ORIGINAL ARTICLE

Evaluation of Periodontal Inflammation and Its Association with Serum IL-6 and IL-1β Levels in Orthodontic Patients

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Key words:
Orthodontic patients, Orthodontic treatment, Cytokines, IL-6, IL-1β, ELISA.

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ABSTRACT

Background: *Maintaining periodontal health is essential for the stability of orthodontic treatment. Fixed orthodontic appliances can increase dental plaque accumulation, complicate oral hygiene, and consequently lead to gingival inflammation. Cytokines such as interleukin-6 (IL-6) and interleukin-1β (IL-1β) are key inflammatory mediators involved in periodontal tissue response.* ***Objective:*** *The aim of this study was to evaluate the asossiation between periodontal inflammation and serum levels of IL-6 and IL-1β in orthodontic patients.* ***Methodology:*** *This study was conducted at the Specialized Dental Center in Tikrit, Salah al-Din, between October 2024 and June 2025. A total of 90 participants aged 13–30 years were divided into four groups: control (n=30), baseline pre-treatment (n=20), post-treatment at 3 months (n=20), and post-treatment at 6 months (n=20). Blood samples were collected to measure serum IL-6 and IL-1β levels using ELISA. Gingival swabs were obtained from patients aged 13–21 years with orthodontic-related inflammation to identify bacterial pathogens through culture and microscopy.* ***Results:*** *A total of 52 bacterial isolates were identified. The predominant pathogens were Staphylococcus aureus (23.1%), Streptococcus sanguinis (19.2%), and Klebsiella pneumoniae (11.5%). IL-6 levels were highest at baseline (233.1 ± 102.6 pg/mL) and decreased progressively at 3 months (215.1 ± 111.2) and 6 months (148.7 ± 51.4), approaching control values (146.2 ± 58.9). Similarly, IL-1β levels declined from 255 ± 101.8 at baseline to 215.3 ± 67.09 at 3 months and 194 ± 60.91 at 6 months.* ***Conclusion:*** *Orthodontic treatment is associated with transient increases in inflammatory cytokines and bacterial accumulation. Over time, IL-6 and IL-1β levels decreased, indicating periodontal adaptation. These findings highlight the interaction between microbial factors and host immune response during orthodontic therapy.*

INTRODUCTION

Gingivitis is a mild form of gum disease. Daily brushing, flossing, and regular dental cleaning along with appropriate healthcare may treat gingivitis, here is no bone or tissue with gingivitis. Therefore, it is important that gingivitis be treated right away as possible in order to avoid the advanced gum disease known as periodontitis[1].

The disease is characterized by Gum bleeding or swelling (gingivitis), smelly breath and pain. Gums may separate from the teeth and the bones that support them, causing the teeth to become loose and occasionally fall off and this is the most advanced stages of the condition. Smoking and poor dental hygiene is the primary causes of gum disease[2].

Two important cytokines implicated in both periodontal disease and orthodontically induced tissue remodeling are interleukin-1 beta (IL-1β) and interleukin-6 (IL-6)[3].

IL-6 plays a crucial part in the pathogenesis of PD by promoting osteoclast development and bone resorption and preventing bone formation[4].

The pro-inflammatory cytokine interleukin (IL-1β), which contributes to periodontitis, was increased. Clinical data has demonstrated not just the correlation between IL-1β and periodontitis, but also that elevated IL-1β causes a cascade of inflammatory responses and stimulates bone resorption[5].

When mechanical forces are applied during orthodontic treatment, the periodontal ligament triggers a localized inflammatory response. Shortly after the force is applied, the gingival crevicular fluid (GCF) levels of IL-1β and IL-6 rise, usually reaching a peak in 24 to 72 hours, and then gradually declining back to baseline.

Plaque is a structured microbial biofilm salivary protein, extracellular polysaccharides, and bacteria combine to form the organized microbial biofilm known as plaque. When it builds up around orthodontic appliances, the mouth cavity's natural balance is changed[6,7].

During orthodontic treatment, gingival irritation is largely caused by dental plaque. Daily dental hygiene is made more difficult by the multiple plaque-retentive regions created by fixed orthodontic appliances,

especially around brackets, bands, and wires[7]. The aim of this study was to evaluate the relationship between fixed orthodontic appliances and gingival inflammation as well as to assess the immunological parameters associated with bacterial colonization during orthodontic treatment.

METHODOLOGY

Study population

A total of 90 participants aged (13-31) comprising males and females who visited Orthodontic Clinic at the Specialized Dental Center in Tikrit, Salah al-Din Governorate, between October 2024 and June 2025, were enrolled and divided into four groups: Control (n = 30), Pre-treatment (n = 20), post-treatment 3 months (n = 20), and post-treatment 6 months (n = 20). The control group consisted of Healthy individuals without gingival inflammation or malocclusion. The pre-treatment group comprises patients with malocclusion but without clinical signs of gingival inflammation. Patients in the post-treatment 3- and 6-month groups presented moderate to severe gingivitis related to orthodontic appliance. Venous blood (5 mL) was collected from each participant into serum separator tubes. Samples were centrifuged for 10 minutes at 3,000×g. After 30 minutes of clotting at room temperature, before analysis, the serum was aliquoted and kept at -80°C. Serum concentrations of human IL-1β and IL-6 were measured using commercial ELISA kits (Sun Long Biotech, China).

Ethical Approval:

This study was reviewed and approved by the Ethics Committee of the college of Science, Tikrit University, Iraq. (Approval No.035/ TUCOS / ETH / 2024/ 10 – CLIN). Clinical samples were collected from Orthodontics clinic, Specialized Dental Center, Salah Al-Din Health directorate, Tikrit, Iraq. all participants and when applicable their legal guardians were informed, the study objectives, sampling procedures, and confidentiality assurances. Prior to participation, each participant provided written informed consent. The study was conducted in accordance with the 2013 version of the Declaration of Helsinki's guidelines for biomedical research involving human subject.

Gingival swab collection and Microbiological culture

A total of 40 gingival swabs were collected from patients in the post-treatment groups (3 and 6 months) who presented with moderate to severe orthodontic-related gingival inflammation, the patient's ages ranged from (13–21 years). samples were obtained to evaluate the local inflammatory response and establish a correlation between the microbiological results and serum interleukin levels, IL6 and IL1β. Each swab was placed in Gel transport medium (Oxoid, UK) and transported to the laboratory, where it was cultured on Blood Agar (HI Media, India), Mannitol Salt Agar (HI Media, India), and MacConkey Agar plates (HI Media, India). In order to recover aerobic Gram-positive and Gram-negative bacteria, plates were incubated for 18 to 24 hours. Gram staining was performed using a Gram Stain Kit (Bio Research, Gordon) following incubation.

concentrations of interleukin-1β (IL-1β) and interleukin-6 (IL-6) were determined, using commercially available ELISA kits (Sun Long Biotechnology, China), following the manufacturer's instructions.

Assay Principle

Microplate wells were pre-coated with monoclonal antibodies specific to each cytokine. Following this, standards and serum samples were introduced into the wells, allowing the target cytokines to bind to the immobilized antibodies. After incubation, a horseradish peroxidase (HRP)-labeled detection antibody, also specific to IL-6, IL-1β was added to form an antibody–antigen–antibody complex. Unbound materials were removed by thorough washing. Subsequently, the chromogenic substrate (TMB) was added, producing a color change proportional to the cytokine concentration. The reaction was terminated using a stop solution, resulting in a yellow color. The optical density (OD) was recorded at 450 nm using a microplate reader. The concentrations of IL-6, IL-1β in the samples were then determined by plotting the OD values against the respective standard curves. Serum levels of (IL-6) and (IL-1β) were determined using enzyme_ linked immunosorbent assay (ELISA) kits (Sun Long Biotechnology, China, Catalogue Nos SL1001Hu_1 , SL0984Hu, respectively) according to the manufacturer's instructions.

RESULTS

Gingival inflammation caused by orthodontics is a common adverse effect of fixed orthodontic treatment, primarily because of increased plaque retention around brackets and bands. Data were collected from 40 patients with orthodontic infections.

1- Distribution of Cases by Gender

The distribution of orthodontic infection cases among the 40 patients revealed a gender difference in the incidence rate. The current study showed that the highest rate of orthodontic patients was observed among females, with 28 cases (70%), while males had lower incidence with12 cases (30%), as shown in Fig. 1.

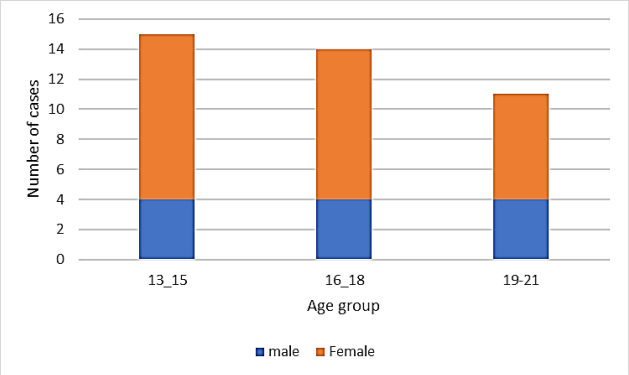

Fig. 1: Distribution of patients with orthodontic infections according to sex and age.

2- Distribution of Cases by Age Group

Patients were classified into three age groups 13–15 years, 16–18 years, and 19–21 years. The results indicate that orthodontic infections are common among all age groups, with little variation in the distribution of cases across age groups. This study showed that Regular examinations and parents monitoring of dental care may result in significantly fewer medical conditions in the 13–15 age group. The result of changes in dental hygiene practices and greater exposure to plaque accumulation, the 16–18 age group, which represents late adolescence, may be more vulnerable to infections.

3- Bacterial isolates in Gingival Inflammation

52 bacterial isolates were found in patients with gingival inflammation, according to the current investigation. Staphylococcus aureus (23.1%) and Streptococcus sanguinis (19.2%) were the most common species, with Staphylococcus epidermidis (19.2%) coming in second. Pseudomonas aeruginosa (9.6%), Staphylococcus saprophyticus (5.8%), Streptococcus pneumoniae (11.5%), and Klebsiella pneumoniae (11.5%) were among the other isolates.

Table 1: Number and percentage of bacterial isolates

Type of bacteria	Number	%
Staphylococcus aureus	12	23.1%
Staphylococcus epidermidis	10	19.2%
Streptococcus sanguinis	10	19.2%
Streptococcus pneumoniae	6	11.5%
Klebsiella pneumoniae	6	11.5%
Pseudomonas aeruginosa	5	9.6%
Staphylococcus saprophyticus	3	5.8%

4- Serum IL-6 levels

After performing immunological tests, using the ELISA technique, serum, IL-6 concentrations showed significant variation across the study groups. According to the findings, the IL-6 level in the pre-treatment baseline group was significantly high (233.1 ± 102.6). While the level in the 3-month group was (215.1 ± 111.2). The 6-month group mean± SD IL-6 levels (148.7 ± 51.4), followed by the control group (146.2 ± 58.9). According to the present findings, IL-6 levels decreased over time from baseline to three months and then again at six months, reaching values close to those observed in the control group.

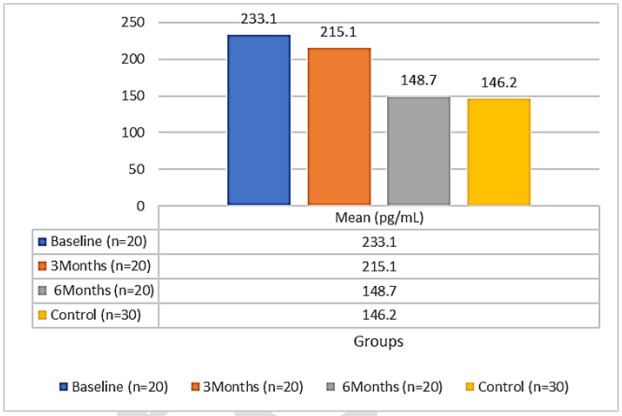

Fig. 2: Mean IL-6 levels among different Groups.

Table 2: Serum IL-6 levels among study Groups

SD	Mean (pg/mL)	Groups	Cytokine
102.6	233.1	Baseline (n = 20)	IL-6
111.2	215.1	3Months (n=20)	
51.37	148.7	6 months (n=20)	
58.9	146.2	Control (n = 30)	

5- Serum IL-1β levels

After conducting immunological tests using ELISA technique for samples the result showed that IL-1β levels in Baseline group is (255 ± 101.8), slightly higher than the control group (246.6 ± 99.54). After 3 months of treatment, the mean decreased with a narrower standard deviation to (215.3± 67.09). After 6 months, further decline to (194 ± 60.91) with continued reduction in variability, implying adaptation of periodontal tissues over time. The Results showed that serum IL-1β levels gradually dropped over time.

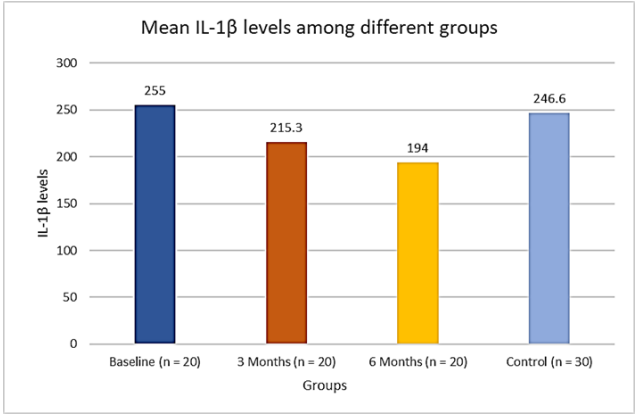

Fig. 3: Mean IL- 1β levels among different groups.

Table 3: Serum IL-1β levels among study Groups

SD	Mean (pg/mL)	Groups	Cytokine
101.8	255.0	Baseline (n = 20)	IL-1β
67.09	215.3	3Months (n=20)	
60.91	194	6 months (n=20)	
99.54	246.6	Control (n = 30)	

DISCUSSION

Gingival inflammation caused by orthodontics is a common adverse effect of fixed orthodontic treatment, primarily because to increased plaque retention around brackets and bands. Professional care and good oral hygiene may typically reverse it, but chronic inflammation might make periodontal issues more likely[8]. The current study showed that the highest rate of orthodontic patients was observed among females, with 28 cases (70%), while males had a lower incidence with12 cases (30%).

This difference may be attributed to biological and hormonal changes that occur in women, especially during pregnancy, childbirth, and even menopause, in addition to stress, that significant affects immunity and thus increases orthodontic infections, as hormones affect the tissues that support the teeth[9]. This difference may be attributed to biological and hormonal changes that occur in women, especially during pregnancy, childbirth, and even menopause .Pregnancy-related hormonal changes, such as increased levels of progesterone and estrogen, can impact local immunity and make people more susceptible to periodontal and gingival diseases[9]. Increased blood flow in the gums due to these hormonal changes leads to inflammation and increases microbial changes toward anaerobic species, increasing the risk of gingivitis and dental caries[10,11].

This study showed that Regular examinations and parents monitoring of dental care may result in significantly fewer medical conditions in the 13–15 age group. The result of changes in dental hygiene practices and greater exposure to plaque accumulation, the 16–18 age group, which represents late adolescence, may be more vulnerable to infections. Oral health is affected by orthodontics. Every patient who has received orthodontic treatment has reported that having orthodontic appliances makes it difficult to clean teeth and increases plaque accumulation. Regardless of age, this raises the risk of gum disease, but the severity of the condition can change based on dental hygiene habits and age. Compared to adolescents, patients in the 19–21 group had an orthodontic infection rate that was similar to or occasionally higher. This group is more susceptible to gum disease and plaque accumulation since they have frequently received orthodontic treatment for longer periods of time, exposing their teeth to orthodontic appliances for longer.

The mean serum IL-6 levels showed significant variation across the study groups. IL-6 levels decreased over time from baseline to three months and then again at six months, reaching values close to those observed in the control group. The findings of the present study align with those of a study by Jayaprakash et al [12]. This pattern suggests that the mechanical forces generated by orthodontic appliances initially trigger an immune response characterized by elevated cytokine production, particularly during the early stages of tooth movement. As treatment progresses, however, IL-6 levels gradually decline, reflecting the adaptive capacity of the periodontal tissues under sustained and controlled pressure. Ultimately, cytokine levels eventually tend to decrease to approximately normal levels, indicating the gingival environment has stabilized and the inflammatory response has decrease.

Microbiological analysis indicated that both Gram-positive cocci (especially Staphylococcus and Streptococcus spp.) and Gram-negative bacilli (Klebsiella and Pseudomonas). Staphylococcus aureus and Streptococcus sanguinis are prevalent, which suggests that they may play an opportunistic role in periodontal diseases.

Cytokine analysis revealed that IL-6 levels decreased over time from baseline to three months and then again at six months, reaching values close to those observed in the control group.

The findings of the present study align with those of a study by Jayaprakash et al.[12]. This pattern suggests that the mechanical forces generated by orthodontic appliances initially trigger an immune response characterized by elevated cytokine production, particularly during the early stages of tooth movement. As treatment progresses, however, IL-6 levels gradually decline, reflecting the adaptive capacity of the periodontal tissues under sustained and controlled pressure. Ultimately, cytokine levels eventually tend to decrease to approximately normal levels, indicating the gingival environment has stabilized and the inflammatory response has decrease.

B cells, T cells, monocytes, macrophages, endothelial cells, fibroblasts, keratinocytes, and various tumor cells are among the cell types that release IL6. Inflammation, diabetes, atherosclerosis, autoimmune, cancer, trauma, and rheumatoid arthritis are all strongly affected it[13]. Increased levels of interleukin-6 in serum and gingival crevicular fluid have been associated to the severity of periodontal disease, according to research on the role of IL-6 in periodontitis and its complications[14].

According to the findings, this gradual decrease in mean levels and variability indicates that periodontal tissues' response to prolonged mechanical stress, which is similar with findings seen in previous studies[15]. Studies examining at the inflammatory and

microbiological changes that occur during orthodontic treatment reported similar adaptive responses. Periodontal tissues and the microbiota that is linked with them may eventually adapt to prolonged orthodontic stresses, as suggested by Kim et al.[7], who observed transient increases in subgingival bacterial counts followed by gradual microbial stabilization.

It has been shown that the pattern of systemic reflections of local inflammation is similar, initially elevated, then gradually declining as tissues recover. This adaptation, in which gradual inflammatory resolution is caused by controlled and maintained orthodontic forces, is highlighted by the gradual release shown in our study.

The findings of the present study align with those of a study by Chelărescu et al.[16]. Orthodontic force is known to cause an immediate, localized inflammatory reaction.

After mechanical stimulation, IL-1β, a crucial mediator of bone remodeling, usually peaks in a matter of hours to days. Interleukins levels rise to high levels and then gradually decline as tissues adapt[16].

IL-1β is essential because it stimulates osteoclast genesis and matrix remodeling especially in the early stages of orthodontic tooth movement (OTM). In response to mechanical stimuli, the periodontal ligament (PDL) and surrounding tissues release a powerful amount of IL-1β, which both triggers and regulates bone resorption and remodeling[17].

IL-1β production decreases when the course of treatment continues by feedback mechanisms, such as the activation of anti-inflammatory signals, which causes levels to return to normal[18]. Bacterial lipopolysaccharides cause the release of IL-1β, triggering a cascade of responses from inflammatory mediators. It promotes vascular permeability, encourages the recruitment of neutrophils and macrophages, and boosts the synthesis of prostaglandins and other cytokines[19].

CONCLUSION

The present study demonstrates indicate that changes in serum IL-6 and IL-1β levels indicate that orthodontic treatment affects periodontal inflammation. Baseline elevated cytokine levels were in line with gingival inflammation, which is closely associated with oral microbiota dysbiosis. Several bacterial species have been detected in association with orthodontic-related gingival inflammation. After three and six months, the cytokine levels gradually decreased, which may indicate a change toward a less pathogenic microbial composition as well as an adaptive host response. These results highlight the intricate interactions between several bacterial species and host immune mediators in the pathophysiology of gingival inflammation after orthodontic therapy.

Ethical Approval:
This study was reviewed and approved by the Ethics Committee of the college of Science, Tikrit University, Iraq. (Approval No.035/ TUCOS / ETH / 2024/ 10 – CLIN). Clinical samples were collected from Orthodontics clinic, Specialized Dental Center, Salah Al-Din Health directorate, Tikrit, Iraq. all participants and when applicable their legal guardians were informed, the study objectives, sampling procedures, and confidentiality assurances.

Conflict of Interest:
The authors declare no financial or personal relationships with people or organizations that might inappropriately influence their work.

Financial Disclosures:
The authors declare no specific financial interests, relationships, or affiliations relevant to the subject of the manuscript, including employment, consultancy, honoraria, or stock ownership.

Consent of Patients:
Prior to sample collection written informed consent was obtained from all participants. The study was conducted in accordance with the Declaration of Helsinki's principles and the ethical guidelines of the College of Science's Ethics Committee at Tikrit University.

Publication of issue:
All authors have read and approved the final version of manuscript and consent its publication.

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