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Effect of Orthodontic Treatment on Salivary Immunoglobulin A Levels among a group of healthy Egyptian Children

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Abstract: *It has been reported that the immune system undergoes changes due to various factors, inflammation, surgery, medication, age and gender. The aim of this study was to investigate changes of salivary IgA (S-IgA) among healthy subjects undergoing active orthodontic treatment with fixed and removable appliance. The levels of S- IgA were determined before, 3 and 6 months after active orthodontic treatment. A total of 38 healthy individuals (aged 8-14 years) were enrolled in the study. They were divided into two equal groups. Group A were treated with fixed orthodontic appliances and group B were treated with removable orthodontic appliances. Two milliliters of saliva were collected from all participants before ,three and six months after treatment. Salivary IgA levels were measured by the ELISA technique.*

Results: *The average values of S-IgA in the saliva of group A and group B three and six months after treatment were (160.4 ±3.5 and162.0±3.3 µg/ml) and (145.8 ±6.0 and 146.7±5.2 µg/ml), respectively.*
The average values of S-IgA in the saliva in both group A and group B showed statistically significant higher values after treatment than before. The average values of S-IgA in group A showed statistically significant higher values than those of group B. There was no significant differences between both groups regarding age and sex.

Conclusions: *The average values of S-IgA in both group A and group B showed statistically significant higher values after treatment than before. The average values of S-IgA in group A showed statistically significant higher values than those of group B.*
Removable and fixed orthodontic appliances appeared to be a local immunogenic factor, which provided a stronger stimulus for oral secretory immunity. There isa significant positive correlation between S-IgA and active orthodontic treatment.

Keywords*: Secretory Immunoglobulin A, Removable Orthodontic Appliances, fixed orthodontic appliances.*
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I. Introduction

Saliva plays an important role in the oral environment and contributes to protection and homeostasis in the oral cavity [1]. Secretory immunoglobulin A (S-IgA) is the most frequently immunoglobulin found in mixed saliva and is considered to be a secretory factor for acquired immunity in the oral cavity. Antibodies of this type participate in the preservation of the integrity of the oral surfaces (enamel and mucous membrane) and, through restriction of microbial adhesion, become part of the first line of defense. S-IgA antibodies independently, or in complexes, participate in antigen-antibody reactions on the mucous membrane (and partly on the enamel too), thus limiting the penetration of bacteria and toxins [2,3,4].

The largest amount (90%) of S-IgA is produced by the parotid and submandibular salivary glands. The plasma cells of these glands secrete dimeric immunoglobulin A (IgA-dimer), which

associates with a secretory particle to proteolysis, and is secreted by the epithelial cells of the acini [5].

The amounts of immunoglobulins in saliva and in serum are different. In some pathological processes, this relationship changes, and could have diagnostic value. In other situations, the reduced production of S-IgA, as a consequence of altered oral immunity [6,7], may be the cause of some oral pathological processes.

Secretory IgA does not enter the gingival sulcus and so cannot control subgingival plaque. However, it is possible for S-IgA to modulate the accumulation of subgingival plaque, and thus control the latter's formation and composition. During gingival inflammation, there is increased permeability of the gingival blood vessels and of the gingivae. As a result, larger quantities of serum IgA antibodies are found in the gingival sulcus [8].

It is clear that S-IgA plays an important role in oral homeostasis and is an important indicator of

the defensive status of the oral cavity, where the rich oral microbiota has antigenic potential and can stimulate secretory antibodies [5].

S-IgA is also influenced by the status of the immune system of the individual and can be used as a marker for general health status and for the relationship between this status and that of the oral environment [6].

Aim

The aim of this study was to assess:

1. Determination of the average values of S-IgA in group A and group B before, three and six months after treatment.
2. Evaluate S-IgA level changes between group A and group B after treatment.

II. Subjects and Methods

Study design

This is a prospective clinical study comparing the S-IgA levels in children treated with removable orthodontic appliances and children treated with fixed orthodontic appliances before, three and six months after treatment and the S-IgA levels between both groups after treatment.

The study was designed and carried out in a private orthodontic clinic in collaboration with Department of Medical Biochemistry in National Research Center.

Study population

A total of thirty eight children were selected from those attended the Outpatient Clinic of a private orthodontic clinic.

The age group of the children ranges from eight to fourteen years. Sex equally distributed in both groups(9 female &10 male in each group). All children included in this study were free from any apparent genetic disorders or dental anomalies, apparently healthy, free from any systemic or chronic diseases. They were caries free and have good oral hygein.

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:

Subjects were informed in advance not to eat or drink (except for water) or chew gum for one hour before saliva collection. Obtaining an informed consent from the parents, unstimulated saliva was collected from each child in the morning between 10 to11 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 in The Department of Biochemistry in National Research Centre where they kept frozen at the deep freezer (Samsung RZ90EERS) at -20^{o} C.

Methods of detection of S-IgA in saliva

The S-IgA levels in saliva were measured by ELISA (Enzyme Linked Immunosorbant Assay) Kit (AssayMax, Human IgA ELISA Kit, USA, catalog No. E17001-1).

Statistical methods

The collected data were tabulated, and statistically analyzed using IBM SPSS statistics (Statistical Package for Social Sciences) (V. 22.0) software version 22.0,IBMCorp., USA, 2013.

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 two independent groups with parametric data and paired t-test in cases of two 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.

III. Results

Mean value of S-IgA after 3 month of treatment in the saliva of children in group B and group A were (146.7±5.2) and (162.0±3.3) µg/ml respectively. While mean value of S-IgA after 6 months of treatment in group B and group A were (145.8±6.0) and (160.4±3.5) µg/ml respectively.

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

Group	Measure	Group A (N=14)	Group B (N=14)	$P_{A/B}$
Before treatment	**Mean±SD**	139.5±2.5	140.3±2.3	^0.367
	Range	136.0–145.1	137.0–145.3	
3 months after treatment	**Mean±SD**	162.0±3.3	146.7±5.2	^<**0.001**
	Range	156.3–169.2	139.0–154.1	
6 months after treatment	**Mean±SD**	160.4±3.5	145.8±6.0	^<**0.001**
	Range	154.3–168.2	137.0–156.3	
Difference between 3ms and Before	**Mean±SD**	22.5±3.8	6.3±4.8	^<**0.001**
	Range	13.6–26.2	-3.9–12.2	
	$P_{Before/3m}$	#<**0.001***	#<**0.001***	
Difference between 6ms and Before	**Mean±SD**	20.9±3.9	5.5±5.3	^<**0.001**
	Range	12.0–25.3	-5.8–11.2	
	$P_{Before/6m}$	#<**0.001***	#**0.002***	
Difference between 6ms and 3ms	**Mean±SD**	-1.6±0.8	-0.8±1.8	^0.147
	Range	-3.0– -0.2	-2.1–5.0	
	$P_{3m/6m}$	#<**0.001***	#0.112	

^Independent t-test, #Paired t-test, *Significant,
&Negative values indicate reduction,

Table (1) and figure (1) show that: No significant difference between groups regarding IgA before treatment. After then the mean value of S-IgA levels were significantly higher in both groups (A&B) 3 and 6 months after treatment as compared to before. Degree of elevation was significantly higher in group A. Mean value of S-IgA level increased and reached the highest value 3months after treatment. after 6 month; there was no significant change in IgA from 3 month level in both groups.

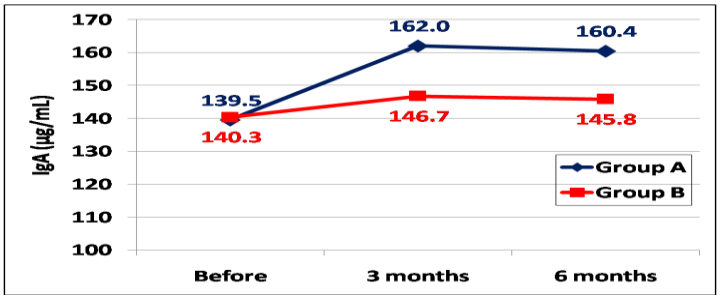

Figure (1): Comparison between study groups regarding IgA

There was no significant difference between study groups regarding age and sex as shown in table (2).

Table (2): Comparison between study groups regarding age (years), and sex

Group	Measure	Group A (N=19)	Group B (N=19)	$P_{A/B}$
Age (years)	**Mean±SD**	10.8±1.3	10.7±1.4	^0.890
	Range	7.0–13.0	8.0–13.0	
Sex (n, %)	**Male**	10 (42.9%)	10 (42.9%)	†1.000
	Female	9 (57.1%)	9 (57.1%)	

^Independent t-test ,†Chi square test.

IV. Discussion

Secretory immunoglobulin A (SIgA) constitutes the predominant immunoglobulin isotype in secretions, including saliva. It is considered to be the first line of defense against pathogens which colonize or invade surfaces bathed by external secretions. The main function of SIgA antibodies seems to be to limit microbial adherence as well as penetration of foreign antigens into the mucosa. Naturally occurring SIgA antibodies reactive with a variety of indigenous bacteria have been detected in saliva .Furthermore, indigenous bacteria of the oral cavity have been found to be coated with SIgA[6].

The effect of active orthodontic treatment and S-IgA levels was not yet throughouly investigated. There is only few studies in the literature where the relation between S-IgA and active orthodontic treatment was investigated in one of them [9]and the others investigated the relation between S-IgA and root resorbtion. In these studies, the authors concluded that increased S-IgA after treatment than before."In this study we compare between two study groups treated with two types orthodontic appliances. In other studies the authors compare between one or more study groups and control group.

Unstimulated saliva was collected by the passive drool method, passive drool is highly recommended because it is both cost effective and approved for the use with almost all analytes also it was easy to obtain the child's cooperation; in addition unstimulated saliva was collected because stimulated saliva increases the salivary flow rate which in turn decreases the concentration of S-IgA[10,11].

In this study, each subject was asked to accumulate the saliva in the floor of the oral cavity and asked to spit it into a pre-labeled sterile container and 1.5 ml saliva was collected. Subjects were informed in advance not to eat or drink (except for water) one hour before saliva collection to minimize possible food debris and stimulation of salivary secretion. Also all the salivary samples were collected between 10-11 AM. in order to prevent any differences in the concentration of the saliva due to the circadian rhythm[12].

ELISA technique was used in this study to measure the levels of S-IgA as it is considered highly sensitive, specific to detect analytes (S-IgA) in the body, also ELISA possesses the advantages of not needing radioisotopes (radioactive substances)[13]

The multivalence of S-IgA enhances its potential to agglutinate bacteria and neutralize toxins, enzymes, and viruses . The decreased ability of S-IgA to activate complement and opsonize bacteria for phagocytosis may limit local inflammatory reactions and mucosal tissue damage[6].

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 this 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. The result of some studies had shown that the secretion rate of saliva is different between both sex. We have demonstrated that the mean S-IgA levels increased significantly during active orthodontic treatment in both gender. No significant differences were observed between girls and boys regarding S-IgA levels which is consistant with finding recently reported in Swedish subjects by Eliasson et al [15].

In the current study, there was no significant differences of the average values of S-IgA for group A and group B before treatment. 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. Different authors have studied the influence of orthodontic appliances on the oral environment of children [5,16,17]. Most have investigated the toxic effects of orthodontic materials and have been conducted in vitro on a chosen cell population. 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. Fixed appliances promote continuous accumulation and retention of microbial growth. Available reports suggests, it is difficult to remove the microbial growth or clean the orthodontic appliances fixed 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].

Analysis of the results suggests that removable orthodontic appliances may provide a significant stimulus for oral secretory immunity. In a previous unpublished study [20], S-IgA is not affected by the quality of the saliva and is stimulated by acrylic removable orthodontic appliances which exert a strong influence on oral secretory immunity.

Orthodontic composites are often used by orthodontists for the bracket-banding process in children with both primary and permanent dentition. However, some components of the orthodontic composites may be released into the oral environment and saliva during fixed-appliance treatment, and even following polymerization[21].

The release of these components and their diffusion may cause various adverse effects in the organism,such as allergic reactions, systemic toxicity, cytotoxicity, mutagenicity, and carcinogenicity[22].

The appliances in fixed orthodontic treatments are fabricated from different alloys. These alloys include nickel, cobalt, and chromium. These metallic ions and monomers released from orthodontic composites

have harmful effects on the adjacent oral tissues. These effects exert a stronger influence on oral secretory immunity[23]. Nickel is a strong immunologic sensitizer, although nickel sensitivity has been reported to be lower in subjects who have received orthodontic treatment; perhaps they develop immunological tolerance over the long period of treatment. On the other hand, chromium and cobalt ions can also cause hypersensivity, dermatitis[24]. The installation of metal orthodontic devices inside the oral cavity leads to an increase in concentration of metalions which cause increase in biofilm biomass[25].

In our study we succeeded to provide significantly the mean S-IgA levels increased due to active orthodontic treatment.

V. 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 stimulous for oral secretory immunity system. There is a significant positive correlation between S-IgA and active orthodontic treatment

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