

Predictive Value of Salivary and GCF Biomarkers for Resorption of The Root During Orthodontic Treatment: A Systematic Review

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ABSTRACT

Introduction: Root resorption due to induced inflammation by orthodontics (OIIRR) is a frequent yet often undetected complication. Radiographs detect OIIRR only after substantial mineral loss has occurred, limiting the ability to intervene early. Biomarkers present in gingival crevicular fluid (GCF) and saliva may offer a more sensitive diagnostic approach.

Aim: To evaluate the biomarkers present in the saliva and GCF to identify the occurrence of OIIRR in the patients undergoing orthodontic treatment.

Material and methods: The study was conducted prospectively following PRISMA guideline and it was registered under PROSPERO (CRD420251129166). Various databases were searched which included PubMed, EBSCO, Web of science and Scopus. Clinical trials, case-control designs, and cohort studies were considered for assessing biomarkers associated with the movement of the tooth with orthodontics treatment and confirmed root resorption. Data extracted included biomarker types, sampling media, timing, study methodologies, and outcomes. The bias towards risk was assessed using optimum tools.

Results: As per the inclusion criteria eight studies were found a good fit. The patients who showed presence of Dentin derived protein such as dentin sialprotein, dentin phosphoprotein and Dentin sialophosphoprotein. Inflammatory cytokines, including IL-1 β , IL-7, , IL-6 IL-10, IFN- γ , TNF- α , and IL-12p70 were elevated in resorption cases, reflecting their role in the immunoinflammatory cascade. Proteomic studies identified additional biomarkers such as S100A9, AGT, IGKC, APOH, and HSPA1A, supporting multi-marker diagnostic approaches. However, there was heterogeneous data sampling and limited availability of longitudinal data.

Conclusion: Current evidence supports salivary and GCF biomarkers as a reliable diagnostic tool for early identification of OIIRR, offering greater sensitivity than radiographs. Standardization of sampling, validated biomarker thresholds, and extended clinical validation before clinical integration.

Keywords: Root resorption, Gingival crevicular fluid, Saliva, Cytokines, Proteomics, Biomarkers, Orthodontic tooth movement

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INTRODUCTION

Orthodontics treatment often causes an irreversible adverse effect known as root resorption. During treatment the mechanical force applied causes disturbance in the structure of dentin and microstructures [1]. Due to the irreversible nature of the adverse effect it progresses further and causes the loss of periodontal support [2].

Conventionally, 2D or 3D cone beam tomography has been used to study the structures of the dentin [3]. However the diagnostic methods could identify the macrostructural damage, due to the loss of mineralized tissue but it could not identify any changes

that might occur due to cellular cascades and upregulation of the cytokines which follows resorption [4]. The identification of the

biomarkers that are present before the resorption in the gingival fluid can serve as diagnostic markers for non-invasive yet earlier detection of the resorption [5]. The proteins involved in the cascade can be found in fluid as it passes through the periodontal spaces and periodontal ligaments, it carries the proteins with it [6]. The root resorption occurring because of mechanical pressure leads to catabolic metabolism causing the cellular cascades with

the release of the proteins which changes the microenvironment of the apical region

In the recent literature the proteins of the dentin and cementum were found to be associated with the resorption. When mechanical pressure is applied, it causes the breakdown of the dentin, dentin has a protein complex known as dentin sialophosphoproteins. When the dentin degrades the complex breaks down into dentin sialoproteins and phosphoproteins. An investigation reported that the cementum protein that is CEMP-1 and other dentin proteins were directly associated with the volume of resorption occurred in the dentin [7,8]. It was also found that patients with higher levels of RANKL and decreased level of OPG, had root resorption [9]. The level of phosphoproteins is also influenced by biomechanical parameters, it is reported that when load is applied the DPP increases significantly compared to other phosphoproteins [10].

A study assessed the enzymatic mediators for its eligibility in detecting resorption, however it was found that proteins were reliable biomarkers comparatively [11]. It was recently reported that a certain protein is involved in the interactive microenvironment which plays a crucial role in bone-remodelling, the proteins can be identified using mass spectrometry [12]. IL-10, IL-12p70, and secretory IgA levels in GCF were also found in increased number in patients prone towards root resorption [13]. The levels of these proteins are lower in gingival fluid under normal condition, making it an effective biomarkers for root resorption when detected in increased numbers.

The biomolecular profile can aid in understanding of the radiographic changes, that is the overall macrostructural damage can be caused by root resorption [14]. However, recognition of a effective biomarker requires a substantial cohort with large sample size to validate its efficacy [15]. Hence a systemic literature analysis is required to identify the biomarkers which can be validated. Consequently, this study aims to synthesize current evidence evaluating the predictive validity of salivary and GCF biomarkers for OIIRR. It specifically analyses the biomolecule profile causing root resorption and it maps the relative expression of the profile with occurrence of mild or moderate to severe resorption.

MATERIALS AND METHODS

Adhering to the PRISMA guidelines [16], this systematic review addresses the research question: *What is the association between salivary or fluid in the gingival cervical(GCF) biomarkers and the severity or presence of root resorption due to orthodontics in human patients?* The protocol is registered in the PROSPERO database under number CRD420251129166.

ELIGIBILITY CRITERIA

The PEO/PICOS framework was used to assess the eligibility criteria of the studies:

Population (P): patients undergoing fixed or removable orthodontic treatment, where root resorption was assessed during or after active orthodontic tooth movement.

Exposure / Intervention (E / I): Presence or measurement of salivary biomarkers and/or fluid from gingival crevicular f(GCF) biomarkers sampled during orthodontic treatment. Biomarkers included inflammatory cytokines, and proteomic markers.

Comparator (C): Patients without clinically or radiographically demonstrated root resorption, baseline (pre-treatment) samples, contralateral/within-patient controls (e.g., split-mouth), or other appropriate comparison groups as defined by each study. When no explicit comparator was used, studies were considered if they reported associations between biomarker levels and extent/severity of resorption.

Outcomes (O): Primary — presence or severity of root resorption in the external apical region due to orthodontic treatment (OIERR) confirmed by radiography (periapical, panoramic) or CBCT and quantified by accepted measures (length, volume, or

categorical grading). Secondary — statistical association between salivary/GCF biomarker concentrations and resorption measures; temporal changes in biomarker levels related to treatment phases.

Study designs (S): Clinical observational studies (cross sectional, comparison of case and control, and cohort study involving prospective and retrospective analysis where longitudinal exposure/outcome is appropriately reported), and randomized controlled trials (including split-mouth RCTs) that measured salivary or GCF biomarkers and used radiographic/CBCT assessment for root resorption.

EXCLUSION CRITERIA

Studies which were case report or did not used biomolecular profile or did not demonstrate the occurrence resorption by radiographs were not included

DATABASES AND SEARCH STRATEGY

The systematic search using Boolean string strings were conducted across several databases such as PUBMED/MEDLINE, EBCSO, Scopus, and Web of Sciences from database inception to 31 May 2026. The electronic search was performed on 31 May 2026. (Table 1). The strategy for Boolean search is illustrated in the table

Selection of Studies

- The investigators conducted an independent search and identified the studies for evaluation. Initially they studied the abstracts and the metadata obtained to consider a particular study eligible for consideration. Each study considered eligible were evaluated considering the criteria. The studies were analysed by independent reviewers. They assessed the abstract, titles and full text. The discrepancy and agreement was subjected to statistics via Cohen's Kappa, later discrepancy was resolved with focussed discussion if unresolved, by a senior third-reviewer adjudication..
- Screening of Articles

Following compilation of the initial search results, duplicate citations were systematically eliminated based on title, authorship, and publication year metadata. Studies failing to align with defined PICO or PEO frameworks were strictly excluded. Following this initial screening cascade, candidate manuscripts underwent review to assess final inclusion eligibility.

Data Extraction

The data obtained was reviewed all eligible studies using the following parameters:

- **Study Identity:** Author, year in which the study was published, and location.
- **Methodology:** Type of the study, source of the data, and characteristics of both the test and control groups.
- **Analysis:** Evaluation parameters, study duration, and specific results for each group.
- **Synthesis:** Statistical significance and the overall clinical inference.

BIAS AND QUALITY OF EVIDENCE

Each included study was critically evaluated using the JBI (Joanna Briggs Institute) Critical Appraisal Tools for Case-Control Studies [17] and RCTs [18]. Given the mix of study designs, the following appraisal approach was used:

- For case-control and cohort-type observational studies, using JBI, Critical Appraisal Checklists appropriate to the design to evaluate selection, comparability, exposure/outcome measurement, confounding, and statistical analysis.
- For randomized trials (including split-mouth RCTs), using the JBI Checklist for RCT. the tool evaluated four key elements to determine the overall risk of bias
- For cross-sectional studies, the corresponding JBI checklist for Analytical Cross-Sectional Studies was used.
- Responses were categorized.

For case-control and comparative studies, the JBI Critical Appraisal Checklist was applied, revealed consistent strengths in baseline comparability and measurement, alongside remarkable variation were observed in confounding variable management and statistical optimization across the cohorts. Concurrently, randomized controlled trials (RCTs) were appraised using the corresponding JBI RCT checklist, demonstrating methodological similarity and robust baseline similarities, identical treatment protocols, and complete follow-up analyses. Incorporating these standardized JBI criteria minimized risk within this study by providing a transparent, systematic framework to identify specific methodological blind spots such as unaddressed confounders.

Data synthesis

Studies were clinically and methodologically comparable (same biomarker, similar outcome metric and imaging method), results were pooled. Heterogeneity was determined. Where pooling was not feasible, a narrative data synthesis was provided, structured by biomarker class and imaging modality. A planned meta-analysis was not performed because the included studies were heterogeneous in biomarker types/assays, sample timing/matrix (saliva vs GCF), imaging modality and resorption metrics, and study design, and too few studies reported comparable biomarker–outcome data for reliable pooling. Therefore a structured narrative synthesis by biomarker class and imaging modality was presented.

Grading

A formal GRADE assessment was not performed because the small number of included studies and their clinical and methodological heterogeneity precluded meaningful grading; this is a limitation of the overall evidence base.

RESULTS

The eight studies that were considered for this study consisted of participants within the range of 10 to 74. The resorption was assessed with the biomarkers present in the gingival crevicular fluid, saliva, and blood when they were undergoing orthodontic treatment. The studies included were case-control studies, prospective studies, and randomized clinical trials the age range amongst the eight studies were between 14–30 years, mainly focusing on young adults. The studies evaluated the groups which underwent the orthodontic treatment and the group which did not undergo the treatment, they evaluated the force of magnitude applied and the difference found between paediatric patients and adult population. The samples were taken from gingival sulcus and the saliva to assess the effect of mechanical force. The biomarkers were dentin proteins, enzymes, and cytokines. The enzymes were phosphatase and tartaric-acid resistant phosphatase. There were three tier system used in most of the cases to identify biomarkers, the trend then shifted towards identifying proteins that could serve as biomarkers

Data from these studies, which tracked baseline versus final values in intervention groups, highlighted the statistical significance of variations associated with root resorption due to orthodontically induced inflammation. Dentin and matrix-related proteins showed variations linked to resorptive activity; specifically, DSP levels were significantly higher in cases compared to controls, suggesting it is a more sensitive marker than DMP1 or PP, which increased without statistical significance. Inflammatory cytokine profiles also shared a strong relation with root resorption, as cytokines increased significantly in individuals with OIIRR, emphasizing the role of immuno-inflammatory pathways. Enzyme assays yielded mixed results; while ALP and TRAP showed no significant change after force application, AST increased significantly under higher force levels, suggesting cellular stress from heavy mechanical loading. Proteomic studies further identified a broad range of significant alterations in proteins involved in immune function, cellular stress, and extracellular matrix regulation, such as S100 A9 and IGKC, highlighting the complex molecular cascade triggered by treatment. Furthermore,

studies comparing force magnitudes showed that distinct biomarker shifts support the clinical principle that lighter forces will decrease biological risk of root resorption.

The methodological strengths varied significantly across the studies as per JBI scale to assess the root resorption. The robust method validated by Kaczor-Urbanowicz et al. (2017) had clearly defined diagnostic benchmarks. Eventhough there were variables there were robust methods were developed Mandour et al. (2020) and Balducci et al. (2007). Driven by restricted imaging precision, backward-looking data collection, and highly constrained patient cohort sizes, the greatest risk of bias was observed in Yashin et al. (2017). Requiring enhanced management of confounding factors, expanded patient cohorts, and more rigorous structural frameworks, promising detection potential for OIIRR continues to be offered by these biochemical markers

To assess the quality of the four included RCTs—Wahab et al. (2015), Nasri et al. (2020), Huang et al. (2021), and Mohammed-Salih et al. (2023)—we used the JBI Critical Appraisal Checklist. Overall, the trials showed moderate to high methodological quality.

Limitation associated particularly regarding outcome evaluation dependability, treatment uniformity, and allocation randomness, a generally solid structural foundation was demonstrated by the randomized clinical trials. Featuring transparent documentation across all parameters and thorough masking, the highest methodological precision was showcased by Mohammed-Salih et al. (2023) and Huang et al. (2021). Yielding a moderate susceptibility to detection and procedural bias due to inadequate details on group concealment and masking, Nasri et al. (2020) and Wahab et al. (2015) faced specific constraints, even though they were properly designed. Backing the growing diagnostic value of salivary and gingival fluid biological markers for catching orthodontically induced inflammatory root resorption early, cellular reactions tied to mechanical force application are clarified by the combined findings of these clinical trials, notwithstanding these limitations

DISCUSSION

The earlier detection of the root resorption is possible using non-invasive method which does not require radiographs, the review states that biomarkers in the fluids can serve as a reliable diagnostic aid. Radiography can detect resorption but only after substantial damage has occurred, it has limitation such as magnification errors [27,28]. There is a need to develop methods which can detect the resorption earlier and in reliable manner. The studies reported with moderate to severe resorption had higher levels of dentin sialoprotein, dentin sialophosphoprotein which demonstrated that they can be used as a reliable marker for detection of resorption [29–32]. The specificity of these markers makes them ideal tool for earlier detection of resorption.

Immune-signaling proteins

Apart from the phosphoproteins reported the biological chain reactions behind how teeth shift, immune-signaling proteins also play a massive part. In the current review it was found that various immune-signaling proteins particularly, study done by Mohd Nasri et al., 2020 and Yahsin D et al., 2017 reported significant increase in the levels of immune-signaling proteins [22,24]. Matching the chance of root breakdown and inflammatory reactions while teeth are actively shifting, elevated amounts of immune-signaling proteins are consistently found in the gum line fluid [36,37] IFN- γ , IL-12p70, IL-10, and IL-7, were found as at increased levels in the patients with moderate to severe root resorption [37]. Although Huang G, et al.2021 reported the increase in cementum and dentin proteins; various proteomic proteins were detected in other studies such as stress response proteins, immunoglobulins, heat shock proteins, stress mediators response [38,39]. Similarly, Wahab RM, et al. 2015 reported the immune-signaling proteins that is Alkaline Phosphatase, Aspartate Aminotransferase, and Tartrate-Resistant Acid Phosphatase increased significantly in the gingival fluid of

the patients with resorption [20] since there are multiple level cascades having multiple factors it increases the scope of earlier detection [40,41].

Selection of the orthodontic treatment- biomechanical consideration

A recent study conducted by Mohammed- Salih HS, et al.2023 reported the biomechanical difference in the proteins detected in the gingival fluid they found S100A9, CASR, and K2C1 suggested a response to force-related inflammation, chemotactic activities, osteoclastogenesis, and epithelial cell breakdown. Meanwhile, the up-regulation of IGKC, NGAL, and K2C1 indicated a response to the inflammatory process, innate immunity activation, and epithelial cell breakdown. They varied the force applied and reported the findings of varying proteins, this can have severe implication is selection of personalized orthodontic treatments [26] Standard X-rays often miss the early signs of tissue damage until it is too late to fix. However, regular blood or biological marker tests can catch these changes early on a fact that has been proven by multiple comparative studies. [40]. Preventing irreversible root shortening, clinicians can monitor patients more closely, extend the time between adjustments, or lower the applied orthodontic forces when detecting unique protein profile shifts, fluctuating cytokine levels, or elevated dentin markers, demonstrating how tracking these biological signals offers clear opportunities for early clinical intervention [41,42]. Accounting for biological differences linked to patient-specific tissue reactions, periodontal health, genetic factors, and age, individualized clinical care is actively supported by biomarker-guided orthodontic strategies [42–44].

A recent review conducted by Kuruthukulangara J. 2025 et al., has consistent findings. It establishes the dentin phosphoproteins as the diagnostic marker for detection of resorption. [48] However, the studies considered for this systematic review are not limited to only dentin phosphate proteins immune-signaling proteins were found increased severely in multiple studies [24, 22]. The study involved such as Mohammad Salih. et al, (2023) which investigates the biomechanical correlation with the occurrence of biomarkers diverts the course towards the how

technical procedures can increase or decrease the occurrence of root resorption. [27]

Limitation

Diminishing comparability across investigations, inconsistencies in study designs, the timing of biomarker collection, laboratory assays, and sampling techniques ultimately restrict broad clinical implementation. [35,45]. Refining forecasting models, defining different orthodontic treatments and its influence on biomarker, establishing baseline values for interpreting these biological markers ultimately requires extended trials across multiple clinical centers can impact the findings and clinical interpretation [28,42,43,44]. Overall, the convergence of findings across this review and external studies supports the strong potential of dentin-derived proteins, inflammatory cytokines, and proteomic signatures as early, sensitive indicators of OIIRR. Continued research focusing on standardization and validation is essential to bring biomarker-based diagnostics into routine orthodontic practice. To advance clinical translation, future research must Develop optimum analytical method and standardize it, Multicentre studies should be conducted longitudinally to confirm the association of biomarkers with root resorption, Validated the developed method for multimarkers with specific threshold, and To determine the patient specific characters to enable orthodontic care. Further studies are required for validating the data and confirming the potential of biomarker in earlier detection of root resorption.

CONCLUSION

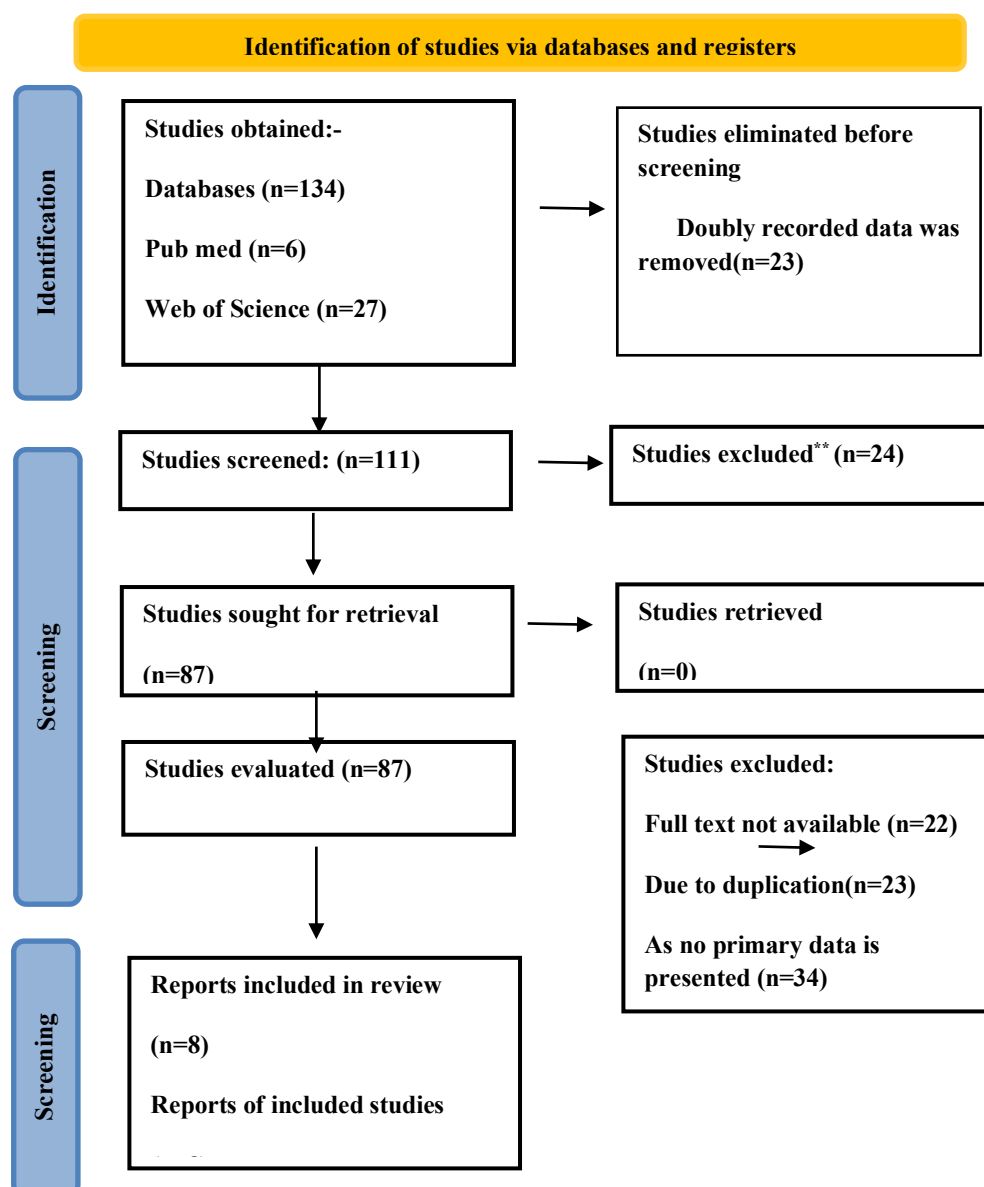
In conclusion, dentin breakdown products (such as phosphoproteins) and immune signaling proteins in gingival fluid show promise as biomarkers for detecting root resorption. However, these findings are limited by small sample sizes and a low number of total studies. Because biomarker identification is heavily influenced by varying orthodontic techniques and research methodologies, larger, standardized studies are needed to confirm their diagnostic validity. Identifying a biomarker depends on the specific orthodontic techniques used and the research methods chosen for that study.

TABLE/ FIGURE 1: SEARCH STRATEGY FOR DATABASES

DATABASES	SEARCH STRATEGY	No. of searches yielded
Pubmed	((("orthodontics"[MeSH Terms] OR "orthodontic treatment"[All Fields] OR "fixed orthodontic treatment"[All Fields] OR "tooth movement"[All Fields] OR "orthodontic retention"[All Fields] OR "retention phase"[All Fields]) AND ("root resorption"[MeSH Terms] OR "external root resorption"[All Fields] OR "dental root resorption"[All Fields] OR "apical root resorption"[All Fields] OR "orthodontically induced inflammatory root resorption"[All Fields] OR "OIIRR"[All Fields]) AND ("gingival crevicular fluid"[MeSH Terms] OR "saliva"[MeSH Terms] OR "crevicular fluid"[All Fields] OR "GCF"[All Fields] OR "gingiva crevicular fluid"[All Fields] OR "saliva"[All Fields] OR "salivary diagnostics"[All Fields] OR "oral fluids"[All Fields] OR "serum"[All Fields] OR "plasma"[All Fields]) AND ("biomarkers"[MeSH Terms] OR "biological marker"[All Fields] OR "biomarker"[All Fields] OR "biomarkers"[All Fields] OR "predictive value"[All Fields] OR "risk factor"[All Fields] OR "risk factors"[All Fields] OR "etiology"[All Fields] OR "iatrogenic"[All Fields] OR "cytokine"[All Fields] OR "cytokines"[All Fields] OR "chemokine"[All Fields] OR "interleukin"[All Fields] OR "proteomic"[All Fields] OR "proteomics"[All Fields] OR "mass spectrometry"[All Fields] OR))	6
WoS	#1: TS=("orthodontic treatment" OR "orthodontics" OR "fixed orthodontic treatment" OR "tooth movement" OR "orthodontic retention" OR "retention phase") #2: TS=("root resorption" OR "external root resorption" OR "dental root resorption" OR "Apical root resorption" OR "orthodontically induced inflammatory root resorption" OR "OIIRR") #3: TS=("gingival crevice fluid" OR "crevicular fluid" OR "GCF" OR "gingiva crevicular fluid" OR "saliva" OR "salivary diagnostics" OR "oral fluids") #4: TS=("biological marker" OR "biomarker" OR "biomarkers" OR "predictive value" OR "risk factor" OR "risk factors" OR "etiology" OR "iatrogenic" OR "cytokine" OR "cytokines" OR "chemokine" OR "interleukin" OR "proteomic" OR "proteomics" OR "mass spectrometry") #5: #1 AND #2 AND #3 AND #4	27

Scopuse	((TI "orthodontic treatment" OR AB "orthodontic treatment" OR SU "orthodontic treatment") OR (TI orthodontics OR AB orthodontics OR SU orthodontics) OR (TI "fixed orthodontic treatment" OR AB "fixed orthodontic treatment" OR SU "fixed orthodontic treatment") OR (TI "tooth movement" OR AB "tooth movement" OR SU "tooth movement") OR (TI "orthodontic retention" OR AB "orthodontic retention" OR SU "orthodontic retention") OR (TI "retention phase" OR AB "retention phase" OR SU "retention phase")) AND ((TI "root resorption" OR AB "root resorption" OR SU "root resorption") OR (TI "external root resorption" OR AB "external root resorption" OR SU "external root resorption") OR (TI "dental root resorption" OR AB "dental root resorption" OR SU "dental root resorption") OR (TI "apical root resorption" OR AB "apical root resorption" OR SU "apical root resorption") OR (TI "orthodontically induced inflammatory root resorption" OR AB "orthodontically induced inflammatory root resorption" OR SU "orthodontically induced inflammatory root resorption") OR (TI "OIIRR" OR AB "OIIRR" OR SU "OIIRR")) AND ((TI "gingival crevice fluid" OR AB "gingival crevice fluid" OR SU "gingival crevice fluid") OR (TI "crevicular fluid" OR AB "crevicular fluid" OR SU "crevicular fluid") OR (TI GCF OR AB GCF OR SU GCF) OR (TI "gingiva crevicular fluid" OR AB "gingiva crevicular fluid" OR SU "gingiva crevicular fluid") OR (TI saliva OR AB saliva OR SU saliva) OR (TI "salivary diagnostics" OR AB "salivary diagnostics" OR SU "salivary diagnostics") OR (TI "oral fluids" OR AB "oral fluids" OR SU "oral fluids") OR AND ((TI "biological marker" OR AB "biological marker" OR SU "biological marker") OR (TI biomarker OR AB biomarker OR SU biomarker) OR (TI biomarkers OR AB biomarkers OR SU biomarkers) OR (TI "predictive value" OR AB "predictive value" OR SU "predictive value") OR (TI "risk factor" OR AB "risk factor" OR SU "risk factor") OR (TI "risk factors" OR AB "risk factors" OR SU "risk factors") OR (TI etiology OR AB etiology OR SU etiology) OR (TI iatrogenic OR AB iatrogenic OR SU iatrogenic) OR (TI cytokine OR AB cytokine OR SU cytokine) OR (TI proteomics OR AB proteomics OR SU proteomics))	59
Embase	('dentin sialoprotein':ti,ab,de OR 'dsp':ti,ab,de OR 'dentin phosphophoryn':ti,ab,de OR 'dpp':ti,ab,de OR 'enolase 1':ti,ab,de OR 'eno1':ti,ab,de OR 'keratin':ti,ab,de OR 'osteocalcin':ti,ab,de OR 'p1np':ti,ab,de) AND ('root resorption':ti,ab,de OR 'external root resorption':ti,ab,de OR 'oiirr':ti,ab,de) AND ('orthodontic':ti,ab,de OR 'orthodontics':ti,ab,de OR 'retention':ti,ab,de)	42

Table/ Figure 2: Flowchart 1: PRISMA Flowchart showing the inclusion process studies that composed in the review



Table/ Figure 3: General characteristics of studies included.

Study ID	Authors & Year	Study Design	Total Sample Size	Case/ Intervention	Control/ Comparison	Mean age group (Mean± SD)	Samples collected	Biomarkers analysed
1	Balducci L, Ramachandran A, Hao J, et al. 2007 [19]	Case control Study	60	Group 1: Mild root resorption Group 2: Severe root resorption	Group 3: No treatment	1.14- 40 years 2.16- 44 years 3.12- 34 years	GCF	Dentin Phosphophoryn (PP), Dentin Sialoprotein (DSP).
2	Wahab RM, Yamamoto Z, Sintian A, et al. 2015 [20]	Prospective study	19	Orthodontic Appliance	None	21.3± 3.3	GCF	Alkaline Phosphatase Aspartate Aminotransferase Tartrate-Resistant Acid Phosphatase
3	Kaczor- Urbanowicz KE, Deutsch O, Zaks B, et al. 2017 [21]	Case control study	72	OIHR	Healthy controls	Age: 10-30 Years	Saliva	P21-ARC, fetuin- A
4	Yashin D, Dalci O, Almuzian M, et al. 2017 [22]	Retrospective Case-Controlled Study	25	Root resorption group	None	23±2.9	Saliva	IL-4, IL-10, IFN-γ, IL-12p70, and IL-7
5	Mandour KA, Tawfeek MA, Montasser MA 2020 [23]	Case control study	74	Group 1: Orthodontic Patients Group 2: Pediatric Patients	Group3: Control group (no orthodontic treatment and root resorption.)	1. 15.6± 2.5 2. 9± 1 3. 18.2± 6.8	GCF	IL1-ra and DSPP
6	Mohd Nasri FA, Zainal Ariffin SH, Karsani SA, Wahab RM 2020 [24]	Clinical Study	10	Orthodontic Appliance	None	20.6± 3.92	GCF	Immune Defense (IGJ, IGHV, IGHG, IGKV), Stress/Repair (S100A9, HSPA1A, TMSB4X), Blood/Fluid Transport (APOD, APOH, HBB, AGT), and Structural Growth (VTN, HRG, TYMP, AZGP1).
7	Huang G, Choi S, Jung H, et al.2021 [25]	Split mouth randomized control trial	11	Orthodontic Treatment	Control group	23.5	GCF	CEMP-1, DPP, and CTX-I
8	Mohammed- Salih HS, Al-lami HA, Saloom HF, et al.2023 [26]	Split mouth Randomized-Controlled Clinical Trial (RCT)	38	Group 1: Heavy force (225g) Group 2: Light Force (25g)	Group 3: Periodontitis	Group 1 & 2: 21.1± 3.1 Group 3: 46.53± 9.5	GCF	Immunoglobulin kappa, Immunoglobulin kappa (isoform), Neutrophil gelatinase associated Lipocalin, Neurolysin, Mitochondrial, Keratin, type II cytoskeletal 1, Keratin, type II cytoskeletal 1 (isoform), Protein S100-A9, Extracellular calcium sensing receptor

Table/ Figure 4: Main outcome and inference from included retrospective studies.

S. No	Authors & Year	Bio markers	Case	Control	Significance
1	Balducci L, Ramachandran A, Hao J, et al. 2007 [19]	DMP1	high concentration	Low concentration	No significance
		PP	high concentration	Low concentration	No significance
		DSP	high concentration	Low concentration	Statistically significant
2	Kaczor-Urbanowicz KE, Deutsch O, Zaks B, et al. 2017 [21]	P21-ARC	---	Increased	Statistically significant
		Fetuin-A	---	Increased	
3	Yashin D, Dalci O, Almuzian M, et al. 2017 [22]	IFN- γ	---	Increased	p = 0.01
		IL-10	---	Increased	p = 0.03
		IL-12p70	---	Increased	p = 0.02
		IL-7	---	Increased	p = .0001
4	Mandour KA, Tawfeek MA, Montasser MA 2020 [23]	IL1-ra	319.9 $\pm$ 181.3	657.4 $\pm$ 257.5	p = 0.01
		DSPP	30.1 $\pm$ 9.6	1.6 $\pm$ 1.0	p = 0.01

Table/ Figure 5: Main outcome and inference from included randomized controlled studies.

S. No	Authors & Year	Bio markers	Base line data	Final data	Significance		
1	Wahab RM, Yamamoto Z, Sintian A, et al. 2015 [20]	ALP	100g	1.67 $\pm$ 0.06	100g	1.94 $\pm$ 0.33	Not significant
			150g	1.67 $\pm$ 0.06	150g	1.86 $\pm$ 0.25	Not significant
		TRAP	100g	2.92 $\pm$ 0.14	100g	4.00 $\pm$ 0.47	Not significant
			150g	2.92 $\pm$ 0.14	150g	3.63 $\pm$ 0.34	Not significant
		AST assays	100g	3.94 $\pm$ 0.23	100g	5.37 $\pm$ 0.34	Not significant
			150g	3.94 $\pm$ 0.23	150g	6.27 $\pm$ 0.54	significant
2	Mohd Nasri FA, Zainal Ariffin SH, Karsani SA, Wahab RM 2020 [24]	angiotensinogen (AGT)	1.03	0.98	0.03		
		apolipoprotein D(APOD)	1.20	0.99	0.05		
		beta-2-glycoprotein 1 (APOH)	1.18	1.12	0.02		
		heat shock protein 1A (HSPA1A)	1.14	1.02	0.01		
		haemoglobin subunit beta (HBB)	1.15	1.07	0.03		
		histidine-rich glycoprotein (HRG)	1.13	1.18	0.02		
		immunoglobulin heavy variable 4–34 (IGHV4–34)	1.02	1.01	0.03		
		immunoglobulin kappa variable 3–20 (IGKV3–20)	1.11	1.01	0.03		

		immunoglobulin heavy variable 3–11 (IGHV3–11)	1.08	1.12	0.04
		immunoglobulin heavy constant gamma 1 (IGHG1)	1.12	0.99	0.03
		immunoglobulin J chain (IGJ)	0.98	1.05	0.03
		thymidine phosphorylase (TYMP)	1.07	0.83	0.03
		S100A9	0.97	1.07	0.02
		thymosin beta-4 (TMSB4X)	1.02	1.04	0.05
		zinc-alpha-2-glycoprotein (AZGP1),	0.95	1.13	0.04
		vitronectin (VTN)	0.99	1.064	0.04
3	Huang G, Choi S, Jung H, et al.2021 [25]	CEMP 1	10.03 $\pm$ 13.62		p < 0.05
		DPP	18.96 $\pm$ 22.56		p < 0.05
		CTX- I	352.37 $\pm$ 209.16		p < 0.05
4	Mohammed-Salih HS, Al-lami HA, Saloom HF, et al.2023 [26]	Immunoglobulin kappa (IGKC)	4.70 $\pm$ 0.24	5.54 $\pm$ 0.39	0.03

		immunoglobulin heavy variable 3–11 (IGHV3–11)	1.08	1.12	0.04
		Immunoglobulin kappa (IGKC) isoform	6.01±0.41	5.75±0.23	0.001
		Neutrophil gelatinase-associated lipocalin	4.78±0.48	4.66±0.10	0.007
		Neurolysin mitochondrial	4.73±0.51	5.48±0.34	0.011
		Keratin, type II cytoskeletal 1 (K2C1)	5.80±0.32	5.62±0.33	0.013
		Keratin, type II cytoskeletal 1 (K2C1) isoform	5.41±0.36	4.98±0.28	0.00001
		S100-A9	6.44±0.21	5.80±0.45	0.0008
		Extracellular calcium-sensing receptor	5.93±0.27	5.21±0.40	0.013

Table/ Figure 6: Results of JBI critical appraisal tool for Case Control Studies

JBI Criterion	Balducci et al., 2007 [19]	Kaczor-Urbanowicz et al., 2017 [21]	Yashin et al., 2017 [22]	Mandour et al., 2020 [23]
Groups comparable at baseline	Y	Y	Partly	Y
Cases & controls appropriately matched	N	Y	N	N
Criteria for identification was same	Y	Y	Y	Y
Exposure measured validly & reliably	Y	Y	Y	Y
Same exposure measurement for groups	Y	Y	Y	Y
Factors which were cofounding were identified	N	N	N	N
Strategies they had to deal with cofounding factors	N	N	N	N
Outcomes measured validly & reliably	Y	Y	Y	Y
Exposure period appropriate	U	Y	N	U
Optimum statistical analysis	N	N	N	N
Risk of bias was	Moderate	Moderate	High	High

*** Y: Yes, N: No, U: Unclear, N/A: Not Applicable***

TABLE/ FIGURE 6: RESULTS OF JBI CRITICAL APPRAISAL TOOL FOR RCT

Question	Rohaya et al., 2015 [34]	Nasri et al., 2020 [24]	Huang et al., 2021 [25]	Mohammed-Salih et al., 2023 [26]
Randomization was true for the participants?	Yes	Yes	Yes	Yes
The treatment group knew about their allocation?	Yes	Unclear	Yes	Yes
The control and treatment group had similar baseline?	Yes	Yes	Yes	Yes
The participants were blind towards the assignment of the treatment?	Unclear	Unclear	Yes	Yes
The professional delivering the treatment were blinded?	Unclear	Unclear	Yes	Yes
All the participants of treatment group received identical treatment?	Yes	Yes	Yes	Yes
The professional who assessed the treatment were blinded?	Unclear	Unclear	Yes	Yes
The measurement of outcome was on similar basis?	Yes	Yes	Yes	Yes
Measurement of outcomes was reliable?	Yes	Yes	Yes	Yes
The follow-up were complete and were the differences analyzed?	Yes	Yes	Yes	Yes
Were participants analyzed in the groups to which randomize?	Yes	Yes	Yes	Yes
The study applied statistical analysis?	Yes	Yes	Yes	Yes

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