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## Biomarker changes in gingival crevicular fluid during orthodontic treatment with clear aligners - a systematic review

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### Abstract

**Introduction.** Clear aligner therapy has gained widespread popularity as an alternative to fixed orthodontic appliances; however, its biological effects on periodontal tissues remain not fully elucidated.

**Aim.** To synthesize the available evidence on cytokine and bone metabolism biomarker changes in gingival crevicular fluid associated with orthodontic treatment using clear aligners.

**Material and methods.** This systematic review was conducted in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines. A comprehensive search was conducted on PubMed, Science Direct, ProQuest, Web of Science and Cochrane Library electronic databases from July 1st until July 31 2025. In accordance with the predefined inclusion and exclusion criteria, studies assessing changes in inflammatory cytokines and bone metabolism biomarkers during orthodontic treatment with clear aligners were included in this review.

**Results.** A total of 403 records were identified through electronic database searches, and after duplicate removal and screening, 9 studies were included in the review. All examined inflammatory cytokines (interleukin-1 beta (IL-1 $\beta$ ), interleukin-1 alpha (IL-1 $\alpha$ ), tumor necrosis factor- alpha (TNF- $\alpha$ ), interleukin-6 (IL-6), interleukin-8 (IL-8), interleukin-2 (IL-2), interleukin-17 (IL-17), and granulocyte-macrophage colony-stimulating factor (GM-CSF)) exhibited elevated levels during the early phase of clear aligner therapy, reflecting an active inflammatory response. Bone metabolism biomarkers, RANKL (receptor activator of nuclear factor kappa-B ligand) and OPN (osteopontin) generally increased, while OPG (osteoprotegerin) showed a decreasing trend.

**Conclusions:** Orthodontic treatment with clear aligners is associated with measurable alterations in inflammatory cytokines and bone metabolism biomarkers during the early phase of treatment. These findings emphasize the biological activity of aligner therapy and highlight the need for further studies to clarify the clinical significance of these molecular changes.

**Keywords:** gingival crevicular fluid, clear aligners, orthodontic treatment, cytokines, inflammatory biomarkers, bone metabolism biomarkers.

## 1. Introduction

Orthodontic tooth movement (OTM) is a biomechanical process in which orthodontic forces disrupt the homeostasis of the periodontal ligament, creating localized hypoxic conditions that initiate an aseptic inflammatory response. This cascade involves the recruitment of immune and bone cells, as well as the release of cytokines and other mediators, which together orchestrate alveolar bone resorption by osteoclasts and bone formation by osteoblasts, ultimately allowing tooth movement [1]. As these molecular changes are reflected in the periodontal environment, gingival crevicular fluid (GCF) serves as a valuable medium for detecting cytokines and other biomarkers related to orthodontic tooth movement [2].

Gingival crevicular fluid (GCF) is an exudate of inflammatory origin that derives from the gingival plexus and adjacent periodontal tissues. It contains proteins, tissue breakdown products, growth factors, electrolytes, cytokines, as well as other low-molecular-weight compounds and enzymes of both host and bacterial origin [3]. Among the numerous components of GCF, cytokines and bone metabolism biomarkers (BMBs) are considered crucial mediators, as they regulate inflammatory responses and bone remodeling processes underlying OTM [4]. Cytokines are low-molecular-weight proteins (<25 kDa) secreted predominantly in an autocrine or paracrine manner, and many of them participate in the recruitment and activation of immune cells at sites of localized force application or stress within the physiological environment [4,5]. Moreover, bone metabolism biomarkers represent valuable tools for monitoring the complex dynamics of bone remodeling. By mirroring the interplay between resorption and formation, they provide essential information on the molecular events that govern bone turnover. Assessing these biomarkers improves our comprehension of the mechanisms

involved in OTM and treatment-induced bone responses [4,6].

The rising demand for aesthetic treatment options has significantly contributed to the widespread use of clear aligner therapy in orthodontics [7,8]. Clear aligners represent one of the most frequently used alternatives to fixed orthodontic appliances, combining advantages such as enhanced aesthetics, better oral hygiene maintenance, and increased patient comfort [9]. Unlike fixed appliances, aligners produce intermittent forces on teeth due to their removability, and these forces tend to fluctuate over time [10]. According to Kuncio et al. [11], tooth movement achieved with aligners may not correspond to the typical stages of orthodontic movement outlined by Krishnan and Davidovitch [12]. However, a major limitation of clear aligners is that they are not suitable for treating all types of malocclusions, particularly those requiring complex tooth movements [13,14].

It is well established that cytokine activity and bone metabolism biomarkers in GCF reflect biological responses during orthodontic treatment with fixed appliances, evidence concerning these biological responses in patients undergoing clear aligner therapy remains limited. This lack of consistent data highlights the need for a comprehensive evaluation of the molecular mechanisms underlying OTM with aligners. Therefore, the aim of this systematic review was to synthesize the available evidence on cytokines and BMBs changes in GCF during orthodontic treatment with clear aligners.

## 2. Materials and Methods

This systematic review was conducted in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines [15]. The protocol was prospectively registered in the International Prospective Register of Systematic

Reviews (PROSPERO) under the identifier CRD420251120919.

## 2.1 Focus Question

The focus question of this review was formulated in accordance with the Population Intervention Comparison Outcome-Study design (PICOS) framework [16]. It investigated patients undergoing orthodontic treatment (P) with clear aligners (I), where changes in gingival crevicular fluid were assessed across multiple treatment time points (C). The outcomes (O) were defined as quantitative alterations in the composition of GCF, particularly in biomarkers related to inflammation and bone metabolism. The review included prospective clinical studies, comprising pilot, comparative and cohort designs (S).

## 2.2 Eligibility Criteria

The inclusion criteria included:

- Patients undergoing orthodontic treatment with clear aligners;
- Studies that evaluate changes in gingival crevicular fluid (GCF) composition, particularly levels of inflammatory or bone metabolism biomarkers;
- Participants without periodontal disease or systemic conditions affecting periodontium;
- Clinical full-text studies on human participants;
- Articles published in English;
- Original prospective clinical studies.

## 2.3 Exclusion criteria

The exclusion criteria included:

- Studies involving only fixed orthodontic appliances or removable appliances other than clear aligners;
- Studies that do not assess gingival crevicular fluid (GCF) or do not report GCF-related outcomes;

- Studies involving participants diagnosed with periodontitis, presenting clinical signs of active periodontal disease, or systemic conditions that could affect periodontal status;
- Systematic reviews, meta-analyses, case reports.

## 2.4 Search strategy and study selection

The search for relevant articles was conducted in five electronic databases, which included PubMed, Science Direct, ProQuest, Web of Science and Cochrane Library from July 1st until July 31, 2025. For PubMed, the following search strategy was applied: (gingival crevicular fluid) AND (*clear aligners* OR *aligners* OR *invisalign*) AND (*inflammatory* OR *cytokine* OR *bone metabolism* OR *biomarkers*). The search strategy was appropriately modified and applied to the other electronic databases included in this review.

The process of study selection was independently conducted by two reviewers. The procedure involved an initial screening of titles and abstracts, followed by full-text assessment of potentially eligible articles. Duplicate records were identified and removed using Zotero reference management software version 7.0.24 (Corporation for Digital Scholarship, USA). Any disagreements that arose during this process were resolved through discussion until consensus was achieved.

## 2.5 Data extraction

From each included study, data were extracted on the main study characteristics, including author, year of publication, country, study design, and study population details (number of participants, sex distribution, and mean age). Information was also collected on aligner type, GCF sampling method, and the GCF collection site. In addition, data regarding the biomarkers analyzed, their temporal changes during aligner therapy, and the main conclusions were extracted.

## 2.6 Risk of bias assessment

Risk of bias in the included non-randomized studies was assessed using the ROBINS-I (Risk Of Bias In Non-randomized Studies – of Interventions) tool [17]. This instrument evaluates seven bias domains and classifies the overall risk of bias for each study as low, moderate, serious, or critical.

## 3.1. Results

### 3.1 Study selection

A total of 403 records were identified through electronic database searches. After duplicates were removed, 374 articles were screened for eligibility based on titles and abstracts. Of these, 360 articles were excluded according to the predefined criteria. In total, 9 studies fulfilled all inclusion criteria and were included in this systematic review.

### 3.2 Study characteristics

The main characteristics of the included studies are summarized in Table 1. All included articles were designed as prospective studies, comprising pilot studies [18-20], comparative studies [4,21,22], observational studies [25,26], and one cohort study

[25]. Collectively, the studies assessed 152 patients undergoing orthodontic treatment with clear aligners. Sample sizes ranged from 10 to 30 participants per study, with an average of approximately 17 participants. Age

and sex reporting were inconsistent across studies: while some studies reported both mean age and age range, others provided only one of these measures, and in several cases, age was presented for overall study population rather than specifically for the clear aligner group. Two studies did not specify the sex distribution of participants [20, 25].

Most studies specified the use of Invisalign® clear aligners, while one study investigated aligners manufactured by K Line [20]. A few studies did not report the manufacturer or brand [21,22,24]. Gingival crevicular fluid (GCF) was collected using either PerioPaper® strips or microcapillary pipettes, with sample isolation achieved using cotton rolls, sterile gauze, or gentle air-drying. Sampling sites varied widely, including maxillary and mandibular incisors, molars, and canines (Table 1).

**Table 1.** Characteristics of included studies.

Publication	Study Design	The Study Sample: Patients (M/F); Mean Age (Years)	Aligner Type	GCF Sampling Protocol	GCF Collection Site	Eligible Outcome
Castroflorio et al., 2016 [18]	Prospective split-mouth clinical pilot study	10 patients; 5/5; mean age 22.3 ± 3.3 years	Invisalign	PerioPaper® strips for 30 seconds; cotton rolls and a gentle stream of air	Mesiobuccal and distobuccal sites of second molar	Quantitative changes in the levels of inflammatory and bone metabolism biomarkers in GCF
Chami et al., 2018 [19]	Prospective pilot study	11 patients; 5/6; mean age 23.63 ± 4.88 years	Invisalign	PerioPaper® strips for 30 seconds; cotton rolls and a gentle stream of air	Vestibular surface of lower central incisors and lateral incisors	Quantitative changes in the levels of inflammatory biomarkers in GCF
Gujar et al., 2019 [21]	Prospective comparative study	20 patients (aligner group); 9/11; mean age not reported for	NR	A micro-capillary pipette	Proximal site of maxillary canine	Quantitative changes in the levels of inflammatory

		aligner group (overall study sample mean age $28 \pm 4$ years)		(1 $\mu$ L); sterile gauze		biomarkers in GCF
Aziz, et al., 2020 [20]	Prospective clinical pilot study	10 patients; M/F not specified; mean age not reported	K line	PerioPaper <sup>®</sup> strips for 60 seconds; cotton roll and a gentle stream of air	Distolabial sulcus of the lower incisor	Quantitative changes in the levels of inflammatory biomarkers in GCF
Chen et al., 2021 [23]	Prospective observational study	14 patients (aligner group); 7/7; mean age $10.8 \pm 0.9$ years	Invisalign	PerioPaper <sup>®</sup> strips for 30 seconds; cotton roll and a gentle stream of air	Mesiobuccal sulcus of the maxillary first molar	Quantitative changes in the levels of inflammatory and bone metabolism biomarkers in GCF
Baeshen, 2022 [25]	Prospective cohort study	20 patients (aligner group); M/F not specified; mean age not reported (age range of 12–32 years reported for overall study sample only)	Invisalign	A micro-capillary pipette (1 $\mu$ L); sterile gauze	Proximal region of canines in the maxillary arch	Quantitative changes in the levels of inflammatory biomarkers in GCF
Kamran et al., 2023 [22]	Prospective comparative study	25 patients (aligner group); 15/10; mean age $25 \pm 4$ years	NR	A micro-capillary pipette (1 $\mu$ L); sterile gauze	Proximal site of maxillary canine	Quantitative changes in the levels of inflammatory and bone metabolism biomarkers in GCF
Alnazeh et al., 2023 [4]	Prospective comparative study	30 patients (aligner group); 10/20; mean age not reported separately (age range of 18–32 years and mean age $25 \pm 3$ years reported for overall study sample only)	Invisalign	A micro-capillary pipette (1 $\mu$ L) under aseptic conditions; sterile gauze	Proximal site of maxillary canine	Quantitative changes in the levels of inflammatory biomarkers in GCF
Altindal et al., 2024 [24]	Prospective observational study	15 patients; 10/5; mean age $27.4 \pm 6.52$ years	NR	PerioPaper <sup>®</sup> strips for 60 seconds; cotton rolls	Right upper first molar and left lower central incisor	Quantitative changes in the levels of inflammatory biomarkers in GCF

F, female; GCF, gingival crevicular fluid; M, male; NR, not reported.

### 3.3 Results of individual studies

The main outcomes of the nine included studies are summarized and presented in Table 2. All studies assessed biomarker changes in GCF during the initial phase of orthodontic treatment with clear aligners. Inflammatory cytokines were assessed in every study, while three studies [18,22,23] additionally evaluated bone metabolism biomarkers. The following sections provide a detailed synthesis of the findings, grouped by biomarker category.

#### 3.3.1 Inflammatory biomarkers

The inflammatory biomarkers investigated across the nine included studies comprised interleukin-1 beta (IL-1 $\beta$ ), interleukin-1 alpha (IL-1 $\alpha$ ), tumor necrosis factor- alpha (TNF- $\alpha$ ), interleukin-6 (IL-6), interleukin-8 (IL-8), interleukin-2 (IL-2), interleukin-17 (IL-17), and granulocyte-macrophage colony-stimulating factor (GM-CSF). IL-1 $\beta$  were the most frequently studied cytokine, evaluated in seven studies. Of these, five studies reported statistically significant increases during clear aligner therapy, though the timing of peak expression varied across studies. Aziz et al. [20] observed a sharp elevation as early as day 1, while Castroflorio et al. [18] reported significant increases at day 7 and day 21. Gujar et al. [21] and Baeshen [25] also identified elevated levels by day 21, and Kamran et al. [22] reported significant changes at day 28.

TNF- $\alpha$ , IL-6, and IL-8 were each investigated in five studies. Gujar et al. [21] and Baeshen [25] reported statistically significant increases in all three cytokines 21 days after treatment initiation. Kamran et al. [22] also evaluated all three markers and observed peak levels at day 28, while Alnazeh et al. [4] identified a marked rise in IL-6 at day 28. Altindal et al. [24] further reported notable elevations in IL-6 and IL-8 during a 21-day observation period.

Other inflammatory cytokines were evaluated less frequently across the included studies. IL-1 $\alpha$  and IL-2 were analyzed in three studies [21,22,25], all of which reported statistically significant increases during the initial phase of aligner therapy. IL-17 and GM-CSF were each evaluated in two studies [4,19]; however, significant changes were observed in only one of these investigations for each biomarker [4]. Two of the nine included studies did not identify any statistically significant changes in the analyzed biomarkers. Chami et al. [19] reported no significant alterations in IL-1 $\beta$ , IL-8, TNF- $\alpha$ , IL-17, or GM-CSF levels during the observation period, while Chen et al. [23] also reported no significant differences in IL-1 $\beta$  or TNF- $\alpha$  concentrations.

#### 3.3.2 Bone metabolism biomarkers

Three of the included studies [18,22,23] evaluated bone metabolism biomarkers in GCF, specifically assessed RANKL (receptor activator of nuclear factor kappa-B ligand), OPG (osteoprotegerin), and OPN (osteopontin). Castroflorio et al. [18] reported significant increases in RANKL at days 7 and 21 after treatment initiation, accompanied by decreased OPG and elevated OPN levels during the same period. Kamran et al. [22] observed a similar pattern, with significant increases in RANKL and OPN and a decrease in OPG at day 28. Chen et al. [23] analyzed RANKL and OPG only and reported no significant changes in either marker throughout the study period.

### 3.4 Quality assessment

The results of the risk of bias assessment for the included non-randomized studies are summarized in Table 3. According to the ROBINS-I tool, two studies were identified as having a serious overall risk of bias due to inadequate control of confounding factors [22,27], while the remaining studies were consistently rated as having moderate overall risk of bias.

**Table 2.** *Outcomes.*

Publication	Stage of Orthodontic Treatment	Biomarkers Analyzed	Biomarker Changes	Conclusions
Castroflorio et al., 2016 [18]	Before treatment 1 hour of treatment 7 days of treatment 21 days of treatment	IL-1 $\beta$ , RANKL, OPG, OPN	Statistically significant increases were observed in IL-1 $\beta$ levels at pressure sites after 7 and 21 days, in RANKL levels at both pressure and tension sites after 1 hour and 7 days, and in TGF- $\beta$ 1 and OPN levels at tension sites after 21 days. OPG levels significantly decreased at both pressure and tension sites after 7 and 21 days compared with baseline.	Clear aligner therapy may induce early increases in inflammatory and bone metabolism biomarkers, while some markers may show a decreasing trend, during the initial treatment phase.
Chami et al., 2018 [19]	Before treatment 1 day of treatment 7 days of treatment 21 days of treatment	IL-1 $\beta$ , IL-8, TNF- $\alpha$ , IL-17, GM-CSF	No statistically significant changes were observed in IL-1 $\beta$ , IL-8, IL-17, TNF- $\alpha$ or GM-CSF levels throughout the 21-day period. Although non-significant, a decreasing trend over time was noted for all these GCF biomarkers.	Clear aligner therapy may induce a limited inflammatory response, with minimal changes observed in biomarker levels during the initial phase of treatment.
Gujar et al., 2019 [21]	Before treatment 21 days of treatment	IL-1 $\beta$ , IL-1 $\alpha$ , IL-2, IL-6, IL-8, TNF- $\alpha$	Statistically significant increases were observed in all six cytokines in the aligner group after 21 days of treatment, with IL-1 $\beta$ and TNF- $\alpha$ showing the most prominent changes, and IL-1 $\alpha$ exhibiting the least.	Clear aligner therapy appears to elicit an inflammatory response during the initial phase of orthodontic treatment.

Aziz et al., 2020 [20]	Before treatment 1 day of treatment 3 days of treatment 7 days of treatment 21 days of treatment	IL-1 $\beta$	IL-1 $\beta$ levels significantly increased at day 1 (peak) compared to before treatment and remained elevated through days 3, 7, and 21.	Clear aligners may induce a sustained short-term inflammatory response during the initial phase of treatment.
Chen et al., 2021 [23]	Before treatment 1 hour of treatment 7 days of treatment 14 days of treatment	IL-1 $\beta$ , TNF- $\alpha$ , RANKL, OPG	No statistically significant changes in IL-1 $\beta$ , TNF- $\alpha$ , RANKL and OPG expression levels were observed in the aligner group across all time points.	Clear aligner therapy did not induce statistically significant changes in either pro-inflammatory cytokines or bone metabolism biomarkers during the initial phase of treatment.
Baeshen, 2022 [25]	Before treatment 21 days of treatment	IL-1 $\beta$ , IL-1 $\alpha$ , IL-2, IL-6, IL-8, TNF- $\alpha$	Statistically significant increases were observed in all six inflammatory cytokines in the aligner group, with IL-1 $\beta$ and TNF- $\alpha$ showing the most prominent changes, and IL-1 $\alpha$ , IL-6 and IL-8 the least.	Clear aligner therapy could be associated with a short-term increase in inflammatory cytokines during the initial treatment phase.
Kamran et al., 2023 [22]	Before treatment 28 days of treatment	IL-1 $\beta$ , IL-1 $\alpha$ , IL-2, IL-6, IL-8, TNF- $\alpha$ , RANKL, OPG, OPN	Statistically significant increases were observed in all six inflammatory cytokines and in two bone metabolism biomarkers in the aligner group, with IL-2 and IL-1 $\beta$ showing the most prominent increases among cytokines. OPG showed the most prominent statistically significant decrease among bone biomarkers, while IL-8 and OPN exhibited the smallest changes.	Clear aligner therapy may induce a short-term inflammatory response and simultaneous alterations in bone metabolism biomarker levels during the initial phase of orthodontic treatment.

Alnazeh et al., 2023 [4]	Before treatment 28 days of treatment	IL-6, IL-17, GM-CSF	IL-6, IL-17 and GM-CSF levels statistically significantly increased after 28 days of clear aligner treatment compared to baseline, with the most prominent change observed in IL-17.	Clear aligner therapy appears to trigger a short-term inflammatory response during the initial phase of treatment.
Altindal et al., 2024 [24]	Before treatment 1 hour of treatment 3 days of treatment 7 days of treatment 14 days of treatment 21 days of treatment	IL-6, IL-8	Statistically significant differences were observed in IL-8 levels between pre-treatment, day 3, and day 7 compared with day 21. IL-6 levels significantly increased at days 14 and 21 compared to pre-treatment.	Clear aligner therapy may induce early inflammatory response, with biomarker levels showing progressive increases over the initial treatment period.

GM-CSF, granulocyte-macrophage colony-stimulating factor; IL 1 $\alpha$ , interleukin-1 alpha; IL 1 $\beta$ , interleukin-1 beta; IL-2, interleukin-2; IL-6, interleukin-6; IL-8, interleukin-8; IL-17, interleukin-17; OPG, osteoprotegerin; OPN, osteopontin; RANKL, receptor activator of nuclear factor kappa-B ligand; TNF  $\alpha$ , tumor necrosis factor alpha.

**Table 3.** RISK of bias assessment using ROBINS-I tool.

		Risk of bias domains							Overall 1
		D1	D2	D3	D4	D5	D6	D7	
Study	Castroflorio et al., 2016 [20]	+	+	+	-	+	+	+	-
	Chami et al., 2018 [21]	+	+	+	-	+	+	-	-
	Gujar et al., 2019 [23]	-	+	-	-	+	+	+	-
	Aziz et al., 2020 [22]	X	-	+	-	+	+	-	X
	Chen et al., 2021 [25]	+	+	+	-	+	-	-	-
	Baeshen, 2022 [27]	X	+	+	-	+	+	+	X
	Kamran et al., 2023 [24]	+	+	-	-	+	+	+	-
	Alnazeh et al., 2023 [4]	-	-	+	-	+	+	+	-
	Altindal et al., 2024 [24]	+	+	-	-	+	+	+	-

**Domains:**

- D1: Bias due to confounding.
- D2: Bias due to selection of participants.
- D3: Bias in classification of interventions.
- D4: Bias due to deviations from intended interventions.
- D5: Bias due to missing data.
- D6: Bias in measurement of outcomes.
- D7: Bias in selection of reported result.

**Judgement**

- X Serious
- Moderate
- + Low

#### 4. Discussion

This systematic review aimed to synthesize the available evidence on cytokine and bone metabolism biomarker levels in gingival crevicular fluid (GCF) following the application of orthodontic forces by clear aligners. Analysis of GCF provides an advantageous approach for investigating such responses in human *in vivo* studies, as it is noninvasive and allows repetitive sampling from the same site without limitations on the number of collections. This enables longitudinal monitoring of molecular changes at a single site over time [26]. Fluctuations in inflammatory and bone metabolism biomarker levels in GCF may reflect not only the response to orthodontic forces but also the presence of subclinical periodontal inflammation [27]. Since the composition and quantity of GCF are altered during periodontal inflammation, ensuring optimal periodontal conditions is essential to avoid bias in biomarker quantification. In this review, all the included studies examined participants with clinically healthy periodontal tissues, thereby minimizing this potential confounding factor.

Cytokine fluctuations collectively reflect the inflammatory and bone remodeling response to orthodontic forces, but analysis of individual mediators allows a more precise interpretation of mechanisms underlying clear aligner treatment. Among the cytokines examined across the included studies, IL-1 $\beta$  emerged as the most frequently investigated [18-23,25], consistent with its recognized role as a central mediator of orthodontic tooth movement [5]. As a pro-inflammatory cytokine, IL-1 $\beta$  specifically promotes osteoclast differentiation, enhances their fusion and survival, and stimulates their resorptive activity [5]. Clinically, this activity initiates bone resorption and contributes to the rapid phase of early tooth movement following the application of orthodontic force [28]. In the present review, the majority of

included studies reported a statistically significant increase in IL-1 $\beta$  levels during clear aligner therapy [18,20-22,25], whereas two studies did not observe such changes [19,23]. Among the included studies, Aziz et al. [20] uniquely reported a clear peak at day one, with the concept of an early-phase IL-1 $\beta$  response. An early peak in IL-1 $\beta$  levels at around 24 hours has also been reported with other orthodontic force systems, including elastic separators [29], microimplants [30], and cantilever springs [31], supporting its role as a general early mediator of mechanical stress. When compared across orthodontic systems, clear aligners generally induced a lower magnitude of IL-1 $\beta$  expression. Gujar et al. [21] and Kamran et al. [22] reported greater IL-1 $\beta$  release with fixed labial appliances, while Chen et al. [23] found a more pronounced increase with pendulum appliances. Baeshen et al. [25] similarly observed higher levels with lingual appliances, and in this case the differences reached statistical significance. Taken together, these findings suggest that although clear aligners trigger IL-1 $\beta$  release, the response appears weaker than that elicited by conventional or alternative fixed systems. Other pro-inflammatory cytokines investigated in the included studies, such as IL-6, IL-8, IL-2, TNF- $\alpha$ , and IL-1 $\alpha$ , also participate in the orchestration of periodontal inflammation and bone remodeling during the early phase of OTM [32,33]. Among these, IL-6 was the most frequently assessed cytokine, showing significant increases in all studies [4,21,22,24,25], typically within the first 2–4 weeks of clear aligner therapy. This pattern contrasts with evidence from fixed appliance studies, where IL-6 has frequently been shown to peak much earlier, within 1-3 days after force application [26,34,35]. IL-8, a chemokine involved in neutrophil recruitment [28], showed a response pattern similar to IL-6, with most studies reporting significant increases at later stages of aligner therapy

[21,22,25]. While Altindal et al. [24] observed earlier IL-8 elevations at days 3 and 7, studies employing other orthodontic force systems [36,37] reported significant increases within 24–48 hours. IL-8 is secreted by various cells including monocytes, endothelial cells, and fibroblasts as a response to TNF- $\alpha$  and IL-1 $\alpha$  [22]. In addition, TNF- $\alpha$ , IL-1 $\alpha$ , and IL-2 were evaluated across the same set of studies, with statistically significant increases consistently reported by Gujar et al. [21], Kamran et al. [22], and Baeshen et al. [25]. In studies employing other orthodontic force systems, TNF- $\alpha$  and IL-2 typically exhibited an early peak within 24 hours after force application [35,36,38], while IL-1 $\alpha$  showed a transient rise that did not reach statistical significance [39]. Across comparative studies, IL-1 $\alpha$  and TNF- $\alpha$  tended to show greater increases with fixed appliances (labial or lingual) than with aligners [21,25], whereas IL-6, IL-2 and IL-8 responses were in some reports more pronounced with aligners [4,22]. These divergent patterns likely reflect differences in the timing of cytokine release, with fixed appliances inducing stronger immediate pro-inflammatory signals, whereas aligner studies predominantly report elevations at later time points, possibly because early peaks were not captured due to sampling schedules. Additionally, Bařaran et al. [40] reported that the expression of IL-6, IL-2, and IL-8 was influenced by both the duration and magnitude of orthodontic force, highlighting the role of force parameters in modulating cytokine responses.

IL-17 is a relatively recently identified cytokine with a pivotal role in bone remodeling and in modulating cell-mediated immune responses, particularly by directing the shift toward antigen-specific effector responses [5]. Across the included studies, findings were inconsistent: one trial demonstrated a significant late increase after 28 days of aligner therapy [4], whereas another did not detect relevant

changes [19]. Similar early, transient increases in IL-17 have also been observed with other orthodontic force systems, such as the Hyrax appliance, suggesting that this cytokine may participate in the early phase of mechanical stress-induced remodeling [41]. GM-CSF, another pro-inflammatory mediator implicated in bone remodeling [42], was assessed in the same two studies and showed a response pattern consistent with IL-17 [4,19]. Overall, the evidence regarding these cytokines remains scarce, and further research is required to clarify their roles during orthodontic tooth movement.

Beyond pro-inflammatory cytokines, biomarkers of bone metabolism offer additional insights into the molecular mechanism of orthodontic tooth movement. The remodeling process is regulated by the equilibrium between RANKL-mediated signaling and osteoprotegerin (OPG) production [43]. OPG is secreted by osteoblasts as well as RANKL and functions as a decoy receptor, competing with RANK for RANKL binding [44]. Application of compressive forces to human periodontal ligament (PDL) cells has been demonstrated to increase RANKL and reduce OPG secretion, with these responses varying according to both force magnitude and duration [44]. These biomarkers were assessed in several studies with partly divergent results [18,22,23]. OPG levels showed a decreasing trend: Castroflorio et al. [18] observed significant reductions after 7 and 21 days, while Kamran et al. [22] – at 28 days. Regarding RANKL, Castroflorio et al. [18] detected a significant rise as early as one hour after force application and again at day 7, whereas Kamran et al. [22] found a significant elevation after 28 days. In the study by Chen et al. [23] a decrease in OPG together with an increase in RANKL was noted after 7 days, although these changes did not reach statistical significance. Alongside these mediators,

osteopontin (OPN) has been studied as another indicator of bone remodeling. It is a non-collagenous bone protein that participates in early osteoblast differentiation, contributes to biomineralization, regulates RANKL expression, and can inhibit osteoclast activity [18]. In our review, OPN levels showed a significant increase 21 days after force application in Castroflorio et al. [18] and at 28 days in Kamran et al. [22].

Several limitations should be considered when interpreting the present findings, as the overall methodological quality of the included studies ranged from moderate to serious. All authors reported certain constraints, most related to relatively small sample sizes and short observation periods, which limited the evaluation of longer-term biomarker dynamics. In addition, there was considerable heterogeneity in the timing and frequency of GCF sampling – in some studies, samples were collected only before treatment and at a single late time point (e.g., 21 or 28 days) – potentially overlooking early transient cytokine fluctuations. Individual biological responses to mechanical loading are known to vary with factors such as age, sex, and bone density; however, some studies lacked detailed demographic information, such as the age range and mean age of participants in the aligner group, and some did not specify the distribution of males and females, which may limit the interpretation of biological variability in biomarker responses. Although all studies included participants with clinically healthy periodontal tissues, the possibility of subclinical, bacteria-induced inflammatory changes in GCF composition cannot be entirely excluded, given that the oral environment is never completely free of microbial activity [41]. Moreover, variations in study design, applied orthodontic force levels, and aligner wear protocols may have further contributed to the heterogeneity observed in biomarker responses. To

strengthen the current evidence base, future research should focus on larger, methodologically consistent trials with standardized protocols and longer follow-up intervals.

## 5. Conclusion

Based on the current evidence, orthodontic treatment with clear aligners is associated with measurable alterations in inflammatory cytokines and bone metabolism biomarkers during the early phase of treatment. These findings support the concept that aligner therapy elicits a biologically active response within periodontal tissues. Although the clinical implications of these molecular changes remain incompletely understood, their presence underscores the biological relevance of aligner-mediated tooth movement and highlights the need for well-designed longitudinal studies to clarify their impact on treatment outcomes and patient well-being.

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