

A Comparative Study in Tumor Necrosis Factor- α and Interlukin-1beta Levels Between Fixed and Removable Orthodontics Appliances

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Abstract

Background: Orthodontic is a branch of dentistry that deals with the identification, avoidance, treatment, and correction of misaligned jaws, teeth, and bite patterns. The oral environment, periodontal tissue, and oral microbiome are all greatly affected by orthodontic appliances. The level of tumor necrosis factor- α (TNF- α) and interleukin-1beta (IL-1 β) change following force application by orthodontic appliances. **Objective:** This study aimed to study IL-1 β , TNF- α in patients with fixed and removable orthodontic appliances in different time and evaluate the immune response for patients to IL-1 β , TNF- α in different time. **Materials and Methods:** A total of 186 study saliva samples from (30 patients with fixed appliance; 32 patients with removable type), 29 male and 59 female patients, aged 15–35 years, whose wear fixed and removable orthodontics appliances in the beginning of the healing process and after 1 month and 2 months. The saliva samples were centrifuged after being collected and measured by enzyme-linked immune sorbent assay (ELISA). Statistical package for the social sciences (SPSS) version 29.0 was used to export all data from this study for statistical analysis. **Results:** Although there were no significant differences, but the mean values of TNF- α in fixed appliances are more than removable type whereas the mean values of IL-1 β in removable appliances was slightly higher than fixed type with significant difference only after 1 month of treatment about 102.55, 130.61 ($P \leq 0.05$) respectively. **Conclusion:** Proinflammatory IL-1 β and TNF- α were shown to be increased in both appliances in all time intervals, with high amount of TNF- α in fixed appliances while IL-1 β show elevated amount in removable types. The statistical software for the social sciences, edition 29 (SPSS) program was used for the descriptive statistics.

Keywords: Fixed appliances, IL-1 β , removable appliances, TNF- α

INTRODUCTION

Orthodontics is a branch of dentistry concerned with correcting and improving irregularities of the teeth and jaws. This may involve correcting crooked teeth and improper bites with orthodontic devices like braces.^[1] A typical course of orthodontic treatment can be finished in 12–24 months, depending on the patient's individual needs and the length of time required for retention with appliances or fixed splints following treatment.^[2] There are generally four different kinds of orthodontic appliances that can be used singly or in combination to correct malocclusions.

- i. Removable orthodontic appliances.
- ii. Fixed orthodontic appliances.

- iii. Functional appliances.
- iv. Orthopedic and extra-oral force appliances.^[3]

Lack of compliance may have an impact on the effectiveness of both fixed and removable appliances. For example, poor oral hygiene during fixed orthodontic treatment may result in periodontal disease and caries. Disregarding the wearing time of a removable appliance could lead

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to treatment delays, thus slowing the treatment process and compromising the achievement of the treatment goals.^[4] One of the challenges of orthodontics is to finish the treatment with the least effect on the tooth root and periodontium. Periodontal health is an important factor that may be used to evaluate the success of orthodontic therapy, periodontal complications are reported to be one of the most common side effect linked to orthodontics.^[5] Dehiscence, fenestrations, periodontal pocket, dark triangles, gingival recession or hypertrophy, periodontitis, and alveolar bone loss are the main periodontal side effects of orthodontic treatment.^[6] Recent research has focused on pro-inflammatory cytokines due to the possibility that they can be used as biomarkers in periodontal health assessments, allowing for earlier detection of inflammation and periodontal diseases.^[7] Cytokines and eicosanoids are involved in every step of inflammation, from the earliest to the latter stages. Pro-inflammatory cytokines, such as interleukin-1beta (IL-1 β), IL-2, IL-5, IL-6, IL-8, TNF- α , interferon-gamma, and granulocyte-macrophage colony-stimulating factor, are responsible for the inflammatory response.^[8] Pro-inflammatory cytokines act primarily at the onset of orthodontic tooth movement by inducing vasodilatation, increasing vascular permeability, and inflammatory response.^[9] At the regions of tension and pressure, these mediators result in bone resorption and deposition.^[10] TNF is the common mediator of inflammatory responses of bone resorption process.^[11] After 4 h IL-1 β and IL-8 levels in compression sites raised, but after applying force, IL-1beta, IL-8, and TNF levels significantly increased in tension sites. These findings suggest that the application of orthodontic force is related with elevated markers of tissue and bone metabolism as well as pro-inflammatory cytokine levels were measured in gingival crevicular fluid.^[12]

MATERIALS AND METHODS

A total of 186 study saliva samples (30 patients with fixed appliance; 32 patients with removable type), 29 male and 33 female aged 15-35 years whose wear fixed and removable orthodontic during the initial stage of treatment and after 1 month and 2 months. Exclusion criteria was systemic or orally diseased patient, previous orthodontics treatment, pregnant women, missing 1st molar, mouth breath, smokers, sever skeletal anomalies. All saliva samples were collected from the private orthodontic clinic and the specialized health center of dentistry in Babylon City, Iraq during the period from October 2022 to February 2023.

Specimen collection methods

The participants were asked not to brush their teeth from the night which preceded the time of the samples collection, and they had to fast for at least an hour before giving a sample. Saliva was collected into a specimen tube at regular intervals of 60s, with at least 2mL of

unstimulated saliva obtained each time.^[13] Saliva samples were centrifuged at 10,000 rpm for 5 min to remove bacteria and other impurities before being used for IL-1 β and TNF assays. The supernatant was then frozen at -20°C and used for immunological analysis using (ELISA) method.^[14]

Detecting of interleukin-1beta and tumor necrosis factor- α by ELISA technique

The technique was followed in accordance with the assembly instructions, and the ELISA sample included in the kit was pre-coated with just an antibody particular for IL-1 β and TNF- α . The specific antibody is combined with the references or information in the relevant micro-ELISA sample wells, and then each microplate is thoroughly processed before the adding of a biotinylated detection antibody and an Avidin Horseradish Peroxidase (HRP) substance. The substrate solution was then poured into each well after the unattached components had been removed by washing. The biotinylated detection antibodies and the Avidin HRP conjugate would both be blue in color. The introduction of a sulfuric acid solution causes the color of the enzyme-substrate interactions to change to yellow.

Statistical analysis

The statistical software for the social sciences, edition 29 (SPSS, IBM Company, Chicago, IL, USA.) program was used for the descriptive statistics. Means and standard deviations were used to illustrate persistent variables. Because the data were not normally distributed, the Mann-Whitney *U* test was employed to determine the mean differences in TNF and IL-1 β between patients with fixed and removable orthodontics. With *P* value 0.05, statistical significance will be taken into account.^[15]

Ethical consideration

The investigation was done in conformity with the ethical standards outlined in the Helsinki Declaration. Before taking the sample, the patients verbal, and analytical consent were obtained. To obtain this permission, a local ethics committee evaluated and approved the study protocol, subject information, and consent form using document number 566 (containing the number and date in December 14, 2022).

RESULTS

Correlation amount of tumor necrosis factor between patients with fixed and removable appliances

In Table 1 shows the mean values of TNF at each time intervals between patients with fixed and removable orthodontics. Although the mean values in fixed appliances were higher than removable type but there were no significant differences (*P* $\leq$ 0.05) [Table 1]. Mean and SD demonstrate the amount of TNF at the initial time (T0), 1 month (T1), and after 2 months (T2) between patients with fixed and removable orthodontics.

Correlation amount of interleukin-1beta between patients wearing fixed and removable appliances

Table 2 shows mean and SD demonstrate the amount of IL-1 β at the initial time between patients with fixed and removable orthodontics. There were significant differences of IL-1 β after 1 months (T1) of treatment between patients with fixed and removable orthodontics treatment ($P \leq 0.05$). In Figure 1, shows the distribution of biomarkers in each time intervals with continuous increasing if compared to initial time of treatment.

Table 1: The mean values of TNF at (T0,T1,T2) between patients with fixed and removable orthodontics

	Groups	N	Mean $\pm$ Std. Deviation	P value
TNF, T0	Fixed	30	112.78 $\pm$ 50.03	0.098
	Removable	32	98.18 $\pm$ 65.16	
TNF, T1	Fixed	30	114.68 $\pm$ 58.28	0.678
	Removable	32	106.28 $\pm$ 55.72	
TNF, T2	Fixed	30	142.86 $\pm$ 98.49	0.838
	Removable	32	118.89 $\pm$ 71.35	

*P value of ≤ 0.05 is significant

Table 2: The mean values of IL-1 β at (T0,T1,T2) between patients with fixed and removable orthodontics

	Groups	N	Mean $\pm$ Std. deviation	P value
IL-1 β , T0	Fixed	30	114.68 $\pm$ 58.28	0.678
	Removable	32	106.28 $\pm$ 55.72	
IL-1 β , T1	Fixed	30	102.55 $\pm$ 48.89	0.050*
	Removable	32	130.61 $\pm$ 61.47	
IL-1 β , T2	Fixed	30	116.60 $\pm$ 51.97	0.382
	Removable	32	135.05 $\pm$ 81.77	

*P value of ≤ 0.05 is significant

Distribution of patients with fixed and removable orthodontic treatment according to the gender and age

Overall, 62 patients were included in this study, in that 30 patients were wearing fixed orthodontics and another 32 were wearing removable. In Figure 2, most patients wear fixed orthodontic treatment were women (63.3%), while majority (56.3%) of the patients wearing removable orthodontics were males. Figure 3 shows the mean $\pm$ SD for the patients ages were 20.38 ± 5.30 years old, there was no significant difference ($P > 0.05$) between the age of the patients with fixed and removable orthodontics, 20.13 ± 5.90 and 20.62 ± 4.67 years, respectively.

DISCUSSION

The result shown in Table 1 revealed that continuous increase of concentration of TNF from T0 to T2 in both type of treatment; this is a normal result as it is a

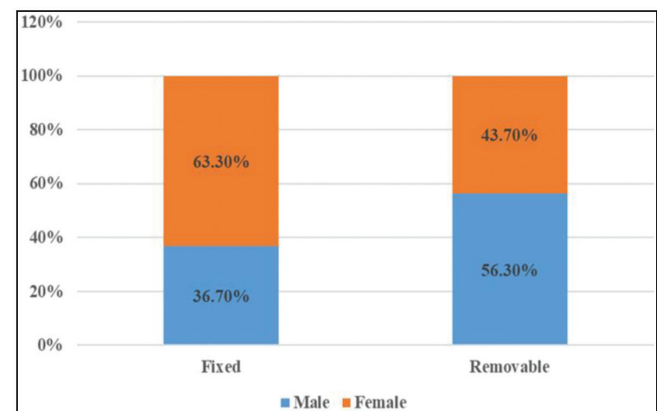


Figure 2: Distribution of gender between patient of fixed and removable orthodontic treatment

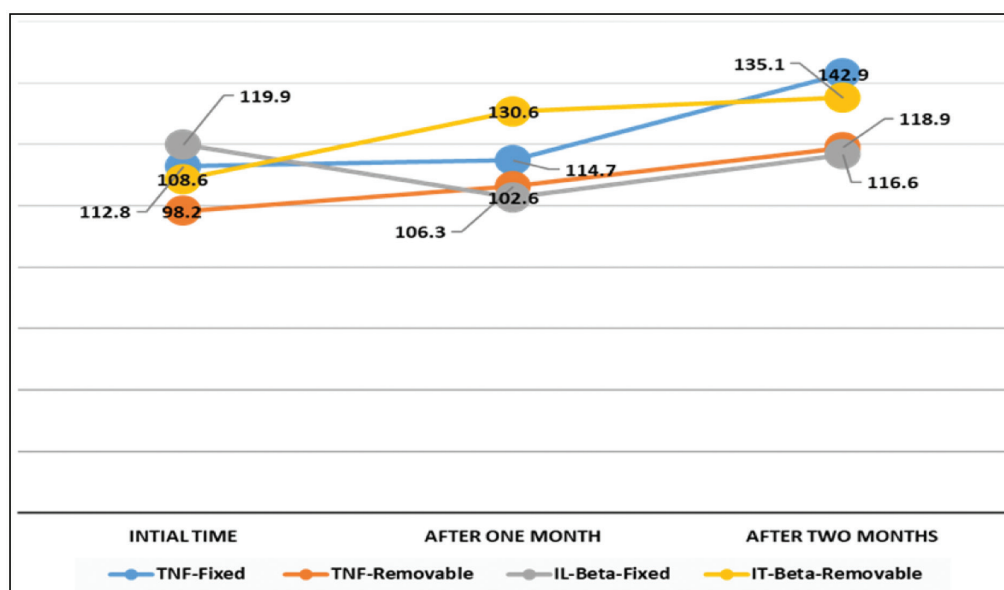


Figure 1: Distribution of oral biomarker among time

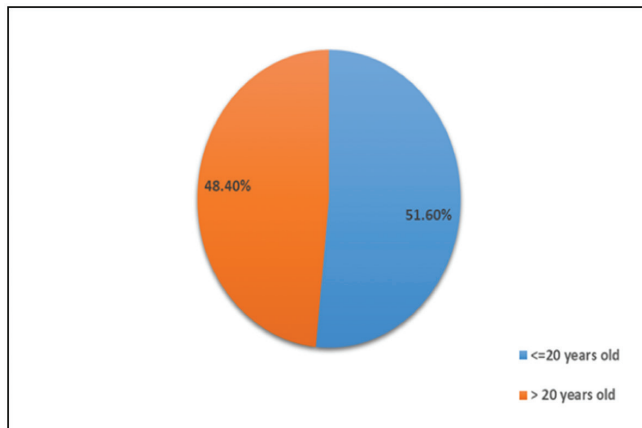


Figure 3: Distribution of age between patient of fixed and removable orthodontic treatment

proinflammatory cytokine. The outcome closely resembles that of the following researchers, who discovered a rise in TNF concentration. Padisar *et al.*^[10] who measured TNF at baseline, after 1 h, and after 28 days, and revealed the increasing amount of it, also Afshar *et al.*^[16] who discovered that the concentration of TNF- and IL-1 β increased following treatment in their study, comparison of TNF- α and IL-1 β concentrations in gingival crevicular fluid in early orthodontic treatment in adults and adolescents. At the same time, Shaik and Reddy^[9] found that after using orthodontic force for 1 and 24 h, TNF concentration increased in both type of treatment, whereas Baeshen^[17] found that the alteration in amount of TNF was great differ in fixed appliance if it compared with removable type, in Chowdhury and Chakraborty^[18] who found in their study at 24 h after the appliances were inserted. The levels of IL-1 β and tumor necrosis factor were considerably greater in the experimental group than in the controls. But it declined to be the same that found in base line.

Table 2 demonstrates that the mean values of IL-1 β for fixed appliance from T0 to T1 were decreased than its level in T0; this may be because of many variables such as sample size, patient gender, age, and salivary sample type analysis and is incoordinate with Saadi and Ghaib^[19] and Çevik-Aras *et al.*^[20] found that the results of their studies on monitoring salivary levels of IL-1 β during orthodontic treatment showed significant increase in the mean values of clinical periodontal parameters and salivary cytokines levels of it after 1 and 2 months of treatment, whereas Leethanakul *et al.*^[21] found increasing of IL-1 β in pressure site in appliance with time then return to normal value after the area was accustomed to amount of pressure while Castroflorio *et al.*^[22] found in their studies for measuring the amount of interleukin in orthodontic patient were lowered after 1 month of application and this finding is agreed with the result in it the IL-1 β dropped after 1 month of application. In removable type, De Aguiar *et al.*^[23] showed in their evaluation of saliva Biomarkers to enhance efficiency of orthodontic patient that IL-1 β levels

were increased in the early stage of treatment but it was not significant, but a statistically significant increase in the level of IL-1 β was observed in Gujar *et al.*,^[24] Shirozaki *et al.*^[25] and Motyl *et al.*^[26] whom found from T0 to T2 which agreed with our result in it mean value raised. The concentrations of IL-1 β , TNF- α at 24 h following the start of the trial, were considerably greater in the experimental group than in the controls, but it declined to be the same that found in base line was presented by Chowdhury and Chakraborty.^[18] In this study, the results indicated that the prevalence of female (63.3%) is more than male wearing fixed orthodontic treatment which might be attributed to the fact that females place a higher value on attractiveness. As a result, women are more prone than men to pay attention to the finer features of their appearance and their attractiveness, this result in good agreement with that found by Siddiqui *et al.*^[27] whereas the majority (56.3%) of the patients wearing removable orthodontics were men for many reasons. One of these is easy to remove, not give an ugly appearance, easy to brush, and clean teeth than do fixed type. This partially agreed with Saccomanno *et al.*^[28] who saw that women prefer more of removable treatment than men. In this study, the patients were selected at open aged to include all cases that prefer in fixed and removable treatments. This agreement with study made in Turkey by Article^[29] but disagreed with Lucchese *et al.*^[30] who selected age group according to their need.

CONCLUSION

We conclude this study that orthodontic force causes interleukin-1 beta and tumor necrosis factor-alpha levels in unstimulated whole saliva to increase during orthodontic tooth movement. The chance of increasing inflammation is observed in patient wearing fixed appliances than in removable one which reflect both TNF- α and IL-1 β as they have proinflammatory cytokine but not in specific form specially with IL-1 β .

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Conflicts of interest

There are no conflicts of interest.

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