Original Article

Comparative evaluation of salivary immunoglobulin a levels between pedodontic subjects

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Abstract

Background and Aims: Host immune response is altered by a series of physiologic and pathologic factors like age, gender, inflammation, surgery, medication etc., The present study was conducted to evaluate differences in salivary IgA (S-IgA) levels among pedodontic subjects undergoing active orthodontic treatment with fixed and removable appliance. The levels of S- IgA were determined before 3 months and 6 months post active orthodontic treatment. **Methods:** A total of 40 healthy pedodontic subjects (aged 8-15 years) were recruited in the present study. They were equally divided into Group A (fixed orthodontic group) and Group B (removable orthodontic group) with 20 subjects each. 1.5 mL of saliva per subject was obtained before 3 and 6 months after treatment. Enzyme Linked Immunosorbent Assay (ELISA) technique was used for measurement of Salivary IgA levels. **Results:** Group A and B both showed significant rise in S-IgA levels 3 months and 6 months post active orthodontic treatment. Mean value of S-IgA 3 months post treatment in the saliva of children in group B and group A were (144.27 ± 5.32) and (164.0 ± 3.23) µg/ml respectively. While mean value of S-IgA after 6 months of treatment in group B and group A were (149.8 ± 6.02) and (166.4 ± 3.65) µg/ml respectively. **Conclusion:** Salivary Immunoglobulin A level values were significantly higher statistically in both group A and group B post active orthodontic treatment than before. The results however, showed that Group A (fixed orthodontic group) showed statistically significant higher levels of S-IgA than Group B (removable orthodontic group). Active orthodontic treatment triggered a stronger stimulus for oral secretory immunity, hence the increase in levels were detected. There is a significant positive correlation between S-IgA and active fixed as well as removable orthodontic treatment. Orthodontic treatment is hence a local immunogenic factor.

Keywords: Pedodontic, removable and fixed orthodontic treatment, secretory immunoglobulin A

Introduction

Oral environment is well protected by salivary immunoglobulins.[1] Immunoglobulin A (IgA) is the most frequently occurring secretory immunoglobulin in mixed saliva. This secretory Ig assures the acquired immunity of the host. Oral surfaces maintain their integrity through this salivary antibody. These surfaces include enamel and mucous membranes which serve as first line of defence. S-IgA actively participate in prevention of any microbial ingress in the deeper tissues and also prevent their adhesion to the aforementioned surfaces. This IgA also plays an important role in Ag-Ab reactions hence prevents bacterial toxins like lipopolysaccharide to penetrate deeper tissues.[2-4]

Parotid and submandibular salivary gland produce the maximum amount (90%) of salivary IgA and the dimeric form of this antibody is formed by the plasma cells present in these glands. This IgA-dimer further attaches to a secretory particle and

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undergoes proteolysis and is secreted by the epithelial cells of acini.[5] Saliva and serum produce different amount of immunoglobulins. These dynamics change during an active inflammation or pathology. Hence saliva is used as biomarker and has a diagnostic value. In other conditions, there might be reduction in IgA production due to alteration in oral immunity.[6,7] This may stem into some oral pathologies.

Subgingival plaque is mainly governed by IgG a principal immunoglobulin of gingival crevicular fluid (GCF) as S-IgA does not enter the sulcus. However, it has been reported in studies that S-IgA can possibly cause alteration or modulation in subgingival plaque accumulation. So, indirectly S-IgA controls the formation and composition of subgingival plaque.[8] Gingival inflammation results in increased gingival blood vessels. Consequently, during active inflammation larger quantities of serum IgA antibodies are found in the gingival sulcus.[8] It is clear that S-IgA plays a contributing role in oral homeostasis. It is an important indicator of the acquired immunity of the oral cavity, where the rich oral microflora has antigenic potential and can stimulate the precipitation of secretory antibodies.[5,6]

The current study is of importance for general public in a way that there is always a misconception about the gingival inflammation caused by fixed and removable orthodontic treatment, as being a cause for post treatment periodontitis. The point of importance and the primary care is to control the gingival inflammation caused by orthodontic treatment and failure to do so might not be irreversible losses, and can be treated with prophylaxis and curettage.

Aim

The aim of this study was to assess:

1. Investigation of the average values of S-IgA in group A (fixed orthodontic group) and group B (removable orthodontic group) before, three and six months after treatment.
2. Evaluation of S-IgA level changes between group A and group B after treatment.

Methods

Study design

This is a prospective clinical study comparing the S-IgA levels in healthy pedodontic patients treated with removable orthodontic appliances and subjects treated with fixed orthodontic appliances before, three and six months after treatment and the S-IgA levels between both groups after treatment (Ethical approval 18-10-2019). A total of 40 healthy pedodontic subjects (aged 8-15 years) were recruited in the present study. They were equally divided into Group A (fixed orthodontic group) and Group B (removable orthodontic group) with 20 subjects each. 2 mL of saliva per subject wasobtained before 3 and 6 months after treatment. Enzyme Linked Immunosorbent Assay (ELISA) technique was used for measurement of Salivary IgA levels.

Inclusion criteria

1. Healthy pedodontic subjects
2. Age range (8-15 years)
3. Compatible with the study design
4. Free of any systemic or chronic diseases
5. Free of caries
6. Able to maintain good oral hygiene.

Exclusion criteria

1. Adults
2. Patients above 15 years of age
3. Undergoing other dental treatment besides orthodontic treatment.

All children included in this study were free from any apparent genetic disorders or dental anomalies, apparently healthy, free from. They were caries free and have good oral hygiene.

The children were divided equally into two study groups:

1- Group A containing children treated with fixed orthodontic appliances.
2- Group B containing children treated with removable orthodontic appliances.

Collection of saliva

An informed consent was procured from parents prior to enrolment of subjects into the study. The children were asked not to eat or drink one hour before collection of unstimulated saliva. To prevent any discrepancy in the saliva concentrations due to the effect of circadian rhythm, a morning appointment was given (10-11 am). All salivary samples were collected in sterile containers, saliva was collected by passive drool method; the participant was asked to accumulate the saliva in the floor of the mouth and then spit it into a pre-labeled sterile container. Then 1.5 ml of saliva was taken by a dropper and stored in test tubes. Salivary samples were stored on dry ice and were carried immediately to immunologic laboratory where they kept frozen at the deep freezer (Samsung RZ90EERS) at -20°C.

Methods of detection of S-IgA in saliva

The S-IgA levels in saliva were measured by ELISA (Enzyme Linked Immunosorbant Assay) Kit.

Statistical methods

The collected data were tabulated, and statistically analyzed using IBM SPSS statistics (Statistical Package for Social Sciences) software version 22. Descriptive statistics were presented for quantitative data as mean ± (standard deviation SD), minimum, maximum and range, while it was presented for qualitative data as number and percentage. Inferential analyses were done for quantitative variables using independent *t*-test in cases of 2 independent groups with parametric data and paired *t*-test in cases of 2 dependent groups with parametric data. In qualitative data, inferential analyses for independent variables were done using Chi square test for differences between proportions. The

level of significance was taken at *P* value < 0.050 is significant, otherwise is non-significant. The *P* value is a statistical measure for the probability that the results observed in a study could have occurred by chance.

Results

Group A and B both showed significant rise in S-IgA levels 3 months and 6 months post active orthodontic treatment. Mean value of S-IgA 3 monthspost treatment in the saliva of children in group B and group A were (144.27 ± 5.32) and (164.0 ± 3.23) μg/ml, respectively [Table 1]. While mean value of S-IgA after 6 months of treatment in group B and group A were (149.8 ± 6.02) and (166.4 ± 3.65) μg/ml, respectively.

Discussion

Saliva is one of the many secretions that are predominantly rich in secretory immunoglobulin A isotype. S-IgA is regarded as the first line of defence which protects against the assault by microbes that inhibit the oral cavity which is constantly flushed by saliva secreted by salivary glands. There have been evidence reporting detection of indigenous pathogens of oral microbiota to be coating S-IgA.[6] The present study is one of a kind as there is still limited data available on evaluation of S-IgA during orthodontic treatment. Another peculiar feature is enrolment of young pedodontic subjects in the study.

Literature reviews are limited on such studies that investigate co-relation of immunogenic activity of active orthodontic treatment that trigger a stimulus for increase release of S-IgA.[9] Some studies have also attempted to investigate relation between root resorption and S-IgA. The conclusion drawn by these studies reflect a statistically significant increase in levels of S-IgA post orthodontic treatment compared to pre-treatment data. In the present study, a comparison is drawn between co-relation of S-IgA and fixed versus removable orthodontic treatment groups.

Rationale behind collecting unstimulated saliva was to obtain S-IgA in adequate concentration. While stimulated saliva results in increased salivary flow, it further reduces the concentration of S-IgA.[10,11]

In the present study, individual child in each group (A and B) was instructed to accumulate their saliva in the floor of the mouth followed by spiting the same into sterile container that was already pre-labelled. About 2 mL of unstimulated saliva was collected and 1.5 ml used for testing. Children were advised in advance not to eat or drink (except for water) an hour prior to saliva collection. This ensured minimisation of probable food debris or any kind of salivary stimulation. It is a well-known fact that circadian rhythm affect salivary flow rate and concentration too so all samples were collected between 10-11 am, so as to ensure any discrepancy in salivary concentration.[12]

Measurements of S-IgA levels were done through ELISA technique. Favourable attributes of ELISA:

1. Highly sensitive
2. No need for radioisotopes (radioactive substances)[13]
3. Specific for detection of analytes.

The rationale for the selection of a group of healthy children with removable and fixed orthodontic appliances was that their antigenic action has been shown to have a strong antigenic stimulus.[14] The focus on saliva studies was still now on evaluating the influence on the secretion rates of saliva and IgA levels induced by inflammation, systemic diseases, surgery, medication, sport, various syndromes with gene mutation. In spite of these studies, the interrelation between IgA, age and sex are being the most investigated among them, it has been reported that salivary secretion rate may inversely influence the IgA concentration in saliva. In the current study, before treatment there was no significant differences in the average values of S-IgA for group A and group B. The average of S-IgA values become significantly elevated with time. There were significantly higher values of S-IgA for both groups 3 and 6 months after treatment. After treatment the average values of S-IgA were significantly higher in group A than the values recorded in group B at the two different time points. This finding can be explained by the stimulatory effect exerted directly by the conditions created in the mouth by the presence of the removable and fixed appliances, which make good oral hygiene more difficult to achieve, and thus change the microflora and oral homeostasis. Various authors have studied the influence of orthodontic appliances on the oral environment of children.[15-17] Furthermore, in orthodontic therapy, different materials are used and subjected to a damp oral environment. The materials used in orthodontic therapy are liable for microbial adhesion, greatly inhibit oral hygiene and create new retentive areas for plaque and debris, which in turn predisposes the wearer to increased microbial burden and possibility of subsequent infection. Available reports suggests that it is difficult to remove the microbial growth or clean the orthodontic appliances fixed

Table 1: Comparison between study groups regarding IgA (μg/mL)

Group	Measure	Group A (*n*=14)	Group B (*n*=14)	PA/B
Before treatment	Mean±SD	137.45±2.5	139.73±2.3	^0.367
3 months after treatment	Mean±SD	164.0±3.23	144.27±5.32	^<0.001
6 months after treatment	Mean±SD	166.4±3.65	145.8±6.02	^<0.001
Difference between 3 ms and Before	Mean±SD	26.55±0.73	4.54±3.02	^<0.001
Difference between 6 ms and Before	Mean±SD	28.95±1.15	6.07±3.72	^<0.001
Difference between 6 ms and 3 ms	Mean±SD	−2.40±0.42	−1.53±0.70	^0.147

^Statistically significant

at the critical sites.[18] The use of biomaterial components in orthodontic practice was shown to release potential allergens such as metal ions from base metal alloys in fixed appliances, methylmethacrylate monomers and other organic substances from chemically-curing removable appliances and resin-based bonding materials. The results of preliminary investigations indicated that allergic patients with orthodontic appliances exhibit changes in the morphology and composition of salivary cells as compared to control patients. Intra-oral orthodontic appliances, frequently used in the treatment of malocclusions, may cause pathomorphological changes in the mouth and can be a potential source of antigen stimulation.[19,20] In our study, we succeeded to provide significantly the mean S-IgA levels increased due to active orthodontic treatment.

Conclusions

Removable and fixed orthodontic appliances appeared to be a local immunogenic factor, which provided a stronger stimulus for oral secretory immunity. Secretory immunity as a marker for local acquired immunity in the oral cavity may be affected by local factors which provided a stronger stimulus for the oral secretory immunity system.

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Conflict of interest

There is no conflict of interest.

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