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USE OF REMOVABLE ORTHODONTIC APPLIANCE: DOES IT INFLUENCE THE SALIVARY COMPONENTS?

Marwa Sameh Shamaa* *and* Mostafa Mohamed Mansour**

ABSTRACT

Introduction: Saliva plays a key role in the oral cavity health. It contains many defense elements and is considered a cornerstone in the oral metabolism.

Objective: The aim of this study was to investigate the changes in the salivary concentrations of calcium, glucose, total protein, lactate dehydrogenase and alkaline phosphatase in patients undergoing removable orthodontic appliances treatment.

Methods: Ninety saliva samples were collected from thirty subjects ranging in age from 8 to 11 years. An initial sample was attained before starting treatment with removable orthodontic appliances; a second sample 1 month after treatment and a third sample was obtained three months following treatment. The saliva was collected from each patient in pre-labeled sterile containers using the passive drool method.

Results: Salivary lactate dehydrogenase and alkaline phosphatase concentrations were significantly increased in patients undergoing removable orthodontic treatment after as compared to before treatment. There was also an increase in the calcium, glucose and total protein concentrations but the differences were insignificant.

Conclusion: Removable orthodontic treatment changes the oral fluid contents, promotes an increase in the levels of salivary lactate dehydrogenase and alkaline phosphatase enzymes after one month of treatment with increased values after three months. These oral changes emphasize the importance of maintaining proper oral hygiene measures during treatment**.**

KEY WORDS: Saliva, Interceptive orthodontics, Salivary proteins and peptides.

INTRODUCTION

Saliva plays a fundamental role in the oral environment and it assumes a critical part in oral health protection [1]. The interest in using saliva as a diagnostic medium has increased dramatically in the modern era[2]. It has been utilized as a tool for periodontal diagnosis and to assess the caries risk in orthodontic patients. These diseases may necessitate premature removal of the appliances. Salivary tests

* Lecturer of Orthodontics, Faculty of Dentistry, Mansoura University.
** Lecturer of Clinical Pathology, Faculty of Medicine, Mansoura University.

are valuable both in assessment of the treatment result and in planning the preventive measures [3].

The salivary calcium has high affinity to be easily taken up by plaque. Thus, it is an imperative factor not just concerning the beginning of periodontitis but significantly with respect to the dental health [4].

The salivary glucose level increases with increasing DMFT index [Decayed Missing Filled teeth Index] and it assumes a fundamental role in high caries incidence[5].

The salivary total protein was reported to increase in patients undergoing orthodontic treatment [6] and in patients with active caries than caries free individuals [7].

Lactate dehydrogenase (LDH) is an enzyme that is released extracellularly only after cell death as it is normally limited to the cytoplasm. Thus its extracellular detection is attributed to tissue breakdown and cell necrosis[8]. The enzyme Alkaline Phosphatase (ALP) is considered as a potential marker for periodontal disease. The increased activity of ALP was observed in the acute phase of periodontal disease in many studies[9].

Removable appliances have played an important role in contemporary orthodontic treatment. It is the type of orthodontic appliances that is not permanently attached to the teeth and can be removed by the patient without orthodontist supervision. It can be effectively used for treatment of a great number of minor malocclusions[10].

Previous studies have focused on evaluating the changes in the saliva of patients undergoing orthodontic treatment with fixed appliances and no investigations have been made on the effect of removable orthodontic appliances treatment. Thus, the objective of this prospective study was to examine the changes in the salivary: calcium, glucose, total protein, LDH and ALP in patients undergoing removable appliances treatment with the null hypothesis that there is no difference between the salivary parameters at baseline, after one and three months of treatment.

MATERIALS AND METHODS

Study design and patient selection

This is a prospective clinical study comparing the levels of salivary calcium, glucose, total protein, LDH and ALP in children treated with removable orthodontic appliances before treatment and one and three months after treatment. A total of thirty patients (19 females and 11 males) aging from 8 to 11 years were selected from the patients who required orthodontic treatment with removable appliances at the Department of Orthodontics, Faculty of Dentistry, Mansoura University. The selection was based on the following criteria:(1) free from any systemic or chronic diseases, (2) no previous orthodontic or orthopedic treatment, (3) good oral health, (4) didn't receive any medications one month before and throughout the study period, (5) patient cooperation. The study was initiated after receiving signed informed consent forms from the participants keeping all their rights. . The research protocol was reviewed and approved by the Ethical Committee, Faculty of Dentistry, Mansoura University (No. 05130818).

All the patients received oral hygiene instructions and they were guided to the proper tooth brushing technique before the beginning of treatment to ensure good oral hygiene at the onset of the study. Patients were instructed to wear the appliances all times except during eating and tooth brushing which should be done three times daily. They were also instructed to clean the appliances by brushing them and rinsing with running tap water. The appliances were in passive contact with the mucosa, even though slight pressure could be produced from chewing.

Salivary Analysis

Salivary samples were taken from each patient in three stages, the first before insertion of the

removable appliance, the second stage one month after the appliance placement and the third stage three months following treatment.

The participants were instructed not to eat or drink and avoid tooth brushing at least two hours before sample collection in the three stages. The saliva was collected from each patient in the morning between 9 and 11 a.m in pre-labeled sterile containers. It was collected using the passive drool method. The patients were asked to accumulate the saliva in the mouth floor and spit it into the containers. 1.5ml of saliva was then taken by a micropipette and stored in eppendorf tubes. The salivary samples were placed on dry ice and sent immediately to the laboratory where they were preserved frozen at -20°c.

The Calcium concentrations were measured with calium_MTB (BioSytem S. A Costa Brava, 30.08030 Barcelona, Spain) and the absorbance values were detected with spectrophotometer (Erba Chem 7 Mannheim) at 600 nm.

Glugose was estimated using Glugose - TR (SPINREACT. Ctra. Santa Coloma, 7 E-17176 SANT ESTEVE DE BAS (GI) SPAIN) and its readings were detected using spectrophotometer (Erba Chem 7 Mannheim) at 505 nm.

Total protein was assessed using Chromarest (LINEAR CHEMICALS S.L Joaquim Costa 18 2 planta. 08390 Montgat, Barcelona, Spain) and the absorbance values were detected with spectrophotometer (Erba Chem 7 Mannheim) at 600 nm.

The LDH activity was measured with LDH SCE mod. liqui UV (Human Geselischaft fur Biochemica und Diagnostica mbH-Max-Planck-ring 21.65205 Wiesbaden Germany). The absorbance readings were detected with spectrophotometer (Erba Chem 7 Mannheim) at 340 nm.

The ALP activity was measured with ALP (DEA) SL (EliTech Clinical Systems SAS-Zone Industrielle - 61500 SEES FRANCE) and the absorbance values were detected with spectrophotometer (Erba Chem 7 Mannheim) at 405 nm.

Data entry and analysis were done using SPSS (version 20) under windows. Test for data distribution was done by one sample Kolmogorov- Smirnov test. Normally distributed data were described as mean ± SD. Non normal data were described by median, minimum and maximum. Comparison of matched pairs was done by paired t test and Wilcoxon signed rank test. P value was considered significant when less than 0.05.

RESULTS

In the current study, thirty participants (19 females and 11 males) were involved. The mean age of patients was 9.066 years with a range from 8 to 11 years.

Table 1 shows the changes in the salivary concentrations of calcium, glucose and total protein in patients undergoing removable orthodontic appliances treatment. There was no statistically significant difference in calcium, glucose and total protein levels after one month compared to base line values (p 0.1, 0.4 and 0.7 respectively). The same was also detected after three months compared to baseline readings (p 0.08, 0.4 and 0.2 respectively). Furthermore, no significant difference was found in these salivary parameters after three months of treatment compared to one month (p 0.7, 0.08 and 0.1 respectively) as shown in figures (1 & 2).

Treatment using removable orthodontic appliances exhibited significant ($p<0.05$) increase in both lactate dehydrogenase (figure 3) and alkaline phosphatase enzymes after 1 month ($p<0.001$) and 3 months of treatment ($p<0.001$) compared to baseline readings. The values of salivary LDH and ALP enzymes at 3 months were significantly higher compared to those after 1 month of treatment ($p<0.001$ and 0.001 respectively) (Table 2).

TABLE (1): Comparison between the salivary concentrations of calcium, glucose and total protein in patients treated with removable orthodontic appliances.

	Baseline	**1Month**	**3 Months**	**P1**	**P2**	**P3**
	Mean ± SD	**Mean ± SD**	**Mean ±SD**			
Calcium (mg/dL)	4.55**±** 1.01	4.45**±** 1.01	4.42 ± 0.9	0.1	0.08	0.7
Glucose (mg/dL)	3.18± 1.58	3.17± 1.57	3.24± 1.5	0.4	0.4	0.08
Total protein (g/dL)	5.9± 2.23	5.9± 2.28	5.9± 2.24	0.7	0.2	0.1

P 1: p values of the salivary parameters after 1 month of treatment compared to baseline.

P2: p values of the salivary parameters after 3 months of treatment compared to baseline.

P3: p values of the salivary parameters after 3 months of treatment compared to 1 month.

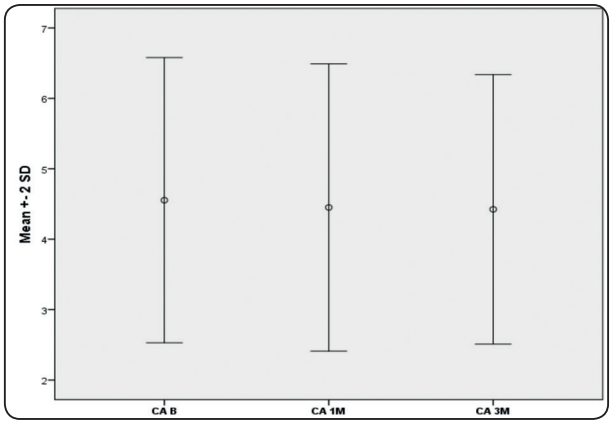

Fig. (1): Error bar chart for mean values of calcium at baseline, 1 month and three months of treatment.

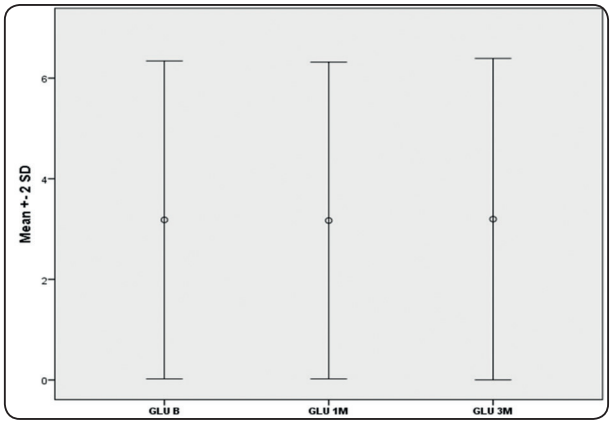

Fig. (2): Error bar chart for mean values of glucose at baseline, 1 month and three months of treatment.

TABLE (2): Comparison between the salivary concentrations of lactate dehydrogenase and alkaline phosphatase in patients treated with removable orthodontic appliances.

	Baseline	**1 month**	**3 Months**	**P1**	**P2**	**P3**
LDH Median (min-max) (U/L)	408.5 114-1459	426.5 202-1501	509.5 211-1650	**<0.001***	**<0.001***	**<0.001***
Alkaline phosphatase Mean ± SD (U/L)	44.6 ± 15.1	49.4 ± 15.6	64.6 ± 23.6	**<0.001***	**<0.001***	**0.001***

P 1: p values of the salivary parameters after 1 month of treatment compared to baseline

P2: p values of the salivary parameters after 3 months of treatment compared to baseline

P3: p values of the salivary parameters after 3 months of treatment compared to 1 month.

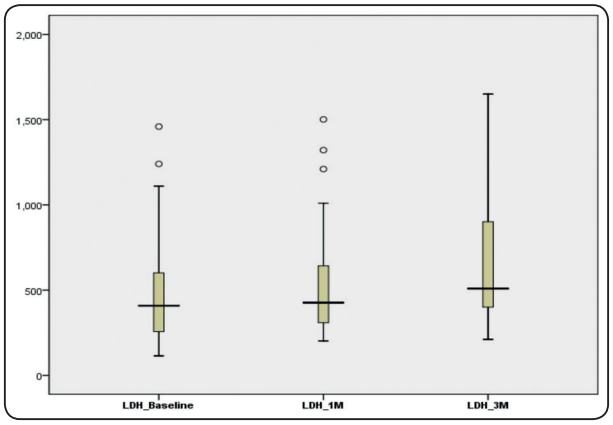

Fig. (3): Box plot for median LDH at baseline, 1month and 3 months.

DISCUSSION

Removable appliances could be considered as a mean of interceptive orthodontic treatment and it is mostly used in the mixed dentition period. It essentially either corrects the forthcoming dental malocclusion or decreases its severity [11].

Various tooth movements can be accomplished with removable orthodontic appliances, either individually or on group of teeth such as tipping, cross bite correction, overbite reduction, intrusion and extrusion. A removable appliance is considered a successful way for reduction of deep overbite in a growing child as it could increase the vertical dimension by allowing differential eruption of the posterior teeth through comprising anterior bite plane. If there is an available space, a removable appliance with an activated screw or palatal spring can be used for treatment of cross bite in the anterior teeth. Removable appliances are also effective in treatment of posterior dental cross bites through incorporating a midline expansion screw or spring resulting in widening of the maxillary arch in the mixed dentition [12].

Saliva is a complex mixture of fluids formed in the mouth and secreted by the salivary glands. The human saliva consists mainly of water in addition to leukocytes, electrolytes, epithelial cells, glycoproteins, antimicrobial agents and enzymes (such as amylase and lipase)[13] .

The salivary enzymes play a fundamental role in the process of digestion of dietary fat and starch. They also help in breaking down the entrapped food particles within dental crevices, thus they protect the teeth from bacterial decay. Moreover, saliva has a lubricative function and prevents dryness of the mucosal surfaces of the oral cavity [14].

Saliva has been utilized as a diagnostic tool in dentistry and medicine [15]. The key to successful prognostic outcome is the detection of disease in its early stage. Early detection tools should be non-invasive and easy to attain, this makes saliva a suitable diagnostic alternative to blood. There are many advantages of using the salivary fluid as a diagnostic tool compared to tissues or serum. The good patient compliance as a result of its non-invasive collection method, the correlation with levels in blood, greater sensitivity and easy storage and transportation are the advantages of using saliva for diagnostic purpose [16].

Calcium is one of the most extensively studied salivary components as it is the fifth most copious mineral in the human body which plays an important role in both dental and gingival health [17]. In our study, there was non-significant increase in the salivary calcium in the experimental group undergoing removable appliances treatment. Corega et al [4] found a significant correlation between higher salivary calcium and orthodontic treatment. Bhavsar et al [6] detected that the salivary calcium was significantly increased in patients undergoing fixed orthodontic treatment. This increase in the concentration of salivary calcium may result from demineralisation of the teeth subjected to fixed orthodontic appliances treatment. The salivary calcium affects the dissolution or precipitation of the enamel hydroxyapatite. In the present study, the non-significant change in the salivary calcium which is considered a marker of dimeneralisation suggests

that using removable appliances for a period of three months did not affect tooth dimineralisation.

The saliva of the experimental group revealed a greater glucose concentration after three months of treatment, even though this difference was not significant. This is in consistency with Forsberg et al [18] who found an increase in the salivary glucose concentration after insertion of orthodontic appliances. This finding may be correlated with the increased salivary flow rate.

Salivary proteins are contributed in multiple biological processes including the immune response, cellular support, tissue flexibility and strain. They have different functions like soft tissue repair, regulation of both antimicrobial activity and pH and maintenance of the dental and mucosal integrity. The concentration of the salivary proteins may be affected by many factors such as tooth brushing and/or exercise, psychological disorders and the presence of hormones [19].

In our study, there was non-significant increase in the salivary total protein concentration in the treated cases. However, Bhavsar et al [6] reported that in patients undergoing fixed orthodontic treatment, the total protein concentration was increased in saliva. Also, Arash et al [20]found an increase in the salivary total protein in patients after orthodontic tooth movement. They stated that the increase in the salivary total protein may be due to mechanical stresses that modify the concentration of the secretory proteins at the local level in the oral cavity. So, removable appliances treatment for that period of time may have little effect on the salivary total protein in comparison to fixed appliances.

Among the important constituents of saliva, there are various enzymes. Lactate dehydrogenase is a prevalent enzyme that plays an important role in the clinical diagnosis of pathologic processes. LDH was believed to have the capability to be utilized as a marker for inflammation process during orthodontic treatment[21] . In our study, LDH activity in saliva was significantly increased in patients undergoing removable appliances treatment both at 1 and 3 months. Values were significantly higher after 3 months compared to after 1month, meaning that there was a progressive rise with increased treatment duration. The present study was in line with that of Husin et al [22] who reported that orthodontic interrupted force application caused an increase in the salivary LDH. The increase in LDH activity in the saliva of our patients during removable appliances treatment may be explained by having increased retention sites for microbial samples compared to before treatment which may be responsible for gingivitis. The result of the current work is in accordance with another study that related the increased lactate dehydrogenase activity to tissue inflammation and damage mostly caused by gingivitis and periodontitis respectively [23].

Alkaline phosphatase is an enzyme secreted by neutrophils and its level is markedly increased with inflammation and plaque accumulation. Some investigators recorded high salivary ALP level in the acute phases of periodontal disease and they found that its level was returned to normal after periodontal therapy [9]. Therefore, change in the ALP level in saliva has been utilized as an inflammatory marker of the periodontium as well as bone metabolism [24]. In the current study, ALP activity was significantly increased in the saliva of patients undergoing removable appliances treatment both at 1 and 3 months, also the values at 3 months were significantly higher than at 1 month. This may be attributed to that removable appliances produce forces that are of intermittent type. An intermittent pressure may act as an irritant and usually produces formative changes, especially in young individuals. Also, tipping movements are produced when a single force is applied against the crown of a tooth leading to concentration of pressure in limited areas of the periodontal ligament. Furthermore, experimental studies in

beagles have demonstrated that orthodontic tipping forces could shift a supragingivally located plaque subgingivally resuling in the infrabony pockets formation [25]. Our results agree with that of Ameer et al [26] who reported a significant increase in the level of alkaline phosphatase enzyme during orthodontic treatment. They concluded that the level of ALP can reflect the biological activity occurring in the periodontium during orthodontic tooth movement. Thus, clinical and experimental researches stress the importance of adequate oral hygiene during orthodontic treatment.

CONCLUSION

According to our results, we can conclude that orthodontic treatment with removable appliances didn't induce significant changes in the salivary concentrations of calcium, glucose and total protein, while the inflammatory markers like LDH and ALP increased significantly which may reflect the effect of treatment on gingiva and periodontium. This emphasizes the importance of maintaining proper oral hygiene measures throughout the treatment period.

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