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ORIGINAL ARTICLE

The Relationship Between Orthodontic Force Applied by Monoblock and Salivary Levels of Alkaline Phosphatase and Lactate Dehydrogenase Enzymes

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ABSTRACT

Aim: The current study was aimed to determine the relationship between the orthodontic force applied by monobloc and the salivary level of alkaline phosphatase (ALP) and lactate dehydrogenase (LDH) enzymes, considering the time factor after insertion of the appliance and whether there is a correlation between these enzymes.

Materials and methods: A sample of 28 growing patients requiring orthodontic treatment with myofunctional appliance (Monoblock) was taken for the current study with an age range 9 to 12 years,all patients had Angle's class II division 1 malocclusion with no or mild crowding, the sample was selected using simple random sampling. Only 16 subjects (10 males and 6 females) were included who follow certain inclusion criteria. Unstimulated saliva was collected from the patients before monoblock insertion, then 1 hour after insertion, followed by 14 days and 28 days. Salivary levels of ALP and LDH were measured using a spectrophotometer and compared with the base line.

Results: The results revealed that ALP and LDH levels increased with increasing time after monoblock insertion, and there was the statistically insignificant difference after 1-hour post-insertion for ALP enzyme level, but highly significant after 14 and 28 days. While for LDH level,there was the statistically significant difference after 1-hour post-insertion, but highly significant difference after 14 and 28 days post-insertion. In this regard to the relation between salivary ALP and LDH enzymes levels at different time intervals, showed that there were no significant correlations between the enzymes using Pearson's correlation test.

Conclusion: The ALP and LDH salivary enzymes activity is affected by mechanical forces generated by monobloc activator and these enzymes activities can also be increased during the rapid growth phase of childhood such as late infancy and early puberty where the age of subjects was selected in the current study.

Clinical significance: The determination of ALP and LDH salivary enzymes activities during the skeletal maturity is crucial for the success of myofunctional monobloc treatment; therefore, saliva can be used as a noninvasive diagnostic tool for determination of chemical biomarkers for detection of bone remodeling process during myofunctional monoblock treatment

Keywords: Alkaline phosphatase (ALP), Lactate dehydrogenase (LDH), Monoblock, Orthodontic force, Salivary levels.

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INTRODUCTION

During orthodontic treatment, to get movement of the teeth, orthodontic treatment needs an optimum force, this optimum force should produce a maximum rate of tooth movement with minimum irreversible jeopardization of the periodontium.[1]

Orthodontic tooth movement is characterized by tissue responses and reactions, which consists of an inflammatory response in periodontal ligament and followed by bone remodeling in the periodontal tissues depending on the amount, type, direction, and period of forces applied, these processes trigger the production of various proteins and enzymes into the saliva. During orthodontic treatment, an orthodontic force results in alveolar bone remodeling that are represented by alveolar bone resorption in the pressure side and bone formation in the tension side.[2]

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To monitor, orthodontic tooth movement noninvasively in human beings, changes have been examined in the patient profile, the levels of various enzymes, cytokines, growth factors, biomarkers and proteoglycans in gingival crevicular fluid and saliva. Among those components that change and response to orthodontic force, ALP, TRAP, LDH, and aspartate aminotransferase (AST).[3,4] Although the clinical and radiographic follow-up examination remains the basis for patient's evaluation, an investigation of saliva, that is a fluid that contains local and systemically derived markers, may provide the basis for a phase-specific screening of orthodontic tooth movement.[5] Appropriate timing of the interception of skeletal malocclusion is the key to success in dentofacial orthopedics. In Class II growing subjects, the amount of supplementary mandibular growth induced by functional appliances appears to be significantly greater when the functional treatment is performed during the pubertal growth spurt.[6] The increase in osteoblastic activity during bone formation will be accompanied by an increased expression of an enzyme called alkaline phosphatase.[7] To investigate the bone remodeling pattern based on ALP activity during an orthodontic treatment, body fluids such as saliva can be used.[8]

The identification of salivary biomarkers and its use as a diagnostic tool has many advantages. It is much easier to collect; sufficient quantities can easily have obtained for analysis and no specific laboratory devices are necessary. The collection of saliva is also far less invasive compared to other body fluids such as gingival crevicular fluid and serum.[9] To the best of authors knowledge, no studies to date have described the ALP and LDH enzymatic activity in saliva during myofunctional treatment with monoblock activator. The aim of the current study was to determine and evaluate the changes in ALP and LDH enzymes activity in saliva when orthodontic force was applied by monoblock, considering the time factor after insertion of the appliance and whether there is a correlation between these enzymes, and if these observations can be used in the future for assessing the improvement of the bone remodeling process during orthodontic myofunctional treatment with monoblock and achieving the optimal treatment results at such age group of subjects.

MATERIALS AND METHODS

A sample of 28 growing patients requiring orthodontic treatment with myofunctional appliances (Monoblock) was taken for the current study with age ranged 9 to 12 years. Patients involved were attending the postgraduate clinic of the Orthodontic Department in the College of the Dentistry/University of Baghdad. All patients had Angle's class II division 1 malocclusion with no or mild crowding (about 2–3 mm), the sample was selected using simple random sampling.[10-12]

Only 16 subjects (10 males and 6 females) were included, all of them follow the inclusion criteria, which were free of oral and systemic diseases, had no periodontal problems, and had not been on a regimen of antibiotic therapy for at least 3 months before the commencement of the study. The subjects were willing to follow the oral hygiene instructions provided by the investigator strictly and agreed to follow the oral hygiene program, and orthodontic treatment prescribed for them with informed consents were obtained from the parents before the study. To standardize the sample, all the orthodontic appliances (Monoblock) were made by the same technician in the orthodontic laboratory. The selected patients were instructed to brush and use dental floss three times a day. For standardization, the oral hygiene of all the patients was provided by their parents throughout the study.

The patient was instructed not to drink or eat for at least 1 hour before the sample collection. The patient was instructed to sit in a comfortable position and spit unstimulated saliva into sterile plane plastic test tube within 10 minutes. About 5 mL of unstimulated whole saliva were collected into 10 mL sterile plastic plane test tube and was put in a cooling box to stop the growth of bacteria, as shown in Figure 1.

The samples were taken from each patient immediately before fitting the orthodontic appliance (Monoblock) at baseline, then after one hour, 14 days and 28 days after insertion of the appliance. The whole saliva was undergone a process of centrifugation for 20 minutes at 3000 RPM to get a supernatants saliva that was free from insoluble materials, after salivary collection by a pipette into Eppendorf tubes and frozen at minus 20°C until biochemical analysis, as shown in Figure 2A to C.[13-15]

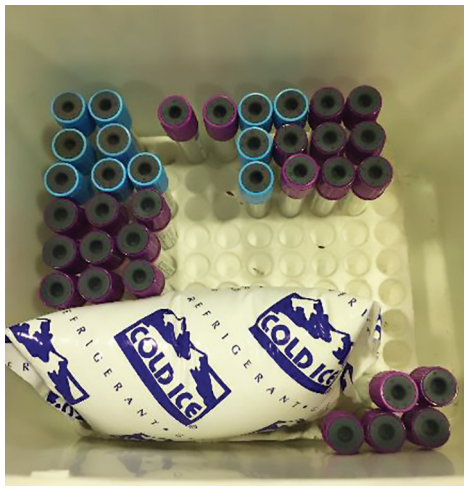


Fig. 1: Cooling box

The samples' analyzes were done in a private laboratory (Beirut Laboratory) to measure the concentration of ALP and LDH enzymes in saliva by kinetic method (spectrophotometrically) Mindray, semi-auto chemistry analyzer, model BA–88A Nanshan, Shenzhen 518057, P. R. China at constant temperature of 37° C, with less than 0.05° C fluctuation, as shown in Figure 3.[16-20]

Statistical Analysis

The data were analyzed using the Statistical Package for the Social Sciences program (SPSS), version IBM SPSS Statistics 24. The analyses including descriptive statistics (means and standard deviation) and inferential statistics [one-way analysis of variance (ANOVA)] were used to analyze the differences among mean salivary enzymes ALP and LDH activities (IU/L) at different time intervals, and if ANOVA revealed a significant difference, the least significant difference test (LSD) was used. Pearson's correlation test evaluated the correlation between salivary enzymes at different time intervals.

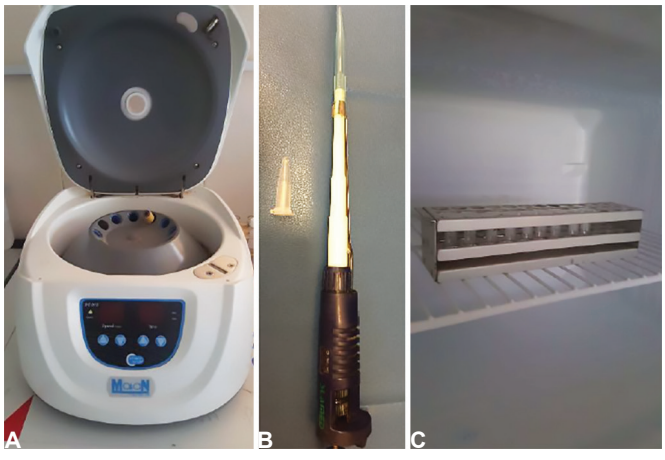
RESULTS

The mean salivary enzymes ALP level in IU/L was 33.94 ± 7.36 at the baseline before insertion of the monoblock, the level increased to a value of 42.38 ± 8.98 after one-hour post-insertion, while after 14 days of wearing the monoblock the level also increased to the value of 64.00 ± 15.50, after 28 days the level of the enzyme revealed a peak of 104.06 ± 28.47. The difference between time intervals was highly significant (p = 0.000) using F-test analysis of variance, as shown in Table 1.

The difference between time intervals for salivary enzyme ALP level using LSD showed that there was a statistically insignificant difference (p > 0.05) between baseline time and 1-hour post-insertion of monoblock appliance, while there were highly significant differences (p < 0.01) between one hour and 14 days, and 14days and 28 days, as shown in Table 2.

The mean salivary enzymes LDH level in IU/L was 179.63 ± 22.55 at the baseline before insertion of the monobloc, the level increased to 225.81 ± 33.06 after 1-hour post-insertion of the appliance, while after 14 days of wearing the monoblock the level also increased to the value of 320.19 ± 60.64, and the level of the LDH enzyme reached to the peak with a value of 496.31 ± 98.04. The difference between time intervals was also highly significant (p = 0.000) using F-test analysis of variance, as shown in Table 1.

The difference between time intervals for salivary enzyme LDH level using least significant difference test (LSD) showed that there was a statistically significant difference (p < 0.05) between base line time and one-hour post-insertion of monoblock appliance, while there were



Figs 2A to C: Centrifuge, pipette and eppendorf tube, and samples are frozen at minus 20° C

Table 1: Descriptive and inferential statistics for salivary enzymes ALP and LDH activities (IU/L) at different time intervals

Enzyme	*ALP*		*LDH*	
Duration	*Mean (IU/L)*	*SD*	*Mean (IU/L)*	*SD*
Baseline	33.94	7.36	179.63	22.55
1 hour	42.38	8.98	225.81	33.06
14 days	64.00	15.50	320.19	60.64
28 days	104.06	28.47	496.31	98.04
F-test	52.96		84.28	
df	63		63	
p-value	0.00		0.00	
Sig.	HS		HS	

Fig. 3: Spectrophotometry

Table 2: Difference between time intervals for salivary enzymes ALP and LDH levels using least significant difference test

		ALP		*LDH*	
Duration		*p-value*	*Sig.*	*p-value*	*Sig.*
Baseline	1 hour	0.171	NS	0.036	S
1 hour	14 days	0.001	HS	0	HS
14 days	28 days	0	HS	0	HS

Table 3: Correlation Between salivary enzymes ALP and LDH levels at different time intervals

ALP and LDH	*Pearson correlation*	*p-value*	*Sig.*
Baseline	0.259	0.351	NS
1 hour	0.407	0.133	NS
14 days	0.127	0.651	NS
28 days	0.083	0.767	NS

highly significant differences ($p = 0.00$) between one hour and 14 days, and 14 days and 28 days, as shown in Table 2.

Regarding the relation between salivary ALP and LDH enzymes levels at different time intervals, Table 3 shows that there were no significant correlations ($p > 0.05$) between the enzymes at different time intervals using Pearson's correlation test.

DISCUSSION

The myofunctional monoblock activator used in the current study consisted of a plastic device fitted on the lingual side of both upper and lower dentition and constructed to a bite interdentation block which will alter the mandible's functioning position.[21] this appliance is passive itself but serves as transmitter of forces generated by the oral and facial musculature when used in the oral cavity, so the principle of orthodontic treatment by monoblock myofunctional activator is based on redirection the pressures of the facial and masticatory muscles on the tooth and supporting structures to produce improvements in the tooth arrangements and occlusal relations by improving mainly jaws relations.[22] Various amounts of force magnitude, duration, and frequency applied a great effect on the surrounding tissues involving a bone remodeling process.[23]

Generally, many researchers have described the role of biomarkers during orthodontic force application such as ALP and LDH salivary enzymes activities, which have been associated with the bone remodeling process,[24-28] but this is the first ever research done to describe the activity of ALP and LDH enzymes expression in the saliva during myofunctional treatment with monoblock activator, the age at which the myofunctional appliance used is of a prime importance for successful treatment of Class II malocclusion, it should be initiated during the middle to the late mixed dentition period where the active growth is occurring, and the success is also totally dependent on patient cooperation and the appliance should be worn for a prolong period,[29] Ever since such type of appliances had an effect on teeth, researchers[30,31] found significant dentoalveolar change,a class I occlusion was achieved by distal tipping of maxillary dentition and mesial and vertical tipping of mandibular teeth by inhibiting vertical maxillary dentoalveolar development, while promoting vertical and mesial mandibular dentoalveolar development, if the Monobloc used during the growth spirit of the patient, it can cause an inhibition in the horizontal growth of maxilla and a significant decrease of the SNA angle,[32-34] and an increase in the condylar growth and a bone remodeling at the glenoid fossa and articular eminence, thus the combination of these effects resulting in a permanent forward mandibular posture,[33] on the other hand, investigators observed upper lip retrusion and soft tissue pogonion was further anteriorly positioned after the treatment with the monoblock.[34]

Since ALP is very important enzyme, and it is considered as a part of normal turnover of periodontal membrane, cementum, and bone, because it is produced by many cells including fibroblasts, cementoblasts, and osteoblasts, so all these forming cells show to have ALP activity, and the main source in saliva and gingival crevicular fluid is the neutrophils,[24,35] while LDH is an enzyme which reflects a tissue destruction and necrosis which were demonstrated during heavy orthodontic tooth movement.[36,37]

Regarding the enzymatic profile in saliva which reflects the process that occurs after monoblock insertion, both enzymes ALP and LDH showed a highly significant difference at different periods of time (1 hour, 14 days, and 28 days) after insertion of the monoblock (baseline time), this reveals a bone remodeling can occur after wearing the monoblock appliance, this can be supported by other studies that generally stated when any orthodontic force is applied, there will be a ruptured osteoblast and fibroblast, and their cellular content will be released extracellularly and tissue destruction occurred, thus resulting in the release of the ALP and LDH in saliva from gingival crevicular fluid,[24,25,38-43] current study finding showed that the level of ALP salivary enzyme not significantly increased after one hour of monoblockinsertion, while the level of LDH salivary enzyme significantly increased after one hour from the monoblock insertion, this occurrence is in accordance with the process of starting bone remodeling after insertion of monoblock, our finding further demonstrates that the bone destruction can occur as early as one hour post-insertion of monoblock, which can confirm the bone turnover process interpreted by Perinetti et al,[24] who described that the bone resorption can occur before bone formation during application of orthodontic force, and this principle is congruent with our result.

While the level of ALP and LDH enzymes activities showed highly significant differences after 14 days and 28 days, these findings demonstrated that there were overlaps between bone destruction and bone formation processes during wearing time of the monoblock, our result is in accordance with the basic principle of bone

remodeling process that stated a bone remodeling is carried out by a functional and anatomic structure known as the basic multicellular bone unit that works in a coordinated overlapping manner.[44,45]

There were no significant correlations between ALP and LDH salivary enzymes at different periods of time after wearing the appliance and these correlations were not strong (weak, "r <0,4"), this enzymatic protein expression may require more accurate and sensitive investigations and procedures to obtain a more obvious view to the real relation of active salivary biomarker that precisely reflects bone remodeling cycle during usage of monoblock.

CONCLUSION AND CLINICAL CONSIDERATION

It can be concluded from the results of the current study that ALP and LDH salivary enzymes activity is affected by mechanical forces generated by monobloc myofunction activator that can cause a bone remodeling process around the teeth and at the growth center in the temporomandibular joint, although these enzymes might be fluctuated by factors other than orthodontic forces such as gingival and periodontal inflammation, and oral hygiene of the subjects involved, where these other factors were kept under control during the course of the study, furthermore the ALP and LDH salivary enzymes activities can also be increased during rapid growth phase of childhood such as late infancy and early puberty, where the age of subjects sample was selected in the current study, so the determination of the skeletal maturity is crucial for the success of myofunctional monoblock treatment; therefore, saliva can be used as a noninvasive diagnostic tool for determination of chemical biomarkers for detection of bone remodeling process during myofunctional monoblock treatment and to a lesser extent can identify how much is the bone remodeling improvement and how much is the skeletal maturity at such age group subjects. Consequently, the ALP and LDH salivary enzymes can be promising bone remodeling biomarkers to assess the biological alterations and improvement in the bone remodeling process that occurs during treatment with monoblock activator and achieving the optimal treatment results at such age group of subjects.

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