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Effect of orthodontic treatment on systemic inflammatory biomarkers in patients with malocclusion: A Systematic Review of Randomized Controlled Trials

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ABSTRACT

Background

Orthodontic tooth movement instigates an inflammatory response in periodontal tissue, but beyond the local compartment, whether this response is detectable remains unclear . To assess the changes in inflammatory and immune biomarkers following orthodontically induced tooth movement in patients with malocclusion, and to investigate if these changes differ by sampling point, biological specimen, and orthodontic force magnitude.

METHODOLOGY

PubMed (including MEDLINE), Scopus, Web of Science, and the Cochrane Central Register of Controlled Trials (CENTRAL) from inception to August 2026. Screening, data extraction, and Risk of bias (RoB 2) were performed by three reviewers, and any disagreements were resolved by a fourth reviewer. A systematic review was undertaken; heterogeneity across interventions, comparators, force magnitudes, sampling sites, assays, and follow-up periods precluded meta-analysis.

Results

383 records were identified and 380 were screened, and three trials were included, comprising 70 participants aged 8-25 years, published from 2018 to 2022. One of the included trials used a parallel-group design, and two used a split-mouth design. All three trials sampled gingival crevicular fluid only; none of them measured C-reactive protein. IL-1β showed an early rise after acute stimuli, peaking at day 1 after separator placement (54.06 ± 42.41 versus 22.93 ± 29.97 pg/ml in control teeth, $P = 0.011$) and within one week returning to baseline, with a comparable transient rise after extraction with a leukocyte-platelet-rich fibrin plug. TNF-α showed different time courses, increasing at 28 days under continuous distalizing force but at 1 and 2 weeks after extraction ($P < 0.001$ and $P = 0.006$). Only in one trial was IL-6 measured; however, no effect estimate could be extracted. A significant force × side × time interaction ($P = 0.0016$) was observed for IL-8, with lower concentrations at pressure sites under 150 g than at 75 g at 24 hours, resolving by 28 days. Increasing force from 75 g to 150 g produced no greater biomarker response and no faster tooth movement (1.13 ± 0.91 versus 0.94 ± 0.87 mm, $P = 0.41$). Out of the three trials, only one was at high risk, and two of them raised some concerns. Out of the three articles, none of them was at low risk.

Conclusions

Orthodontic force is associated with an early, self-limiting rise in local pro-inflammatory mediators in gingival crevicular fluid, generally peaking within 1 day to 1 week after an acute stimulus and returning toward baseline within 1 to 4 weeks. Neither a greater local response nor faster tooth movement is produced by greater force. Of the three

trials, none measured circulating biomarkers, and it remained unanswered whether orthodontic treatment alters systemic inflammation.
Registration
PROSPERO with registration number CRD420261494225.
Keywords
orthodontics; tooth movement techniques; malocclusion; gingival crevicular fluid; inflammation mediators; interleukin-1beta; tumor necrosis factor-alpha; systematic review.

INTRODUCTION

Orthodontic treatment is a dental specialty focusing on controlling the mechanical forces that cause changes in the periodontal ligament and coordinated removal, correction of malpositioned teeth, and abnormalities of dental and skeletal relationships.[1] Orthodontic treatment uses appliances, such as braces, to manage mild cases and to reduce pressure on the teeth. Sustained pressure can stimulate changes in the tissues surrounding the teeth, specifically the periodontal ligament and the alveolar bone, allowing the teeth to gradually change position.[2]

Malocclusion is a dental condition in which there is an abnormal relationship between the upper teeth and the lower teeth. The teeth may also have an unusual or aberrant relation with the jaw and surrounding bone.[1] Malocclusion not only affects the teeth but also affects the symmetry of the face and smile, oral hygiene, and the increased risk of gingival inflammation.[3]

Orthodontic tooth movement is not simply a mechanical process but a biological process that requires remodeling of the periodontal ligament and surrounding tissue to change to a new position, allowing the teeth to move the alveolar bone gradually.[4]

Orthodontic force is applied via braces or aligners, which apply sustained pressure to the tooth, triggering an inflammatory response in the surrounding tissue due to mechanical stress and tissue change. This mechanical stress is the reason that mediators such as IL-1β, IL-6, TNF-α, and prostaglandins are released, causing changes in the periodontal ligament and activating cells involved in tissue remodeling, allowing the tooth to move.[5]

IL-1β, IL-6, and TNF-α are released when the orthodontic force is applied, which helps in the regulation of cells responsible for the formation and breakdown of bone.[6]

Orthodontic tooth movement around the teeth causes inflammation, yet the researchers are not quite sure whether this inflammation is strong enough to be detectable throughout the body.[7] Maclaine et al. found no significant increases in CRP, IL-6, or TNF-α during fixed appliance treatment, despite clear evidence of inflammation in the tissue immediately around the teeth.

A prospective cohort study suggested that inflammation caused by orthodontic treatment may remain local rather than causing a substantial systemic inflammatory response.[8] Some studies have reported that a temporary increase in high-sensitivity C-reactive protein can occur after orthodontic treatment, implying that orthodontic therapy can trigger a brief, measurable inflammatory response in the body.[9]

Previous studies showed inconsistent results. However, the purpose of our systematic review is to evaluate changes in inflammatory and immune biomarkers in response to orthodontically induced tooth movement in patients with malocclusion, and to explain

these changes by sampling time points, biological specimens, and orthodontic force characteristics.

OBJECTIVE

This systematic review assessed whether orthodontically induced tooth movement alters inflammatory and immune biomarkers in patients with malocclusion. It further examined the magnitude and duration of any such changes, and whether they differed according to the sampling time point, the biological specimen analysed, and the magnitude of the orthodontic force applied.

MATERIALS AND METHODS

Protocol and registration

This review was conducted and reported according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA 2020) guidelines.[10] The review has been registered with PROSPERO with registration number CRD420261494225.

Inclusion criteria :

Eligibility was defined using the PICO framework.

Population. Children, adolescents, and adults with malocclusion or a dentofacial abnormality who were undergoing orthodontic treatment or orthodontically induced tooth movement.

Intervention. Any orthodontic intervention involving appliance placement, force application, or appliance activation intended to correct malocclusion or to move teeth orthodontically. Fixed appliances, removable appliances, functional appliances, and clear aligners were all eligible.

Comparator. Participants receiving no orthodontic treatment, contralateral or within-patient control teeth or sites, or pre-treatment baseline measurements recorded before force application.

Outcomes. The primary outcomes were changes in the concentrations of IL-1β, IL-6, IL-8/CXCL8, and TNF-α measured after orthodontic force application relative to an eligible comparator. The secondary outcomes were changes in C-reactive protein and in any other reported inflammatory or immune biomarker, together with differences in biomarker concentrations according to sampling time point, biological specimen (gingival crevicular fluid, saliva, serum, or plasma), and the magnitude or duration of orthodontic force, where sufficient quantitative data were reported. Reporting of at least one eligible biomarker was sufficient for inclusion.

Study design. Only randomized controlled trials were eligible. All other study designs were excluded.

Information sources and search strategy

A comprehensive literature search was conducted in PubMed/MEDLINE, Scopus, Web of Science, Embase, Cochrane (CENTRAL), and Google Scholar from inception to August 2026.The PubMed string combined ("Orthodontics"[Mesh] OR orthodontic*[tiab]) AND ("Malocclusion"[Mesh] OR malocclusion*[tiab]) AND ("C-Reactive Protein"[Mesh] OR "c-reactive protein"[tiab] OR CRP[tiab] OR "Interleukin-1beta"[Mesh] OR "interleukin-1beta"[tiab] OR "IL-1beta"[tiab] OR "Interleukin-6"[Mesh] OR "interleukin-6"[tiab] OR "IL-6"[tiab] OR "Interleukin-8"[Mesh] OR "interleukin-8"[tiab] OR "IL-8"[tiab] OR "Tumor Necrosis Factor-alpha"[Mesh] OR "tumor necrosis factor-alpha"[tiab] OR "TNF-alpha"[tiab]). The relevant systematic reviews and their reference lists were screened together to identify potentially eligible studies and to avoid re-examining them in the electronic database search.

Study selection

Screening was done in two phases. In the first stage of screening, the title and abstract were assessed against the PICO criteria. Studies were not excluded at this early stage solely because of a lack of methodological details in the abstract; instead, questionable studies were retained for full-text assessment to avoid potential bias that could result from excluding randomized controlled trials (RCTs).

In the second screening, full-text articles were assessed for final eligibility. Studies that did not meet the PICO (s) criteria were excluded and were assigned the primary reason of exclusion. Separate categorization of those studies was done for major internal inconsistencies between reported results and tabulated data, test statistics that were irreconcilable with the reported means and standard deviations, or reference lists that did not correspond to in-text citations. Screening was performed by three independent reviewers, and disagreements were resolved through discussion or by a fourth reviewer. Three reviewers screened the articles via Rayyan. The potentially eligible studies were assessed separately by the three reviewers through full-text screening. Disagreements at any stage were resolved via discussion and consensus, and with consultation of a fourth reviewer.

Data extraction

Data extraction was performed independently by three reviewers using a piloted extraction form. Extracted data consisted of authorship and year of publication, type of study, sex, periodontal status at baseline, orthodontic treatment movement, force and magnitude, duration, biomarker measured, sampling time point, and associated conditions together with the reported means, standard deviations, and p-values.

Risk of bias assessment

Risk of bias was assessed via the revised Cochrane risk-of-bias tool for randomized trials (RoB 2) by two reviewers, covering five domains: randomization process, deviations from intended interventions, missing outcome data, measurement of the outcome, and selection of the reported result. Each domain was judged as low risk, with some concerns, or high risk.

Data synthesis

A narrative synthesis was undertaken due to substantive clinical, methodological, and statistical heterogeneity. Participants' characteristics, orthodontic appliance type, and assay performance and reporting units will be synthesized narratively, and a meta-analysis will not be conducted because the available studies did not provide sufficiently comparable outcomes or data for meaningful statistical pooling.

RESULTS

Study selection

The search identified 383 records (PubMed 300, Cochrane CENTRAL 58, and Google Scholar 25). After 3 duplicate records were removed, 380 records were screened by title and abstract, of which 375 were excluded. Five reports were sought for retrieval, all of which were obtained and assessed in full text. Two reports were excluded because they did not meet the PICO criteria, leaving three trials that were included in the review (Figure 1).

Characteristics of included articles

Table 1 states the characteristics of the included articles. The included articles were published between 2018 and 2022, conducted in Switzerland, India, and Turkey . Among the included articles, one used a parallel-group design, and two used a split

(within-patient) design. The three trials that met the eligibility criteria sampled gingival crevicular fluid (GCF). No trials in the selected study reported serum, plasma, or salivary biomarkers. The biomarkers reported were IL-1β, TNF-α, IL-6, IL-8, and others , which were quantified by multiplex immunoassay in two trials and ELISA in one trial.

Risk of bias in included studies

Risk was assessed independently by two reviewers using ROB 2, and in case of disagreement, it was resolved through discussion with a third reviewer. Domain-level and overall judgments are presented in Figures 2 and 3. In the three included trials, one of them was judged at high risk of bias, and two raised some concerns; no trial was at low risk of bias overall. Antonarakis et al. (2020) was rated at high risk because of self-reported pain by the unblinded participants against an untreated control group; the outcome could be influenced plausibly by the knowledge of allocation. For biomarker outcomes, which were qualified to bind to allocation, this domain would be at low risk. The two split-mouth trials raised some concern in randomization because it was carried out by treating clinicians (Afacan et al., 2018), or masking was not described (Barhate et al., 2022). In the latter trial, some concerns were also raised about deviations from the Deliberate omission intervention, as neither the orthodontist nor the patient was blinded. Some concerns were raised regarding selection of reported results, reflecting absence of a prospectively registered protocol or pre-specified analysis plan in all three trials . No concern in any trial was regarding the missing outcome data with attrition of 2.5% or less.

Results of individual studies and syntheses

Significant clinical and methodological heterogeneity was observed in included trials, specifically in intervention type, comparator, force magnitude, sampling site, assay method, and follow-up timeline. However, quantitative pooling was considered inappropriate, and results were synthesized narratively according to biomarker and sampling time. Individual study results are presented in Table 2.

Interleukin-1β

Among all three trials, only two reported usable data of prespecified IL-1β in gingival crevicular fluid. A peak value of 54.06 ± 42.41 pg/ml in the treated maxillary molars one day after elastic separator placement was observed in Antonarakis et al. (2020), compared with 22.93 ± 29.97 pg/ml in the within-patient mandibular control teeth, representing a significant rise from the baseline values 23.47 ± 33.21 pg/ml (P = 0.015). Within one week, the values returned to the baseline (22.87 ± 34.71 pg/ml, P = 0.015 for the fall from day 1). No significant difference was noted between the untreated control group and the headgear at any shared time point (T9 months, 17.11 ± 33.51 vs 17.97 ± 32.31 pg/ml).

Barhate et al. (2022) reported that L-PRF site caused a temporary increase in the biomarker from 155.91 ± 9.29 pg/µl at the baseline to a peak of 165.73 ± 9.33 pg/µl at one week, which remained elevated for two weeks (162.26 ± 8.55 pg/µl), and at eight weeks before returning to its baseline (155.95 ± 8.18 pg/µl);. These within-group changes were significant against the baseline. There was no statistically significant difference between the LPRF and the control socket at any time point.

Afacan et al. (2018) measured IL-1β, but it fell below the assay detection limit of 1.34 pg/ mL and was not sufficiently reliable for statistical analysis. It was detected in fewer than 35% of the samples. So, the author attributed it to the high concurrent abundance of IL-1Ra.

Interleukin-6

Of the three trials, only one included interleukin-6. A 30-analyte multiplex panel was applied to gingival crevicular fluid collected from tension and pressure sites of mandibular canines, subjected to 150 g or 75 g tension and pressure at baseline for 24 hours and 28 days. Interleukin-6 was detected in sufficient samples for statistical analysis, but according to the Brunner-Langer model, it showed no significant effect on force, side, or sampling time, nor on the interaction of force × side × time. Neither intra-group nor inter-group comparisons were significant, and underlying interleukin-6 concentration was not provided. So no mean, standard deviation, or effect estimates could be extracted from the outcomes. Antonarakis et al. (2020) and Barhate et al. (2022) did not measure interleukin-6.

Interleukin-8 / CXCL8

Only one trial by Afacan et al. (2018) assessed patients for IL-8 in gingival crevicular fluid and identified about 180 samples. Neither the overall effect of force(P = 0.5714) nor sampling time (P = 0.9782) was significant, but the force × side × time interaction observed (P = 0.0016) was significant. At 24 hours, pressure site IL-8 showed an increase of 15% with 75g and decreased by 38% with 150 g compared with baseline; neither change from baseline was significant. Nevertheless, the concentration of IL-8 was lower with 150g as compared to 75g at 24 hours ($P < 0.05$). This difference was no longer observed at day 28. Because the concentrations were reported only graphically, no effect estimate could be calculated. No IL-8 was assessed by Antonarakis et al. (2020) and Barhate et al. (2022).

Tumor necrosis factor alpha

Two trials assessed TNF-α and demonstrated divergent time courses. Afacan et al. (2018) reported a significant effect of sampling time (P = 0.0066), which survives Bonferroni correction, with a significant increase from baseline to 28 days (P = 0.017) and no significant change at 24 hours (P = 0.087); this increase was observed at both sides and force group with no significant difference between 75g and 150 g (P = 0.5553). An early transient rise was found by Barhate et al. (2022) . TNF-α was comparable between sides at baseline (L-PRF 6.40 ± 0.73 vs control 6.27 ± 0.75 pg/ml, P = 0.634), then significantly higher on the L-PRF side at one week (8.78 ± 1.21 vs 6.95 ± 0.67 pg/ml, P < 0.001) and two weeks (7.96 ± 1.06 vs 6.83 ± 1.01 pg/ml, P = 0.006), returning to comparable values by four weeks (P = 0.505) and eight weeks (P = 0.862). TNF-α was not measured by Antonarakis et al. (2020). The two trials reported that they responded to the intervention, but the disagreement was due to timing, which is consistent with their different stimuli: continuous distalizing orthodontic force without reactivation in one, and a surgical extraction with an L-PRF plug in the other.

Secondary outcomes

The main finding was negative for this subsection. No included trials measured C-reactive protein (CRP), and no serum, plasma, or saliva samples were assessed. All biomarker values were obtained from gingival crevicular fluid (GCF). The available randomized evidence did not address the systemic component of the review questions. Three local mediators yielded extractable data and are summarized in Table 3. In Antonarakis et al. (2020), substance P peaked one day after separator placement and settled to baseline within a week.IL-1RA behaved differently, climbing progressively up to 28 days, irrespective of force magnitude, as reported by Afacan et al. (2018). It's the only biomarker in the trials that showed a difference in pressure from the tension

site.MCP-1 showed a pattern similar to IL-8: both were suppressed at pressure sites under higher force at 24 hours, and both coincided by 28 days.
A larger group of remaining analytes did not yield usable data. IL-12, GCSF, IFN-α, HGF, and VEGF show a fourfold increase at the pressure site by 28 days, but none of them survived correction for multiple comparisons. IL-2R, IL-6, IL-13, MIP-1α, MIP-1β, MIG, and IP-10 did not provide a significant force, side, or time and were reported as "data not shown".A final 13 analytes, including IL-1β, were detected in fewer than 35% of samples and were excluded from the statistical analysis. Of the 30 analytes measured, fewer than one-third yielded a reportable result.
For two of the three pre-specified moderators, subgroup analysis was possible. Sampling time had the clearest influence on biomarker responses. Acute stimulus produced an early, transient change, with peak response on day 1 following separator placement and at approximately one week following extraction with L-PRF, while a significant change at 28 days was produced by unreactive forces. Pressure and tension site differences were reported only for IL-1RA and IL-8 (P = 0.0440 and P = 0.0430, respectively). The authors attributed this variation to the type of tooth movement and possible diffusion of GCF between adjacent sites, and to limited site-specific differences. At 24 hours, the force magnitude and pressure site: only two chemokines were moved, with no accompanying differences in cumulative canine movements (1.13 ± 0.91 mm at 150 g vs 0.94 ± 0.87 mm at 75 g, P = 0.41). Specimen type could not be assessed as a moderator, as all the trials used the same fluid .

DISSCUSSION

The review was conducted to determine whether orthodontic treatment can reduce systemic inflammation associated with malocclusion. Three randomized trials were included in the review, meeting the inclusion criteria, and comprised 70 participants aged 8 to 25 years.[11,12] The most important findings were negative. Circulating systemic biomarkers were not measured by any of the included trials. None of the included trials sampled serum, plasma, or saliva, and none of them measured C-reactive protein. All the biomarkers were derived from the gingiva, a local exudate of the gingival sulcus within the local compartment. The orthodontic forces were followed by a transient increase in inflammatory mediators in response to the applied stimulus. The magnitude, timing, and statistical significance of the rise varied.

Local versus systemic inflammation

The central issue for interpretation is the gap between the review question and the available evidence. Gingival crevicular fluid reflects the inflammatory activity in the periodontal tissue and alveolar bone surrounding the sampled tooth, collected from the gingival sulcus.[13] Consequently, a rise in cytokines such as interleukin-1 beta in GCF following orthodontic force indicates a local tissue response and should not be interpreted as evidence of altered systemic inflammation. Local periodontal remodeling, mechanical stimulation, and site-specific periodontal status can be influenced by the GCF cytokine concentrations.[14] Among the included trials, only two of them made this definite by using within-patient control teeth or contralateral control sites[11,15]. However, these included trials provide evidence of local inflammatory responses but do not directly address whether orthodontic treatment influences systemic inflammation. Two outcomes resulted from the Discrepancy[11,15]. Firstly, no direct answers were provided on whether the orthodontic treatment altered systemic inflammation. This downgrade does not emphasize that the study was conducted

improperly, but the outcomes they measured did not correspond directly to our review question. The lack of randomized trials that measure circulatory inflammatory markers also epitomizes an important research gap. The aforementioned systematic review of cytokines and receptor responses to orthodontic forces mainly focused on the locally measured cervical mediators.[16] Therefore, whether fixed orthodontic appliance therapy causes a measurable systemic inflammatory response remains uncertain and insufficiently investigated.

Timing of the inflammatory response

Sufficient findings available suggested that the inflammatory responses had temporary variation to the orthodontic forces. Among the three trials, those involving acute orthodontic stimuli reported an early but transient response to local mediators. The concentration of IL-1β and Substance P peaked one day after elastic separator placement and returned toward baseline within one week[11], while TNF-α increased on the L-PRF side at one week but was no longer different from the control side at four weeks[12]. However, delayed changes were associated with a continuous, unreactivated distalizing force, with significant alterations becoming apparent only at 28 days, with nothing detectable at 24 hours in TNF-α and L-PRF. This difference in timing may suggest that the inflammatory response depends not only on the biomarkers assessed but also on the duration and nature of the orthodontic stimulus.[17,18]

Taken together, these results report a biphasic local response to orthodontic force, followed by an early inflammatory response, followed by a more prolonged remodeling-related response to orthodontic forces. Therefore, the restricted number of trials and sampling schedules and variation in force protocols make the Subsistence uncertain of a consistent biphasic pattern. However, the apparent time dependence of biomarker responses suggests that studies measuring the same analyte at different time points may not be directly comparable. As a result, meta-analyses should consider sampling time specifically rather than presuming that measurements obtained at different stages describe the same biological consequences.

Force magnitude and the chemokine response

Among the three included trials, only one directly examined different orthodontic force magnitudes, and the results were unexpected.[15] Cervical concentrations of assessed mediators did not increase as the destalizing forces were increased from 150g to 75g. In lieu, IL-8 and MCP-1 were markedly lower at the pressure sites exposed to the higher force after 24 hours, although cumulative canine movement was comparable between groups. Consistent with these results, the systematic review did not report any reliable association between greater orthodontic force and faster tooth movement,[17] and earlier included split-mouth trials also reported comparable canine movement with lighter and heavier continuous forces.[19,20] However, there is a divergence between the experimental evidence showing that IL-8 and MCP-1 expression increased following excessive mechanical loading and the evidence of cytokine upregulation during orthodontic tooth movement.[21,22] One proposed explanation is that cytokine expression may be suppressed by increased force, limiting leukocyte recruitment and ensuring tissue degradation.[15] Nonetheless, due to limited evidence and biological findings, crevicular chemokine concentrations should not be elucidated as reliable proxies for the adequacy of orthodontic force.

An important result reported was that IL-1RA progressively increased over 28 days at pressure and tension sites, and it was the only biomarker that varied predominantly at

these two sites.[15] An association between GCF IL-1RA levels and the rate of tooth movement has been reported in previous research.[23] Since IL-1RA acts as an antagonist of IL-1β signaling,[24] the absence of detectable IL-1β in the trial may be due to the contribution of a sustained increase in IL-1RA.

Heterogeneity, assay sensitivity and reporting

Among the three included trials, there was substantial clinical and methodological heterogeneity. The included trials varied in the orthodontic intervention, comparator biomarker assay, sampling schedule, and follow-up duration. However, among the three included trials, only one directly compared orthodontic forces with no orthodontic forces.[11] These dissimilarities limit the comparability of the outcomes and caution against interpreting the trials as estimating a common treatment effect. This heterogeneity rules out meta-analysis and resulted in narrative synthesis.

assay sensitivity, which was one of the important determinants of biomarker evidence available for synthesis, rather than a technical consideration. Among the three included trials, only one did not measure IL-1β because its concentration fell below the multiplex platform's detection limit.[15] Therefore, the present data do not allow a direct comparison of assay sensitivity between multiplex immunoassays and ELISA. Across all trials, 13 of the 32 measured biomarkers fell below the detection limit, while the outcomes for another seven biomarkers were not reported because they were null.

The quality of reporting compounded the problem. Among the included trials, no trial reported a confidence interval for any biomarker; all reported means, standard deviations, and p-values only. Standard deviation surpassed the means in one of the trials, indicating variability in a sample of 20 per arm. Cochrane RoB 2 tool was used to assess the risk of bias,[25] which was high in one trial and raised some concern in the other two, and there was no trial at low risk; some concerns were raised about selective reporting, with none having a prospectively registered protocol available for comparison in all three trials.

Limitations

Several limitations were present in this review. Restricting the study to randomized trials was appropriate for evaluating the effects of orthodontic intervention, but this restriction may have reduced the available evidence by excluding a large body of non-randomized trials investigating crevicular biomarkers during tooth movement. Although the larger number of studies may have increased the amount of local biomarker evidence, it would not necessarily have addressed the limited evidence regarding the systemic inflammatory responses. Due to limitations in the search strategy and the number of databases searched, some studies may have been missed. Moreover, the grey literature and the trial registries were not searched. That means that unpublished studies, those reporting null results or outcomes, may not have been identified. Publication bias could not be assessed as it included fewer than ten trials.

The further limitation concerned the available evidence and the review question. Since the review focused mainly on the systemic inflammatory biomarker, the included studies determined local inflammatory mediators in gingival cervical fluid. Therefore, the results provided evidence regarding local periodontal inflammation during orthodontic treatment. But it cannot be used to conclude effects on systemic inflammatory status.

CONCLUSION

An association with a transient, self-limiting increase in pro-inflammatory mediators in gingival crevicular fluid (GCF) is observed with orthodontic force. The responses generally increase within 1 day to 1 week following an acute stimulus and return towards baseline within 1 to 4 weeks. Neither greater local biomarker response nor faster tooth movement was produced by greater force. No circulatory inflammatory biomarkers were assessed by the included trials. On systemic inflammation, the effect of orthodontic treatment remains unresolved by the available randomized evidence. Before drawing conclusions about systemic inflammatory effects, sufficiently powered, prospectively registered trials that incorporate serial measurements of circulating inflammatory biomarkers are needed.

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TABLES

Table 1. Baseline characteristic table

Table 2. Biomarker concentrations at the time point of maximum effect in each trial.

Table 3. Secondary biomarkers with extractable data.

Table 1. *BASELINE TABLE*

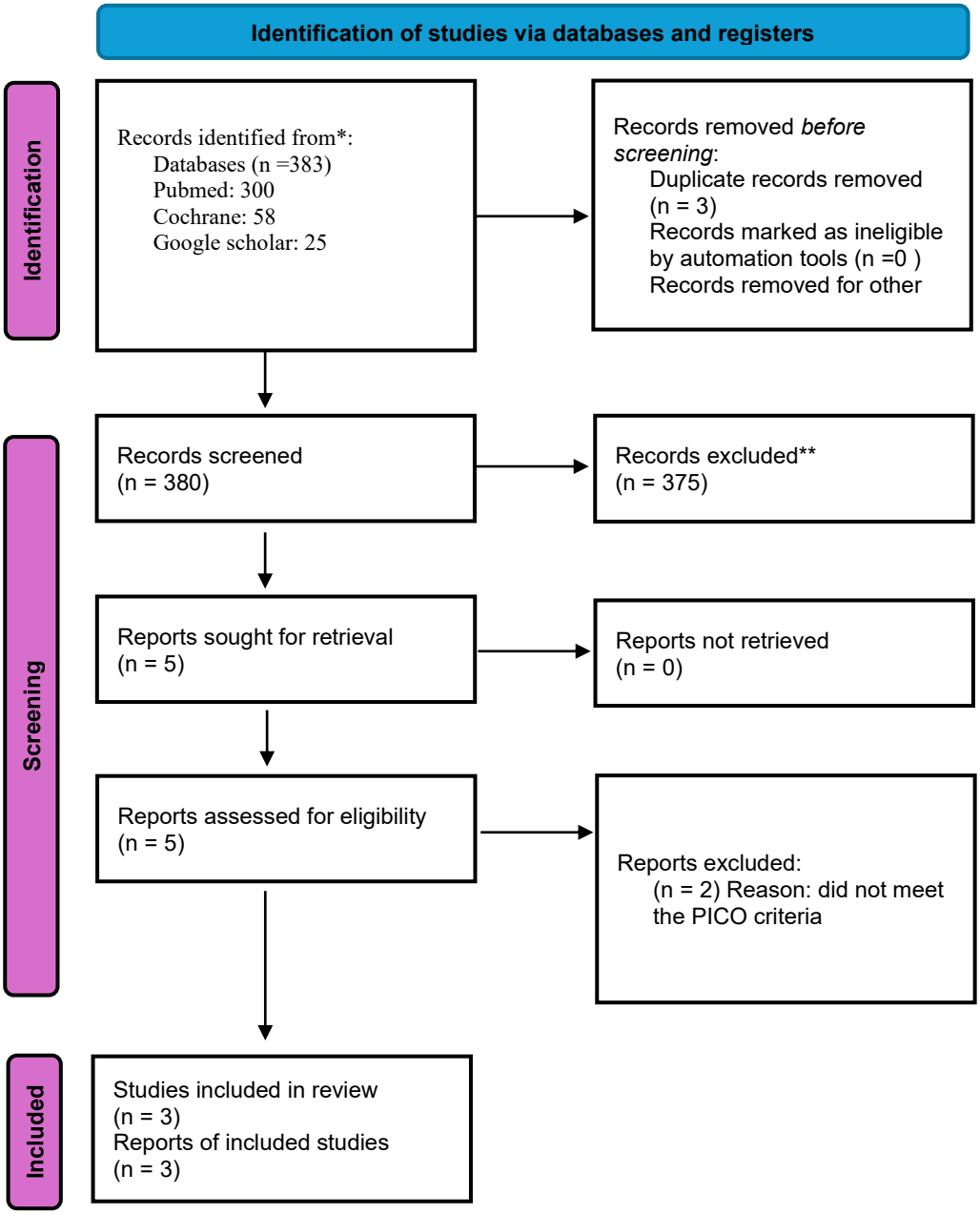
Study, country	Design; sample	Participants	Intervention	Comparator	Specimen; biomarkers; assay	Sampling time points
Antonarakis 2020, Switzerland	Parallel-group RCT; n = 40 (20 headgear / 20 control)	Children 8–12 y; mean 10.2 ± 1.3 vs 9.4 ± 1.2 y; Class II malocclusion, mixed dentition	Elastic separators (1 wk), then molar bands + cervical headgear, 250–300 cN, prescribed 12 h/day for 9 months	No orthodontic treatment during the 9-month study period	GCF, maxillary first molars (mandibular molars as within-patient control); IL-1β, Substance P; Bio-Plex 200 multiplex	T0, 1 day, 1 wk, 2 wk, 3 wk, 6 wk, 9 mo (control: T0, 2 wk, 6 wk, 9 mo)
Afacan 2018, Turkey	Split-mouth RCT; n = 15 patients / 30 canines	Adolescents 11–16 y; mean 14.53 ± 1.64 y; Class II division 1 malocclusion	Maxillary canine distalization with NiTi closed coil spring at 150 g (miniscrew anchorage)	Contralateral canine distalized at 75 g; baseline values of both canines	GCF, mesial (tension) and distal (pressure) sites; 30-analyte panel incl. TNF-α, IL-1RA, IL-6, IL-8, MCP-1 (IL-1β below detection limit); Luminex 200 multiplex	Baseline, 24 h, 28 days
Barhate 2022, India	Split-mouth RCT; n = 15 patients / 30 sides	Females 18–25 y; mean 20.73 ± 2.34 y; Class I bimaxillary dentoalveolar protrusion	L-PRF plug in first premolar extraction socket + canine retraction, NiTi coil spring 150 g	Contralateral extraction socket without L-PRF, same retraction protocol	GCF, distal aspect of canine; IL-1β, TNF-α; ELISA	T0, 1 wk, 2 wk, 4 wk, 8 wk

Figure legends

Figure 1: PRISMA flow chart

Figure 2: Risk of bias summary

Figure 3: Weighted summary of risk of bias judgments across the five RoB 2 domains


Identification of studies via databases and registers
Identification
Records identified from*:
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Pubmed: 300
Cochrane: 58
Google scholar: 25
Records removed before screening:
Duplicate records removed (n = 3)
Records marked as ineligible by automation tools (n =0)
Records removed for other
Screening
Records screened (n = 380)
Records excluded** (n = 375)
Reports sought for retrieval (n = 5)
Reports not retrieved (n = 0)
Reports assessed for eligibility (n = 5)
Reports excluded:
(n = 2) Reason: did not meet the PICO criteria
Included
Studies included in review (n = 3)
Reports of included studies (n = 3)

Figure 1. PRISMA 2020 flow diagram of the study selection process.

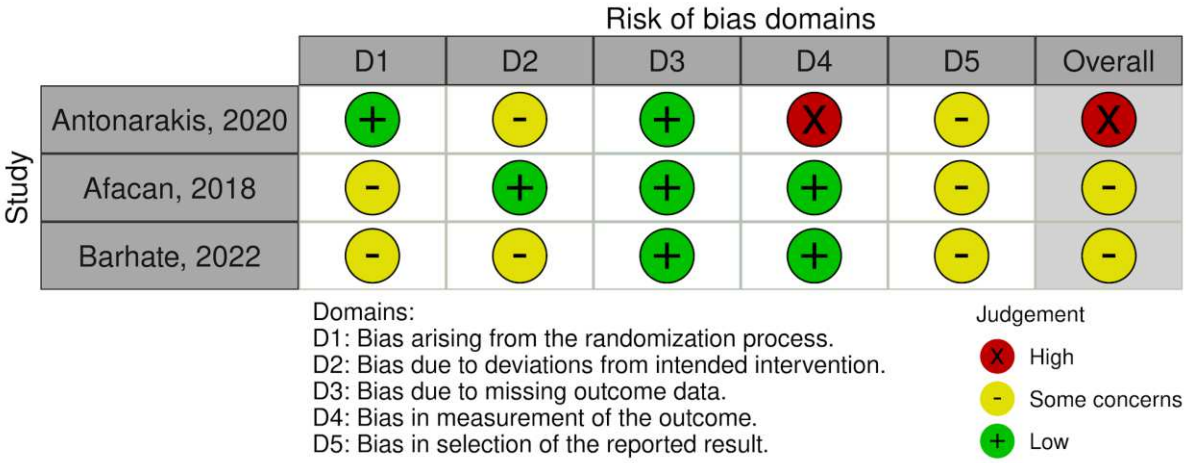

Figure 2. Risk of bias summary *(traffic-light plot) for the three included randomized controlled trials.*

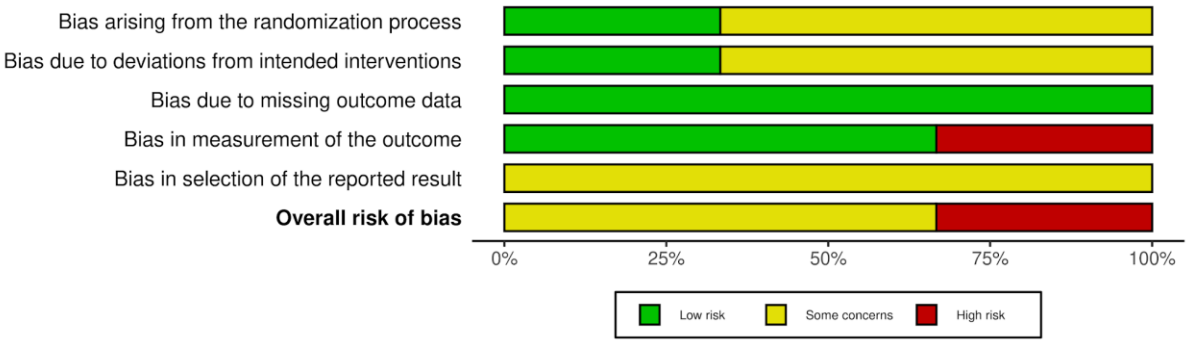

Figure 3. Weighted summary *of risk of bias judgments across the five RoB 2 domains (n = 3 trials).*

Table 2. *Biomarker concentrations at the time point of maximum effect in each trial.*

Study	Biomarker	Specimen / site	Time point	Intervention, mean ± SD	Comparator, mean ± SD	Between-group effect (P)
Antonarakis 2020	IL-1β (pg/ml)	GCF, maxillary (test) vs mandibular (control) molars	T1 day	54.06 ± 42.41	22.93 ± 29.97	P = 0.011

Study	Biomarker	Specimen / site	Time point	Intervention, mean ± SD	Comparator, mean ± SD	Between-group effect (P)
Antonarakis 2020	Substance P (pg/ml)	GCF, maxillary (test) vs mandibular (control) molars	T1 day	53.04 ± 36.15	27.66 ± 29.75	P = 0.020
Antonarakis 2020	IL-1β (pg/ml)	GCF, maxillary molars; headgear vs untreated group	T9 months	17.11 ± 33.51	17.97 ± 32.31	NS (exact P NR)
Afacan 2018	IL-8 (pg/ml)	GCF, pressure site; 150 g vs 75 g	24 hours	−38% vs baseline (means NR)	+15% vs baseline (means NR)	P < 0.05 (exact P NR)
Afacan 2018	MCP-1 (pg/ml)	GCF, pressure site; 150 g vs 75 g	24 hours	−33% vs baseline (means NR)	+74% vs baseline (means NR)	P < 0.05 (exact P NR)
Afacan 2018	TNF-α (pg/ml)	GCF, pressure site; 150 g vs 75 g	28 days	0.66 ± 0.37	0.84 ± 0.68	P = 0.555
Afacan 2018	IL-1RA (pg/ml)	GCF, pressure site; 150 g vs 75 g	28 days	11778.24 ± 7894.56	17616.76 ± 19667.83	P = 0.320
Afacan 2018	IL-1β; IL-6	GCF, tension and pressure sites	All	Not estimable	Not estimable	IL-1β below detection limit; IL-6 “data not shown”
Barhate 2022	IL-1β (pg/µl)	GCF, distal of canine; L-PRF vs control socket	T1 (1 week)	165.73 ± 9.33	159.36 ± 7.87	P = 0.091
Barhate 2022	TNF-α (pg/µl)	GCF, distal of canine; L-PRF vs control socket	T1 (1 week)	8.78 ± 1.21	6.95 ± 0.67	P < 0.001

Table 3. *Secondary biomarkers with extractable data*.

Biomarker	Trial	Site / comparison	Peak intervention value	Comparator value	Effect
Substance P	Antonarakis 2020	GCF, maxillary (test) vs mandibular (control) molars	53.04 ± 36.15 pg/ml at T1 day	27.66 ± 29.75 pg/ml	P = 0.020; returned to baseline by 1 week
IL-1RA	Afacan 2018	GCF, pressure site; 150 g vs 75 g	11 778.24 ± 7894.56 pg/ml at 28 days	17 616.76 ± 19 667.83 pg/ml	No force effect (P = 0.3200); rise over time P = 0.045; higher at pressure than tension sites (P = 0.0440)
MCP-1	Afacan 2018	GCF, pressure site; 150 g vs 75 g	−33% from baseline at 24 h	+74% from baseline	Lower with 150 g, P < 0.05; resolved by 28 days

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SUPPLEMENTARY INDEX

Supplementary Table S1: Search strategy

PubMed	Google Scholar	Cochrane Central
("Orthodontic Tooth Movement"[MeSH] OR "orthodontic tooth movement" OR "orthodontic treatment" OR orthodont* OR "tooth movement") AND ("Inflammatory Biomarkers"[MeSH] OR biomarker* OR cytokine* OR "inflammatory marker*") AND ("Interleukin-1 beta" OR IL-1β OR "Interleukin-6" OR IL-6 OR "Interleukin-8" OR IL-8 OR CXCL8 OR "Tumor Necrosis Factor-alpha" OR TNF-α OR CRP OR "C-reactive protein")	"orthodontic tooth movement" AND inflammatory biomarkers; "orthodontic treatment" AND cytokines AND gingival crevicular fluid; "orthodontic tooth movement" AND IL-1β OR IL-6 OR IL-8 OR TNF-α	(orthodont* OR "orthodontic tooth movement" OR "tooth movement") AND (biomarker* OR cytokine* OR "inflammatory marker*") AND ("IL-1β" OR "IL-6" OR "IL-8" OR CXCL8 OR "TNF-α" OR CRP)

Supplementary Files

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- TABLES.docx