



## Evaluation of Gingival Crevicular Interleukin- 1 $\beta$ Levels in Patients Undergoing Clear Aligners vs. Fixed Orthodontic Therapy

Areeba Khan<sup>1</sup>, Rabiya Saif<sup>2</sup>, Muhammad Mohsin Kamal<sup>3</sup>, Muhammad Iftikhar Ahsen<sup>4</sup>, Ghazala Hassan<sup>5</sup>, Eesha Muneeb<sup>6</sup>, Ejaz Ahmed<sup>7</sup>

<sup>1</sup>Department of Dentistry, Superior University Lahore, <sup>2</sup>Department of Oral Pathology, de'Montmorency College of Dentistry, Lahore, <sup>3</sup>Department of Community and Preventive Dentistry, Fatima Memorial Dental Hospital, Lahore, <sup>4</sup>Department of Periodontology Rahbar College of Dentistry, Lahore, <sup>5</sup>Department of Oral Biology, Fatima Memorial College of Medicine and Dentistry, FMHCMD, Lahore, <sup>6</sup>Department of Orthodontics, Rahbar College of Dentistry, Lahore, Pakistan, <sup>7</sup>School of Pathology, International Max Planck Research Institute, Berlin, Germany.

### ABSTRACT

**Background:** Tooth movements induced by orthodontic force cause an inflammatory response in the periodontum that is mediated by cytokines, including Interleukin-1beta (IL-1 $\beta$ ). Fixed appliances produce different biomechanical forces that may modulate GCF cytokines compared with clear aligners. The study aimed to determine the levels of gingival crevicular fluid (GCF) IL-1 $\beta$  in patients receiving clear aligner treatment compared to those receiving fixed appliances.

**Methods:** This cross-sectional analytical study was conducted at a tertiary care dental hospital from February to June 2025. 100 (50 clear aligner patients and 50 fixed appliance patients), 18-30 years old participants were recruited. Samples of GCF were obtained as a baseline and 1 month after treatment. IL-1 $\beta$  levels were measured by Enzyme-Linked Immunosorbent Assay (ELISA). The significance level was set as  $p < 0.05$ .

**Results:** No significant difference was found between baseline IL-1 levels ( $54.7 \pm 9.3$  vs.  $56.1 \pm 10.1$  pg/L, CI: 2.5-5.3,  $p = 0.47$ ). At 1 month, IL-1 $\beta$  levels were significantly elevated in the non-MAR group ( $92.4 \pm 12.6$  vs.  $71.8 \pm 10.9$  pg/L, CI: 16.3-24.9,  $p < 0.001$ ). Increases within the group were noted in both groups (clear aligners:  $17.1 \pm 6.2$ ; fixed appliances:  $36.3 \pm 7.8$ , both  $p < 0.001$ ). The difference between the groups persisted after controlling for confounders (CI: 15.7-23.0,  $p < 0.001$ ).

**Conclusion:** Both appliance systems increased GCF IL-1 $\beta$  levels, but fixed appliances produced a significantly larger inflammatory response than clear aligners, even after adjustment for potential confounders.

**Keywords:** Orthodontic Appliances, Gingival Crevicular Fluid, Interleukin-1beta, Periodontal Index

**\*Corresponding Author:** Ejaz Ahmed

**Email:** [ejazahmedpatho@gmail.com](mailto:ejazahmedpatho@gmail.com)

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## INTRODUCTION

Orthodontic treatment triggers a complex biological response in the periodontal tissues, where mechanical forces stimulate cytokine release, specifically interleukin-1 $\beta$  (IL-1 $\beta$ ), a key mediator of bone resorption and tooth movement <sup>1</sup>. The orthodontic appliance type may also impact periodontal health, with clear aligners generally associated with decreased plaque accumulation and gingival inflammation compared to conventional fixed appliances <sup>2</sup>.

Gingival crevicular fluid (GCF) is a promising medium for tracking biomarkers, and IL-1 $\beta$  levels are commonly used to determine inflammatory activity during orthodontic treatment <sup>3</sup>. Clinical comparisons have also indicated that aligner therapy yields more favorable periodontal indices at the initial stages of treatment compared to fixed appliances <sup>4</sup>. Oral microbiome analysis also reveals a lower extent of ecological disturbances with aligners, possibly linked to reduced levels of inflammatory cytokines <sup>5</sup>. Moreover, IL-1 $\beta$  concentrations in GCF typically rise within days of force application, reflecting the acute inflammatory response necessary for tissue remodeling <sup>6</sup>.

Regardless of these findings, existing evidence is limited by inconsistencies in sampling methodologies, force magnitudes, and the timing of biomarker measurement <sup>7</sup>. Reports of appliance-related variations in cytokine expression remain inconsistent and inconclusive <sup>8</sup>. Systematic reviews support the necessity of standardized, sufficiently powered trials examining the cytokine dynamics during various orthodontic mechanics <sup>9</sup>. Addressing this knowledge gap may help improve evidence-based appliance choice and strategies to reduce the risk of periodontal disease during therapy <sup>10</sup>.

The purpose of this study was to compare the concentration of IL-1 $\beta$  in GCF between fixed and clear aligner users. It measured changes at baseline and after 1 month to compare the development of inflammation. Furthermore, the aim was to investigate whether these differences persisted after adjustment for periodontal indices and potential confounders.

## METHODS

A cross-sectional comparative study determined the IL-1 $\beta$  concentrations in GCF and periodontal characteristics between individuals using orthodontic clear aligners and fixed appliances, conducted at the Dental College of Superior University and SZH Lahore, From February to June 2025 (Ref: 4421/25), after informed consent.

A consecutive non-probability sampling method was used to select eligible patients. A sample size of 100 was determined using OpenEpi version 3.0.0 (released 2013, Atlanta, GA, USA) with an assumed prevalence of 30%, 95% confidence level, a power of 80%, and a 5% margin error <sup>11</sup>.

The inclusion criteria were: 18-30-year-old age, good overall health without any systemic condition, indication to orthodontic treatment via either clear aligners or conventional braces, and absence of antibiotics, anti-

inflammatory drugs, or steroid use in the last 3 months. Exclusion criteria were smoking or tobacco use, systemic diseases, including diabetes, immunodeficiency, or cardiovascular disease, pregnancy or lactation, or prior orthodontic treatment in the past 6 months.

An elevated IL-1 $\beta$  was defined as a GCF concentration above baseline (>10 pg/l), and periodontal health was identified as a Plaque Index  $\leq$ 1.0, Gingival Index  $\leq$ 1.0, and probing depth  $\leq$ 3 mm. Participants were classified into two groups: clear aligners (n = 50) and fixed appliances (n = 50). GCF samples were collected by sterile microcapillary pipette at the mesio-buccal region of the maxillary central incisors at baseline and after 1 month. The IL-1 $\beta$  concentrations were measured using Enzyme-linked Immunosorbent Assay (ELISA). Periodontal indices, including plaque index, gingival index, bleeding on probing, and pocket depth, were recorded. Adherence to the appliance was confirmed using patient logs and monthly follow-ups.

SPSS version 26.0 (released 2019, IBM Corp., Armonk, NY) was used to analyze data. Inter-and intra-group differences were compared using independent and paired t-tests, respectively, and ANCOVA adjusted for confounders. Statistically significant results were defined as p-value <0.05.

## RESULTS

**Table 1: Baseline Demographic Characteristics of the Study Population**

Variable	Clear Aligners (n = 50)	Fixed Appliances (n = 50)	Test Value	p-value
Age (years), mean $\pm$ SD	22.6 $\pm$ 3.1	23.1 $\pm$ 2.9	t = 0.79	0.43
Gender (Male) n (%)	18 (36.0%)	20 (40.0%)	$\chi^2$ = 0.16	0.68
Smoking, n (%)	3 (6.0%)	4 (8.0%)	$\chi^2$ = 0.16	0.68
Brushing $\geq$ 2/day, n (%)	38 (76.0%)	36 (72.0%)	$\chi^2$ = 0.18	0.67
Mouthwash use, n (%)	20 (40.0%)	17 (34.0%)	$\chi^2$ = 0.36	0.55

n = Number of Participants, SD = Standard Deviation, % = Percentage, \* = Significance at p<0.05

This study compared periodontal status and GCF IL-1 $\beta$  levels in 100 subjects (50 with clear aligners, 50 with fixed appliances). Baseline demographics and oral hygiene practices were similar across groups. Plaque, gingival index, and bleeding on probing were significantly higher in the fixed appliance group after one month. Both groups demonstrated increased IL-1 $\beta$ , although levels were higher among the fixed appliance users. Baseline demographic characteristics of the study participants are indicated in **Table 1**.

**Table 2: Clinical and Periodontal Characteristics**

Variable	Clear Aligners (n = 50)	Fixed Appliances (n = 50)	Test Value	p-value
Plaque Index, mean $\pm$ SD	0.72 $\pm$ 0.21	0.89 $\pm$ 0.26	t = 3.45	0.001*

Gingival Index, mean $\pm$ SD	0.65 $\pm$ 0.18	0.84 $\pm$ 0.22	t = 4.23	<0.001*
Bleeding on Probing (% sites)	12.4 $\pm$ 4.3	15.6 $\pm$ 5.2	t = 3.12	0.002*
Mean Pocket Depth (mm)	2.2 $\pm$ 0.4	2.3 $\pm$ 0.5	t = 1.07	0.28

n = Number of Participants, SD = Standard Deviation, \* = Significance at  $p < 0.05$

Both groups were similar in their baseline characteristics with no significant differences in age (22.6  $\pm$  3.1 vs. 23.1  $\pm$  2.9 years,  $p = 0.43$ ), gender proportions (36% vs. 40% males,  $p = 0.68$ ), smoking, 3 (6%) vs. 4 (8%),  $p = 0.68$ , brushing frequency, 38 (76%) vs. 36 (72%),  $p = 0.67$ , and mouthwash use 20 (40%) vs. 17 (34%),  $p = 0.55$ . These similarities imply that potential confounders were balanced. Clinical and periodontal features of the study population are illustrated in **Table 2**.

**Table 3: Comparison of GCF IL-1 $\beta$  Levels between Groups**

Time-point / Group	Clear Aligners (n = 50)	Fixed Appliances (n = 50)	95% CI of Difference	Test Value	p-value
Baseline IL-1 $\beta$ (pg/ $\mu$ L)	54.7 $\pm$ 9.3	56.1 $\pm$ 10.1	-2.5 to 5.3	t = 0.72	0.47
1 month IL-1 $\beta$ (pg/ $\mu$ L)	71.8 $\pm$ 10.9	92.4 $\pm$ 12.6	16.3 to 24.9	t = 8.78	<0.001*
Within-group change ( $\Delta$ )	17.1 $\pm$ 6.2	36.3 $\pm$ 7.8	17.2 to 22.1	t = 15.4 / 21.2	<0.001*
Adjusted IL-1 $\beta$	70.5 $\pm$ 11.2	91.1 $\pm$ 12.3	15.7 to 23.0	F = 21.6	<0.001*

n = Number of Participants, IL = Interleukin, SD = Standard Deviation, % = Percentage, \* = Significance at  $p < 0.05$

The fixed appliance group showed significantly higher plaque index (0.89  $\pm$  0.26 vs. 0.72  $\pm$  0.21,  $p = 0.001$ ), gingival index (0.84  $\pm$  0.22 ns 0.65  $\pm$  0.18,  $p < 0.001$ ), and bleeding on probing (15.6  $\pm$  5.2 vs. 12.4  $\pm$  4.3,  $p = 0.002$ , suggesting that fixed appliances are linked to higher levels of plaque deposits and gingival inflammation. Table 3 presents the comparison of IL- $\beta$  levels between the two groups.

There was no difference in basal IL-1 $\beta$  levels ( $p = 0.47$ ), but after one month, fixed appliances had higher levels than clear aligners (92.4  $\pm$  12.6 vs 71.8  $\pm$  10.9 pg/ $\mu$ L;  $p < 0.001$ ). The increase was more significant in fixed appliances (36.3  $\pm$  7.8 vs. +17.1  $\pm$  6.2 pg/uL,  $p < 0.001$ ) and reconfirmed after adjusting ( $p < 0.001$ ). This reflects the higher inflammatory levels associated with fixed appliances, suggesting careful monitoring of periodontal health.

## DISCUSSION

This study aimed to compare the inflammatory response of periodontal tissues in patients with fixed orthodontic appliances and clear aligners, measuring IL-1 $\beta$  in GCF, and to evaluate the relationship between this biomarker and periodontal indices. The results support that fixed appliances induce a stronger inflammatory response compared to aligners.

The biomarker analysis revealed that IL-1 $\beta$  is an important mediator linking mechanical loading and osteoclastic activity<sup>12</sup>. Aligner therapy generated more favorable oral-hygiene markers and lower inflammatory signals in clinical cohorts, reducing the IL-1 $\beta$  response<sup>13</sup>. Comparative studies demonstrated decreased gingival inflammation and enhanced plaque control with removable devices, validating periodontal measures<sup>14</sup>.

An orthodontic study indicated increased levels of pro-inflammatory mediators such as IL-1 $\beta$  in the first weeks of fixed appliance activation, consistent with the acute cytokine dynamics<sup>15</sup>. Multi-biomarker studies demonstrated IL-1 $\beta$  elevation with bone-remodeling markers early in treatment, supporting IL-1 $\beta$  as an early outcome variable<sup>16</sup>. Interventional studies show that measures such as micropulse vibration or low-level laser can change biomarker kinetics, indicating the inflammatory response to appliances is modifiable<sup>17</sup>.

Mechanical and microbiological factors were major contributors to variation<sup>18</sup>. Large microbiome studies showed ecological shifts were more common in fixed appliances, increasing bacterial stimulation of IL-1 $\beta$  secretion<sup>19</sup>. Proteomic and bioinformatic analyses of GCF revealed dissimilar molecular signatures corresponding with appliance mechanisms, providing mechanistic support to distinct cytokine profiles<sup>20</sup>. Clinical trials comparing inflammatory and bone-metabolic markers at week 4 demonstrated differences across appliances, supporting that the device choice influences tissue biology<sup>21</sup>.

Nevertheless, mixed results have been found in systematic reviews and narrative studies, often attributed to variability in GCF<sup>22</sup>. Cytokine-profile analyses found greater pro-inflammatory load among some fixed treatment groups, consistent with these findings<sup>23</sup>. Transient elevations of IL-1 $\beta$  and IL-6 in pediatric and adolescent cohorts, within days to weeks of force application, support the temporal plausibility of one-month sampling. Prospective reviews also demonstrated IL-1 $\beta$  as one of the most reactive to orthodontic force, supporting its use as an early clinical outcome<sup>24</sup>. These results support the potential to improve periodontal outcomes in orthodontics through reinforced oral-hygiene instructions to fixed appliance patients<sup>25,26</sup>.

Limitations include a cross-sectional design, a brief one-month follow-up, and the single biomarker of focus, which constrains causal inference and mechanistic understanding. Other confounders, including hygiene, diet, smoking, genetics, fluoride use, and force variability, may have affected results. Randomized, longitudinal multicenter studies and multi-marker GCF panels, microbiome analysis, and standardized force testing should be used to optimize force-specific periodontal care strategies with appliances.

## CONCLUSION

The present study revealed that both clear aligners and fixed appliances increased levels of GCF IL-1 $\beta$  after one month, but with fixed appliances, the effect was stronger on the inflammatory response and poorer periodontal indices. These findings demonstrate the role of appliance type in determining periodontal inflammation, with aligners presenting a comparative advantage. Clinically, an aligner may be more beneficial in patients who are more susceptible to gingival disease, and future prospective trials including multi-marker and microbiome studies are advised to inform evidence-based appliance and preventive strategies.

## LIST OF ABBREVIATIONS

**ANCOVA:** Analysis of Covariance

**ELISA:** Enzyme-Linked Immunosorbent Assay

**GCF:** Gingival Crevicular Fluid

**IL-1 $\beta$ :** Interleukin-1 beta

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## CONFLICT OF INTEREST

None

## ETHICAL APPROVAL

A cross-sectional comparative study conducted at the Dental College, Superior University, and SZH Lahore, from February to June 2025 (Ref: 4421/25), after informed consent.

## AUTHORS' CONTRIBUTION

All authors contributed equally as per ICMJE policy.

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