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The Effect of Fixed Space Maintainer Appliances on Salivary Level of Immunoglobulin A and other Saliva Properties

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Abstract: Secretary immunoglobulin A SIgA , play an important role in mucosal immunity, and the salivary level change in response to infection ,social , psychological and physical stress, also the change in level of salivary IgA and other saliva properties include salivary pH, salivary flow rate SFR, and body mass index BMI in children with fixed appliances were measured .The aim of this work was to compare the values of SIgA, PH, SFR and BMI in children with and without fixed appliances. This work included 20 children (10 males and 10 females) with mean age 6 years old who were users of fixed space maintainer for at least 12 weeks, and 20 children (12 males and 8 females) without fixed appliances and with mean age = 6.7 years. The level of SIgA in saliva were assessed by ELISA test. The results revealed that the mean values of SIgA and SFR were higher in fixed appliance group than the group without fixed appliance, with statistical significant difference, but no statistical significant differences were reported for salivary pH and BMI between the studied groups. The results also showed a weak positive correlation between SIgA and SFR (r = 0.07) while negative correlation between SIgA and pH in fixed appliance children (r = 0.1).
In conclusion, children with fixed appliances associated with high level of SIgA and SFR and the level of salivary pH and BMI do not correlated with fixed appliance.

Key words: Secretary immunoglobulin A, fixed appliances, body mass, flow rate.

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Introduction

The premature loss of primary molars may lead to mesial drifting of erupting posterior teeth, crowding in the dental arches, changes in the arch line, and inadequate space for the eruption of permanent teeth, all these eventually lead to malooclusions that are considered as the third most common of oral health problem, and are associated with number of complications (1,2).Thus the use of fixed space maintainer (SM) is one well-known method that can prevent these problems from happening (3). As well as, during treatment with fixed appliance there is an increased chance of plaque retention and greater difficulty in optimal oral hygiene maintenance which may lead to gingival inflammation (4). Secretory immunoglobulin A (SIgA) is the main immunoglobulin isotype found in saliva and other body secretion (5). Biologically, SIgA provides the first line of immune defense in the oral environment. It is responsible for inhibiting the bacterial adhesion on the enamel and epithelial cells, acting in synergy with other defense mechanisms, making inactive bacterial enzymes and toxins and activating the complement (6). It is partially involved in cell-mediated immune responses. Thus, SIgA limits the invasion of various antigens

in the mucosal epithelium and is involved in the maintenance of bacterial environment in the mouth and in the formation of biofilm on the enamel surface, also it has been reported that the immune system undergoes changes due to various factors, inflammation, surgery, medication, age, and gender (7).To evaluate the oral changes with fixed appliances, studies have used a variety of methodology designs. The way salivary parameters are investigated also varies from study to study, and many changes such as increased PH, buffer capacity and flow rate, may contribute to decreased susceptibility to dental caries (8,9). All these salivary properties become of utmost importance during treatment with fixed appliance when the cariogenic environment result from plaque accumulation is thought to predispose to enamel demineralization and white spot formation (10). Some authors compare the salivary parameters over time in a fixed appliance group without a control group (11,12), and time of follow-up also varies across studies (13). Also studies of the immunologic function in obese humans and experimental models indicate that excess body mass is associated with impairments in host defense mechanisms, increase body weight was also related to suppressed levels of monocyte as well as other immune markers, supporting the concept that obesity is associated with alterations in, and even suppression of immunity (14). The aim of this study was to evaluate the level of salivary IgA, flow rate, pH, and body mass of the patients wearing the fixed appliances and compare it with that of patients without fixed appliances.

Materials and Methods

The study population consisted of two groups of normal, healthy and cooperative children between (5 – 7) years. The first group comprised 20 children (10 males and 10 females) wearing fixed space maintainer (Band and loop B and L), the second group (20) was made up of children without space maintainers (12 males and 8 females). For both groups, non inclusion criteria were diagnosed diabetes mellitus, autoimmune disease, epilepsy, any syndrome, systemic or prolonged prescribed medication, antibiotic or local antiseptic therapy within the three weeks preceding the observation and presence of clinical signs of unhealthy gingival conditions. The research protocol was approved by Al-Mustansiriya University, Dentistry college from November , 2014 till February , 2015 . All patients and their parents were informed of the purpose of the study, non declined to participate.

The subjects of the first group were treated with band and loop space maintainer by using stainless steel band (Dentarum Stainless-steal band, Dentaform) appropriate for the tooth size was selected and contoured to adapt closely to the abutment teeth. The band was then seated on the tooth, and a full arch impression was taken using alginate impression material. Next, the band was removed from the mouth and placed in the impression, and a working model was prepared from dental stone. All B&L SMs were constructed by the same dental technician. Prior to cementation, abutment teeth were cleaned with prophylaxis paste and the B&L SM was cemented using glass ionomer cement (GC Fuji plus, resin reinforced multipurpose cement) (15). Oral hygiene instructions were given for both patients and parents after fixed appliance placement.

Salivary samples (5ml) were collected from children of both groups, and for those wearing SM the samples were collected 12 weeks after the placement of fixed appliances. The time of day for

sample collection was consistent for each subject during the study, and the subjects were instructed not to eat or to drink for at least one hour before sample collection and to brush their teeth once in the morning on the day of salivary collection (16). The saliva was collected by having the subjects spit for some minutes into a sterile plastic container, the volume of the collected saliva was measured in milliliters, and the calculation of the salivary flow rate was based on a collection time (milliliter per minutes).The samples were centrifuged and analyzed for the level of SIgA using enzyme linked immunesorbent assay (ELISA) in both study groups according to the manufacture protocol by using kit supplied by immunotech- company- France.

Estimation of SIgA by ELISA Procedure of the Test

1- Standard was constituted to 1000 pg/ ml with standard dilution buffer. a serial diluents of the standard were prepared from original standard .
2- One hundred ml of standard or sample was added per well, then 100 ml of diluent were added for each well. The plate was incubated for 4 hours.
3- The contents of well were discarded and washed by washing solution.
4- Two hundred ml of conjugate reconstituted was added into each well and incubated at 25 C° and then washed three times.
5- Two hundred ml of substrate was added into each well, followed by incubation for 15 minutes at 25 C° with continuous shaking in the dark.
6- Fifty ml of stopping solution H_2SO_4 was added into each well and mixed gently.
7- Absorbance was measured by spectrophotometer at 450 nm within two hours.

The flow rate of saliva (SFR) ml/min was estimated by dividing the total collected saliva volume (ml) by collecting time (min) that was measured by sample collection: SFR ml/min = saliva sample volume (ml)/ collection time (min). Salivary pH was measured with a digital pH-meter (Hanna Instruments, USA) 30 to 60 minutes after saliva samples were collected, and pH was considered as a quantative variable. The BMI for a person is defined as their body mass divided by the square of their height with the value universally being given in units of Kg/m^2. Statistical analysis was performed using SPSS (SPSS Inc. Chicago USA), independent t-test and correlation were used to analyses the data, p- value < 0.05 was considered as significant.

Results

Figure (1) described the sex distribution of the studied groups which showed that 40% of non wearing SM and 50% of wearing group were female and 60% of non wearing SM and 50% of wearing SM were male.

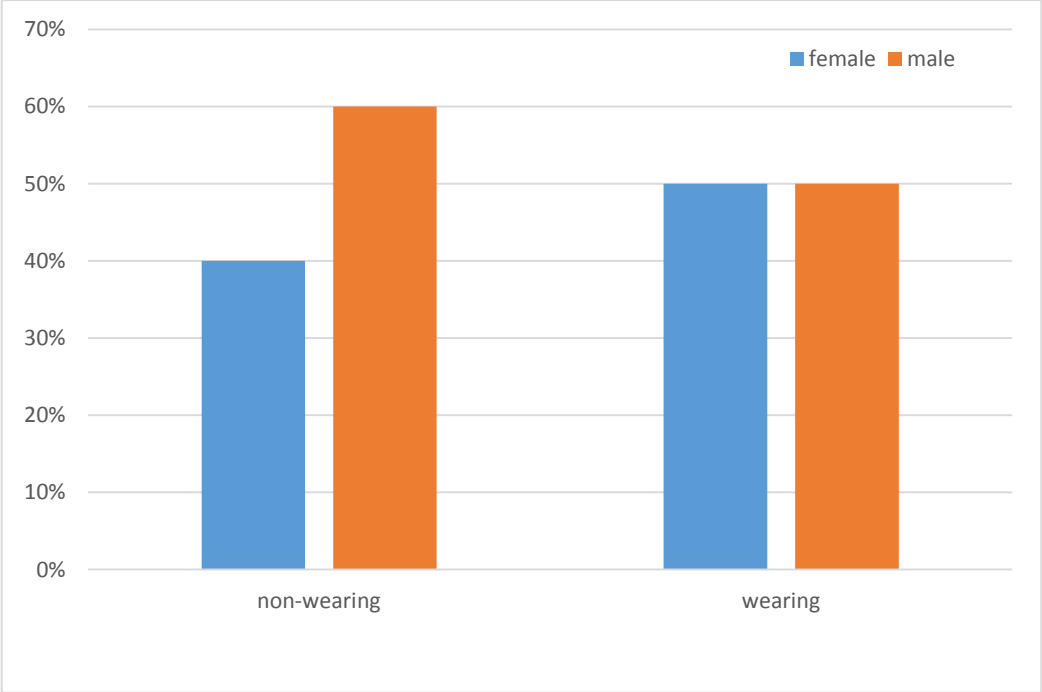

Figure (1) Sex distribution of the studied group

Table (1) represents the descriptive criteria of age and BMI of the studied groups, where the results showed that the mean of age (6.0) and BMI (22.1) of wearing group is higher than non-wearing group in which the mean of age was (5.7) and BMI (21.8).

Table (1) Descriptive criteria of age and BMI of the studied groups

Study groups		Age				BMI			
		Mean	Standard Deviation	Minimum	Maximum	Mean	Standard Deviation	Minimum	Maximum
Wearing state	Non-wearing	5.7	0.5	5.0	6.7	21.8	2.3	18.0	26.0
	Wearing	6.0	0.6	5.0	7.0	22.1	2.8	16.0	26.0

In Table (2), The results showed the difference in the mean level of salivary IgA between studied groups, where the mean level of wearing group is higher than non-wearing with statistical significant difference (p value=0.04), the same difference had been noted for the salivary flow rate with high statistical significant difference (p value=0.001), no statistical significant difference had been noted for the salivary pH (P value=0.18) by using T-test.

Table (2) The difference in mean level of different variables of the studied groups

Parameters	Wearing state	N	Mean	Std. Deviation	t-test	p-value
S IgA	Non-wearing	20	400.6	268.5	-2.047	0.04
	Wearing	20	617.6	390.5		
Salivary PH	Non-wearing	20	7.3	0.2	1.352	0.18w
	Wearing	20	7.1	0.2		
SFR	Non-wearing	20	1.8	0.06	20.465	0.001
	Wearing	20	2.2	0.05		

Results in table (3) showed no statistical significant difference in the mean level of all tested parameters in wearing group regarding the gender (p value $\geq$ 0.05).

Table (3) Mean value of different parameters of wearing group regarding gender

	Gender	N	Mean	Std. Deviation	T test	p-value
S IgA	Male	10	619.3	486.1	0.018	0.9
	Female	10	616.0	292.8		
Salivary PH	Male	10	7.09	0.24	1.628	0.1
	Female	10	7.29	0.30		
SFR	Male	10	2.27	0.04	1.852	0.08
	Female	10	2.23	0.048		

In table (4) the result of correlation showed weak positive correlation between salivary IgA (SIgA), salivary flow rate (SFR) equal to (0.07), and BMI equal to (0.1) with no statistical significant difference, also weak negative correlation between SIgA and salivary pH (- 0.1) had been noted with no statistical significant difference.

Table (4) Correlation between different parameters in wearing group

		S IgA	SFR	Salivary PH	BMI
S IgA	**Pearson Correlation**	1	0.07	-0.1	0.1
	Sig. (2-tailed)		0.7	0.4	0.1
	N	20	20	20	20
SFR	**Pearson Correlation**	0.07	1	0.1	0.3
	Sig. (2-tailed)	0.7		0.6	0.1
	N	20	20	20	20
Salivary PH	**Pearson Correlation**	-0.1	0.1	1	0.2
	Sig. (2-tailed)	0.4	0.6		0.3
	N	20	20	20	20
BMI	**Pearson Correlation**	0.1	0.3	0.2	1
	Sig. (2-tailed)	0.5	0.1	0.3	
	N	20	20	20	20

Discussion

Oral secretory immunity is characterized by antibodies of the SIgA type which are secreted in saliva and are the result of local antigenic stimuli. Wearing fixed appliances has been known to induce intraoral changes, such as increased plaque accumulation and elevated bacterial colonization along with potential enamel demineralization and harmful effect on periodontal tissues (17).

Over time people experience changes in salivary function, and these changes have a long-term clinical significance (16). Although some studies have detected association between fixed appliances and measures of salivary function, the results are not consistent. Chang *et al*., (18) found an increase in stimulated salivary flow rate, pH, and buffer capacity after 3 months of active treatment. The findings of kanaya *et al*., (19) were similar, except that salivary pH decreased in their study. Li *et al*., (20) found that during the first month of fixed treatment, the whole saliva flow rate and concentrations of some saliva ions increased significantly, but were at normal levels after 3 months. In this study the salivary flow rate and SIgA appeared to be higher in the group wearing fixed appliance after 3 months than non wearing group, while PH almost the same when comparing the two groups, highlighting that the period of fixed therapy is important in successful host defense.

Previous studies showed that the application of fixed appliances, dentures, and maxillary occlusal splints increased the salivary flow rate (21,22). Our findings presented an interesting correlation in SIgA during fixed appliance treatment and are in accordance with those of Corega *et al*., (23), which showed that the levels of

salivary IgA were significantly higher in subjects with fixed treatment than those observed in the control group**.**

It arises from these data that the increase in the salivary properties (SIgA, flow rate) of the wearing group when compared to the non wearing group can be considered as a physiological response to the mechanical stimulation resulting from the presence of fixed appliances, as a result of a disturbed intra oral homeostasis (24). No significant difference was noted in PH between the two groups, these results are in agreement with those by Sanpei *et al.*, (25), reporting no significant difference in this salivary parameter.

There were no significant differences between SIgA, salivary flow rate, and body mass. This is in contrast to the results of Niemanet *et al*., (26) who found that there was a correlation between body fat levels and SIgA in children.

From our results it is well known the placement of a foreign body into the oral cavity will causes a stimulation indicated by the increase in mean of the SIgA, and flow rate.

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