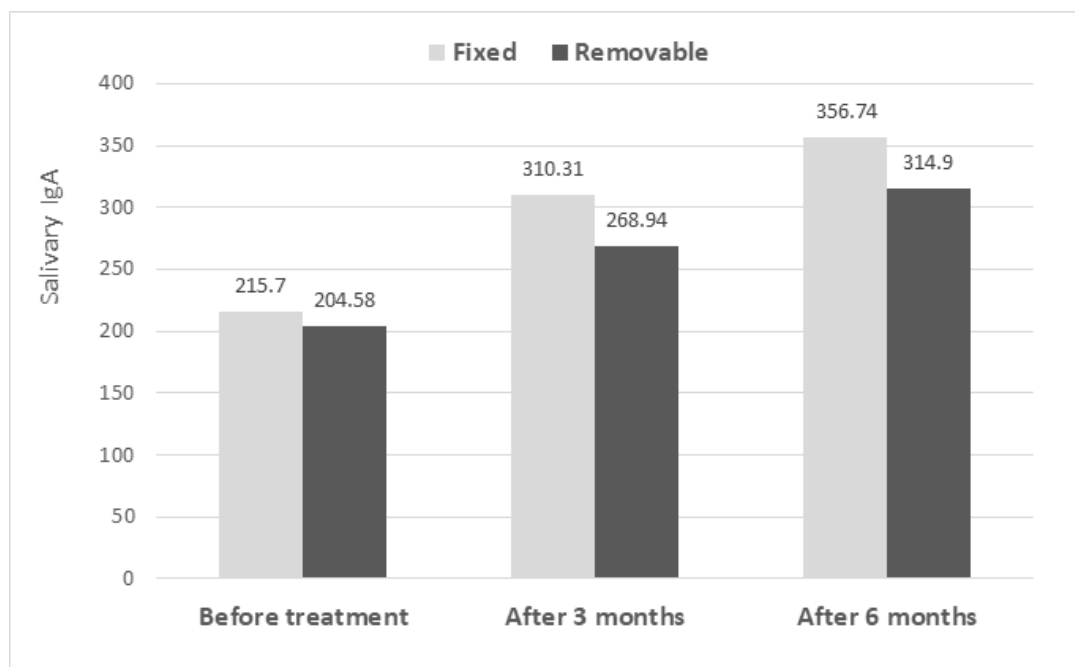
Time	No.	Mean ($\mu\text{g/mL}$)	SD	Min	Max
Before treatment	20	204.5	70.09	115.4	309.3
After 3 months	20	268.94	151.98	123.1	550.6
After 6 months	20	314.90	170.16	120.0	723.1
P-value			0.046		

Table 4. Two-by-two comparisons of time intervals in terms of salivary IgA levels in removable orthodontic treatment patients.

Time	(J)	Mean difference (I-J)	P-value	95% confidence interval	
				Lower bound	Upper bound
Before treatment	After 3 months	-64.365	.309	-169.22	40.49
Before treatment	After 6 months	-110.960	.037	-215.18	-5.46
After 3 months	After 6 months	-45.960	.546	-150.82	58.90

Table 5. Comparison of salivary IgA levels in fixed and removable orthodontic treatment patients at the three time intervals.

Time	Fixed		Removable		P-value
	Mean	SD	Mean	SD	
Before treatment	215.70	96.37	204.58	70.09	.679
After 3 months	310.31	140.07	268.94	151.98	.376
After 6 months	356.74	152.56	314.90	170.16	.418

**Figure 1.** Salivary IgA levels in fixed and removable orthodontic treatment patients at the three time intervals.

Discussion

Secretory IgA antibodies play an important role in oral homeostasis. They are a sign of adaptive immunity in the mouth and interact with oral microorganisms. In this study, 20 patients with fixed orthodontic treatment and 20 patients with removable orthodontic treatment were examined three times: before treatment and three and six months after treatment regarding salivary IgA levels. The frequency of boys and girls in the two groups was similar, and the age range of the patients was 8–15 years. The rationale for selecting a group of children with removable and fixed orthodontic appliances was that they have a strong stimulatory antigenic effect [17].

In our study, the level of salivary IgA increased significantly in fixed orthodontic treatment after three months and six months compared to before treatment. Youness et al. (2015) showed a positive and significant relationship between S-IgA and the type of orthodontic treatment. In the study of these researchers, the mean level of saliva S-IgA in two groups (under fixed and removable orthodontic treatment) was significantly higher after treatment than before treatment, and the level of salivary IgA in the fixed orthodontic group was significantly higher than the removable one [14], which might be attributed to the lower access to maintain oral hygiene and the presence of more areas of plaque retention during fixed orthodontic treatment [18]. Also, in 2016, Shaymaa et al. studied 30 patients aged 18–25 and showed that the level of S-IgA was significantly different between the two time intervals of T0 (before treatment) and T3 (one year after treatment [19]. In 2020, a study was conducted to measure the amount of salivary IgA in two treatment groups with fixed and mobile orthodontic appliances in children. The results showed increased S-IgA levels in both groups after 3 and 6 months [20].

The results of these three studies were consistent with our study. Fixed orthodontics and appliances in the oral cavity make it more difficult to achieve oral and dental hygiene, changing the microflora and homeostasis of the oral cavity. The materials used in orthodontics cause the adhesion of microorganisms, create new areas for plaque formation, and increase the microbial load and the possibility of infection [21]. The appliances in fixed orthodontic treatments are made of different alloys, including nickel, cobalt, and chromium. These metal ions and monomers have detrimental effects on the adjacent oral tissues, cause pathomorphological changes in the oral cavity, and can be a potential

source of antigenic stimulation. It can be concluded that fixed orthodontic appliances serve as an immunological stimulus in the oral cavity, change the level of S-IgA, and change the immune status. Also, removable orthodontic appliances may provide a significant stimulus to the safety of oral secretions [22]. It has been shown that using removable appliances causes the release of allergens from methylmethacrylate monomers and other organic substances from chemically cured removable appliances and resin-based bonding materials. The preliminary investigations showed that allergic patients with orthodontic appliances show changes in the morphology and composition of salivary cells compared to control patients [23,24].

In 2019, Jing et al. showed that the salivary IgA levels did not change significantly during the 18-month period of fixed orthodontic treatment, and no correlation was observed between S-IgA and bacterial counts. The results of the above study were contrary to our study [25]. The difference in the gender ratio of the participants in this study and our study and, consequently, the hormonal differences affecting the immune system can justify this difference in the results. Also, the age difference of the participants in the two studies and the fact that the humoral immune mechanism changes with age increases [4] is another possible reason for the differences in the results. In 2021, a study was conducted on orthodontic patients treated with premolar extraction and canine distalization, leading to the conclusion that there was no significant difference in the salivary IgA level before and after canine distalization [26]. The reasons for the differences in the results might be the type of orthodontic appliances (the type of the metal), the age range of the patients, the gender of the patients, the presence of stress factors, etc., all of which affect the salivary IgA levels.

In our study, in removable orthodontics, the salivary IgA levels three months after treatment were not significantly different from the baseline; however, six months after treatment, they increased significantly compared to the baseline. The longer the orthodontic treatment is, the patient's motivation and cooperation to comply with oral hygiene decreases [27]. Also, the surface roughness of the materials used and the accumulation of bacterial plaque that occurs as a result of it increase with increased duration of treatment [28]. Rashkova et al. (2009) evaluated S-IgA in children with different periodontal statuses, reporting that gingivitis was observed in 50% of children with diabetes and 30% of children undergoing orthodontic treatment. However, the S-IgA of children with gingivitis was not

significantly different from the S-IgA of children without gingivitis. The results of this research are somewhat different from the present study; however, it should be noted that in this study, salivary IgA was investigated in orthodontic children with gingivitis and not in children undergoing orthodontic treatment without gingivitis [15]. In the present study, the salivary IgA levels between fixed and removable orthodontic patients were almost the same three and six months after treatment. In 2022, Al-Khafaji et al. conducted a study on microbial accumulation and secretory IgA in crossbite patients undergoing comprehensive orthodontic treatment. They concluded that crossbite treatment in the early stages reduced the risk of microbial infection, and secretory IgA levels increased as one of the first lines of defense, consistent with the present study [29].

Conclusion

1. In fixed orthodontics, the salivary IgA levels increased significantly three months after treatment and six months after treatment compared to the baseline.
2. In removable orthodontics, the salivary IgA levels three months after treatment were not significantly different from the baseline. However, six months after treatment, there was a significant increase compared to the baseline.
3. The salivary IgA level was almost similar between fixed and removable orthodontic patients three and six months after treatment.

Conflict of Interest

There is no conflict of interest to declare.

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