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EFFECT OF ORTHODONTIC THERAPY ON THE IMMUNE PROFILE OF ADULT INDIVIDUALS DURING PERIODONTAL MAINTENANCE PHASE

EFEITO DA TERAPIA ORTODÔNTICA SOBRE O PERFIL IMUNOLÓGICO DE INDIVÍDUOS ADULTOS EM FASE DE MANUTENÇÃO PERIODONTAL

EFECTO DE LA TERAPIA DE ORTODONCIA EN EL PERFIL INMUNITARIO DE INDIVIDUOS ADULTOS DURANTE LA FASE DE MANTENIMIENTO PERIODONTAL

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ABSTRACT

Interest among adults in improving oral esthetics through orthodontic treatment has increased in recent years. The status of periodontal support before and during orthodontic therapy is critical for identifying patients susceptible to more severe periodontal breakdown. Therefore, the aim of this study was to evaluate the effect of approximately 1 year of orthodontic therapy on the immunological profile of pro-inflammatory cytokines (INF, IL-1, and IL-6) and the anti-inflammatory cytokine IL-10 in adults undergoing periodontal maintenance. Twenty individuals were recruited from the Dental Clinic of UNIVERITAS UNG University and allocated to two groups: Periodontal Maintenance (PM) and Periodontally Healthy (PH). All participants underwent clinical and immunological monitoring. After 12 months, the PH group showed reductions in mean probing depth and clinical attachment level, along with an increase in the percentage of sites with visible biofilm. In the PM group, only a reduction in mean clinical attachment level was observed. Immunological findings showed no significant changes in the PM group. In the PH group, final IL-10 and IL-1 levels were lower than baseline levels ($p<0.05$, Wilcoxon test). IL-6 also decreased from baseline (4.49 ± 5.80) to the final assessment (1.66 ± 1.28), although without statistical significance. In conclusion, orthodontic therapy lasting approximately 1 year did not alter the immunological profile of pro-inflammatory cytokines (INF, IL-1, and IL-6) or the anti-inflammatory cytokine IL-10 in adults undergoing periodontal maintenance.

KEYWORDS: *Periodontitis. Interleukin. Cytokines. Tooth Movement Techniques. Orthodontic appliance. Orthodontic movement. Microbiological changes.*

RESUMO

O interesse da população adulta na estética oral por meio da ortodontia aumentou nos últimos anos. A condição do suporte periodontal antes e durante a terapia ortodôntica é extremamente importante para verificar e identificar os pacientes suscetíveis às manifestações mais severas da doença. Assim, o objetivo deste estudo foi avaliar o efeito da terapia ortodôntica com duração de aproximadamente um ano sobre o perfil imunológico de citocinas pró-inflamatórias (INF, IL-1, IL-6) e anti-inflamatória (IL-10)

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de adultos em manutenção periodontal. Foram selecionados 20 indivíduos na Clínica Odontológica da Universidade UNIVERITAS UNG, sendo um grupo de indivíduos em Manutenção Periodontal (MP) e um Periodontalmente Saudável (PS). Todos receberam monitoramento clínico e imunológico. Aos 12 meses, observou-se que o grupo PS apresentou redução dos valores médios da profundidade de sondagem, do nível clínico de inserção, bem como o aumento do percentual de sítios com biofilme visível. No grupo MP, notou-se apenas redução na média do nível clínico de inserção. Os resultados imunológicos demonstraram que não houve alteração significante no grupo MP. Em relação ao grupo PS, os níveis finais de IL-10 e IL-1 foram menores do que os níveis observados no início do estudo ($p<0,05$, teste de Wilcoxon). A IL-6 também mostrou redução dos níveis médios inicial (4,49 ± 5,80) e final (1,66 ± 1,28), porém sem diferença estatística. Em conclusão, a terapia ortodôntica, com duração de aproximadamente um ano, não alterou o perfil imunológico de citocinas pró-inflamatórias (INF, IL-1, IL-6) e anti-inflamatórias (IL-10) de indivíduos adultos em fase de manutenção periodontal.

PALAVRAS-CHAVE: Periodontite. Interleucina. Citocinas. Técnicas de Movimentação Dentária. Aparelho ortodôntico. Movimentação ortodôntica. Alterações microbiológicas.

RESUMEN

El interés de la población adulta por mejorar la estética oral mediante el tratamiento ortodóncico ha aumentado en los últimos años. El estado del soporte periodontal antes y durante la terapia ortodóncica es fundamental para identificar a los pacientes susceptibles a una destrucción periodontal más severa. Por lo tanto, el objetivo de este estudio fue evaluar el efecto de aproximadamente un año de terapia ortodóncica sobre el perfil inmunológico de citocinas proinflamatorias (INF, IL-1 e IL-6) y de la citocina antiinflamatoria IL-10 en adultos en fase de mantenimiento periodontal. Veinte individuos fueron reclutados en la Clínica Odontológica de la Universidad UNIVERITAS UNG y distribuidos en dos grupos: Mantenimiento Periodontal (MP) y Periodontalmente Sano (PS). Todos los participantes fueron sometidos a seguimiento clínico e inmunológico. Después de 12 meses, el grupo PS mostró reducciones en los valores medios de profundidad de sondaje y nivel clínico de inserción, junto con un aumento en el porcentaje de sitios con biofilm visible. En el grupo MP, solo se observó una reducción en el nivel clínico medio de inserción. Los hallazgos inmunológicos no mostraron cambios significativos en el grupo MP. En el grupo PS, los niveles finales de IL-10 e IL-1 fueron menores que los niveles iniciales ($p<0,05$, prueba de Wilcoxon). La IL-6 también disminuyó desde el valor inicial (4,49 ± 5,80) hasta la evaluación final (1,66 ± 1,28), aunque sin significación estadística. En conclusión, la terapia ortodóncica de aproximadamente un año no alteró el perfil inmunológico de citocinas proinflamatorias (INF, IL-1 e IL-6) ni de la citocina antiinflamatoria IL-10 en adultos en fase de mantenimiento periodontal.

***PALABRAS CLAVE**: Periodontitis. Interleucina. Citocinas. Técnicas de movimiento dentario. Aparato ortodóncico. Movimiento ortodóncico. Cambios microbiológicos.*

1. INTRODUCTION

The importance attributed to esthetics has increased substantially in recent years. Among adults, the demand for orthodontic treatment has become increasingly prominent. Clinical conditions

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such as tooth loss, pathologic tooth migration, reduced width of attached gingiva, osseous defects, loss of vertical dimension, and periodontal problems, among others, have required an interdisciplinary approach involving Periodontics and Orthodontics (Dannan, 2010). Once periodontal health has been reestablished and the patient has been enrolled in a periodic periodontal maintenance program, the first stage of integrated treatment is considered complete. The second stage involves planning orthodontic therapy aimed at tooth repositioning, while considering the adjustments required for individualized treatment finishing (Cirelli et al., 2006; van Gastel et al., 2011).

The establishment of periodontal health or disease is directly related to the dynamic balance among the microbiota and the host immune and inflammatory responses. With the presence of brackets, biofilm accumulation and colonization by periodontopathogenic bacteria occur. In response to microbial challenge, gingival enlargement may be developed, which can further hinder oral hygiene procedures. As a consequence, increased levels of gingival bleeding are also observed (Liu et al., 2014; Yanez-Vico et al., 2015; Sun et al., 2018; Jepsen et al., 2023; Villamil-Jaramillo et al., 2024). In adult individuals, mean probing depth may increase slightly during orthodontic treatment. This change should not be considered clinically relevant, as it is likely a response to increased supragingival biofilm accumulation and gingival inflammation during the 12 months of orthodontic therapy in areas adjacent to bonded attachments. Nevertheless, it clearly underscores the importance of periodontal maintenance therapy (Verrusio et al., 2018; Erbe et al., 2023; Gül et al., 2025; Martin et al., 2022; Jiang et al., 2026).

The recommended management involves periodontal therapy, often with temporary removal of the orthodontic appliance. A reduction in the levels of periodontopathogenic microorganisms is observed, such as *Porphyromonas gingivalis, Aggregatibacter actinomycetemcomitans, Fusobacterium nucleatum, Campylobacter rectus*, and *Treponema denticola*. As a result of tissue repair and resolution of the inflammatory signs, probing depth values also tend to return to baseline levels (Kruk et al., 2018; Gujar et al., 2020; Lemos et al., 2020; Nassar et al., 2021; Martin et al., 2022; Peterson et al., 2024).

With regard to orthodontic tooth movement and immunological changes, the scientific literature includes several review articles (Domenico et al., 2012; Kitaura et al., 2020; Han et al., 2023; Luchian et al., 2022; Alghamdi et al., 2023) as well as clinical studies. Giannopoulou et al. (2008) investigated the expression of IL-1β, IL-4, and IL-8 in gingival crevicular fluid (GCF) from children, adolescents, and young adults with and without fixed orthodontic appliances. The authors suggested that fixed orthodontic appliances result in increased expression of IL-1β and IL-8, which may reflect biological activity in the periodontium during orthodontic tooth movement. In a controlled longitudinal interventional study, Grant et al. (2013) investigated changes in cytokines and biomarkers of bone and tissue metabolism in the GCF of patients undergoing orthodontic treatment. Elevated levels were evident as early as 4 hours and persisted for periods of up to 6 weeks. These findings suggest that

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the analysis of biomarkers in GCF may help optimize orthodontic treatment with regard to the application of individualized forces for each patient. In 2015, Gameiro et al. evaluated whether orthodontic pain reflected differences in objective masticatory performance and in the levels of pro-inflammatory cytokines in the crevicular fluid of patients undergoing orthodontic treatment. Patients' masticatory performance was significantly reduced 24 hours after bracket placement, a period during which they reported higher pain scores and exhibited increased interleukin-1β levels. Prostaglandin E2 levels did not change over the periods evaluated, and no correlations were found between cytokine levels and the observed functional limitations. In 2017, Zhang et al. investigated the effect of combined periodontal-orthodontic treatment on inflammatory cytokine levels in individuals with periodontitis over a 2-year period.

The authors concluded that combined periodontal-orthodontic treatment showed the greatest clinical efficacy in the management of periodontitis and may effectively reduce inflammatory cytokine levels, including high-sensitivity C-reactive protein, interleukin-1β, interleukin-5, interleukin-6, interleukin-8, tumor necrosis factor-α, and prostaglandin E2. More recently, Lin et al. (2020) provided preliminary evidence of the involvement of the Th17 pathway (IL-17, IL-23, and IL-27) in the regulation of orthodontic tooth movement. In 2021, Lin et al. demonstrated that IL-17 promotes the expression of MMP-1, MMP-2, and MMP-9 by human periodontal ligament cells (hPDL cells), suggesting that IL-17 plays a crucial role in remodeling during orthodontic tooth movement. Also in 2021, Chen et al. showed that salivary IL-1β levels were positively correlated with oral bacterial load in orthodontic patients; however, the relationship between inflammatory cytokines and the oral microbiota still requires further investigation. In 2024, Zheng et al. investigated the application of periodontal tissue regeneration combined or not combined with orthodontic treatment in the restoration of oral function and esthetics and explored its effect and significance on the expression of interleukin-1β (IL-1β), tumor necrosis factor-α (TNF-α), and interleukin-5 (IL-5) in periodontal tissues.

Scores for masticatory function, speech, esthetics, social function, emotional status, cognitive function, and general health were higher after treatment, with superior outcomes in the group receiving the two therapies in combination (test group). Periodontal parameters and inflammatory mediator levels decreased after treatment and were lower in the same test group. In 2025, Liang et al. evaluated the clinical parameters and inflammatory mediators (IL-6, MMP-8, and TGF-β), the efficacy of guided tissue regeneration (GTR) combined with a miniscrew anchorage technique in the treatment of patients with periodontitis and malocclusion. After 6 weeks, combined therapy with miniscrew anchorage was found to provide superior therapeutic outcomes in patients with periodontitis and malocclusion, with reduced levels of IL-6 and MMP-8 in gingival crevicular fluid and increased levels of TGF-β.

To date, few studies have performed longitudinal evaluations. Therefore, the aim of this clinical study is to assess the effect of approximately 1 year of orthodontic therapy on the immunological profile

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of pro-inflammatory cytokines (INF, IL-1, and IL-6) and the anti-inflammatory cytokine IL-10 in adult individuals undergoing periodontal maintenance.

2. METHODOLOGY

2.1. Sample Selection

Twenty individuals attending the Dental Clinic of UNIVERITAS UNG University (Guarulhos, SP, Brazil) were selected to participate in the study. Participant selection was carried out by a single trained professional. From this sample, 10 individuals had treated generalized Stage III periodontitis and were enrolled in a periodontal maintenance program (PM), whereas 10 were periodontally healthy (PH) individuals with no history of periodontal disease. All participants were informed about the study objectives, as well as its risks and benefits, including the types of clinical and radiographic assessments, sample collection procedures, and therapies involved. They agreed to participate and signed an Informed Consent Form, completed a health questionnaire/anamnesis, and received periodontal maintenance therapy and orthodontic treatment free of charge, in accordance with the guidelines and regulations of the Brazilian National Health Council (Resolution No. 466/2012). The study protocol was approved by the Human Research Ethics Committee of Guarulhos University (SISNEP 691).

To be included in the study, participants were required to meet the following criteria: completion of periodontal treatment at least 6 months earlier and enrollment in a maintenance program (PM group); or periodontally healthy status, with no indication for periodontal therapy (PH group); age 30 years or older; presence of at least 20 teeth, excluding third molars; and diagnosis of malocclusion Angle Class I, II, or III, with indication for corrective orthodontic therapy and the presence of mild to moderate crowding, without anchorage requirements.

The exclusion criteria were: current smokers and former smokers who had quit less than 5 years previously; a history of systemic disease that could compromise host response or require prophylactic medication before treatment (e.g., diabetes, immunodeficiencies, hypertension, among others); pregnancy or lactation; history of antibiotic therapy within the previous 3 months; regular use of mouthrinses within the previous 3 months; previous orthodontic treatment; and extensive prosthetic rehabilitations.

2.2. Experimental Design

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At baseline, all participants underwent anamnesis and periodontal clinical examination and were allocated to 1 of the 2 study groups according to their clinical characteristics. The participants received clinical and immunological monitoring at baseline and again 12 months after therapy.

- Group 1 (n = 10): individuals who had completed periodontal treatment at least 6 months earlier and were in the periodontal maintenance phase.
- Group 2 (n = 10): periodontally healthy individuals.

2.3. Clinical Assessment

Two examiners were trained and calibrated to achieve the highest possible reproducibility of the measurements obtained. The calibration methodology followed that proposed by Araujo et al. (2003), in which the standard error of measurement (SEM) and the mean percentage error (MPE) were assessed for the continuous periodontal clinical parameters, namely probing depth and clinical attachment level. The intraexaminer and interexaminer SEM and MPE indicated acceptable reproducibility within the standards of periodontal clinical research. For the categorical variables visible plaque index and gingival bleeding index, considering only the presence or absence of the clinical parameter, the mean level of agreement was calculated, and the examiners showed 92% agreement (Kappa test).

Clinical measurements were performed at six sites per tooth (mesiobuccal, midbuccal, distobuccal, mesiolingual, midlingual, and distolingual) on all teeth, except third molars, using a manual millimeter periodontal probe (PCPUNC-BR15, HuFriedy do Brasil, RJ, Brazil). The following clinical parameters were evaluated:

- Visible Plaque Index (VPI) (Ainamo & Bay, 1975): The presence or absence of visible supragingival dental plaque was recorded after rinsing and drying the teeth. Absence of plaque was scored as 0 (not visible), and presence of plaque was scored as 1 (visible).
- Gingival Bleeding Index (GBI) (Ainamo & Bay, 1975): The presence or absence of bleeding in the marginal gingiva was recorded after gently passing the periodontal probe along the gingival sulcus. Absence of bleeding was scored as 0, and presence of bleeding was scored as 1.
- Probing Depth (PD): Distance, in millimeters, between the free gingival margin and the most apical probing depth of the periodontal sulcus/pocket.
- Clinical Attachment Level (CAL): Distance, in millimeters, between the cementoenamel junction and the most apical probing depth of the periodontal sulcus/pocket.
- Bleeding on Probing (BOP): Presence (score 1) or absence (score 0) of bleeding within 20 seconds after probing with a millimeter periodontal probe.

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- Suppuration (SUP): Presence (score 1) or absence (score 0) of suppuration within 20 seconds after probing with a millimeter periodontal probe.

2.4. Immunological Assessment

Two contralateral quadrants were randomly selected by means of a random numbered table generated using computer software (IBM SPSS 17.0, New York, NY, USA). These two quadrants required to include at least nine teeth. If this requirement was not met, the other two quadrants were considered. Two teeth per quadrant, one posterior and one anterior, were selected for mesial gingival crevicular fluid (GCF) sampling at baseline and at the 12-month evaluation.

After removal of supragingival biofilm, the sites were isolated with sterile cotton rolls and dried with an air jet to eliminate any possibility of saliva contamination. After a 2-minute interval to allow crevicular fluid accumulation, a sterile paper point (#30, Tanariman Indústria Ltda, Manacaru, AM, Brazil) was inserted at least 3 mm into the periodontal sulcus/pocket for 10 seconds. This procedure was repeated four consecutive times at the same site, totaling 40 seconds of sampling. The paper points were immediately placed into dry plastic microtubes and promptly stored at −80°C until sample processing.

Inflammatory mediators (INF, IL-10, IL-1, and IL-6) were analyzed by multiplex immunoassay at UNIVERITAS UNG University. Concentrations of INF, IL-10, IL-1, and IL-6 were determined in GCF samples collected from each subgingival site using customized commercial kits (MILLIPLEX® Kits, Merck Millipore, Billerica, MA, USA). The results were acquired using a multiplex analyzer (MAGPIX® System, Merck Millipore, Billerica, MA, USA) and analyzed with dedicated software (MILLIPLEX® Analyst Software, Merck Millipore, Billerica, MA, USA), based on standard curves and a five-parameter logistic model, in accordance with the manufacturer's instructions. The Bradford method (Bradford, 1976) was used to determine the total protein concentration in each sample according to the manufacturer's recommendations (Bradford Reagent, Sigma-Aldrich, St. Louis, MO, USA). The results were expressed as the amount of each analyte (pg) per mg of total protein in the gingival crevicular fluid sample.

2.5. Orthodontic Therapy

The orthodontic records for each participant were requested from the same imaging center (Cury Radiologia e Documentação Odontológica, located in Guarulhos, SP, Brazil) and included extraoral photographs (frontal, profile, and smiling views), intraoral photographs (frontal, right, and left views), a lateral cephalometric radiograph with two standard cephalometric tracings (USP and Ricketts), a panoramic radiograph, study casts (maxillary and mandibular), cast analysis, a record file, a storage box for the casts, digital images stored on compact disc (CD), and cone-beam computed tomography (CBCT), also provided on CD.

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For all patients, the orthodontic appliance was placed using the straight-wire technique with Roth prescription, employing only bonded attachments with 0.022" × 0.028" slots (Roth Max, Morelli Ortodontia; Morelli®: REF 10.15.400, 10.15.401, 10.15.402, 10.15.403, 10.15.409, 10.15.411, 10.15.413, 10.15.415, 10.15.416, 10.15.418, 10.15.420, 10.15.422, 10.15.426, 10.15.424, 10.15.428, 20.11.211, 20.11.212, 20.11.221, and 20.11.222).

The leveling sequence was performed with NiTi and 40°C thermoactivated CuNiTi archwires, which provide lighter, more constant, and lower force levels (Dalstra & Melsen, 2004; Sakima, Dalstra, Melsen, 2006). Progress during leveling was assessed at orthodontic maintenance appointments every 4 to 5 weeks. Standardization of care was sought for both groups, without introducing or postponing additional procedures such as semi-fixed anchorage devices (transpalatal arch), temporary anchorage devices (TADs; mini-implants), intermaxillary elastics, or other devices that could produce isolated changes in some individuals.

During this period, treatment was performed with the following materials:

- Maxillary arch: superelastic maxillary standard round NiTi intraoral archwires, diameter 0.30 mm (.012"), REF 50.60.001, and diameter 0.35 mm (.014"), REF 50.60.012 (Morelli Ortodontia, Morelli®); Thermo-Plus maxillary large round NiTi intraoral archwires, diameter 0.40 mm (.016"), REF 50.60.226, round diameter 0.45 mm (.018"), REF 50.60.227, rectangular 0.43 × 0.63 mm (.017" × .025"), REF 50.62.225 (Morelli Ortodontia, Morelli®); Thermo-Active Copper NiTi® .019" × .025" 40°C, REF 211-0955 (Ormco Corp., Thermodynamic); and maxillary CrNi rectangular intraoral archwire, 0.48 × 0.63 mm (.019" × .025"), REF 50.62.004 (Morelli Ortodontia, Morelli®).
- Mandibular arch: superelastic mandibular medium round NiTi intraoral archwires, diameter 0.30 mm (.012"), REF 50.70.000, and diameter 0.35 mm (.014"), REF 50.70.001 (Morelli Ortodontia, Morelli®); Thermo-Plus mandibular medium round NiTi intraoral archwires, diameter 0.40 mm (.016"), REF 50.70.226, round diameter 0.45 mm (.018"), REF 50.70.227, rectangular 0.43 × 0.63 mm (.017" × .025"), REF 50.72.225 (Morelli Ortodontia, Morelli®); Thermo-Active Copper NiTi® .019" × .025" 40°C, REF 211-0945 (Ormco Corp., Thermodynamic); and mandibular CrNi rectangular intraoral archwire, 0.48 × 0.63 mm (.019" × .025"), REF 50.72.004 (Morelli Ortodontia, Morelli®).
- Ligature materials: CrNi ligature wire in spool form, round diameter 0.20 mm (.008"), REF 55.01.208, and diameter 0.25 mm (.010"), REF 55.01.210 (Morelli Ortodontia, Morelli®), used for metallic ligatures and tie-backs. Orthodontic elastic chain, medium, gray, length 4.5 m, REF 60.05.511 (Morelli Ortodontia, Morelli®).

It should be emphasized that, for all participants, the standard approach during the first 12 months was the placement of bonded attachments in an attempt to standardize treatment within the group (Gkantidis et al., 2010). Subsequently, orthodontic tooth movement was initiated with placement of the first 0.014-in NiTi leveling archwire, which was secured to the brackets with elastomeric modules or metallic ligatures. After this procedure, the participant was dismissed and instructed to schedule the

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next appointment after 28 days. At each follow-up visit, orthodontic appliance maintenance and replacement of the leveling archwires were performed according to the previous treatment plan established for each participant. It is important to emphasize that all participants were treated according to the same leveling sequence, thereby standardizing force application at equivalent levels during each archwire change.

2.6. Periodontal Maintenance Therapy

Throughout the study period, all participants received periodontal maintenance therapy. Individuals included in the PH group received prophylaxis and reinforcement of oral hygiene instructions at each follow-up visit. Participants included in the PM group received the same care described above at each return visit; in addition, they also underwent periodontal maintenance therapy approximately every 3 months. All sites with probing depth of 5 mm or greater were subjected to scaling and root planning in a single session using Gracey curettes # 5/6, 7/8, 11/12, and 13/14 (Hu-Friedy, Chicago, IL, USA) for disruption and removal of newly formed biofilm. Whenever other dental treatment needs were identified, the participants were referred to the other disciplines of the Dental Clinic of UNIVERITAS UNG University.

Statistical Analysis: The sample size was defined considering the methodological complexity of the immunological analyses and the characteristics of the sample group, as well as the preliminary purpose of evaluating the biological response to the treatments. The immunological data, expressed as the levels of each cytokine (INF, IL-10, IL-1, and IL-6) by group (healthy or diseased) at time points 1 and 2, were analyzed using the Mann–Whitney test. The Wilcoxon test was used to compare data between the different time points within groups. Statistical analyses were performed using BioStat software, and the significance level was set at 5%.

3. RESULTS

3.1. Clinical Assessment

During the 12-month study period, no participant was excluded or withdrew from the study. Table 1 presents the epidemiological characteristics and the mean values of the clinical parameters assessed at baseline and after 12 months of orthodontic treatment in the two experimental groups. With regard to the changes observed over the course of orthodontic therapy, the periodontally healthy group showed reductions in mean probing depth (from 2.8 ± 0.3 mm to 2.3 ± 0.3 mm, $p = 0.001$) and clinical attachment level (from 2.9 ± 0.4 mm to 2.4 ± 0.3 mm, $p = 0.001$), as well as an increase in the percentage of sites with visible biofilm (from 12.4 ± 17.3% to 29.5 ± 13.5%, $p= 0.003$). In contrast, in the periodontal maintenance group, only a reduction in mean clinical attachment level was observed (from 3.6 ± 1.1 mm to 3.2 ± 0.7 mm, $p= 0.03$).

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Table 2 shows the percentage of sites that gained attachment (> 2 mm), lost attachment (> 2 mm), or remained stable (variation from −1 mm to 1 mm) during orthodontic therapy in the two experimental groups. No statistically significant differences were observed between the groups regarding the percentage of sites that gained clinical attachment or remained stable. However, the percentage of sites that lost attachment was statistically significantly higher in the periodontal maintenance group.

During the 12-month period of orthodontic treatment, only two teeth (molars) were indicated for extraction, both from two individuals included in the periodontal maintenance group. It is important to emphasize that, at baseline, these teeth already exhibited probing depths greater than 5 mm and furcation involvement and were therefore not included in the orthodontic treatment plan.

Table 1. Epidemiological characteristics and mean periodontal clinical parameters (± standard deviation) at baseline and after 12 months of orthodontic therapy in the two experimental groups

Variable	**Time**	**Groups**	
		Periodontally healthy	**Periodontal maintenance**
Age (years)	*Baseline*	38.3 ± 6.3	42.1 ± 8.1
Angle Class (%)			
I	*Baseline*	17.7 %	25.0 %
II	*Baseline*	52.9 %	58.3 %
III	*Baseline*	29.4 %	16.7 %
Probing Depth (mm)	*Baseline*	2.8 ± 0.3 [a]	3.0 ± 0.8 [a]
	12 months	2.3 ± 0.3 [b]	2.8 ± 0.5 [a]
Clinical Attachment Level (mm)	*Baseline* *	2.9 ± 0.4 [a]	3.6 ± 1.1 [a]
	12 months	2.4 ± 0.3 [b]	3.2 ± 0.7 [b]
% of sites with:			
Visible Biofilm	*Baseline* *	12.4 ± 17.3[a]	20.4 ± 17.8 [a]
	12 months	29.5 ± 13.5 [b]	35.6 ± 18.0 [a]
Gingival Bleeding	*Baseline* *	2.4 ± 3.9 [a]	4.6 ± 11.5 [a]
	12 months	2.5 ± 2.9 [a]	3.4 ± 3.8 [a]
Bleeding on Probing	*Baseline*	9.4 ± 6.5 [a]	7.5 ± 4.5 [a]
	12 months	8.2 ± 12.0 [a]	14.6 ± 23.5 [a]
Suppuration	*Baseline*	0.0 ± 0.0 [a]	0.2 ± 0.6 [a]
	12 months	0.0 ± 0.2 [a]	0.1 ± 0.2 [a]

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Different letters indicate differences between time points within the same group (Wilcoxon test). * Mann–Whitney U test (differences between groups at baseline; p < 0.05).

Table 2. Mean (± standard deviation) percentage of sites that gained clinical attachment (> 2 mm), lost clinical attachment (> 2 mm), or remained stable (variation from −1 mm to 1 mm) during orthodontic therapy in the two experimental groups

Variable	Condition	Groups		MW * P value
		Periodontally healthy	Periodontal maintenance	
% of sites:	Gained CAL	11.0 ± 9.0	19.0 ± 14.0	0.076
	Lost CAL	3.0 ± 5.0	6.0 ± 3.0	*0.009*
	Stable	86.0 ± 9.0	75.0 ± 14.0	0.102

*MW: Mann–Whitney U test (differences between groups; italicized values indicate p < 0.05).

3.2. Immunological Assessment

The analysis of inflammatory mediators (INF, IL-10, IL-1, and IL-6), reported as the amount of each analyte per mg of total protein (pg) in the gingival crevicular fluid sample, is presented in Table 3. Considering the data obtained at baseline and at the end of the study (12 months), no statistically significant differences were found between the groups for any of the four inflammatory mediators evaluated. The within-group analysis of each inflammatory mediator showed that, in the periodontal maintenance group, there were no statistically significant changes between baseline and 12 months in the levels of any of the mediators assessed. In the periodontally healthy group, final IL-10 and IL-1 levels were lower than those observed at baseline (p<0.05, Wilcoxon test). A reduction in IL-6 levels was also observed; however, the difference between baseline (4.49 ± 5.80) and final values (1.66 ± 1.28) did not reach statistical significance.

Table 3. Mean (± standard deviation) inflammatory mediator levels expressed as pg/mg (INF, IL-10, IL-1, and IL-6) at baseline and after 12 months of orthodontic therapy in the two experimental groups

Variable	Time	Groups	
		Periodontally healthy	Periodontal maintenance
INF	*Baseline*	3.06 ± 0.90 ^{aA}	3.92 ± 3.10 ^{a}A
	12 months	2.36 ± 0.92 ^{aA}	3.08 ± 2.07^{a}A
IL-10	*Baseline*	11.57 ± 4.04 ^{aA}	9.13 ± 6.87 ^{a}A
	12 months	6.72 ± 1.77 ^{bA}	8.67 ± 4.24 ^{a}A
IL-1	*Baseline*	16.87 ± 7.82 ^{aA}	26.79 ± 22.81 ^{a}A
	12 months	8.33 ± 5.96 ^{bA}	25.46 ± 36.47 ^{a}A
IL-6	*Baseline*	4.49 ± 5.80 ^{aA}	2.77 ± 2.95 ^{a}A
	12 months	1.66 ± 1.28 ^{aA}	1.77 ± 1.70 ^{a}A

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Different lowercase letters indicate differences between time points within the same group (Wilcoxon test). Different uppercase letters indicate differences between groups at each experimental time point (Mann–Whitney test)

4. DISCUSSION

Orthodontic tooth movement depends on tissue resorption and apposition in the surrounding bone and periodontal ligament. Compression and tension are associated with specific signaling factors, thereby establishing local gradients that regulate bone and periodontal ligament remodeling for tooth displacement. The inflammation that occurs during tooth movement must be carefully controlled, as a dysregulated inflammatory process leads to tissue destruction manifested by root resorption, which may be induced both by orthodontic treatment and by periodontal disease. Given that biology has profound clinical implications, particularly in orthodontic tooth movement, several surgical, pharmacological, and physical interventions have been investigated to accelerate tooth movement, shorten treatment duration, and reduce time-dependent adverse outcomes (Li et al., 2018).

Some general aspects of the experimental design of this study should be highlighted, including the difficulty in standardizing malocclusion cases because of the limitations of Angle's classification when first molars are missing. Another important factor to be considered is patient age and the magnitude of the forces applied. Aging is known to cause a slower tissue response to orthodontic forces, involving displacement and turnover of collagen fibers. In this process, it is important to emphasize the reduction in cellular activity and the presence of greater amounts of collagen in the tissues (Ong and Wang, 2002). In general, these cases are also associated with a reduced periodontium; therefore, the application of light, continuous forces should always be considered in treatments involving extrusion, intrusion, and buccal tooth movement. It must be considered that patients with a reduced periodontium may exhibit minimal or no bone loss during orthodontic tooth movement and, in some cases, may even show gain in bony support (Melsen, 1991; Erbe et al., 2023; Gül et al., 2025; Martin et al., 2022; Jiang et al., 2026).

With regard to the choice of inflammatory mediators, it is known that several mediators are involved in the inflammatory process and in tissue turnover, including the pro-inflammatory cytokines IFN-γ, IL-1, and IL-6, as well as the anti-inflammatory cytokine IL-10. IFN-γ is a cytokine produced by T cells and is predominantly inflammatory, contributing to both acute and, especially, chronic inflammatory responses. It is recognized as a major mediator of innate and adaptive immunity, attracting macrophages and increasing the number of phagocytic cells at the site of inflammation. Given its potent immunoregulatory role, IFN-γ has been suggested to be involved in periodontal remodeling during orthodontic tooth movement, contributing to inflammatory and autoimmune processes, and is found at high concentrations in patients during periods of disease exacerbation (Silveira et al., 2009). IL-1 is an important cytokine and a key mediator of the immune response against bacterial invasion, inflammation, infection, and tissue injury. It has also been described as a product

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of active phagocytic cells and is secreted by a variety of cell types, including fibroblasts, keratinocytes, Langerhans cells, kidney mesangial cells, certain B- and T-cell lineages, natural killer (NK) cells, mast cells, vascular endothelial cells, microglial cells, and smooth muscle cells. In addition, cytokines such as tumor necrosis factor-alpha (TNF-α), interferons alpha, beta, and gamma (IFN-α, IFN-β, and IFN-γ), lipopolysaccharides (LPS), viruses, and immunogens are also responsible for inducing IL-1 synthesis (Arend, 1991). Studies have shown that IL-1 cytokines are involved in bone deposition and resorption both in vivo and in vitro (Silveira et al., 2009). IL-6 is a pro-inflammatory cytokine involved in the regulation of innate and adaptive immune responses and acute-phase reactions. It is produced by several different cell types, including activated monocytes, fibroblasts, macrophages, and T and B cells, and one of its functions is to induce B-cell maturation into immunoglobulin-secreting plasma cells (Oliveira et al., 2011). IL-10 is a cytokine with anti-inflammatory and suppressive effects on most hematopoietic cells and indirectly suppresses the production of other cytokines as well as the proliferation of antigen-specific effector CD4+ T cells by inhibiting the function of antigen-presenting cells, including dendritic cells, Langerhans cells, and macrophages. In contrast, IL-10 also exerts stimulatory effects on effector CD8+ T cells by increasing their cytotoxic capacity and proliferative potential (Roncarolo et al., 2006). IL-10 production is impaired by several cytokines, such as IL-4, IL-13, and IFN-γ, as well as by its own autoregulation. It inhibits pro-inflammatory cytokines, particularly TNF, IL-1, and IL-6, produced by activated macrophages and monocytes, while stimulating the endogenous production of anti-inflammatory cytokines. In addition, it increases mast cell proliferation and prevents IFN-γ production by natural killer cells (Oliveira et al., 2011).

The results of the present study showed no statistically significant differences between the groups, either at baseline or at the final follow-up, for any of the four inflammatory mediators evaluated. In addition, in the periodontal maintenance group, no statistically significant changes were observed between baseline and 12 months. In contrast, in the periodontally healthy group, final IL-10 and IL-1 levels were lower than those observed at baseline ($p<0.05$). Although IL-6 levels decreased (from 4.49 ± 5.80 to 1.66 ± 1.28), this difference did not reach statistical significance.

Several authors have investigated the relationship between inflammatory mediators and the process of orthodontic tooth movement (Giannopoulou et al., 2008; Domenico et al., 2012; Grant et al., 2013; Hazan-Molina et al., 2015; Gameiro et al., 2015; Zhang et al., 2017; Lin et al., 2020; Kitaura et al., 2020; Lin et al., 2021; Chen et al., 2021; Baeshen, 2021; Liu et al., 2024; Zheng et al., 2024; Liang et al., 2025). However, only Zhang et al. (2017) followed patients longitudinally over an 18-month period. These authors also observed no worsening of clinical or immunological parameters, suggesting that combined periodontal-orthodontic treatment showed the greatest clinical efficacy in the management of periodontitis and in reducing inflammatory cytokine levels.

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Considering the clinical and immunological findings, it may be more broadly inferred that the indication for orthodontic treatment in patients undergoing periodontal maintenance does not result in harm to the periodontium and may be considered safe when combined with proper treatment planning.

5. CONCLUSION

In conclusion, orthodontic therapy lasting approximately 1 year did not alter the immunological profile of pro-inflammatory cytokines (IFN, IL-1, and IL-6) or the anti-inflammatory cytokine IL-10 in adult individuals undergoing periodontal maintenance.

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