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ORIGINAL ARTICLE

EFFECT OF TIME INTERVALS ON *S. MUTANS* AND IL-2 LEVELS FOR PATIENTS WEARING REMOVABLE ORTHODONTICS

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ABSTRACT

Background: *Streptococcus mutans* is the primary organism in dental plaque that contributes to caries development, particularly in individuals wearing orthodontic appliances. This study aimed to identify and quantify *S. mutans* using a molecular (qPCR) technique and evaluate IL-2 levels in an Iraqi sample population wearing removable orthodontic appliances.

Materials & Methods: This cross-sectional descriptive study involved 60 patients with removable orthodontic appliances (36 women and 24 men) aged 14 to 30 years. Participants were selected using the census method. The study sample was divided into three phases: immediate insertion (T0), three weeks (T1), and six weeks (T2) post-insertion. Gingival crevicular fluid (GCF) samples were collected from the proximal surfaces of the first molars using sterilized paper points. Additionally, 2–3 mL of unstimulated saliva was collected from each patient to evaluate IL-2 levels using ELISA.

Results: The mean values of *S. mutans* increased significantly from the time of appliance insertion (T0) to the subsequent follow-ups at T1 and T2. Similarly, IL-2 levels showed significant elevation at T1 and T2 compared to baseline ($P \leq 0.001$).

Conclusion: The study highlights the inflammatory role of IL-2 and the infectious mechanisms of *S. mutans*, emphasizing the direct impact of removable orthodontic appliances on oral and dental health. These findings underscore the necessity of maintaining effective oral hygiene practices among orthodontic patients to mitigate these effects.

KEY WORDS: Real Time PCR; *S. Mutans*; IL-2; Removable Orthodontic Appliance.

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INTRODUCTION

The oral cavity hosts a complex microbiome, harboring a wide variety of bacterial species, many of which remain uncultured and reside in hidden niches within the mouth.[1,2] Numerous factors influence the oral microbiome both qualitatively and quantitatively, including plaque accumulation, metal corrosion, and tooth movement.[3] Malocclusion, a common dental anomaly, negatively impacts aesthetics, oral health, and social well-being, often necessitating orthodontic treatment. Orthodontic therapy not only addresses dental misalignment but also enhances mastication, speech, facial aesthetics, self-esteem, and overall health.[4] However, despite its benefits, orthodontic treatment can contribute to various oral disorders and tissue damage.[5]

Orthodontic appliances, whether fixed or removable, create retention sites for dental plaque and food debris, complicating oral hygiene and increasing the risk of gingivitis, periodontitis, white spot lesions, dental caries, and halitosis.[6-9] Plaque accumulation during orthodontic treatment is a well-documented factor in the development of gingivitis and enamel demineralization.[10]

Dental plaque, a biofilm comprising diverse bacterial species, evolves based on its site and maturity, with significant roles in conditions such as caries and periodontitis.[11]

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Supragingival plaque, in particular, is a primary cause of enamel and dentin demineralization due to Streptococcus mutans (SM) activity.[12] SM is a key microorganism in dental plaque, initiating caries lesions and serving as an indicator of poor oral hygiene and caries susceptibility.[13,14]

Beyond oral health, SM is implicated in systemic conditions such as infective endocarditis and cardiovascular diseases due to its virulence factors.[15,16] It has been classified as a Gram-positive pathogen closely associated with the human host.[17] Orthodontic braces also influence the oral immune response, leading to the release of inflammatory mediators, including interleukins (IL-1, IL-2, IL-6, IL-8) and tumor necrosis factor, which contribute to local inflammation and tissue remodeling.[18] IL-2, in particular, plays a pivotal role in periodontal pathogenesis by stimulating T-cells, macrophages, and osteoclasts involved in bone resorption.[19]

Accurate identification of pathogens like SM is crucial for effective treatment strategies. Various techniques, including culturing, direct microscopy [20], biochemical assays, ELISA, and PCR, have been used for SM identification, with PCR offering superior simplicity, speed, and reliability.[21-23] Although prior studies have linked orthodontic appliances to increased bacterial pathogens,[24,25] and pro-inflammatory mediators, [26, 27] the specific effects of removable orthodontic appliances on the oral environment and immune response over time remain inadequately explored. This study aimed to identify and quantify *S. mutans* using qPCR with specific primers and evaluate IL-2 levels in Iraqi patients wearing removable orthodontic appliances over multiple time intervals.

MATERIALS AND METHODS

This cross-sectional descriptive study included 60 patients (36 females and 24 males) aged 14 to 30 years, all wearing removable orthodontic appliances. Patients were selected using a census method during the study period, which extended from December 2022 to April 2023. Participants were recruited from those seeking orthodontic treatment at the College of Dentistry, Babylon University, and a specialized dental health center.

Inclusion criteria:

Participants were required to meet the following criteria:

- Age between 14 and 30 years.
- No antibiotic use for more than five days.
- Good oral hygiene.
- Clinically healthy status.
- Recorded plaque, gingival, and bleeding-on-probing indices.
- Both male and female patients were included.

Exclusion criteria:

- Exclusion criteria Patients were excluded if they had:
- Systemic or oral diseases.
- Previous orthodontic treatment within the last two years.
- Pregnancy.
- Habitual mouth breathing.
- Smoking habits.
- Severe jaw abnormalities.

Study Design and Sampling:

The study was structured into three follow-up phases:

T0: Immediately after appliance insertion.

T1: Three weeks post-insertion.

T2: Six weeks post-insertion.

At each phase, gingival crevicular fluid (GCF) samples were collected from the mesial buccal aspect of the first molars using sterilized paper points (size 30, 4 mm). Each point was inserted obliquely for approximately 30 seconds, then removed and placed in tubes containing normal saline. Samples were frozen at -20°C until molecular analysis.

Additionally, 2–3 ml of unstimulated saliva was collected from each patient during each phase to assess IL-2 levels using the ELISA technique.[28] Saliva samples were collected with patients seated upright in a dental chair, refraining from eating or drinking for at least one hour before sampling.[29]

Molecular Analysis:

Quantitative PCR (qPCR) was used to detect *Streptococcus mutans* (*S. mutans*). The primer sequences for conventional and real-time PCR are shown in Table 1.

Table 1: Primer Sequence of *S. Mutans*.

Primer	Sequence (5′-3′)	Product Size
16S rRNA	Smf: GCCTACAGCTCAGAGATGCTATTC	144 bp (70)
	Smr: GCCATACACCACTCATGAATTGA	

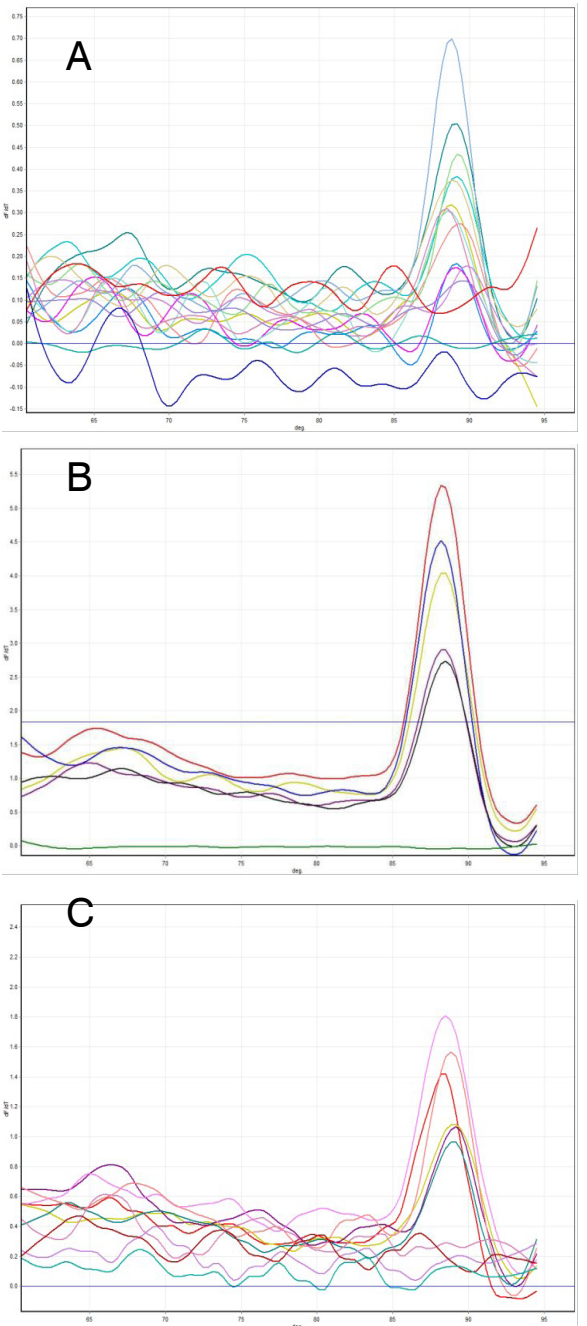

Figure 1: The Melt of *S. mutans* by real time PCR at different times, (A)at T0, (B) at T1, (C) at T2.

Statistical Analysis

SPSS version 29 was used to export all data for statistical analysis. Frequency (percentage) was used to report qualitative variables and mean ± (SD) was used for quantitative variables; and inferential statistics, represented by T-test and repeated ANOVA. The level of significance was taken by probability value (P- value), which ≤ 0.05 or < 0.001 is considered statisticallysignificant.

RESULTS

Figure 2 illustrates the percentage of patients with *S. mutans* at three time points: immediately after appliance insertion (T0), after three weeks (T1), and after six weeks (T2). The highest percentage of *S. mutans* was observed at T2, followed by T1, and the lowest percentage was noted at T0.

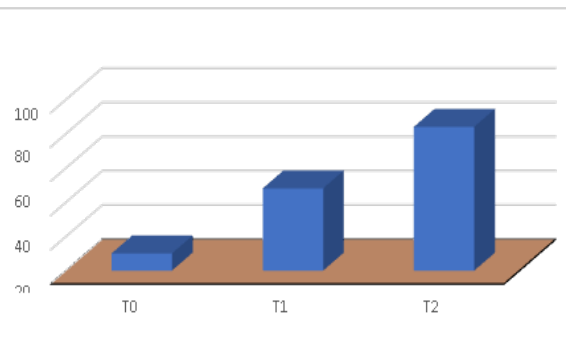

Figure 2: Percentage of patients with *S. Mutans* in (T0, T1, T2)

Table 2 presents the mean ± SD values of *S. mutans* at T0 and T1. At T0, the mean ±SD was 4293.27 ±3497.63, while at T1, it increased to 13186.03 ±9011.38. This difference was statistically significant (P ≤0.027), indicating a substantial rise in *S. mutans* levels after three weeks of appliance use compared to the immediate post-insertion phase.

Table 3 shows the mean ±SD values of *S. mutans* at T0 and T2. The mean ±SD at T0 was 4293.27 ±3497.63, whereas at T2, it significantly increased to 20522.18 ±15807.26 (P ≤0.024). These findings demonstrate a notable increase in *S. mutans* levels after six weeks of appliance use compared to the immediate phase.

Table 2: Mean ±(SD) streptococcus for patients with removable orthodontic appliances in (T0 and T1)

Bacteria	Time	No.	Mean ±SD	P value
S. Mutans	T0	5	4293.27 ±3497.63	0.027*
	T1	24	13186.03 ±9011.38	

* P- value ≤0.05 is significant

Table 3: Mean ±(SD) streptococcus for patients with removable orthodontic appliances in (T0 and T2)

Bacteria	Time	No.	Mean ±SD	P- value
S. Mutans	T0	5	4293.27 ±3497.63	0.024*
	T2	42	20522.18 ±15807.26	

* P- value ≤0.05 is significant.

Table 4: Mean ±(SD) streptococcus for patients with removable orthodontic appliances in (T1 and T2)

Bacteria	Time	No.	Mean ± SD	P- value
S.Mutans	T1	24	13186.03 ±9011.38	0.128
	T2	42	20522.18 ±15807.26	

Table 5: IL-2 levels in patients with removable orthodontic appliances in (T0, T1 and T2)

Parameter	Groups (n=60)	Mean ±SD	Significance*
IL-2/ pg/ml	T0	2.6997 ±1.327	< 0.001*
	T1	137.03 ±80.742	
	T2	272.57 ±109.142	

*Repeated measure ANOVA

Table 4 compares the mean ±SD values of *S. mutans* between T1 and T2. At T1, the mean ±SD was 13186.03 ±9011.38, increasing to 20522.18 ±15807.26 at T2. Although there was an observable rise in *S. mutans* levels from T1 to T2, the difference was not statistically significant (P >0.05).

Table 5 reports IL-2 levels at T0, T1, and T2. At T0, the mean IL-2 level was 2.6997 ±1.327, which increased significantly to 137.03 ±80.742 at T1 and further to 272.57 ±109.142 at T2 (P ≤0.001).

DISCUSSION

The use of removable orthodontic appliances addresses aesthetic, functional, and psychological concerns. However, their presence alters the oral microbiome equilibrium by creating additional retention sites for debris and new adhesion surfaces for oral flora.[30]

This disruption can lead to decreased salivary buffering capacity, encouraging the growth of cariogenic bacteria like *S. mutans* in saliva and dental plaque.[31] Early identification of these bacterial species is crucial for designing appropriate therapeutic strategies.[32] Among detection methods, quantitative PCR (qPCR) has proven to be more sensitive and reliable than conventional techniques for species-specific identification, particularly due to primers like 16S rRNA.[33-34]

In the present study, the prevalence of *S. mutans* increased over time. Initially, at appliance insertion (T0), only a small percentage of patients tested positive. After three weeks (T1), *S. mutans* was detected in 48% of participants, and by six weeks (T2), most patients tested positive (Figure 2). Similarly, mean *S. mutans* values showed a significant rise, increasing from T0 to T1 and reaching their highest levels at T2, where values were five times higher than at T0 (Tables 2 and 3). Although *S. mutans* levels at T2 were higher than at T1, the difference was not statistically significant (Table 4). These findings align with Lucchese et al.[35], who reported a continuous rise in *S. mutans* colony counts during the first month of removable orthodontic appliance use. However, their review noted a decline in bacterial levels after 1-2 months, which contrasts with this study. This discrepancy may be attributed to differences in ethnicity, sample age, appliance materials, and sampling methods.

Similarly, Agarwal et al.[25] found a significant increase in *S. mutans* counts during the first month of both fixed and removable orthodontic appliance use. While removable appliances allow for some maintenance of oral hygiene, achieving the same level of cleanliness as at T0 becomes challenging during follow-up visits. Factors such as appliance porosity, surface roughness, and prolonged wearing time contribute to increased bacterial adhesion and plaque formation. As appliances remain in the oral cavity for extended periods, they provide a conducive environment for the overgrowth of *S. mutans*, a dominant oral microflora.

In addition to bacteriological analysis, this study assessed immune responses by measuring IL-2 levels in patients treated with removable orthodontic appliances. IL-2 was selected for its role as an inflammatory marker

and its involvement in osteoclastic activity during bone remodeling.[36-37]

Over the course of treatment, IL-2 levels showed a significant increase from baseline (T0) to both T1 and T2 ($P \leq 0.001$) (Table 5). These findings are consistent with Gujar et al.[38], who reported a significant rise in IL-2 levels in patients using removable orthodontic aligners compared to those with fixed appliances. Aligners, although different in mechanism, share similarities with removable appliances in terms of fabrication and use. Similarly, Başaran et al.[39] observed increased IL-2 levels around treated cuspid teeth during orthodontic therapy.

The elevation in IL-2 levels during T1 and T2 likely corresponds to the initial tooth movement phase, characterized by acute inflammation, periodontal vasodilation, and cellular migration to inflamed sites. These migratory cells release cytokines that stimulate the native cells involved in tooth movement. By T2, the continued inflammatory response, transitioning from acute to chronic inflammation, further amplifies cytokine concentrations. In contrast, low IL-2 levels at T0 may reflect a refractory period before the onset of appliance-induced inflammation.[40, 41]

This study highlights the interplay between orthodontic appliances and changes in both the microbiological and immunological landscapes of the oral cavity. However, its limitations include a small sample size and a relatively short follow-up period. Future studies should consider longer follow-ups and larger, more diverse samples to validate these findings.

CONCLUSION

Real-time PCR is a simple and accurate molecular tool for the rapid detection of *S. mutans* in the oral cavities of patients treated with removable orthodontic appliances. Initially, the mean levels of *S. mutans* and IL-2 are minimal; however, as the appliance is worn for three to six weeks, there is a significant and progressive increase in both parameters. These findings underscore the impact of removable orthodontic appliances on oral and dental health, emphasizing the necessity for rigorous oral hygiene practices among orthodontic patients.

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CONFLICT OF INTEREST
Authors declare no conflict of interest.
GRANT SUPPORT AND FINANCIAL DISCLOSURE
None declared.

AUTHORS' CONTRIBUTION

The following authors have made substantial contributions to the manuscript as under:

Conception or Design:	RJA, KAA
Acquisition, Analysis or Interpretation of Data:	RJA, KAA
Manuscript Writing & Approval:	RJA, KAA

All the authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.