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Estimation of Biomarkers in Gingival Crevicular Fluid during Accelerated Orthodontics

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Abstract

This study is to estimate the biomarker during canine retraction through a non-invasive procedure (GCF) to compare the level of biomarkers during canine retraction done through accelerated procedure by microosteo perforation.

Keywords: Orthodontics; Microosteo perforation

Introduction

Orthodontic tooth movement is based on the principle that an applied force to a tooth. Bone turnover during orthodontic tooth movement characterized by continual bone deposition on the tension side and continual bone resorption at sites of pressure. However, recent Histomorphometric data suggest that this process may be more complex than this classical paradigm.

The forces exerted during orthodontic treatment cause distortion of the periodontal ligament (PDL) extra-cellular matrix, resulting the synthesis and secretion of extracellular matrix components, tissue-degrading enzymes, acids, and local factors; induce cellular proliferation and differentiation; and promote wound healing and tissue remodelling [1]. Application of continuous force produces concomitant bone resorption and formation with increased activities of both osteoclast and osteoblast [2]. The increase in osteoblastic activity during bone formation is accompanied by an increased expression of an enzymealkaline phosphatase (ALP) [3]. The presence of TRAP and ALP activity signifies osteoclastic and osteoblastic activity, respectively. Therefore, TRAP and ALP can act as biomarkers for bone resorption and bone formation. Today it is a challenge to reduce the duration of orthodontic treatment. Many biological and surgical approaches have been introduced. Treixeira, et al. [4,5] has shown that biological principles can be activated to accelerate bone remodelling using micro-osteo perforations. Most of the studies to evaluate the inflammatory response to accelerate orthodontic tooth movement were done in animals (rats). Mani alikhani, et al. [6] also evaluated the level of inflammatory response by measuring CCL-2 (MCP1), CCL-3, CCL-5 (RANTES), IL-8 (CXCL8), IL-1a, IL-1b, IL-6, and TNF-a in GCF of humans. This study is to estimate the biomarker during canine retraction through a non-invasive procedure (GCF). To compare the level of biomarkers during canine retraction done through accelerated procedure by microosteo perforation.

Material and Method

A 15 orthodontic patients (Mean age: 19years, Range: 15-23years) were included in the study. The patients were not divided on the basis of sex. The study was a split mouth design. Inclusion criteria are, both Male and female with age range 15 to 23 years, Class I and class II bimaxillary malocclusion requiring 1st premolar extraction with no canine root prominence, 2q1`radiographic evidence of bone loss, systemic disease, no history of periodontal therapy, current active periodontal disease no smoking, gingivitis with no untreated caries. Also probing depth should be < 4mm in all teeth, gingival index ≤ 1mm and plaque index less than or equal to 1. And exclusion criteria are long term use of antibiotics, phenytoin, cyclosporine, anti-inflammatory drugs, systemic corticosteroids, and calcium channel blockers. Extreme skeletal class ll malocclusion with Overjet > 10mm, Bone diseases and Probing depth>4mm in any tooth. Materials used Rubber separators (Ortho Organizers) for separation, band material (RMO

.018x .006), welder, MBT kit of 0.018" slot (American Orthodontics), Pilot Lance Drill (Alpha Bio Tec, Simplantology Alpha Bio Tec LTD), micro motor, contra-angle hand piece. Etchant, bonding agent, composite (Transbond XT, 3M, Unitek) for bonding, LED curing light (unicorn, Easy Light II). Archwire (0.014", 0.016", 0.016 × 0.022NiTi, 0.016 × 0.022"SS), 0.009" ligature wire. NiTi closed coil spring 9.0 mm, 150 gm force (Optima, Desire). Armamentarium required for GCF sampling. Endodontic paper point (No-30) and Eppendorf tube. For biochemical assay UV Spectrophotometer (Shimadzu, Japan), Centrifuge machine (REMI centrifuge; S.no EMLC -4532), Chemical for biomarkers (ALP) (Figures 1-15).

***Figure 1**: Performing MOPs.*

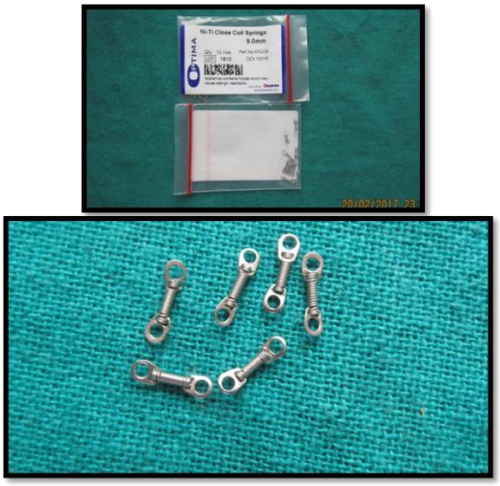
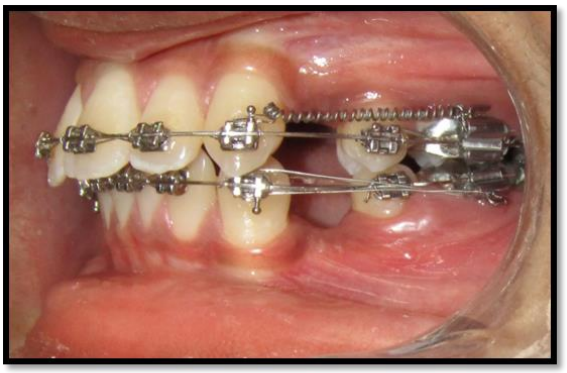


***Figure 2:** NiTi Coil Springs.*

***Figure 3:** Measuring Gauge.*



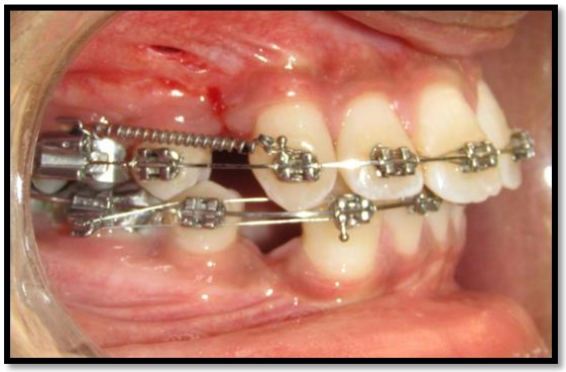


***Figure 4:** Canine retraction using NiTi Coil Spring (Right side).*

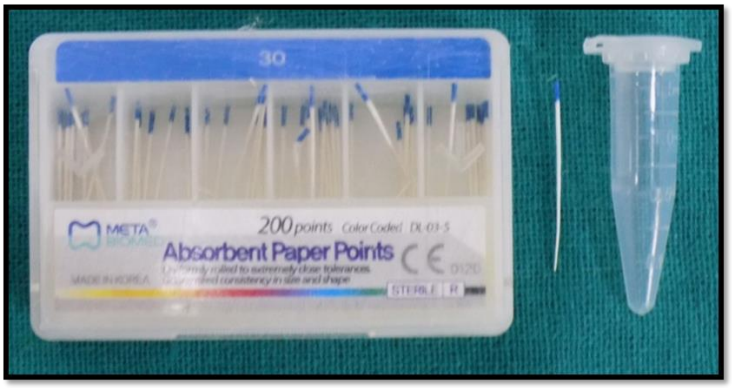


***Figure 6:** Endodonticpaperpoint (No 30)&eppendrof tube.*

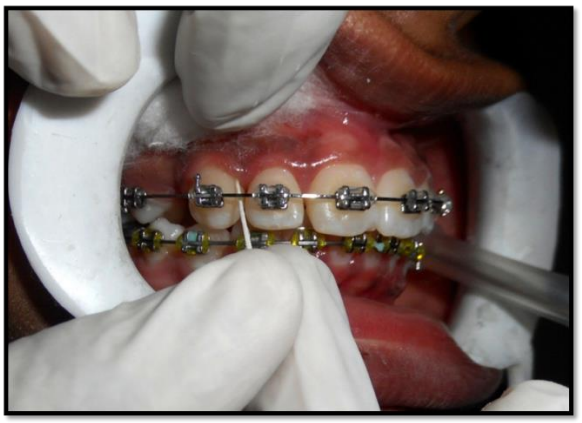


***Figure 7:** Loe and Holm Pedersen technique of collection.*

Figure 8: *UV spectrometer (Shimadzu,Japan).*

Figure 9: *Solutions of the salts required for the alkaline phosphatase assay.*

Figure 10: *Solutions of the salts required for the TRAP assay: A: Ascorbic acid B: Potassium chloride; C: Para Nitropheynl Phosphate; D: Sodium acetate buffer E: Triton X-100 F: ferric chlorideG: sodium tartrate*

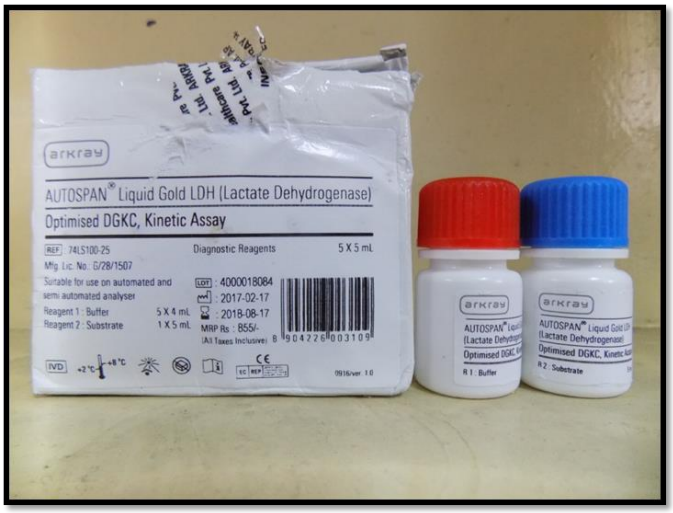


Figure 11: *Estimation kit (ArkrayhealthcarePvt.Ltd) for Lactate dehydrogenase.*

Figure 12: *Estimation kit (Erba Mannheim germany) for Aspartate aminotransferase.*

Figure 13: *centrifuge machine (REMI centrifuge;S.no EMLC – 4532).*

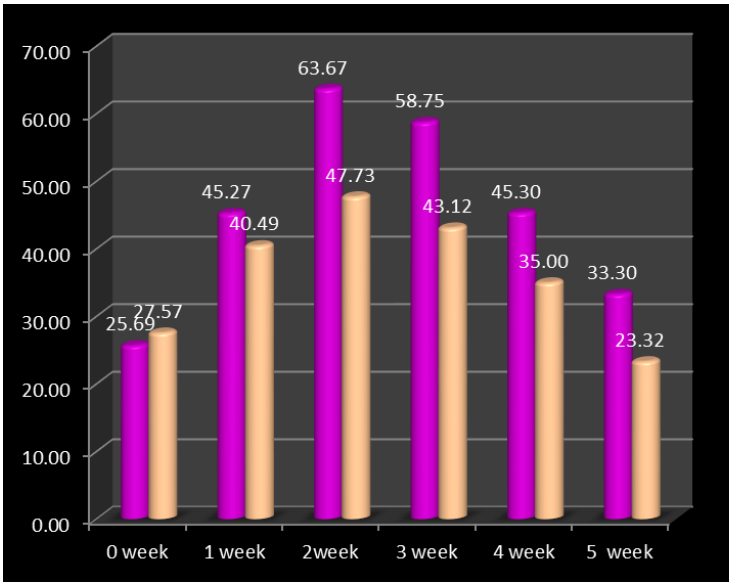


Figure 14: *The average score of ALP biomarker B/w MOPS and Control groups at different time-intervals at mesial side.*

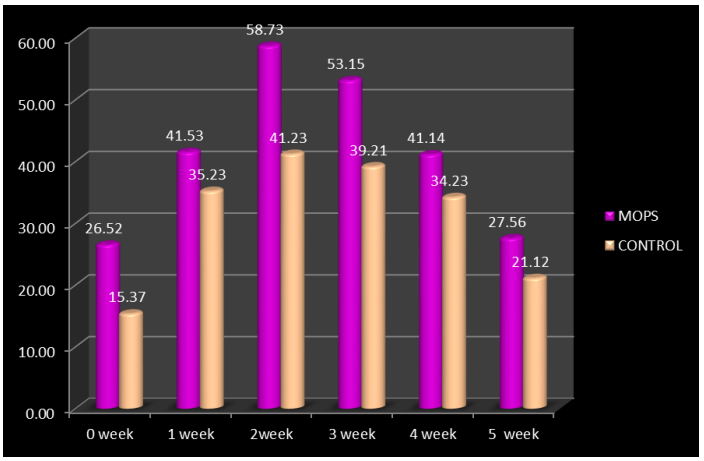


Figure 15: *The average score of ALP biomarker B/w MOPS and Control groups at different time-intervals at distal side.*

Experimental Design

This study was a split mouth design. After the extraction of 1st premolars, Preadjusted edgewise appliance with 0.018" slot of MBT prescription was used. Anterior and posterior teeth were bonded and banded with conventional orthodontic attachments and an initial arch wire was placed to achieve levelling and alignment in each patient before going for canine retraction. Before starting canine retraction, the root was checked for its prominence. This was done by a simple palpation method. If the canine root was found to be in cortical bone then either the subject was removed from our study or it was torqued to shift into the cancellous bone. Patient was referred to an oral surgeon in oral surgery department, to perform the MOPS with lance pilot drill. Before perforating, 0.2% lignocaine was injected, the marking present on the pilot drill were used to reach the accurate depth. After anaesthesia was achieved, a complete sterile environment was created. Microosteo perforation was performed with micro motor, a contra-angle hand piece and Lance Pilot Drill (Alpha Bio Tec, Simplantology Alpha Bio Tec LTD) and 3 perforations were performed distal to canine root with a perforation width and depth of 2 mm and 5 mm respectively under copious saline irrigation. Retraction was done on 0.016 x 0.022 inch stainless steel wire with NiTi closed coil spring of length 9 mm and a force of 150 gm attaching from the canine power arm to the hook of molar tube. At each appointment a Dontrixgauge was used to check the retraction force. Bite was raised in those subjects where occlusal interferences were present. Patients were asked to visit a 0,1,2,3,4,5 week. The force produced by the coil spring was checked and the appliance was monitored for any breakage or deformation. Until complete canine retraction, the study was continued (Tables 1,2).

GCF Sampling

The sample collection was done at 0 week, 1stweek, 2ndweek, 3rdweek, 4thweek and 5thweek. The patients were asked to gargle vigorously with a glass of sterile water to cleanse the oral cavity. The maxillary canines of both experimental and control side was also cleaned with cotton pellet to remove any supragingival plaque, it was then isolated using cotton roll and dried using gentle air stream. Three standard endodontic paper (Meta biomed) points (size 30) was inserted 1 mm into the crevice of canine from mesial and distal sides of the experiment and control site for 30 sec. Each site was samples 3 times at a minute interval. Immediately, after this, 3 dipped paper points (per site) were placed in 2 mL Eppendorf tube containing 200 µL of soreness medium containing 0.05% bovine serum albumin in phosphate buffered saline (pH=7.0). The Eppendorf tube containing 3 dipped paper points per site was then centrifuged using the centrifuge machine for 2 min at 2000rpm in order to elute the GCF components completely. Finally the paper points were removed and the supernatant was stored at –400C until analysed.

Assay of alkaline phosphatase (ALP) activity

ALP activities in GCF samples were measured by spectrophotometrically (UV Spectrophotometer Shimadzu, Japan) at 405 nm, Out of the total sample, 50µLof the solution was added into 1000 µLof reagent which contain the mixture of p-nitrophenyl phosphate (1mM), mannitol (20mM), magnesium chloride (0.3 mM) and carbonate buffer (pH 9.8) and incubated for 5 minutes. Readings were noted immediately after initiation of the reaction (A1) and 1 min later (A2).The change in absorbance was noted by summation of the changes over the 1 min period stating f, om A1 to A2 (A2 - A1) and was designated as delta. Mean change in

absorbance per minute was calculated (delta A/min). Total alkaline phosphatase activity was calculated using the formula: U/L = 2712 × ΔA / min.

Data Processing

A random sample size of 15 patients was taken by simple random sampling without using systematic sampling technique. The estimation of biomarkers level was observed at 6 different time point for each patients in control group as well as in experimental group respectively. The power of study was 82% with .05 level of significance/ type-1 error. The mean scores of the two observers were taken into consideration in the study. Thus, the data/ observations were subjected to SPSS (Statistical Package for Social Sciences) 22.0 version for analysis. The unpaired "t"test was applied to find the level of significance in estimation of biomarkers level in GCF during accerated orthodontics b/w control & experimental group at different time points at.05 level of significance (Tables 3-6).

Table 1: *Mean & standard deviation of ALP biomarker for mesial side in MOPS & control groups at different time intervals.*

ALP BIO MARKER-MESIAL SIDE		0 week	1 week	2 week	3 week	4 week	5 week
MOPS	MEAN	25.69	45.27	63.67	58.75	45.30	33.30
	S.D.	5.12	6.12	7.83	6.12	5.13	4.12
CONTROL	MEAN	27.57	40.49	47.73	43.12	35.00	23.32
	S.D.	4.12	5.73	6.12	5.13	4.12	4.12

Table 2: *Mean & standard deviation of ALP biomarker for distal side in MOPS & control groups at different time intervals.*

Alp Bio Marker Distal Side		0 Week	1 Week	2 week	3 Week	4 Week	5 Week
Mops	Mean	26.52	41.53	58.73	53.15	41.14	27.56
	S.D.	5.12	6.12	6.12	5.12	4.12	4.12
Control	Mean	15.37	35.23	41.23	39.21	34.23	21.12
	S.D.	4.12	5.12	6.12	5.12	4.12	4.12

Table 3: *Inter-Interval comparison of ALP level in MOPS on mesial side at different time intervals.*

S.No.	Alp (Mops) Mesial Site	Time Points	Mean Difference	P-Value
1	**0 Week**	**1 Week**	-45.87	.0002*
2		**2 Week**	2.02	.0011*
3		**3 Week**	-157.93	.0000*
4		**4 Week**	-26.37	.0000*
5		**5 Week**	-27.46	.0003*
6	**1 Week**	**2 Week**	47.60	.0001*
7		**3 Week**	-112.05	.0001*
8		**4 Week**	19.50	.0006*
9		**5 Week**	18.42	.0024*

10	**2 Week**	**3 Week**	-159.95	.0000*
11		**4 Week**	-28.40	.0003*
12		**5 Week**	-29.48	.0001*
13	**3 Week**	**4 Week**	131.55	.0000*
14		**5 Week**	130.47	.0002*
15	**4 Week**	**5 Week**	-1.09	.0028*
*Shows A Significant Difference At .05 Level Of Significance. I.E.P<.05				

Table 4: *Inter-Interval comparison of ALP level in MOPS on distal side at different time intervals.*

S.No.	Alp (Mops) Distal Site	Time Points	Mean Difference	P-Value
1	0 Week	1 Week	-9.56	.0000*
2		2 Week	-7.05	.0004*
3		3 Week	-21.70	.0000*
4		4 Week	-76.82	.0000*
5		5 Week	-22.06	.0004*
6	1 Week	2 Week	2.50	.0006*
7		3 Week	-12.14	.0000*
8		4 Week	-67.26	.0000*
9		5 Week	-12.50	.0002*
10	2 Week	3 Week	-14.65	.0003*
11		4 Week	-69.77	.0001*
12		5 Week	-15.01	.0001*
13	3 Week	4 Week	-55.12	.0000*
14		5 Week	-.36	.0004*
15	4 Week	5 Week	54.76	.0001*
*Shows A Significant Difference At .05 Level Of Significance. I.E.P<.05				

Table 5: *Inter-Interval comparison of ALP level in control on mesial side at different time intervals.*

S.No.	Alp (Control) Mesial Site	Time Points	Mean Difference	P-Value
1	**0 Week**	**1 Week**	-20.16	.0000*
2		**2 Week**	-12.92	.0002*
3		**3 Week**	-85.18	.0000*
4		**4 Week**	-49.63	.0002*
5		**5 Week**	4.25	.0005*
6	**1 Week**	**2 Week**	7.23	.0004*
7		**3 Week**	-65.03	.0000*

8		**4 Week**	-29.47	.0003*
9		**5 Week**	24.47	.0004*
10	**2 Week**	**3 Week**	-72.26	.0006*
11		**4 Week**	-36.70	.0000*
12		**5 Week**	17.18	.0000*
13	**3 Week**	**4 Week**	35.55	.0000*
14		**5 Week**	89.43	.0000*
15	**4 Week**	**5 Week**	53.88	.0000*
*Shows A Significant Difference At .05 Level Of Significance. I.E. P<.05				

Table 6: *Inter-Interval comparison of ALP level in control on distal side at different time intervals.*

S.No.	Alp (Control) Distal Site	Time Points	Mean Difference	P-Value
1	**0 Week**	**1 Week**	-47.18	.0000*
2		**2 Week**	-68.88	.0000*
3		**3 Week**	-83.46	.0002*
4		**4 Week**	-35.79	.0005*
5		**5 Week**	-9.58	.0009*
6	**1 Week**	**2 Week**	-21.70	.0000*
7		**3 Week**	-36.28	.0000*
8		**4 Week**	11.39	.0002*
9		**5 Week**	37.61	.0008*
10	**2 Week**	**3 Week**	-14.58	.0004*
11		**4 Week**	33.09	.0000*
12		**5 Week**	59.36	.0006*
13	**3 Week**	**4 Week**	47.67	.0009*
14		**5 Week**	73.88	.0002*
15	**4 Week**	**5 Week**	26.22	.0000*
*Shows A Significant Difference At .05 Level Of Significance .I.E. P<.05				

Results

The inter-interval comparison of mean ALP on Mesial Distal side of MOPS group. There was a statistically significant difference in mean ALP level from 0 week to 5week.The mean ALP level increased from 0 to 2 week and significantly decreased from 2 to 5 weeks. The inter-interval comparison of mean ALP on Mesial and Distal side of control group .There was a statistically significant difference in mean ALP level from 0 week to 5week.The mean ALP level increased from 0 to 2 week and significantly decreased from 2 to 5 weeks.

Discussion

A controlled clinical trial was designed to estimate biomarkers in GCF during accelerated orthodontics through micro-osteo perforation assisted technique. This study was a split mouth design. Split-mouth designs first appeared in dental clinical trials in the late sixties. In split-mouth designs, each intervention is randomly

allocated to a different site or sites within the mouth of each individual. A total of 15 sample were selected for this study .After the extraction of 1st premolars, Canine retraction was done on the both sides of maxillary arch. Microosteo perforation (MOPS) was performed on one side of maxillary arch in extraction space that side act as a experimental side (MOPS group) and other side of maxillary arch canine retraction was done without microsite perforation that side act as control side (control group). The biomarker of tooth movement can be detected in blood, saliva and GCF. In the present study, we used GCF as a reservoir for the determination of alkaline phosphatase, tartrate resistant acid phosphatase, aspartate aminotransferase and lactate dehydrogenase. A good biomarker should be specific and sensitive and have the ability to inform about the biological condition in terms of periodontal tissue changes and their relationships with the particular phase of orthodontic tooth movement. Bone metabolism is associated with alkaline phosphatase (ALP) and tartrate resistant acid phosphatase is a cytoplasmic enzyme present in many cells (Paolantonio et al., 2000). The duration of this study was 0week, 1week, 2week, 3week, 4week, 5week and the time interval of collection for GCF was programmed so as to identify and understand the enzymatic changes occurring during the early stages of accelerated orthodontics through microosteo perforation technique**.** Alkaline phosphatase is commonly associated with bone metabolism with osteoblasts showing high alkaline phosphatase activity. As a result of orthodontic force application, these enzymes produced in the periodontium diffuse into the GCF. Thus monitoring of phosphatase activities in the GCF could be suggestive of the tissue changes occurring during orthodontic tooth movement. Alkaline Phosphatase activities reflect bone turnover in orthodontic ally treated tissues. In the present study, there was variation in alkaline phosphatase level among the patients at the baseline level (week 0, before the initiation of canine retraction). This could be due to difference in the time required to achieve levelling and alignment and also bone remodelling may continue for long period after the appliance decay. In the present study, the activity of ALP in MOPS and control group was increase up to 2nd week from 0 week and then decreased up to 5th week. The peak of ALP level occur at 2nd week on mesial and distal side in both MOPS and control groups. The result of our study was in accordance to the study done by P Batra et.al (2006)30 they also found that ALP activity peak occurred on the 14th day of initiation of reaction followed by significant fall in activity especially on the mesial aspect as compare to distal aspect. The fall in activity is related to removal of the hyalinized zone. This study was also in accordance to the study done by Perinetti G (2002) and King GJ (1995) et al which showed that in the early phases of bone remodelling, a resorption activity (3-5 days) is followed by its reversal (5-7 days) and, subsequently, by a late phase of bone deposition (7-14 days) in both tension and pressure sites of the alveolar wall. In the early phase of tooth movement, bone resorption is greater than bone deposition, but in a later phase, resorption and deposition can become synchronous. This might be due to the high acid phosphatase activity that has been observed in the early period of tooth movement; high levels of ALP activity have been described after 7 days, when bone deposition begins. The study was in accordance to the study done by in soft et al which showed that alkaline phosphatase peaked between the first and third weeks and there after declined to constancy. They conducted a study on 3 patients who were longitudinally observed for 4 to 6 weeks, they described the existence of an ALP activity peak at 7 days in 1 of them. Alfaqeeh et al reported peak in the ALP activity on 14th day. Similar findings were reported by Farahani et al. In our study, the ALP activity was more on mesial side as compare distal side in both MOPS and control group. This indicate that ostoblastic activity more in Tension site as compare to pressure site this result could be explain on the basis that the osteogenic cells in the periodontal ligament respond to the tensional forces with an increase in the maturation rate. In the periodontal ligament, the fibroblast proliferation and collagen has been shown to increase in the tension sites. In addition, the osteoprogenator cell pool responds by increased proliferation and differentiation. The second messengers thus transmit the responses from the periodontal ligament fibroblasts to the osteogenic cells. When we compare the level of ALP on mesial and distal sides of canine between MOPS and control group. ALP activity is significantly higher in MOPS group, as compare to control group. These result explained that controlled microtrauma increase expression of inflammatory markers. These inflammatory markers enhance the born turnover rate. In our study, we found that there is a strong relationship between the level of biomarker in GCF and micro-osteoperforation. Future studies targeting on large sample size are required for better insight and understanding of the role of these biomarkers during accelerated orthodontics [7-30].

Conclusion

On estimation of biomarker level in gingival crevicular fluid during accelerated orthodontics the following conclusions were drawn. The higher level of all biomarkers (ALP) found in GCF in MOPS group as compare to control group at all the time intervals. This indicate that these enzymatic-activity increase due to micro-osteo perforation. The ALP in GCF can be considered good indicator for bone deposition and resorption respectively. They maximum change (peak) in ALP activity occurred on 14th day of force application on both tension (mesial) and compression (distal) sites in both MOPS and control groups. The ALP activity was observed

higher on tension (mesial) side of canine as compare to compression (distal) side in both MOPS and control group that indicated osteoblastic activity predominantly occur on tension side.

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