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Impact Of Orthodontic Fixed Retainers On Periodontal Health Assessed By Salivary Biomarkers - An In Vivo Study

[1*]Dr. Anita Bishnoi, [2]Dr. Nandini .V. Kamat, [3]Dr. Kshama Kudchadkar, [4]Dr. Anushka Mesquita,
MDS, [1]Independent Researcher, [2]Professor and Head, [3]Assistant Professor, [4]Lecturer
[2,3,4]Department of Orthodontics, Goa Dental College And Hospital,Goa, India

***Corresponding Author:**
Dr. Anita Bishnoi
Sunshine Apartment, Vishtapit, Rishikesh, Uttarakhand-249202

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Abstract

Introduction: The purpose of this study was to evaluate whether biomarkers of inflammation and periodontal remodeling, such as salivary AST, ALT and ALP are differentially expressed in patients wearing different types of retainers.

Methodology: Thirty patients post fixed mechanotherapy who were about to begin with the retention phase were randomly divided into 3 groups of 10 individuals each: removable retainer group (RRG), multistranded fixed retainer group (FRG) and fiber-reinforced retainer group (FRRG). Unstimulated whole saliva samples were collected at T0 (start of retention) and T1 (4 months after retainer placement). Levels of salivary AST, ALT and ALP were spectrophotometrically determined by the International Federation of Clinical Chemistry (IFCC) method on a fully automatic auto-analyzer. Periodontal measurements such as bleeding on probing, plaque, calculus and gingival indices were also obtained at T0 and T1. Paired t-test and ANOVA were used for comparison of AST, ALT and ALP values among the three groups between T0 and T1.

Results: Statistically significant increase in the salivary biomarker level was observed for all the three groups from T0 to T1 ($p<0.05$) with the greatest increase for the FRRG group.

Conclusions: The presence of orthodontic retainers especially the fiber-reinforced composite retainers may have some detrimental effects on periodontal health as stipulated by the increase in salivary biomarker levels. With longer retention periods, oral hygiene should be carefully monitored.

Keywords: Orthodontic fixed retainers, fiber reinforced reatiner, periodontal health, salivary biomarkers

Introduction

The success of orthodontic treatment is at risk of relapse which occurs after removal of the orthodontic appliance due to periodontal, occlusal, soft tissue forces and continuing dentofacial growth[1]. Hence, orthodontists tend to recommend long periods of retention[2].

Since removable retainers require dependence on patient compliance, fixed retainers bonded to the lingual/palatal surface of anterior teeth were introduced. Various generations of fixed retainers have been introduced till date. The first generation consisted of a round wire (0.032- 0.036 inches) with terminal folds, bonded to the canines only [3,4,5]. The second generation consisted of rigid multi-stranded wire of similar diameter (0.032 inches) bonded to only canines while the third generation consisted of a multi-stranded thinner diameter wire (usually 0.021 inch) bonded on each tooth from canine to canine. Fiber-reinforced composite retainers have been lately introduced in orthodontics with the advantage of superior esthetics and biocompatibility in patients who are allergic to nickel of stainless steel wire [4,5]. However, the disadvantage of placing fixed retainers is the tendency for plaque and calculus accumulation

along the retainer wire leading to a greater incidence of gingival and periodontal problems [3,6].

The traditional diagnostic methods such as periodontal indices used for periodontal health assessment are easier to use and inexpensive, they do not detect the presence of disease until a threshold change of 2-3 mm has been identified and active destruction has occurred [7,8]. Therefore, Rody et al[7,9] evaluated periodontal health using biomarkers like enzymes of tissue degradation released from the damaged cells in response to periodontal infection within the gingival crevicular fluid (GCF).

Recently, it has been shown that these biomarkers can be quantified in the saliva and the use of saliva offers an edge over GCF as it is faster, easier, more convenient to collect, and represents a pooled sample with contributions from all periodontal sites [8].

To the best of our knowledge, only two studies have evaluated the impact of orthodontic retainers on periodontal health using GCF biomarkers[7,9] and none with salivary biomarkers. Keeping this perspective in mind, the present study was designed to evaluate whether biomarkers of inflammation and periodontal remodeling, such as salivary AST (Aspartate Aminotransferase), ALT (Alanine Aminotransferase) and ALP (Alkaline Phosphatase) levels are differentially expressed in patients who have worn different types of retainers.

Materials And Methods:

A study was conducted on 30 patients (11 males, 19 females in the age range of 14-36 years) who had completed their orthodontic treatment and were about to begin with the retention phase in the Department of Orthodontics, Goa Dental College and Hospital, Bambolim. It was approved by the Scientific Ethical Committee of the institution (GDCH, Bambolim) and an informed consent was obtained from all the patients.

Healthy subjects with no history of a systemic disease (diabetes mellitus, hypertension, liver disease, and kidney disease), no history of periodontal disease, and who did not receive any antibiotic treatment for the last 3 months were included in the study while patients on anti-epileptic drugs, pregnant and lactating women were excluded.

All 30 patients were randomly divided into 3 groups. Group 1 consisted of 10 subjects who received removable circumferential retainer as the Control group. Group 2 consisted of 10 subjects who received multistranded retainer (0.0195" Respond®, Omrco, North America). Group 3 consisted of 10 subjects who received fiber-reinforced composite retainer (everStick® ORTHO, GC group, Turku, Finland).

All retainers were fabricated using a standardized protocol. For the control group (Fig. I), the removable retainer was fabricated according to the method described by KC Sahoo[10] using a 21 gauge stainless steel wire (KC SMITH and CO, England) and self-cure acrylic resin (DPI® RR, Mumbai, India).

For group 2 (Fig. II), the multistranded fixed retainers (0.0195" Respond®, Omrco, North America) was first stabilized with a dental floss and bonded with Transbond™ XT Adhesive (3M ESPE, Monrovia, California, USA) using the technique described by Artun and Zachrisson, 1982.

For group 3 (Fig. III), the fiber-reinforced composite retainer (everStick® ORTHO, GC group, Turku, Finland) was bonded using Tetric® N-flow flowable composite (Ivoclar Vivadent, Mumbai, India) using the technique described by Marc Geserick et al [11].

After retainer placement, all patients were given proper oral hygiene instructions with emphasis on flossing below the retainers. Patients were instructed to use dental floss (SuperflossTM, Oral-B®, P&G Manufacturing, Ireland) twice daily.

Clinical periodontal health assessment and salivary biomarker analysis (AST, ALT, and ALP) were carried out at T0 and T1 (T0-start of retention, T1- 4 months after retention).

Clinical parameters like plaque index, calculus index, gingival index, and bleeding on probing were used.

Plaque levels were assessed using a two-tone disclosing solution (Alphaplac®, DPI, Mumbai, India). Plaque scores were recorded for the lingual surfaces of all mandibular anterior teeth using the method described by Silness and Loe (1964). Plaque index for an individual was calculated by adding the plaque scores for lingual surfaces of individual teeth and dividing by the number of teeth examined. The

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scores from the lingual areas were added and divided by 6 to give the lingual score for the individual.

Similarly, gingival index and calculus index were recorded using William's periodontal probe by the methods described by Loe and Silness J (1963) and Greene JC and Vermillion JR (1960) respectively.

The presence or absence of bleeding on probing (BOP) was recorded in a dichotomous manner (0 or 1); if bleeding occurred within 15 seconds after retrieval of the probe, the site was considered BOP positive.

For saliva collection, patients were asked to rinse their mouth with distilled water for 30 seconds to wash out exfoliated cells. Following this, about 2ml of unstimulated whole saliva was collected by passive drooling (allowing saliva to drain off the lower lip) into saliva collecting conical tubes[45] (Fig IV). The saliva was stored in a refrigerator at 2-8º C until it was transported in an ice bag to the laboratory for testing of salivary AST, ALT, and ALP. In the laboratory, the saliva samples were centrifuged at 10,000 rpm for 10 minutes. AST, ALT and ALP levels were spectrophotometrically determined by the International Federation of Clinical Chemistry (IFCC) method on a fully automatic auto-analyzer (EM 200, ERBA Diagnostics Mannheim GmbH, Germany, Fig V)

Statistical Analysis:

The data obtained from salivary AST, ALT, ALP testing and periodontal indices was computerized using Microsoft Excel Worksheet and analyzed using SPSS version 20 (IBM Corporation, SPSS Inc., Chicago, IL, USA). Descriptive and inferential statistical analysis was carried out.

The Kolmogorov-Smirnov and Shapiro-Wilk tests showed that the data obtained from salivary AST, ALT, and ALP testing in all the three groups at both time intervals was normally distributed (p-value > 0.05). Hence, parametric tests paired t-test and ANOVA were used for comparison of AST, ALT and ALP values between the three groups.

The Kolmogorov-Smirnov and Shapiro-Wilk tests showed that the data from clinical parameter testing in all the three groups at both time intervals was not normally distributed (p-value < 0.05). Hence, the non-parametric Wilcoxon signed-rank test was used for comparison of clinical parameters between the three groups. Values were considered to be statistically significant at P<0.05.

Results and observations:

Table I presents concentration of biomarkers for each group at both the time intervals. The mean values of AST at T0 for RRG, FRG and FRRG groups were 16.80 IU/L, 16.20 IU/L and 16.50 IU/L respectively while the mean values of AST at T1 for RRG, FRG and FRRG groups were 20.00 IU/L, 20.20 IU/L and 20.60 IU/L respectively.

The mean values of ALT at T0 for RRG, FRG and FRRG groups were 10.80 IU/L, 11.30 IU/L and 12.50 IU/L respectively while the mean values of ALT at T1 for RRG, FRG and FRRG groups were 13.90 IU/L, 15.90 IU/L and 15.70 IU/L respectively.

The mean values of ALP at T0 for RRG, FRG and FRRG groups were 7.70 IU/L, 7.50 IU/L and 7.90 IU/L respectively while the mean values of ALP at T1 for RRG, FRG and FRRG groups were 10.00 IU/L, 10.10 IU/L and 10.90 IU/L respectively.

Paired t test was used for comparison of AST, ALT and ALP values among the three groups between T0 and T1.The increase in salivary biomarker levels from T0 to T1 was statistically significant for the three groups and greatest for FRRG group (p value < 0.05).

Table II presents periodontal measurements for each group at both time intervals. Wilcoxon signed rank test was used for comparison of the three groups revealed a statistically significant increase from T0 to T1 for the three groups (p value < 0.05).

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TABLE I: Salivary biomarkers at T0 and T1

SALIVARY BIOMARKERS	GROUPS	N	T0 MEAN±SD (IU/L)	T1 MEAN±SD (IU/L)	p VALUE(<0.05) T0-T1
AST	RRG	10	16.80 ± 2.9	20.00 ± 2.6	.046
	FRG	10	16.20 ± 4.1	20.20 ± 2.3	.024
	FRRG	10	16.50 ± 4.4	20.60 ± 3.3	.027
ALT	RRG	10	10.80 ± 4.8	13.90 ± 4.7	.028
	FRG	10	11.30 ± 3.7	15.90 ± 1.8	.024
	FRRG	10	12.50 ± 2.2	15.70 ±2.1	.013
ALP	RRG	10	7.70 ± 2.2	10.00 ± 4.4	.003
	FRG	10	7.50 ± 1.5	10.10 ± 1.3	.015
	FRRG	10	7.90 ± 1.5	10.90 ± 1.2	.038

Table II: Clinical parameters at T0 and T1

CLINICAL PARAMETERS	GROUPS	N	T0 RANGE	T1 RANGE	p VALUE(<0.05) T0-T1
PLAQUE INDEX	RRG	10	0.00-0.004	1.00-3.00	0.005

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	FRG	10	0.00-0.00	1.00-2.00	0.004
	FRRG	10	0.00-0.00	0.00-0.00	0.005
CALCULUS INDEX	RRG	10	0.00-0.04	0.58-1.00	0.007
	FRG	10	0.00-0.00	0.92-1.17	0.004
	FRRG	10	0.00-0.00	0.46-1.00	0.004
GINGIVAL INDEX	RRG	10	0.00-0.00	1.00-1.25	0.004
	FRG	10	0.00-0.00	0.83-1.00	0004
	FRRG	10	0.00-0.00	1.00-1.00	0.002
BLEEDING ON PROBING	RRG	10	0.00-0.00	0.00-1.00	0.017
	FRG	10	0.00-0.00	0.00-1.00	0.017
	FRRG	10	000-0.00	0.33-1.00	0.005

Fig. I Control group- Removable retainer group (RRG)

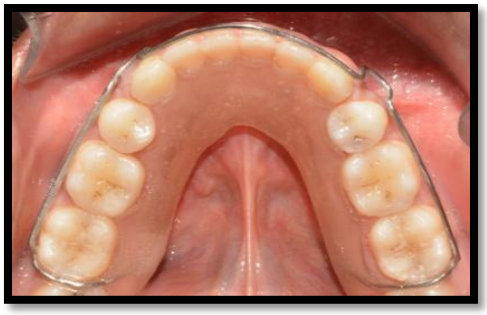

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Fig. II Fixed retainer group (FRG)

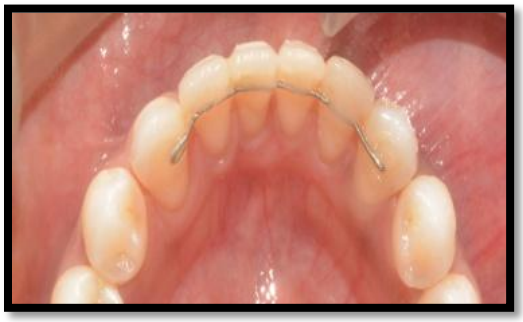

Fig. III Fiber-Reinforced retainer group (FRRG)

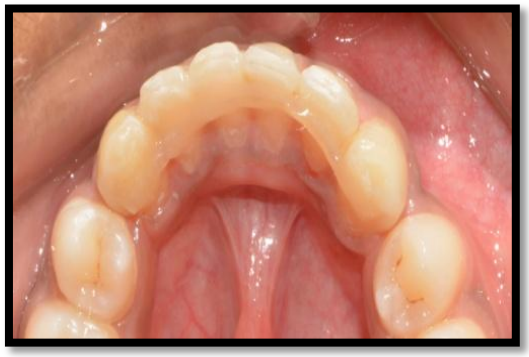

Fig. IV Tubes for saliva collection

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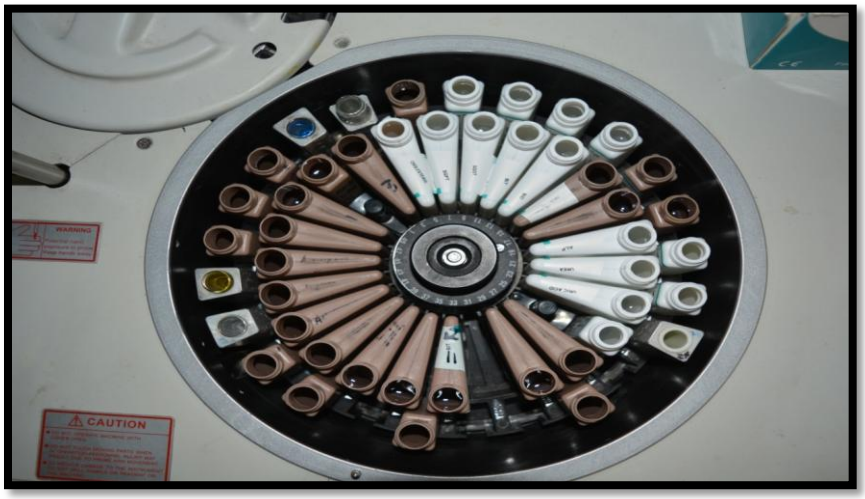
Fig. V Erba automatic analyser



Discussion:

In the present prospective study, 30 patients who were about to begin with the retention phase were divided into 3 groups with 10 patients in each group. Periodontal health in all 3 groups receiving different types of retainers were evaluated at T0 (at the time of retainer placement) and T1 (4 months after retainer placement).

It is a well-known fact that long term use of fixed retainers is recommended by orthodontists to combat the risk of relapse[2].These fixed orthodontic retainers tend to complicate oral hygiene procedures and hence can have a detrimental effect on the health of surrounding periodontal tissues[12,5]. Traditional diagnostic methods used to assess periodontal health after retainer placement[3-6] are easier to use and inexpensive, but their use is limited as they do not detect the presence of disease until a threshold change of 2-3 mm has happened and active destruction has occurred[8,13].

The solution to such problems is offered by biochemical markers which can be measured from various biologic fluids such as blood, saliva, as they represent agents that are involved directly in inflammation and periodontal remodeling[7,8] and GCF, thereby overcoming the subjectivity.

There have been two studies in the literature which have evaluated the effect of retainers on periodontal health using GCF biomarkers. One of which evaluated the effect of retainers 4 years after their placement on periodontal health using GCF biomarkers and found increased GCF MMP-9 concentration in the incisor region for patients with fixed retainers suggesting subclinical inflammation[7] . While the other evaluated the effect of different retainers 6 months after their placement using GCF biomarkers and found multistranded retainer bonded to six anterior teeth was associated with increased expression of MMP-3, MMP-9 and M-CSF as compared to the removable group suggesting its association with gingival inflammation [9].

Enzymes like AST and ALT are intracellular enzymes present within the soft tissue cells of the periodontium. Their increased activity in GCF is a consequence of their increased release from the damaged cells of the periodontium reflecting the metabolic changes in the inflamed gingiva[8]. ALP is an intracellular enzyme present in many of the cells and tissues, particularly in bones. It is also found to be present in neutrophils and is produced by some oral bacteria, including gram-negative microorganisms found in subgingival plaque[14]. Increased ALP activity is probably the consequence of the destructive processes in the alveolar bone seen in advanced stages of periodontal disease.

GCF collection is a complicated and time consuming procedure with a possibility of blood contamination during collection. The collection procedure can cause trauma to the sulcular epithelium and the quantity

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that can be collected is very little[8,15-17]. Compared to GCF, saliva collection is much simpler, easier, does not require specialized instruments and represents contributions from all sites[8].

Moreover, enzymes like AST, ALT and ALP have also been isolated in saliva recently and have been found to be correlating with the periodontal health status [8,15-17] .Since no study was available in the literature so far evaluating the effect of retainers on periodontal health using salivary biomarkers, the present study was carried out.

In addition to the above mentioned biomarkers, periodontal health was also evaluated using traditional methods like plaque index (PI), calculus index (CI), gingival index (GI) and bleeding on probing (BOP) at T0 and T1 for all the three groups.

Periodontal Health Assessment Using Indices

Periodontal Health At The Time Of Retainer Placement

The median values of Plaque index for RRG, FRG and FRRG groups were minimal at the time of retainer placement (Table 12). This is probably because the patients selected for the present study were all healthy individuals with no past history of periodontal disease. Furthermore, prior to retainer placement all patients were advised to undergo oral prophylaxis.

Similarly the median values for calculus index, gingival index and bleeding on probing were also minimal indicating healthy periodontal status. The scores for CI, GI and BOP at the time of retainer placement (T0) are presented in tables 13, 14 and 15 respectively.

Periodontal Health 4 Months After Retainer Placement

The results of the present study showed a statistically significant increase in all the periodontal parameters like plaque index, calculus index, gingival index and bleeding on probing from baseline to 4 months (T1) after retainer placement.

Plaque Index

The median values for plaque index for RRG, FRG and FRRG at T1 were 1, 1.5 and 2 respectively. A statistically significant increase from T0 to T1 was noted for all the groups. A greater tendency for plaque accumulation was seen with fiber reinforced retainer followed by multistranded fixed retainer and the least with removable retainer. The increase in plaque scores was greatest for FRRG group.

This can be attributed to the bulkiness of the fiber reinforced retainer occupying wider span on the lingual surfaces than multistranded retainer thus promoting more plaque accumulation. This is similar to the findings reported by **Torkan et al**[5] where they found more plaque accumulation with fiber reinforced retainer group compared to multistranded retainer group. The next highest plaque scores were recorded in FRG group. This can be attributed to the continuing presence of wire along the lingual surface which increases the retentive sites for microbial colonization and hence more plaque accumulation. This finding is similar to the findings reported by **Årtun**[18]**, Årtun et al**[19]**, Årtun et al**[20]**, Pandis et al**[3]**, Heier et al**[12]**, Al-Moghrabi et al**[21] who observed increased tendency for plaque and calculus to accumulate in cases of fixed retainers as compared to those with removable retainers.

Calculus Index

The median values for calculus index for RRG, FRG and FRRG at T1 were 1, 1 and 1 respectively. A statistically significant increase was noted for all the groups from T0 to T1. However, no significant differences were noted between the three groups at T1.The findings of the present study are in accordance with the findings of **Torkan et al**[5] who compared calculus accumulation between fiber reinforced retainers and multistranded retainers and found increased calculus accumulation after retainer placement but did not find any significant difference between the two. **Heier et al**[12] compared the effects of fixed and removable retainers on periodontal health and concluded that although increased plaque and calculus scores were noted after 6 months of retainer placement with the fixed retainer group, the increase was not statistically significant from baseline to 6 month follow up.

Gingival Index

The median values for gingival index for RRG, FRG and FRRG at T1 were 1, 1 and 1 respectively. A statistically significant increase was noted for the three groups from T0 to T1. However, no significant differences were noted between the groups at T1.The

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findings of the present study were not in accordance with those reported by **Tacken et al**[22] where they compared fiber reinforced retainer with multistranded retainer group and found an increased gingival inflammation associated with fiber reinforced retainer compared to multistranded retainer group. This difference can be attributed to the longer 2 year of observation and the relatively large sample size as compared to the present study. The findings of the present study are similar to the findings of previous studies by **Heier et al**[12] who compared removable and fixed retainers over a 6 month period and found no difference in gingival inflammation between the two groups. **Al-Moghrabi et al**[21] conducted a 4 year follow up RCT to evaluate the effect of removable and multistranded retainers on periodontal health and found that both types of retainers were associated with gingival inflammation and concluded that fixed retainers should be preferred over removable retainers as they maintain better alignment.

Bleeding On Probing

The median values for bleeding on probing for RRG, FRG and FRRG at T1 were 0.42, 0.75 and 0.83 respectively. The increase in scores was highest for FRRG followed by FRG and least for RRG. This can be attributed to the increased plaque accumulation associated with fiber reinforced retainer as compared to other groups. This finding is similar to findings of **Tacken et al**[22] and **Torkan et al**[5].

Salivary Biomarkers (AST, ALT And ALP)

Levels Of Salivary AST, ALT And ALP At The Time Of Retainer Placement

The results of the present study showed that the mean AST values at T0 for RRG (removable retainer group), FRG (fixed retainer group) and FRRG (fiber reinforced retainer group) groups were 16.80 IU/L, 16.20 IU/L and 16.50 IU/L respectively.

The mean values of ALT at T0 for RRG, FRG and FRRG groups were 10.80 IU/L, 11.30 IU/L and 12.50 IU/L respectively.

The mean values of ALP at T0 for RRG, FRG and FRRG groups were 7.70 IU/L, 7.50 IU/L and 7.90 IU/L respectively.

Change In AST, ALT And ALP Levels After 4 Months Of Retainer Placement

At T1 the mean AST values for RRG, FRG and FRRG groups were 20.00 IU/L, 20.20 IU/L and 20.60 IU/L respectively while the mean ALT values at T1 for RRG, FRG and FRRG groups were 13.90 IU/L, 15.90 IU/L and 15.70 IU/L respectively and the mean values of ALP at T1 for RRG, FRG and FRRG groups were 10.00 IU/L, 10.10 IU/L and 10.90 IU/L respectively.

The increase in AST, ALT and ALP values from T0 to T1 was highest and statistically significant for FRRG group. This can be attributed to the bulkiness of the fiber reinforced retainer covering the embrasures thus promoting more plaque and calculus accumulation and thus greater metabolic changes within the periodontium and higher enzyme values. In a histological study, **Oshagh et al**[4] evaluated the effect of fiber reinforced retainer and multistranded retainers on the periodontium of rabbits and found that fiber reinforced retainer inflicted detrimental effects on the periodontal ligament and alveolar bone as greater number of bone resorption lacunae were found in the fiber reinforced retainer group.

The increase in AST, ALT and ALP levels for FRG group was second highest. The increase in three enzyme levels was statistically significant for FRG group. This increase in enzyme levels can be attributed to the fact that fixed retainers tend to complicate oral hygiene procedures[3-5] thus greater tissue damage and increased enzyme levels. This finding is in accordance with the findings of **Rody et al**[7] who evaluated the effect of fixed and removable retainers on periodontal health using GCF biomarkers 4 years after their placement and found increased expression of MMP-9 in the fixed retainer group attributing to more gingival inflammation in the fixed retainer group compared to the removable retainers. A similar trend was reported by **Rody et al**[9] who compared the effect of removable and fixed retainer on periodontal health 6 months after retainer placement using GCF biomarkers and found increased levels of MMP-9, MMP-3 and M-CSF in fixed retainer group suggesting more gingival inflammation.

The increase in AST, ALT and ALP levels was the least for RRG group. This may be attributed to the fact that removable retainers probably do not affect oral hygiene maintenance as much as the fixed retainers do. Although, the increase from T0 to T1

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was statistically significant (p=0.046) it was least as compared to the FRRG and FRG group.

The present study indicated that salivary AST, ALT and ALP levels increased from T0 to T1 in concomitance with the periodontal parameters like plaque index, calculus index, gingival index and bleeding on probing showing a statistically significant increase. The gingival index showed a median value of 1 at T1 for the three groups suggesting minimum amount of inflammation. Similarly, increase in the AST, ALT and ALP values was lesser than that seen in patients with periodontal disease which has been established.

Conclusions:

The following conclusions can be drawn from the present study:

1. Salivary enzymes like AST, ALT and ALP showed a statistically significant increase in the three retainer groups from T0 to T1.
2. The increase in enzyme levels was greatest for FRRG group compared to other groups.
3. The various periodontal parameters like plaque index, calculus index, gingival index and bleeding on probing showed a statistically significant increase from T0 to T1.
4. The increase in plaque scores and gingival bleeding points was highest for FRRG group compared to the other groups.
5. Fiber reinforced retainers can have some deleterious effect on the periodontal effect and oral hygiene in patients with these retainers should be carefully monitored.
6. Salivary enzymes like AST, ALT and ALP can be used as potential biomarkers to assess periodontal health.
7. With longer retention periods, oral hygiene and periodontal health should be regularly evaluated.

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