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IL-1-Beta and TNF-Alpha in Gingival Crevicular Fluid of Patients with Orthodontic Aligners and Application of Vibrations with Sonic Toothbrush: A Pilot Study

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Abstract

Introduction: Non-invasive methods to modulate orthodontic tooth movement have gained interest, particularly those targeting inflammatory mediators such as IL-1β and TNF-α, which regulate osteoclast and osteoblast activity. High-frequency vibrations (HFV), including those delivered by sonic toothbrushes, have been proposed to influence these biological responses. The aim of the study is to assess whether sonic vibrations affect IL-1β and TNF levels in patients undergoing clear aligner therapy. **Materials and Methods**: Twenty Invisalign® patients were evaluated. For each patient, one tooth received HFV via a 285 Hz sonic toothbrush (experimental), while the contralateral served as a control. Gingival crevicular fluid was sampled at baseline (T0), after one week without HFV (T1), and after one week with HFV (T2). Cytokines were measured by ELISA. Because data were non-normally distributed, non-parametric tests were applied. **Results**: No significant differences across T0–T2 were found within the HFV group. At T2, IL-1β levels were significantly lower in the HFV group (mean: 23.04; SD: ± 20.18) than in controls (mean: 44.44; SD: ± 47.14), which showed an IL-1β increase with orthodontic force alone. TNF-α levels remained near the ELISA detection limit. **Conclusions**: Sonic vibrations combined with clear aligners appear to reduce IL-1β secretion and local inflammation without adverse effects. Sonic toothbrushes provide a simple HFV delivery method, though larger studies are needed to confirm these findings.

Keywords: orthodontics; sonic; tooth; inflammatory markers

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1. Introduction

Orthodontic tooth movement is fundamentally governed by the biomechanical forces exerted on dental structures, with both low- and high-magnitude forces inducing remodelling of the alveolar bone via mechanotransduction pathways [1]. Application of orthodontic forces—whether through conventional fixed appliances or removable clear aligners—elicits localized pressure within the periodontal ligament (PDL), initiating a

cascade of vascular and neuroinflammatory events. These mechanobiological stimuli activate intracellular signaling pathways that culminate in the release of pro-inflammatory mediators, including matrix metalloproteinases (MMPs), osteoprotegerin (OPG), lactate dehydrogenase (LDH), and pro-inflammatory cytokines such as tumor necrosis factor-alpha (TNF-α) and interleukin-1 beta (IL-1β) [2,3]. These molecular mediators play pivotal roles in osteoclastogenesis and osteoblastic activity, orchestrating the bone resorption and deposition processes essential to orthodontic tooth displacement [4].

Extended durations of orthodontic treatment are associated with an elevated incidence of adverse effects, including gingival recession, apical root resorption, and both localized and systemic inflammatory responses [5,6]. To attenuate inflammation-induced sequelae, numerous investigations have explored adjunctive therapeutic modalities, one of which involves the application of mechanical vibrational stimuli [7].

Empirical evidence supports the notion that high-frequency vibration (HFV), particularly at a frequency of 120 Hz, exerts dual catabolic and anabolic effects on the alveolar bone microenvironment [4]. The integration of HFV into orthodontic protocols is hypothesized to modulate the inflammatory milieu within the PDL, which is continuously subjected to compressive stress. Mechanistically, vibrational stimuli are postulated to enhance microvascular perfusion and fluid dynamics, thereby diminishing inflammatory signaling [8].

Additionally, several studies have reported that the use of sonic toothbrushes—a form of low-intensity vibrational device—may lead to a downregulation of systemic inflammatory biomarkers, such as pro-inflammatory cytokines and C-reactive protein (CRP) [9]. Given the established correlation between elevated systemic inflammatory markers and the pathogenesis of chronic diseases, including cardiovascular disorders and metabolic syndromes, these findings suggest a potentially broader therapeutic utility of sonic toothbrushes beyond oral hygiene maintenance [10].

Preliminary experimental data, measured in the gingival crevicular fluid (GCF), reveal a statistically significant reduction in IL-1β concentrations, with TNF-α levels remaining minimal across all subjects assessed [11]. These observations suggest that vibratory mechanical stimulation via sonic toothbrushes may constitute a non-invasive and cost-effective strategy to mitigate inflammation associated with orthodontic tooth movement. Furthermore, attenuation of gingival inflammation may contribute to the reduction in systemic inflammatory burden, thereby reinforcing the bidirectional relationship between oral and systemic health. Based on the current literature, although an association between inflammation and orthodontic tooth movement is well established, there is insufficient evidence regarding the potential impact of tooth brushing, particularly the use of sonic toothbrushes, on oral inflammatory markers.

The working hypothesis of the current study posits that regular use of a sonic toothbrush reduces salivary or gingival crevicular fluid levels of IL-1β and TNF-α, conferring both localized periodontal and systemic anti-inflammatory benefits. Conversely, the null hypothesis maintains that sonic toothbrush use exerts no statistically significant impact on these inflammatory biomarkers.

2. Materials and Methods

2.1. Study Design and Ethical Approval

This prospective clinical study was conducted at the Department of Orthodontics, University of Insubria (Varese-Como, Italy), and received ethical approval under protocol number 0111335 from the Institutional Review Board of the Università degli Studi dell'Insubria (22 December 2022). Written informed consent was obtained from all participants or their legal guardians, in accordance with the Declaration of Helsinki. The study was conducted between 2023 and 2024.

2.2. Sample Characteristics

The study can be considered a pilot because of its novelty. However, before planning the study, a revision of the literature was performed. A study has evaluated alterations in cytokine content in GCF under different experimental conditions using a sample size ranging from 10 to 30 samples per group [12]. In addition, an a priori analysis was conducted using G Power 3.1 software. Considering 3 measurements for each of the two experimental conditions, fixed the type I (α) at 0.05 and II error (β) at 0.8 and an effect size of 0.39, we needed 19 samples/group (CTR and HFV). The study population comprised 20 adolescent subjects aged 7–12 years who were undergoing orthodontic treatment with Invisalign® aligners (Align Technology, Santa Clara, CA, USA). Inclusion criteria were: Good general and oral health status; Periodontal health characterized by physiological probing depth ($\leq$2 mm); Presence of dental crowding in one or both dental arches; No antibiotic therapy in the preceding six months; No use of anti-inflammatory medications during the six months prior to enrolment; Absence of radiographic evidence of alveolar bone loss; Bleeding index of zero; No active dental caries or detectable supragingival calculus.

2.3. Experimental Protocol

For each subject, two homologous teeth undergoing similar orthodontic movement (as determined by their individual ClinCheck® digital treatment plan) were selected (Figure 1). One tooth was, according to a computerized method, assigned to the experimental group (HFV stimulation), and the contralateral tooth within the same arch served as a control.

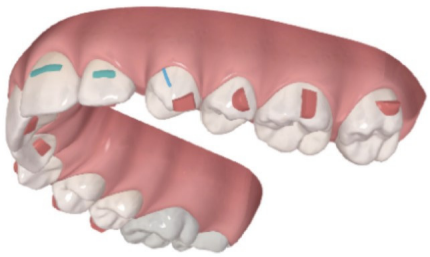

Figure 1. Clincheck simulation.

High-frequency vibrational (HFV) stimulation was administered using the Mentadent Professional 4D TOUCH sonic toothbrush (Mentadent® Professional 4D TOUCH, Unilever, Italy), operating at approximately 285 Hz. The maximal pressure of the toothbrush can be 2.5 Newton.

Mentadent Professional 4D TOUCH is a powered toothbrush designed to enhance mechanical disruption of dental biofilm through high-frequency, multi-directional bristle motion. Its cleaning action is based on high-frequency vibrational technology, which generates micro-shear forces that facilitate plaque detachment beyond direct bristle contact.

The "4D" system integrates these vibrations with controlled motion and pressure-responsive feedback to optimize plaque removal while minimizing enamel and gingival stress.

A flat tongue-cleaning attachment was used to apply localized vibrational input directly to the experimental site. Patients were instructed to use the tongue brush once daily for 5 min, for 7 consecutive days, by occlusally positioning the brush head on the selected tooth. To control for extraneous vibrational exposure, participants were instructed to perform routine oral hygiene exclusively with a manual toothbrush throughout the study period.

2.4. Clinical Assessments and Sample Collection

Clinical evaluations and gingival crevicular fluid (GCF) sampling were performed at three time points: T0: Baseline, prior to the initiation of aligner therapy. T1: After one week of Invisalign® wear without HFV application. T2: After two weeks of Invisalign® wear, including one week of HFV application. At each time point, GCF was collected from both experimental and control sites. Prior to sampling, professional supragingival prophylaxis was performed using ultrasonic instruments.

2.5. Gingival Crevicular Fluid Collection

To minimize sample contamination, the selected teeth were isolated using sterile cotton rolls and a saliva ejector. Supra-gingival plaque was carefully removed with sterile cotton pellets, and the tooth surfaces were air-dried for 10 s using oil- and moisture-free compressed air.

Gingival crevicular fluid (GCF) was collected using standardized absorbent paper strips (PerioPaper®, Harco, Tustin, CA, USA; 20×10^{-2} mm in diameter). Sample collection was performed by a clinician who was different from the investigator responsible for laboratory analysis. Sample collection was performed between 8:00 and 10:00 a.m. to standardize conditions and minimize the effects of circadian rhythm–related fluctuations. For each subject and at each time point, two paper strips were used per site—one for the experimental tooth and one for the contralateral control tooth (Figure 2). The paper strip was gently inserted into the gingival sulcus until mild resistance was felt and left in place for 60 s to allow adequate fluid absorption, while ensuring no mechanical trauma to the surrounding gingival tissues. Strips contaminated with blood or saliva were discarded and replaced.

Figure 2. Collection of Gingival Crevicular Fluid with a Paper Cone.

All samples were immediately stored in sterile microtubes and either processed immediately or frozen under standardized conditions at −30 °C, ensuring uniform preservation across all specimens until biochemical analysis.

2.6. GCF Sample Processing and Cytokine Quantification

Following collection, paper strips were immediately stored at −20 °C until further analysis, which was conducted by the Department of Pharmacological and Biomolecular Sciences at the University of Milan. For each site and time point, the two paper strips were pooled into a single sterile microcentrifuge tube for elution of gingival crevicular fluid (GCF).

To extract the absorbed fluid, 225 µL of 50 mM phosphate buffer (pH 7.2) supplemented with a protease and phosphatase inhibitor cocktail (Roche Complete Mini EDTA-free, catalog no. 1183170001) was added to each tube. The paper strips were fully submerged and incubated at 4 °C for 30 min under continuous agitation. Samples were intermittently vortexed during incubation to enhance elution efficiency.

Following incubation, the tubes were centrifuged at 13,000 rpm for 10 min at 4 °C. The supernatant containing the GCF was transferred to a new sterile tube. To maximize recovery, a second elution was performed using the same procedure, and the resulting supernatants were combined. The extracted GCF was subsequently stored at −80 °C until cytokine analysis.

Quantification of cytokine concentrations in the GCF samples was performed using enzyme-linked immunosorbent assays (ELISA). Tumor necrosis factor-alpha (TNF-α) levels were measured using the Human TNF-alpha ELISA kit (eBioscience, catalog no. 88-7346-88, San Diego, CA, USA), and interleukin-1 beta (IL-1β) concentrations were determined using the Human IL-1 beta ELISA kit (eBioscience, catalog no. 88-7261-88), following the manufacturer's protocols. All evaluations were performed in blind to experimental conditions.

The assay utilized a sandwich ELISA format, wherein the target analyte was captured between two antibodies specific to distinct epitopes of the cytokine. A conjugated detection antibody linked to horseradish peroxidase (HRP) enabled chromogenic detection via a substrate reaction. The intensity of the colorimetric signal, measured spectrophotometrically, was directly proportional to the concentration of the target cytokine in the sample.

2.7. Statistical Analysis

All statistical analyses were conducted using IBM SPSS Statistics software, version 25 (IBM Corp., Armonk, NY, USA) by a researcher blinded to experimental setting. The Shapiro–Wilk test was employed to evaluate the normality of data distributions for each variable at the different time points (T0, T1, and T2). Given that the data did not conform to a normal distribution, non-parametric methods were applied for subsequent analyses. To compare cytokine levels (TNF-α and IL-1β) between experimental and control sites across the three time points, the Wilcoxon signed-rank test was used. A p-value of <0.05 was considered indicative of statistical significance.

3. Results

The concentrations of (IL-1β) and (TNF-α), quantified using enzyme-linked immunosorbent assay (ELISA) and expressed in picograms per millilitres (pg/mL), are summarized in Table 1.

Table 1. Levels of IL-1β and TNF-α.

IL-1 pg/mL	GF	SB	AP	FG	BS	PA	AC	CA	EB	OS	BE	GS	LP	PL	TP	PT	SG	SO	GM	MG
T0	21.53	40.52	12.52	16.77	41.1	55.88	13.04	11.19	44.58	6.21	44.84	1.58	20.29	17.80	5.01	17.17	18.42	6.62	11.60	66.50
T1	60.04	21.70	28.20	22.43	65.16	41.35	14.14	1.20	4.65	11.08	51.28	5.13	30.53	83.07	6.33	39.32	37.06	19.61	17.62	22.10
T2	21.02	18.07	56.86	17.91	30.37	9.33	69.77	36.73	8.33	7.69	64.84	2.33	30.48	13.40	6.56	3.93	7.16	11.37	30.14	14.10
T0CTR	33.47	29.75	36.01	16.93	42.39	28.31	27.47	4.11	5.01	2.08	24.06	2.70	37.34	68.81	24.28	8.98	5.31	29.42	12.93	93.10
T1CTR	69.08	11.60	80.10	11.02	15.17	89.17	17.00	12.52	11.60	12.00	4.17	1.71	40.85	19.55	24.73	19.78	23.11	12.81	25.24	3.10
T2CTR	17.28	29.15	87.53	21.02	26.36	16.65	20.11	122.66	8.98	4.95	8.33	3.62	38.71	148.33	65.64	12.64	10.43	14.02	131.95	101.23
TNF pg/mL	**GF**	**SB**	**AP**	**FG**	**BS**	**PA**	**AC**	**CA**	**EB**	**OS**	**BE**	**GS**	**LP**	**PL**	**TP**	**PT**	**SG**	**SO**	**GM**	**MG**
T0	1.97	2.21	1.81	1.81	2.05	1.85	1.67	1.83	1.74	1.90	1.85	1.81	2.19	1.90	2.22	1.78	1.97	2.00	1.85	1.71
T1	1.95	2.17	2.07	2.07	1.95	2.10	2.10	2.58	1.76	1.97	2.19	1.94	2.02	1.74	1.76	2.07	1.90	1.69	2.15	1.95
T2	1.97	2.05	2.00	1.78	1.90	1.93	1.93	1.83	1.78	1.94	1.97	1.74	2.60	1.74	1.93	1.85	1.93	1.83	2.07	1.69
T0CTR	2.17	1.93	2.42	2.24	2.20	2.05	2.17	1.07	2.05	1.83	2.07	2.50	2.37	2.19	1.76	1.85	1.97	1.90	1.85	2.14
T1CTR	2.02	1.95	1.97	2.30	1.83	2.15	1.97	1.93	1.85	1.81	1.74	1.97	1.74	1.76	1.95	2.19	1.90	1.78	1.81	1.55
T2CTR	2.17	2.15	1.90	2.68	2.22	2.00	1.81	2.22	2.12	2.39	1.85	1.92	2.35	2.02	2.02	1.74	1.85	1.93	2.07	1.60

TNF-α levels detected in the gingival crevicular fluid (GCF) were consistently low across all samples and time points, approaching the lower limit of detection of the assay, indicating minimal expression of this cytokine under the experimental conditions. In

contrast, IL-1β concentrations were notably higher and well within the quantifiable range of the assay, enabling robust statistical comparison between experimental and control groups at each time point.

Further analysis was conducted on IL-1β concentrations, for which the mean values and standard deviations were calculated at each of the three time points (T0, T1, T2) for both the experimental (HFV-treated) and control sites. The resulting data are reported in Table 2 and visually represented in Figure 3. This descriptive analysis allowed for the assessment of temporal trends and intergroup differences in IL-1β expression associated with the application of high-frequency vibration.

Table 2. Descriptive data of IL-1β levels.

	N	Minimum	Maximum	Average	SD
T0	20	1.58	66.50	23.66	18.47
T1	20	1.20	83.07	29.10	22.20
T2	20	2.33	69.77	23.05	20.18
T0C	20	2.08	93.09	26.62	22.86
T1C	20	1.71	89.17	25.26	25.14
T2C	20	3.62	148.33	44.48	47.14

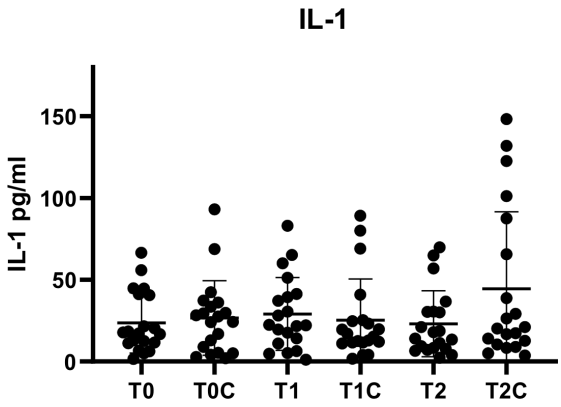

Figure 3. IL-1 distribution.

No statistically significant differences were observed in IL-1β concentrations at T0, T1, or T2 within the experimental group subjected to vibratory stimulation according to the Wilcoxon signed-rank test. However, a statistically significant difference ($p < 0.05$) was noted, through the Wilcoxon signed-rank test, at T2 between the experimental group receiving vibratory stimulation and the control group treated with orthodontic forces alone. At T2, IL-1β levels in the experimental group were significantly lower (23.04 pg/mL; SD: ±20.18) compared to the control group (44.44 pg/mL; SD: ±47.14).

As illustrated in Table 2, vibratory stimulation was associated with sustained low IL-1β levels at T2, while the control group exhibited a marked increase in IL-1β concentrations in the gingival crevicular fluid (GCF) two weeks after the application of orthodontic forces alone. This suggests that vibratory stimulation may modulate the inflammatory response associated with orthodontic treatment.

Regarding safety evaluation, no adverse effects were observed or reported throughout the intervention period. Specifically, no clinical signs or symptoms such as gingival bleeding, mucosal irritation, dental hypersensitivity, or other oral discomfort were recorded in any participant during or after the intervention. Periodontal clinical parameters were routinely monitored as part of standard orthodontic follow-up, and no clinically relevant changes in periodontal probing depth or clinical attachment level were detected over the

study period, indicating the absence of periodontal tissue damage associated with the intervention. Furthermore, all participants reported good tolerance of the procedure, with no complaints of discomfort during use. Collectively, these clinical observations support the conclusion that the intervention was not associated with adverse effects.

4. Discussion

Bone resorption is a physiological process primarily mediated by osteoclasts and intricately regulated by a complex network of molecular signals. Among these signaling molecules, interleukin-1 beta (IL-1β) plays a crucial role in modulating osteoclastogenesis. Evidence has shown that IL-1β promotes the expression of Receptor Activator of Nuclear Factor Kappa-B Ligand (RANKL) in osteoblasts and human periodontal ligament (PDL) cells, which in turn stimulates the differentiation of osteoclast precursors, leading to enhanced bone resorption [13]. This mechanism is particularly relevant in the context of orthodontic tooth movement (OTM), where the application of mechanical forces induces the remodeling of surrounding alveolar bone. Clinical studies have demonstrated elevated IL-1β levels in gingival crevicular fluid (GCF) during orthodontic treatment, indicating a direct correlation between mechanically induced inflammation and bone remodelling processes.

Several studies have examined the relationship between orthodontically induced local inflammation and systemic inflammatory responses, yielding varied results [14–16].

Chronic low-grade inflammation, such as that associated with periodontal disease, has been linked to an increased risk of systemic conditions, including cardiovascular disease and diabetes [17]. It is hypothesized that orthodontically induced inflammation may similarly contribute to these systemic outcomes, although conclusive evidence is still emerging [18]. These results support the hypothesis that localized periodontal inflammation can influence systemic inflammatory status [19,20]. These findings suggest that local and systemic inflammatory responses may not always be directly correlated.

Cytokines such as IL-1β, IL-6, and TNF-α, along with CRP, are key mediators in both orthodontic bone remodelling and systemic inflammatory processes. While CRP is a sensitive marker of systemic inflammation, it can also reflect localized inflammatory events, such as those induced by orthodontic forces. The potential systemic implications of orthodontic treatment are particularly relevant for patients with pre-existing inflammatory conditions like cardiovascular disease or diabetes. In such cases, orthodontic force application may need to be modulated, or adjunctive anti-inflammatory strategies, such as the use of sonic toothbrushes, may be employed to reduce inflammation.

Consistent with previous reports, our data indicate that HFV may attenuate IL-1β elevation during orthodontic treatment, supporting its potential anti-inflammatory effect. Several studies have investigated the effect of vibratory stimuli, such as those produced by electric toothbrushes, on IL-1β levels in GCF. Given that elevated IL-1β is associated with the severity of periodontal disease, IL-1β plays a pivotal role in inflammation-mediated bone pathology. Participants in these studies have reported favourable experiences with electric toothbrushes, noting their comfort and ease of use [8]. Routine vibratory stimulation through electric toothbrushes could thus represent a practical, non-invasive approach to modulate IL-1β expression and reduce local inflammation during orthodontic treatment.

Indeed, findings from the current study suggest that vibratory stimulation may help maintain lower IL-1β levels, which typically rise two weeks after the initiation of orthodontic force.

The therapeutic potential of mechanical vibration has been extensively explored in various clinical disciplines, particularly for its ability to enhance blood and lymphatic circulation [21]. Vibratory stimuli induce muscle contractions that promote vasodilation in small-calibre vessels, thereby increasing tissue perfusion and accelerating tissue repair

and regeneration. These effects are particularly beneficial for bone and soft tissue healing, where adequate vascularization is essential for successful outcomes.

In both animal models and human subjects, prior studies have demonstrated that low-intensity vibration can stimulate angiogenesis and facilitate wound healing [22,23]. In diabetic models, vibratory therapy has been shown to enhance the expression of growth factors, thereby promoting bone regeneration and tissue integration [24]. Moreover, increased blood flow at superficial tissue levels has been observed following vibratory therapy, suggesting potential benefits for periodontal tissue perfusion and for post-extraction or post-orthodontic bone healing [25,26].

Vibratory stimulation also exerts an analgesic effect through the "gate control" mechanism, where activation of fast-conducting nerve fibers inhibits the transmission of nociceptive signals, thereby reducing pain perception [27–29]. This effect may be particularly beneficial in orthodontics, where discomfort is common [30,31]. Since pro-inflammatory cytokines such as IL-1β and TNF-α can sensitize nociceptors and enhance pain signaling, the analgesic effects of vibration may also arise from its ability to suppress IL-1β expression [32].

Given its non-invasive nature, localized vibration therapy has gained acceptance in a variety of medical fields, including physiotherapy, osteopathy, obstetrics, and speech therapy. In orthodontics, vibratory devices are emerging as promising tools for enhancing periodontal and bone tissue responses. These devices are user-friendly and can be easily incorporated into home care routines, offering a convenient adjunctive modality to support the orthodontic treatment process.

4.1. Future Directions

While the present study demonstrates the potential benefits of vibratory stimulation in modulating local inflammation during orthodontic treatment, further investigation is warranted to explore the long-term effects of vibratory devices on both local and systemic inflammatory markers. Future studies should aim to examine whether sustained use of vibratory stimulation can reduce the cumulative effects of chronic low-grade inflammation, particularly in patients with pre-existing systemic conditions. Additionally, it will be important to assess whether the application of vibratory stimuli can mitigate the side effects commonly associated with orthodontic treatment, such as gingival recession, root resorption, and periodontal ligament damage.

Moreover, future clinical trials should explore the optimal parameters for vibratory stimulation, including frequency, duration, and intensity, to identify the most effective protocols for improving patient outcomes. The role of vibratory devices in enhancing orthodontic treatment efficiency, accelerating tooth movement, and promoting bone regeneration also warrants further investigation. Given the promising results observed in preclinical and early-stage clinical studies, the incorporation of vibratory devices as adjuncts to orthodontic therapy may soon become a standard of care.

4.2. Limitations of the Study

Despite the valuable insights gained from this study, several limitations must be acknowledged, which could influence the generalizability and robustness of the findings:

- Sample Size: One of the primary limitations of this study is the relatively small sample size, which may limit the statistical power to detect subtle differences between groups. A larger sample size could increase the reliability of the results and provide more robust evidence regarding the effects of vibratory stimulation on local cytokine levels in gingival crevicular fluid (GCF).

- Patient Compliance: The study relied on patient-reported compliance regarding the use of the vibratory stimulation device. However, the actual adherence to the prescribed protocol was not objectively monitored, leading to potential variability in the level of vibratory stimulation received by individual patients. This limitation may introduce biases in the results, as inconsistent or improper use of the device could impact the outcomes.
- Oral Hygiene Variability: Changes in oral hygiene practices during the study could have influenced the periodontal health of the participants. While instructions were provided for standardized oral hygiene, factors such as variations in brushing technique, frequency, or the introduction of new oral care products could have impacted the presence of supra-gingival plaque and the overall periodontal condition, which in turn may have influenced GCF cytokine levels.
- Fluctuations in General Health Status: Variations in the general health status of participants throughout the study period, including possible illnesses, stress, or fluctuations in medication use, may have introduced confounding variables that could affect both local and systemic inflammatory responses. As the study did not account for these potential fluctuations, the observed effects of vibratory stimulation might have been influenced by factors unrelated to the intervention itself.
- Hormonal Influences in Growing Patients: The potential impact of hormonal fluctuations, particularly in the case of younger, growing patients, represents another limitation. Hormonal changes associated with puberty, menstrual cycles, or other endocrine-related factors could modulate the inflammatory response and influence cytokine levels in GCF. The study did not control for these variables, making it difficult to isolate the effects of vibratory stimulation from the potential influence of hormonal variations, especially in female patients [33].
- These limitations underscore the complexity of studying cytokine modulation during orthodontic treatment, and further research with larger, more controlled populations and long-term follow-up would be necessary to validate and refine the conclusions drawn in this study.

5. Conclusions

The present study focused on the modulation of IL-1β and TNF-α levels in gingival crevicular fluid (GCF) in patients undergoing Invisalign® treatment, with a particular emphasis on the effects of high-frequency vibratory stimuli delivered via a sonic toothbrush. The results suggest that vibratory stimulation has the potential to mitigate local inflammation in the periodontal tissues, which is critical during orthodontic tooth movement. By potentially modulating the inflammatory response in the periodontal ligament, vibratory therapy may assist in maintaining bone health during orthodontic treatment, thus possibly reducing the risk of bone resorption and enhancing the overall efficacy of the treatment.

Nevertheless, the study provides valuable insight into the potential role of vibratory therapy as a non-invasive, easily accessible adjunct to orthodontic treatment. Given its safety profile and ease of use, it could serve as a useful tool for clinicians aiming to enhance patient outcomes by promoting bone health and mitigating inflammation during orthodontic therapy. Vibratory therapy may prove beneficial in preventing bone loss and promoting faster healing in the orthodontic context. However, further research is required to elucidate the underlying biological mechanisms of vibratory stimuli and to determine whether this approach can reliably accelerate orthodontic treatment outcomes. Future studies with larger sample sizes, longer follow-up periods, and more rigorous patient compliance monitoring are essential to confirm and expand upon these preliminary findings.

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Conflicts of Interest: The authors declare no conflict of interest.

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