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# Evaluation of salivary cytokines in patients with fixed orthodontic treatment

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**Abstract**---To evaluate the TNF- $\alpha$  and IL-1 $\beta$  levels in the gingival crevicular fluid (GCF) during orthodontic treatment. This study was carried out on 30 orthodontic patients. Prior to procedure, GCF was collected with a paper strip in gingival sulcus of maxillary right central incisor on the disto labial aspect for 60 seconds, followed by bonding and insertion of initial 14 NiTi archwire. GCF collection was repeated 24 hours, 7 days, and 30 days after bonding. Measurement of TNF- $\alpha$  and IL-1 $\beta$  levels was performed using Enzyme-Linked Immunosorbent Assay (ELISA). Data were evaluated statistically. There was raise in TNF- $\alpha$  and IL-1 $\beta$  concentration after treatment. There was no significant relationship between TNF- $\alpha$  and IL-1 $\beta$  concentrations subsequent to bonding in comparison to the baseline. There was no significant relationship in TNF- $\alpha$  and IL-1 $\beta$  level changes in the two age groups and among males and females. The present study indicated the raise in TNF- $\alpha$  and IL-1 $\beta$  concentration after treatment. There was no statically significant effect of gender and age on the concentration of TNF- $\alpha$  and IL-1 $\beta$ .

**Keywords**---crevicular fluid, cytokine, microbiologic, orthodontic, saliva.

## Introduction

The success of orthodontic treatment is mainly influenced by periodontal health, oral hygiene, and orthodontic forces. <sup>1</sup> The frequency, duration, and force magnitudes of orthodontic treatments influence the adjacent periodontal tissue reaction and bone remodeling. <sup>2, 3</sup> Many differential forces are exerted on the periodontal tissues by all forms of orthodontic appliances. <sup>4</sup> Orthodontic tooth movement is accompanied by the release of numerous regulatory molecules, such as cytokines. <sup>2, 4</sup> It has been observed that the orthodontic forces cause a rapid

release of TNF- $\alpha$ , IL-1 $\beta$ , and IL-8 in the process of tooth movement, the increase of which is clearly visible in GCF. <sup>3</sup> Cytokines are classified as proinflammatory and anti-inflammatory and considered as key mediators of tissue remodeling. Increase in concentrations of cytokines, such as interleukin-6 (IL-6), IL-8, IL-1 $\beta$ , tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ), epidermal growth factor, and b2 microglobulin, are observed during orthodontic tooth movement. The biochemical mediators expressed during orthodontic tooth movement can be isolated from gingival crevicular fluid (GCF). <sup>2</sup> There is increased potential risk for periodontal diseases in patients undergoing fixed orthodontics procedure. Hence there is concern to identify and resolve the condition. The assessment of cytokines during fixed orthodontic procedure warrants consideration in orthodontic patients in understanding their association and pathways for periodontal health. <sup>5</sup> The present study was done to evaluate the TNF- $\alpha$  and IL-1 $\beta$  levels in the gingival crevicular fluid (GCF) during orthodontic treatment.

### **Materials and Method**

This descriptive-analytical study was carried out on 30 orthodontic patients after obtaining the institutional ethical clearance and after obtaining informed consent from the participants and from guardians for participants under 18 years age. The study consists of 15 teenage patients 13- to 18 years and 15 adult patients 21-34 years old. The study was conducted after following the inclusion and exclusion criteria as per standard guidelines. Demographic data related to age and sex was noted. Plaque removal and brushing were applied to all patients one week before bonding and patients were advised about oral health tips. Before starting orthodontic treatment, tooth surface was dried with compressed air and isolated employing cotton roll. GCF was collected at baseline using Paper strip (Periopaper, Medical Electronic Devices Inc., Tustin, CA, USA) from gingival sulcus, 1-2 mm beneath the gingival margin of maxillary central incisor followed by bonding with bracket system MBT 0.022 slot and insertion of initial 14 NiTi archwire (Ortho Technology Inc., Tampa, FL, USA). Patients were recalled after 24 hours, 7 days, and 30 days post operatively and GCF samples were recollected in the similar manner described above. Strips contaminated with blood and/or saliva was excluded from the study. Paper strips were, then, placed in a sterile tube and dispatched to the Lab, where they were stored at -20°C till the day of study. <sup>3</sup>

Enzyme-Linked Immuno sorbent Assay (ELISA) was used to measure TNF- $\alpha$  and IL-1. UK Abcam® ab46052 and ab46088 (Abcam plc, Cambridge, UK) were used to measure IL-1 and for TNF- $\alpha$ , respectively. Samples were prepared as instructed in the company manual. Concentration levels of prepared samples were compared to TNF- $\alpha$  and IL-1 standards. The concentration of biomarkers (pg/mL) was measured with model ELx 808 microplate reader (BioTek Instruments Inc., Winooski, VT, USA). The obtained data was statistically evaluated using SPSS, statistical software version 21 (IBM SPSS Inc., Chicago, IL, USA) with significance level of 0.05.

## Result

Fifteen individual participants (50%) were under 18 and 15 others subjects (50%) were above 18 years of age with a minimum and maximum age of 13 and 34, respectively, and a median age of  $20.14 \pm 5.45$ . Eighteen of the cases (60 %) were female and 12 (40%) were male participants. Table 1 indicates correlation between concentration of IL-1 $\beta$  and TNF $\alpha$  at baseline and treatment interval. There was an increase in IL-1 and TNF- $\alpha$  concentration in different post-treatment time intervals as compared to baseline but there is no significant statistical difference between baseline and post-treatment TNF- $\alpha$  and IL-1 concentrations.

The table 2 indicates correlation between IL-1 $\beta$  concentration with gender and age. No significant variation was found for different age groups and genders in terms of IL-1 $\beta$  concentration at post-treatment. Table 3 indicates mean of TNF- $\alpha$  concentration based on participants' gender and age. There was significant variation was found for different age groups and genders in terms of TNF- $\alpha$  concentration at post-treatment. Table 4 indicates differences between TNF- $\alpha$  and IL-1 $\beta$  in genders and treatments interval. There was no meaningful difference exists between the two groups prior to treatment and during treatment.

## Discussion

Treatment with fixed orthodontic devices such as brackets and bands creates plaque accumulation sites impeding oral hygiene procedures.<sup>6, 7</sup> Gingival inflammation and enlargement are the common side effects of orthodontic treatment.<sup>8</sup> Some enzymes have been identified in the periodontal (pdl) tissue of orthodontically moved teeth. <sup>9</sup> Recently attention has focused on pro-inflammatory cytokines working as biomarkers in periodontal health assessments. Usually patients with periodontal diseases have significantly higher levels of Interleukin-1Beta (IL-1 $\beta$ ) in the gingival crevicular fluid (GCF). <sup>5</sup>Yong Chen et al evaluated the salivary cytokines levels with periodontal and microbiological parameters in patients undergoing fixed orthodontic therapy. They concluded that levels of salivary IL-1 $\beta$  and MIF correlated with oral bacterial load among patients undergoing fixed orthodontic therapy. <sup>5</sup> Nassar et al assessed the microbial flora, alkaline phosphatase and IL-8 Levels in GCF of Orthodontic Patients. Periodontal parameters were recorded and biochemical and microbial analyses were conducted before bonding (baseline), and 14, 30, and 45 days after bonding. They found significantly higher prevalence of gram-negative as well as gram-positive microorganisms was found in the group with conventional brackets than that with self-ligating brackets.<sup>2</sup>

Hosam Ali Baeshen evaluated the salivary pro inflammatory cytokines profile level in patients treated with fixed orthodontic appliances. They concluded that Subjects with conventional lingual appliances had significantly higher salivary protein levels of IL-1 $\beta$ , CCL2, IL17A, and IL-6, higher CD45+ and CD326+ cells and lower defensin levels than subjects with fixed labial appliances. <sup>4</sup> Karimi Afshar et al assessed the levels of TNF- $\alpha$  and IL-1 $\beta$  in the gingival crevicular fluid (GCF) during early leveling stage of orthodontic treatment. They concluded that Concentration of TNF- $\alpha$  and IL-1 $\beta$  increased after treatment. Age and sex had no statically significant effect on the concentration of TNF- $\alpha$  and IL-1 $\beta$ . <sup>3</sup> Levrini et al

evaluated the periodontal health and the microbiological changes via with fixed orthodontic appliances. After 1-month and after 3 months of treatment Invisalign® group showed better results in terms of periodontal health and total biofilm mass compared to the fixed orthodontic appliance group. <sup>6</sup> Kapoor et al had done a systematic review to assess all existing evidence on enzymes in orthodontic tooth movement. They found a strong evidence of variation in enzymes (ALP, AST, ACP TRAP, LDH, MMPs, Cb) in GCF is found in association with different magnitude, stages and sites of orthodontic force application. <sup>9</sup> Kim et al assessed the salivary periodontal pathogens after orthodontic treatment. They found higher salivary levels of Aa with fixed orthodontic appliances. <sup>8</sup>

Çevik-Aras et al evaluated the vascular endothelial growth factor (VEGF) and interleukin 1 beta (IL-1 $\beta$ ) perform functions in orthodontic tooth movement. They concluded that salivary levels of both IL-1 $\beta$  and VEGF increased following orthodontic treatment. <sup>10</sup> Shamaa and Mansour evaluated the biomarkers of oxidative stress in the saliva of patients undergoing fixed orthodontic treatment. They found no oxidative damage by fixed orthodontic procedure. <sup>11</sup> Al-Abdaly et al assessed the influence of fixed orthodontic treatment duration on the severity of inflammatory gingival enlargement. They observed higher inflammatory gingival enlargement associated with a higher plaque index in patients under orthodontic treatment for more than 12 months. <sup>12</sup> Sioustis et al did a study to identify biomarkers that facilitate the assessment of periodontal status during orthodontic treatment. They concluded that there was a strong positive statistically significant correlations between MMP-8 levels and BOP and medium positive statistically significant correlations between MMP-9 and BOP values before and after orthodontic treatment and periodontal treatment. <sup>13</sup> From the present study and supporting it was observed that cytokines has influence on fixed orthodontic treatment. The drawback of the present study was smaller sample size. Further studies are needed to validate the present results.

## Conclusion

According to the findings of this study, TNF- $\alpha$  and IL-1 in the gingival crevicular fluid increase by fixed orthodontic treatment compared to the period prior to treatment. No meaningful statistical difference was observed in TNF- $\alpha$  and IL-1 concentrations at varying post-treatment intervals vs. the pre-treatment period for different adult and adolescent age groups. It is recommended that studies focus on individuals with a higher age average and different treatments.

## Conflict of Interest: Nil

## References

1. Zainal Ariffin SH, Yamamoto Z, Zainol Abidin IZ, Abdul Wahab RM, Zainal Ariffin Z. Cellular and Molecular Changes in Orthodontic Tooth Movement. *The Scientific World Journal*. 2011;11:1788–1803
2. Nassar EA, Almasoud NN, Al- Qurashi MS, Alsulaiman AA, Hassan KS. An Evaluation of Microbial Flora, Alkaline Phosphatase and IL-8 Levels in GCF of Orthodontic Patients with Self-Ligating and Conventional Brackets. *Clinical, Cosmetic and Investigational Dentistry* 2021;13 343–352

3. Karimi Afshar M , Safarian F, Torabi M, Farsinejad A, Mohammadzadeh I. Comparison of TNF- $\alpha$  and IL-1 $\beta$  Concentrations in Gingival Crevicular Fluid During Early Alignment Stage of Orthodontic Treatment in Adults and Adolescents. *Pesquisa Brasileira em Odontopediatria e Clínica Integrada* 2020; 20:e0004
4. Ali Baeshen H. Assessment of salivary pro inflammatory cytokines profile level in patients treated with labial and lingual fixed orthodontic appliances. *Plos One*. 2021;1-10. /doi.org/10.1371/journal.pone.0249999
5. Chen Y, Wing Kit W, Seneviratne JC, Huang S, McGrath C, Hagg U. Associations between Salivary Cytokines and Periodontal and Microbiological Parameters in Patients undergoing Fixed Orthodontic Appliance Treatment. *Research Square*. 2019;1-14. DOI: <https://doi.org/10.21203/rs.2.17349/v1>
6. Levrini L, Mangano A, Montanari P, Margherini S, Caprioglio A, Abbate GM. Periodontal health status in patients treated with the Invisalign® system and fixed orthodontic appliances: A 3 months clinical and microbiological evaluation. *Eur J Dent* 2015;9:404-10.
7. Ahmed A, Hegde A, Gautam R, Kalia A, Nene S, Vairagi S. Analysis of plaque microflora in patients pretreatment and during treatment with fixed orthodontic appliances: An *In vivo* Study. *J Indian Orthod Soc* 2019;53:38-42.
8. Kima K, Jungb WS, Choc S, Ahnd SJ. Changes in salivary eriodontal pathogens after orthodontic treatment:An in vivo prospective study. *Angle Orthodontist*. 2016; 86(6): 998-1003
9. Kapoor P, Monga N, Kharbanda OP, Kapila S, Miglani R, Moganty R. Effect of orthodontic forces on levels of enzymes in gingival crevicular fluid (GCF): A systematic review. *Dental Press J Orthod*. 2019 Mar-Apr;24(2):40.e1-22
10. Çevik-Aras H, Isik-Altun F, Kilic-Tok H , Naoumova J. Monitoring Salivary Levels of Interleukin 1 Beta (IL-1 $\beta$ ) and Vascular Endothelial Growth Factor (VEGF) for Two Years of Orthodontic Treatment: A Prospective Pilot Study. *Mediators of Inflammation*. 2021, Article ID 9967311, 8 pages
11. Shamaa MS, Mansour MM. Long-term assessment of the salivary oxidative stress status during orthodontic treatment with fixed appliances. *Orthodontics, Pediatric and Preventive Dentistry*. 2019; 65, 3151-3157
12. Abdullah Al-Abdaly MMA, Asiri AMA, Al-Abdaly GMM, Ghabri MA, Alqaysi MAH,. Aljathnan AMS, Al Naser YSM, Alshahrani NAN. Evaluation of the Influence of Fixed Orthodontic Treatment Duration on the Severity of Inflammatory Gingival Enlargement (Fixed Orthodontic Induced Gingival Enlargements) and Some Properties of Saliva. *International Journal of Clinical Medicine*.2022;13:132-146
13. Sioustis IA, Martu MA, Aminov L, Pavel M, Cianga P, Kappenberg-Nitescu DC, et al. Salivary Metalloproteinase-8 and Metalloproteinase-9 Evaluation in Patients Undergoing Fixed Orthodontic Treatment before and after Periodontal Therapy. *Int. J. Environ. Res. Public Health*. 2021 18(1583): 1-13

### Legends for illustrations

Table 1  
Correlation between concentration of IL1- $\beta$  and TNF $\alpha$  at baseline and treatment interval

| Variables    | Treatment Time          | Mean  | SD    | p-value |
|--------------|-------------------------|-------|-------|---------|
| IL1- $\beta$ | Baseline                | 12.14 | 5.34  | 0.28    |
|              | 1 Day After Treatment   | 15.45 | 8.67  |         |
|              | 1 Week After Treatment  | 13.08 | 10.12 |         |
|              | 4 Weeks After Treatment | 16.23 | 8.12  |         |
| TNF $\alpha$ | Baseline                | 32.45 | 4.23  | 0.27    |
|              | 1 Day After Treatment   | 36.56 | 2.56  |         |
|              | 1 Week After Treatment  | 39.56 | 9.56  |         |
|              | 4 Weeks After Treatment | 37.45 | 5.67  |         |

Table 2  
Correlation between IL1- $\beta$  concentration with gender and age

| Gender |                     | Treatment Time     |                   |                   |                   | p-value |
|--------|---------------------|--------------------|-------------------|-------------------|-------------------|---------|
|        |                     | Baseline Mean (SD) | 1 Day Mean (SD)   | 1 Week Mean (SD)  | 4 Weeks Mean (SD) |         |
| Male   | <19 years old       | 12.12 $\pm$ 6.45   | 15.27 $\pm$ 7.02  | 13.29 $\pm$ 3.67  | 16.72 $\pm$ 12.45 | >0.05   |
|        | $\geq$ 19 years old | 6.32 $\pm$ 1.45    | 8.45 $\pm$ 2.65   | 10.34 $\pm$ 4.45  | 18.45 $\pm$ 3.56  | >0.05   |
|        | Total               | 12.06 $\pm$ 6.13   | 15.25 $\pm$ 8.76  | 9.23 $\pm$ 3.87   | 17.43 $\pm$ 12.34 |         |
| Female | <19 years old       | 11.23 $\pm$ 5.45   | 17.29 $\pm$ 10.87 | 20.56 $\pm$ 15.56 | 18.67 $\pm$ 12.22 | >0.05   |
|        | $\geq$ 19 years old | 15.78 $\pm$ 6.67   | 18.12 $\pm$ 8.34  | 13.67 $\pm$ 4.76  | 19.34 $\pm$ 6.67  | >0.05   |
|        | Total               | 13.56 $\pm$ 6.34   | 17.74 $\pm$ 9.15  | 17.14 $\pm$ 10.78 | 17.73 $\pm$ 7.13  |         |

Table 3  
Mean of TNF- $\alpha$  concentration based on participants'gender and age

| Gender |                     | Treatment Time     |                  |                   |                   | p-value |
|--------|---------------------|--------------------|------------------|-------------------|-------------------|---------|
|        |                     | Baseline Mean (SD) | 1 Day Mean (SD)  | 1 Week Mean (SD)  | 4 Weeks Mean (SD) |         |
| Male   | <19 years old       | 32.45 $\pm$ 4.45   | 36.28 $\pm$ 2.16 | 39.31 $\pm$ 13.33 | 37.23 $\pm$ 3.03  | >0.05   |
|        | $\geq$ 19 years old | 38.78 $\pm$ 1.34   | 41.25 $\pm$ 7.67 | 40.23 $\pm$ 2.23  | 34.34 $\pm$ 1.44  | >0.05   |
|        | Total               | 38.13 $\pm$ 5.23   | 38.43 $\pm$ 1.45 | 47.53 $\pm$ 15.34 | 35.33 $\pm$ 2.24  |         |
| Female | <19 years old       | 37.89 $\pm$ 3.02   | 39.52 $\pm$ 4.34 | 37.67 $\pm$ 4.12  | 40.45 $\pm$ 4.32  | >0.05   |
|        | $\geq$ 19 years old | 36.16 $\pm$ 2.56   | 37.56 $\pm$ 3.44 | 39.78 $\pm$ 4.65  | 40.55 $\pm$ 7.66  | >0.05   |
|        | Total               | 37.67 $\pm$ 3.78   | 37.65 $\pm$ 3.47 | 38.55 $\pm$ 4.34  | 40.47 $\pm$ 6.78  |         |

Table 4  
Differences between TNF- $\alpha$  and IL-1 $\beta$  in genders and treatments interval

| Gender |                     | Treatment Time        |                    |                     |                      | p-value |
|--------|---------------------|-----------------------|--------------------|---------------------|----------------------|---------|
|        |                     | Baseline<br>Mean (SD) | 1 Day<br>Mean (SD) | 1 Week<br>Mean (SD) | 4 Weeks<br>Mean (SD) |         |
| Male   | <19 years old       | 14.25 $\pm$ 8.24      | 11.46 $\pm$ 6.76   | 10.56 $\pm$ 3.93    | 17.56 $\pm$ 12.56    | >0.05   |
|        | $\geq$ 19 years old | 17.74 $\pm$ 9.53      | 13.45 $\pm$ 6.56   | 16.18 $\pm$ 11.66   | 16.45 $\pm$ 7.15     | >0.05   |
| Female | <19 years old       | 38.12 $\pm$ 5.67      | 38.43 $\pm$ 1.46   | 47.17 $\pm$ 15.89   | 35.56 $\pm$ 2.43     | >0.05   |
|        | $\geq$ 19 years old | 36.72 $\pm$ 4.04      | 37.69 $\pm$ v      | 38.56 $\pm$ 4.56    | 41.77 $\pm$ 6.23     | >0.05   |