Biomarkers in External Apical Root Resorption: An Evidence-based Scoping Review in Biofluids

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ABSTRACT

Background: External apical root resorption (EARR), an unwanted sequela of orthodontic treatment, is difficult to diagnose radiographically. Hence, the current scoping review was planned to generate critical evidence related to biomarkers in oral fluids, i.e. gingival crevicular fluid (GCF), saliva, and blood, of patients showing root resorption, compared to no-resorption or physiologic resorption.

Methods: A literature search was conducted in major databases along with a manual search of relevant articles in the library, and further search from references of the related articles in March 2021. The initial
search was subjected to strict inclusion and exclusion criteria according to the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) guidelines.

**Results:** Following PRISMA guidelines, 20 studies were included in the final review. The studies included human clinical trials and cross-sectional and prospective studies with/without control groups with no date/language restriction. Various biomarkers identified in EARR included dentinal proteins, enzymes, cytokines, and salivary proteins. Severe resorption had higher dentin sialoprotein (DSP) and resorption protein concentrations as well as lower granulocyte-macrophage colony-stimulating factor (GM-CSF) as compared with mild resorption. Increased DSP and dentin phosphophoryn (DPP) expression was found in physiologic resorption. Compared to controls, resorbed teeth showed a higher receptor activator of nuclear factor kappa B ligand/osteoprotegerin (RANKL/OPG) ratio. In contrast, levels of anti-resorptive mediators (IL-1RA, IL-4) was significantly decreased. Differences in force levels (150 g and 100 g) showed no difference in resorption, but a significant rise in biomarkers (aspartate transaminase [AST] and alkaline phosphatase [ALP]) for 150 g force. Moderate to severe resorption in young patients showed a rise in specific salivary proteins, requiring further validation. Limitations of the studies were heterogeneity in study design, biomarker collection, sample selection, and confounding inflammatory conditions.

**Conclusions:** Various biomarkers in biofluids indicate active resorption, while resorption severity was associated with DSP and GM-CSF in GCF, and a few salivary proteins. However, a robust study design in the future is mandated.

**KEY WORDS:** Biomarkers, gingival crevicular fluid, interleukin, orthodontics, root resorption

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**INTRODUCTION**

External apical root resorption (EARR) may occur in a variety of conditions like bacterial invasions, trauma, neoplasms, and systemic or pressure conditions produced by the application of orthodontic forces. External apical root resorption is also known as orthodontically induced root resorption (OIRR), which is an undesirable but common sequela of orthodontic tooth movement. External apical root resorption has a multifactorial etiology and is associated with several risk factors predisposing patients to various degrees of root resorption. The reported EARR incidence is variable: 90% in histological studies, 73% in radiological studies after tooth movement, 6%–13% depending on the type of teeth, and 1%–5% or 1%–2% depending on resorption severity. Nevertheless, any grade of EARR severity is known to limit the outcome of successful orthodontic treatment and also cause oral dysfunction on progression.

The deleterious effects of EARR on tooth movement mandate early detection of resorption. However, early detection is not possible with the currently available diagnostic modalities that rely on two- or three-dimensional radiographs. Radiographs are associated with limitations such as radiation exposure, inability to outline the active resorption process, and limited view and standardization of the resorption process. Hence, there is a great need for non-invasive techniques or determination of biomarkers to detect root resorption early in susceptible patients.

To define the biomarkers in root resorption, a thorough understanding is needed of its pathophysiology in relation to the surrounding bone and the periodontal ligament housing different types of cells, matrix, and biological messengers, as explained in Figure 1. Although the biomarkers released in the paracrine environment in the gingival crevicular fluid (GCF) have been extensively studied in bone resorption during orthodontic tooth movement, a comprehensive study of all body fluid biomarkers (GCF, saliva, and blood) around teeth undergoing resorption is lacking. Various mediums have been evaluated for biomarker collection, of which GCF has the advantages of ease of repeatability, collection, and detection of early resorption. Also, saliva has greater accessibility and ease, but is comparatively less specific to the underlying periodontal condition.

Various biomarkers are indicative of active resorption, with evidence supporting the presence of dentinal proteins including dentin sialophosphoprotein (DSP), dentin sialoprotein (DSP), and dentin phosphophoryn (DPP) in GCF and saliva.
Of these, DSPP shows a continuous expression in amelogenesis and dentinogenesis and is considered a potent resorption marker. Other markers responsible for osteoclastogenesis or extracellular matrix degradation, including pro-inflammatory cytokines (interleukins [IL], tumor necrosis factor, etc.) or matrix metalloproteinases (MMPs), have also been associated with the degree of resorption. Alkaline phosphatase (ALP), an enzyme associated with early deposition of minerals and tissue calcification, may contribute toward pulpal repair and healing after traumatic insults or injury and shows variable expression in root resorption.

Hence, there are multiple mediators having distinct associations in resorption, some in tissue destruction and others in tissue repair, which show variable expression at different stages of resorption. The success of clinical orthodontic treatment in turn...
is dependent on early detection of EARR and on preventing and limiting the extent of this unwanted condition. Tarallo et al. have provided some evidence related to the role of GCF biomarkers in root resorption, but failed to establish an all-inclusive understanding of the dynamics of root resorption markers to identify the most potent biomarker that might show significant association in multiple oral biofluids. Another study by Allen et al. examined salivary protein in orthodontic tooth movement, but it did not specifically target root resorption.

Hence, this scoping review addresses the gap in the literature to generate critical evidence related to biomarkers in all oral fluids (gingival crevicular fluid [GCF], saliva, blood) of patients showing root resorption, compared to no resorption or physiologic resorption.

MATERIAL AND METHODS

Protocol
A scoping review was designed according to the Preferred Reporting Items for Systematic Reviews and Meta-analysis (PRISMA) guidelines specific to scoping reviews (PRISMA-ScR). The inclusion and exclusion criteria were defined (Table 1), and the study was registered in the Open Science Framework (https://osf.io/nep9z/). No funding was received for the study.

Eligibility
The research topic for determining literature eligibility was developed based on the PICOS model, as follows: Population, patients showing EARR on radiographs; Intervention, orthodontic forces; Comparison, no resorption or physiological resorption; Outcomes, change in biomarkers in biofluids. There was no limitation of date or language placed on the literature search.

Based on the above, the research question asked: was the variation in levels of biomarkers in the oral fluids associated with root resorption in patients undergoing orthodontic treatment in comparison to no resorption or physiologic resorption?

Information Sources and Search
In March 2021, a thorough literature search was conducted in the major databases: PubMed, Web of Science, J-Gate, Directory of Open Access Journals, Scopus, and Embase, along with related searches, manual searches, and tracking of references from the manual searches. Both MeSH and free-text terms were used to search most of the databases: “biomarkers,” “root resorption,” and “orthodontics” with the BOOLEAN terminology “AND.” Duplicate results were removed.

Study Selection
The identification, screening, eligibility, and inclusion of studies were performed as detailed in the PRISMA flowchart shown in Figure 2. The search strategy was applied independently by two reviewers (PK and AC) strictly based on the inclusion and exclusion criteria (Table 1).

Any discordance was addressed by two reviewers (DB and DDB) for a final consensus. Duplicates were removed, and articles were screened based on their titles and abstracts. Full texts were then retrieved, and an in-depth review was performed to identify the final studies selected for this review. No quality assessment was done as it is not mandatory for scoping reviews, and the aim of the current scoping review was to present a broad scope of biomarkers identified to date in EARR. Studies related to root resorption by other causes, including traumatic forces or endodontic resorption, were excluded from the final selection. The primary outcome included the variation in expression of different biomarkers.

| Table 1. Inclusion and Exclusion Criteria for Study Selection. |
|---------------------------------------------------------------|
| Inclusion Criteria                                             |
| • All original studies on humans including clinical trials    |
| • Prospective or retrospective cohort studies                 |
| • Studies mentioning both biomarkers and root resorption      |
| • Studies in orthodontics and physiological root resorption   |
| Exclusion Criteria                                            |
| • In vitro studies                                           |
| • Animal studies                                             |
| • Studies on biomarkers but not on resorption                |
| • Studies on resorption but not on biomarkers                |
| • Case reports and reviews/opinions                          |
in root resorption, which was further correlated with their mechanism in the cellular remodeling process.

**Data Charting**
The data charting of these articles was performed by two investigators (PK and AC) independently, and any discordance was addressed by a third researcher (DKB). The criteria for data charting were according to JBI (Joanna Briggs Institute) based on author, reference, and primary outcomes or results relevant to the broad research question.\(^\text{17}\)

**RESULTS**

**Study Selection**
A total of 372 articles were initially identified, duplicate publications were removed, and the inclusion and exclusion criteria were applied, resulting in 20 articles found being included in the final review (Figure 2).\(^\text{2,5,7,9,12,13,15,16,19–30}\)

**Data Extraction**
The data extraction from each study related to participant and study characteristics, the biomarker(s)
studied, the medium and technique of biomarker(s) study, and the outcomes related to biomarker(s) expression. Full details are given in Table 2.

**Participant characteristics**

The majority of studies had 20 or fewer participants. Three studies mentioned participants or teeth in two experimental and one control group with 20 patients in each group.7,13,19 Mah and Prasad mentioned two resorption groups; one group was examined for orthodontic resorption severity at 1–3 mm, while the second group looked at physiologic resorption of primary resorbing molars.13 Balducci et al. classified the two experimental groups as mild (≤2 mm) and severe resorption (>2 mm) groups,7 and George and Evans defined mild resorption (≤2 mm) and severe resorption as >2 mm in their groups.19 A total of 9 studies examined the resorption severity grades measured in mm, or classified it as mild/moderate/severe, or as coronal/apical resorption.5,7,9,12,13,16,20,24,29 Resorption with respect to the duration of orthodontic treatment was considered in 7 studies.7,9,19,20,23,27,30

While the majority of studies had both male and female participants, two studies investigated only female participants.9,15 Most of the studies collected biomarkers for the experimental or control teeth from the maxillary central and lateral incisors. However, controls varied, depending on the study: for example, external (in different subjects),5,7,9,12,15,19,20,24,25,27,29,30 or internal (baseline values),22 antagonistic teeth,15 and contralateral teeth,16,21,26 while one of the studies mentioned no control.28 Only five studies considered physiological resorption of the primary resorbing molars.5,13,16,21,29

**Study characteristics**

The majority of studies were cross-sectional, although six mentioned the collection of samples at more than one observation time.5,24,26–28,30 There were four split-mouth design studies,15,21,22,26 two of which considered 100 g force retraction on one side of the mouth and 150 g force on the other side.15,22 The amount of resorption was judged radiographically in most studies, with intraoral periapical radiograph specified in six,7,15,20,22,24,28 panorex in three,5,12,27 and micro-computed tomography in only one study.26

**Type of biomarkers**

Dentinal proteins were examined in most of the studies, while cytokines were the focus of six studies,9,10,16,17,19,26,29 enzymes in two,15,22 and metabolites in one study.27 Of the various dentinal proteins, DSPP5,25,29,30 and DSP7,16,23 were studied in four studies each. However, dentinal proteins DPP7 and dentin matrix protein-1 (DMP1)5,3 were studied in one study each. Pro-inflammatory cytokines, primarily interleukins (IL-1β, 2, 4, 5, 6, 7, 8, 10, 12, 13), were examined in three studies,5,10,26 and the interleukin-1 receptor antagonist (IL-1ra) in two studies.15,20 Receptor activator of nuclear factor kappa-B ligand (RANKL) and osteoprotegerin (OPG) were looked at in two studies16,19 and osteopontin (OPN) and tumor necrosis factor-α in one study each.10,26 Enzyme ALP was examined in two studies,15,22 Tartrate-resistant acid phosphatase (TRAP),22 aspartate aminotransferase (AST),22 and matrix metalloproteinase (MMP-8)16 were examined in only one study each. Cytokine profile and resorption proteins were evaluated in four studies,12,20,21,28

**Medium and technique of biomarker evaluation**

Biomarkers were evaluated in varied biofluids: the majority of samples were collected from the GCF (n=17 studies), saliva was used in two studies,24,27 and only Yashin et al. evaluated biofluids collected from both saliva and blood.12 Periopaper was used to collect GCF in 11 studies; however, other studies used micro-pipettes,5,23 filter paper,2,23 absorbent paper,20 and endodontic paper points.29 Various methods were used for evaluating biomarkers; the majority of studies used enzyme-linked immunosorbent assay (ELISA), but some studies used sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE),7,19,20 western blotting,5,7,19,24 multiplex bead immunoassay,26 spectroscopy,24,30 liquid chromatography,21,28 spectrophotometry,15,22 and mass spectrometry,21,28

**Upregulation or downregulation of biomarkers**

The amount of DPP in the GCF was found to be significantly higher in resorbing primary molars (11.7±4.1 µg/mg) and orthodontically treated teeth (9.3±4.7 µg/mg) compared to the controls (5.4±4.1 µg/mg).13 Kereshanan et al. also showed increased DSP in the GCF of physiologically resorbing molars compared to non-resorbing teeth but no difference in coronal and apical sites of resorption.5 Other

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Table 2. Evidence Related to Study of Various Biomarkers and Proteins in Various Biofluids Associated to EARR.

| Authors          | Experimental Subjects/Teeth (No./Age/Sex) | Biomarkers Studied   | Condition Analyzed                        | Detection of Root Resorption | Controls/Teeth (No./Age/Sex) | Medium Studied | Technique | Outcomes                                                                                                                                                                            | Conclusions                                                                                                                                                                                                 |
|------------------|------------------------------------------|----------------------|-------------------------------------------|-------------------------------|-----------------------------|---------------------------|-----------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| 2004 Mah and Prasad<sup>13</sup> | Grp 1: Mx central incisor (n=20) with 1-3 mm RR; 13F, 7M, 12-16 y Grp 2: 10 second molars (n=20); 15F, 5M; 9-12 y | DPP                  | Orthodontic Tx (not specified)            | Radiographs (not specified)  | Mx central incisors (n=20) of untreated pts; 12F, 8M, 12-16 y | GCF           | Periopaper, ELISA | Levels of DPP; greatest in resorbing 1<sup>st</sup> molar (11.7±4.1 µg/mg) followed by orthodontically treated tooth (9.3±4.7 µg/mg) and least in controls (5.4±4.1 µg/mg); NS between resorption Grps | DPP can be detected in exfoliating primary teeth and orthodontic root resorption                                                                 |
| 2007 Balducci et al.<sup>7</sup> | 20 pts with mild RR (≤2 mm) (11F, 9M, 14-40 y), 20 pts with severe RR (>2 mm) (15F, 5M, 15-44 y) | DMP1, PP, DSP        | RR in orthodontic pts                     | IOPA                          | 20 pts (13F, 7M, 12-34 y); no RR/orthodontic Tx | GCF           | Periopaper (mesial and distal of Mx central and lateral incisors), SDS-PAGE, stained western blot, ELISA | Molecular weight 77, 66, 55, 50, and 26 kDa proteins identified, NS between control and study Grps in immunoblot; ELISA showed Sig. † of DMP1, PP, DSP in RR vs control Grps and of PP and DSP in severe RR vs mild RR Grps | DMP1, DSP, and PP in GCF proved a biomarker for RR in orthodontic Tx                                                                                                                                   |
| 2008 Kereshanan et al.<sup>3</sup> | Grp 1: 50 second 1<sup>st</sup> molars (9-14 y) (advanced coronal RR [n=33] and apical minimal RR Grp [n=17]) Grp 2: 20 pts (11-15 y), T0=pre-fixed Tx, T1=12 mo post start of Tx | DSP                  | Physiological RR and OTM                  | Orthopantomogram             | Control: 20 pts (10-15 y) erupted second premolars with no RR | GCF           | Micropipettes, slot blot immunobassay, DSP in dentin of 1<sup>st</sup> molars by western blot | DSP levels: greater in physiological RR than non-resorbing teeth, DSP levels NS between coronal RR and apical RR; DSP levels Sig. higher in T1 compared to T0 | DSP in GCF proved a biomarker of root resorption                                                                                                                                                    |
| 2009 George and Evans<sup>19</sup> | Grp 1: mild RR of 2 mm (20 pts, Tx 1 y) Grp 2: severe RR >2 mm (20 pts) | OPN, OPG, RANKL      | Orthodontic Tx (not specified)            | Radiographs (not specified)  | 20 pts: no Tx, no RR         | GCF           | Periopaper (mesial/distal of Mx central and lateral incisors), SDS-PAGE, western blot | Proteins conc greater in severe RR (0.89 µg/µL ±0.32 µg) than mild RR (0.77 µg/µL ±0.21 µg) and least in controls (0.22 µg/µL ±0.05 µg); ELISA showed Sig. higher RANKL antibodies in RR Grps than control Grp; RANKL/OPG ratio in severe RR Sig. greater than in control Grp | Presence of OPN, OPG, and RANKL in root resorption                                                                                                                                                    |
Table 2. Continued (page 2 of 5)

| Authors       | Experimental Subjects/Teeth (No./Age/Sex) | Biomarkers Studied | Condition Analyzed | Detection of Root Resorption | Controls/Teeth (No./Age/Sex) | Medium Studied | Technique | Outcomes | Conclusions |
|---------------|------------------------------------------|-------------------|--------------------|-------------------------------|-----------------------------|----------------|-----------|----------|-------------|
| 2013 Kunii et al. | 5 pts with severe RR (5F, mean age 28.9±6.1 y; mean orthodontic Tx duration of 27.8±3.3 mo) | IL-6 | All 4 extr. orthodontic Tx | Radiographs (not specified) | 15 pts without RR (13F, 2M, mean age, 28.8±5.3 y; mean orthodontic Tx duration of 26.4±3.1 mo) | GCF | Periopaper (mesial/distal of Mx central and lateral incisors), ELISA | IL-6 protein levels Sig. ↑ in RR than non-RR Grp | IL-6 in GCF proved a root resorption biomarker in orthodontic Tx |
| 2013 Wahab et al. | 12F (Mx canines as test teeth), 100 g/150 g force to either side, split mouth design | ALP | Class II div 1 maloccl; upper 4/4 extr with retraction by NITi coil spring | IOPA | Mand canine as control | GCF, collection weekly for 6 wk | Periopaper (mesial/distal of Mx canine, Mand canine), spectrophotometry at 405 nm | ALP at mesial sites peak at wk 1 showing Sig. diff with 100 g force; no RR for test/control teeth in 150/100 g force | Canine movement greater with 150 g than 100 g force and higher ALP at mesial sites with no RR |
| 2014 Sha et al. | 20 pts (12F, 8M, 13-24 y), 8-12 mo of orthodontic Tx | DSPP | Orthodontic Tx (not specified) | Radiographs (not specified) | Same pts for both methods (ELISA with spectrophotometry and electrochemical detection) | GCF | Filter paper strip (mesial/distal sites of left and right Mx central incisors), ELISA | DSPP detection with spectrophotometric ELISA 10 times greater than with electrochemical detection. DSPP conc range NS between methods | DSPP can be sensitively and accurately detected in root resorption |
| 2014 Vieira | Total 60 pts (38F, 22M, 15-30 y with orthodontic Tx of 6 mo); Grp 2: 30 pts, mild to moderate RR | Proteins | Orthodontic Tx (not specified) | IOPA | Grp 1: 30 pts, no RR | GCF | Sterile absorbent paper cones, 2-DE gels, SDS-PAGE with isoelectric focusing | Greatest sharpness to detect protein bands with Milli-Q ultrapure ice-cold water, without GCF protein extraction | Protein extraction protocols tested for accuracy |
| 2014 Rody et al. | 11 pts (7F, 4M, 10-11 y) second1 molars with RR in one quadrant; split mouth design | Proteins | No orthodontic Tx | Radiograph (not specified) | 11 pts (7F, 4M, 10-11 y), permanent 1st molar on contralateral side with no RR | GCF | Periopaper (lingual side of 1st and permanent molars), LC-MS, nanoflow LC system coupled to triple TOF 5600 MS | Total 37 RR proteins upregulated and 59 RR proteins downregulated | RR proteins upregulation and downregulation identified in RR |

Continued on next page.
| Authors                  | Experimental Subjects/Teeth (No./Age/Sex)                                      | Biomarkers Studied | Condition Analyzed          | Detection of Root Resorption | Controls/Teeth (No./Age/Sex) | Medium Studied | Technique                                                                 | Outcomes                                                                 |
|-------------------------|--------------------------------------------------------------------------------|--------------------|-----------------------------|------------------------------|-----------------------------|-----------------|---------------------------------------------------------------------------|--------------------------------------------------------------------------|
| 2015 Wahab et al.       | 19 (13F, 6M), split mouth design, either 100 g or 150 g force                | ALP, TRAP, AST     | All 4 extr and retr         | IOPA                         | Internal control (baseline) | GCF, Baseline    | Periapop (mesial/distal of Mx right and left canine), spectrophotometry  | 100 g Grp: TRAP Sig. ↑ from baseline to 3-5 wk and slight rise of ALP, AST from baseline; 150 g Grp: ALP, TRAP activities ↑ slightly from baseline, AST Sig. ↑ in 5 wk |
| 2016 Lombardo et al.    | 6 pts (5F, 1M), average age 14 y, 12 wk orthodontic Tx                        | DSP                | Radiographs (not specified) | Same pts for both methods (conventional ELISA vs DSP antibody-coated magnetic micro-beads prior to ELISA) | GCF             | Mesial and DS sites of Mx central and lateral incisors, sterile paper strips | Sig. diff between standard ELISA and micro-beads for DSP evaluation in early RR evaluation; results of micro-bead approach are more uniform and highly sensitive | Modified micro-bead approach is more reliable for early detection of RR for DSP evaluation |
| 2016 Rody et al.        | 11 pts (7F, 4M, 10-11 y), second 1st molars with RR in one quadrant          | IL-1β, IL-1RA, MMP-8, DSP, RANKL, OPG | No orthodontic Tx           | Radiograph (not specified)   | Permanent 1st molar on contralateral side with no RR | GCF             | Lingual side of 1st and permanent molars, Perpapaper, immunoassay       | NS in IL-1β, OPG, or MMP-9 between exp and control Grp; RANKL data unreliable; IL-1RA Sig. downregulated in RR |
| 2017 Yashin et al.      | 9 pts (mean age 23±2.9 y), moderate to severe RR                             | Cytokine profile in saliva | Finished orthodontic Tx within 2 y | Orthopantomograms            | Pts with no RR                | Blood and saliva | 10 mL unstimulated saliva collected by expectoration, ELISA             | Saliva: moderate to severe RR show Sig. ↑ in IL-7, IL-10, IL-12p70, and IFN-y, Sig. ↓ in IL-4; blood: control group has higher osteocalcin and P1NP than RR |
| 2017 Kaczor-Urbanowicz et al. | 48 pts with RR (31F, 17M) Grp 1: moderate to severe RR young pts (11F, 6M); Grp 2: moderate to severe RR adult pts (7F, 4M); Grp 3: mild RR young pts (7F, 4M); Grp 4: mild RR adult pts (6F, 3M) | Proteins | Not specified               | IOPA (Mx central and lateral incisors) at T0 (before bonding), T9 (9 mo after bonding) | 24 pts without RR (13F, 11M) Grp 5: control young pts (7F, 6M); Grp 6: control adult pts (6F, 5M) | Saliva          | Unstimulated whole saliva, 2D gel electrophoresis, quantitative mass spectrometry, western blot | 772 proteins identified by qM, 244 highly increased expression profile, Sig. ↑ in moderate to severe young RR Grp compared to controls and 58 proteins in the adult Grp |
| 2017 Thalanany et al.   | 20 pts, 13-22 y; exp Grp: 10 pts undergoing orthodontic Tx                   | DSP                | Radiograph (not specified)  | Control Grp: no orthodontic Tx | GCF, T0= before intrusion, T1=2 mo after intrusion | Mx right and left central and lateral incisors; microcapillary tubes, ELISA | Sig. ↑ in DSP at T1 compared to T0 | Salivary proteins associated with root resorption identified |

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| Authors          | Experimental Subjects/Teeth (No./Age/Sex) | Biomarkers Studied | Condition Analyzed | Detection of Root Resorption | Controls/Teeth (No./Age/Sex) | Medium Studied | Technique                          | Outcomes                                                                                                   | Conclusions                                                                                           |
|------------------|------------------------------------------|--------------------|--------------------|-------------------------------|------------------------------|----------------|-----------------------------------|------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------|
| 2017 Ahuja et al.  | 8 (2F, 6M, age range 13.9–22.9 y) Split mouth design: test vs control sides | IL-18, 2, 4, 5, 6, 7, 8, 10, 12, 13, INF-γ, TNF-α, GM-CSF | 225 g buccal tipping force for 28 d on test side | Micro-CT | Contralateral teeth (control side) | GCF, Time points: 0 h (prior to force), 3 h, 1 d, 3 d, 7 d, 28 d | Peripaper, multiplex bead immunoassay | IL-18: Sig. † peak at days 1 and 7 but NS between test and control side; IL-4: † peak days 1–3; INF-γ: peak at 72 h; TNF-α: † at 3 h, 28 d; IL-7 peak at 28 d; GM-CSF: immediate ↓, † at 7 d, peak at 28 d; Comparison between low and high RR: GM-CSF show Sig. † in low RR; Micro-CT: mesial, distal surface, and middle 3rd showed sig. ↑ RR on test side teeth | Pro-resorptive cytokines (IL-7, TNF-α) ↑ in high orthodontic forces, anti-resorptive cytokines (GM-CSF) ↓ initially |
| 2018 Zhou et al.  | 8F pts with RR, mean age 22.25 y, Tx duration 22.37 mo | Metabolites | Both extr and non-extr Tx | Orthopantomograms | 11F controls, mean age 24.27 y, Tx duration 21 mo | Saliva | Unstimulated saliva collected from occlusal space of right Mand molars without chewing for 3 min | 187 metabolites identified, including butyrate, propionate, lactate, α-linolenic acid (ALA), α-glucose, urea, fumarate, formate, guanine, and purine | Difference in metabolites in saliva of RR pts can be detected by 1 HNMR-based metabolomics method |
| 2020 Mohd Nasri et al. | 10 pts | Protein abundance | Mx and Mand fixed appliances | IOPA at T0 and T6 of Mx central incisors | None | GCF at T0 (pre-Tx), T1 (1 mo), T3 (3 mo), T6 (6 mo) | Mesial and Ds of Mx central incisors | Periopaper, liquid chromatography-tandem mass spectrometry | Increased protein abundance of S100A9, immunoglobulin J chain; heat shock protein 1A, immunoglobulin heavy variable 4-34 and vitronectin at T1; protein abundance of thymidine phosphorylase at T3 | Early RR protein markers identified |
| 2021 Mandour et al. | 74 pts (3 Grps: 2 Tx, 1 control) Grp 1: orthodontic pts (1-3 mm RR); Grp 2: pediatric pts (lower second 1st molars, physiologic RR) | IL-1RA, DSPP | Not specified | Radiograph (not specified) | Grp 3: control (no RR, no orthodontic Tx) | GCF | Endodontic paper points, ELISA | IL-1RA levels in controls greater than orthodontic pts, and least in pediatric Grp; DSPP levels in pediatric group higher than in orthodontic pts, and least in controls; IL-1RA cut-off for OIRR (≤432.6 pg/mL) and DSPP (≥27.33 pg/mL); DSPP reliability (100%) vs IL-1RA (80%) | IL-1RA, DSPP biomarkers for OIRR |

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|---------|------------------------------------------|--------------------|-------------------|-----------------------------|------------------------------|----------------|-----------|----------|-----------|
| 2020 Zain et al. | 7 orthodontic pts, 2 samples taken at 3 and 6 mo into orthodontic Tx and 3 samples at 12 mo of orthodontic Tx | DSPP | Fixed orthodontic Tx | Not specified | 3 non-orthodontic control samples | GCF | Mx central incisors, Periapex, absorption spectroscopy | Control sample showed lower peak in absorption spectrum than exp sample (3, 6, 12 mo); spectrum proportional to Tx duration, 0.91 accuracy | Higher absorption spectrum of DSPP indicates higher resorption |

†, greater/increase; ‡, lower/decrease; HNMR, hydrogen-1 nuclear magnetic resonance; 1º, primary; ALP, alkaline phosphatase; AST, aspartate aminotransferase; conc, concentration; CT, computed tomography; d, day(s); diff, difference; DMP1, dentin matrix protein 1; DPP, dentin phosphophoryn; Ds, distal; DSP, dentin sialoprotein; DSPP, dentin sialophosphoprotein; EARR, external apical root resorption; ELISA, enzyme-linked immunosorbent assay; exp, experimental; extr, extractions; F, female(s); GCF, gingival crevicular fluid; GM-CSF, granulocyte-macrophage colony-stimulating factor; Grp, group(s); h, hour(s); IFN-γ, interferon gamma; IL, interleukin; IL-RA, interleukin-1 receptor antagonist; IOPA, intraoral periapical radiograph; LC, liquid chromatography; LC-MS, liquid chromatography-mass spectrometry; M, male(s); maloccl, malocclusion; Mand, mandibular; min, minute(s); MMP, matrix metalloproteinase; mo, month(s); MS, mass spectrometry; Mx, maxillary; NS, no statistically significant difference; OIRR, orthodontically induced root resorption; OPG, osteoprotegerin; OPN, osteopontin; OTM, orthodontic tooth movement; P1NP, procollagen type 1 N-terminal propeptide; PP, dentin phosphophoryn (alternate abbreviation in the literature); pts, patients; qMS, quadrupole mass analyzer; RANKL, receptor activator of nuclear kappa B ligand; retr, retraction; RR, root resorption; SDS-PAGE, sodium dodecyl-sulfate polyacrylamide gel electrophoresis; Sig., significant; TNF-α, tumor necrosis factor-α; TOF, time of flight; TRAP, tartrate-resistant acid phosphatase; Tx, treatment; vs, versus; wk, week(s); y, year(s).
Biomarkers of Root-resorption in Oral Biofluids

Biomarkers such as the dentinal proteins DMP1, DPP/PP, DSP, DSPP, cytokines IL-6, RANKL, and RANKL/OPG ratio showed better GCF detection in root resorption than in controls. But IL-1RA had higher levels in the controls versus the resorption group. Studies evaluating the difference in resorption severity showed higher levels of DSP, lower levels of granulocyte-macrophage colony-stimulating factor, and higher resorption protein concentrations in severe versus mild resorption (0.89 µg/µL ±0.32 µg versus 0.77 µg/µL ±0.21 µg, respectively). Additionally, in comparison to non-resorbing teeth, mild and severe resorption showed higher RANKL/OPG ratios. Specific protein bands in saliva have also been identified in mild to moderate resorption. In physiologic root resorption of primary molars, upregulation of 37 resorption proteins was seen, as well as downregulation of 59 resorption proteins and IL-1RA levels, compared to no resorption groups in permanent molars.

A few longitudinal studies evaluated the rise or fall of biomarkers in GCF with resorption at different observation times. Kereshanan et al. mentioned the rise of DSP levels in GCF after the start of fixed orthodontic treatment compared with before treatment initiation, while Thalanany et al. showed a significant increase in DSPP after two months of intrusion. Protein abundance was also evaluated in GCF by Mohd Nasri et al. In comparison, Ahuja et al. evaluated multiple markers that peaked at different observation times: IL-1β at 1 and 7 days, IL-4 at 1 and 3 days, interferon gamma (IFN-γ) at day 3, tumor necrosis factor-α at 3 hours and at 28 days, and IL-7 and granulocyte-macrophage colony-stimulating factor (GM-CSF) at 28 days.

Two studies evaluated the efficacy of one method over another in the detection of biomarkers. Sha et al. found that DSPP detection by spectrophotometric ELISA (limit, 5.0 pg/mL) was less sensitive than electrochemical detection (limit, 0.5 pg/mL). Lombardo et al. showed a modified micro-bead approach to be better than standard ELISA for DSP detection in GCF.

However, for salivary detection, Yashin et al. showed a significant increase in IL-7, IL-10, IL-12p70, and IFN-γ, and a significant decrease in IL-4 in moderate to severe resorption compared to controls. That same study also showed lower osteocalcin in the blood for resorption compared to no resorption. Salivary proteins have been shown to vary in young and adult root resorption groups, with an increased expression of 244 proteins in the moderate-to-severe young resorption group and only 58 proteins in the adult group compared to controls. Additionally, 187 metabolites were identified by Zhou et al. in the saliva of root resorption groups compared to their no resorption group.

Cut-off values of biomarkers in orthodontic root resorption were studied by Mandour et al. at less than 432.6 pg/mL for IL-1RA and greater than 7.33 pg/mL for DSPP, with greater reliability for DSPP than IL-1RA. Additionally, Zain et al. proved that treatment duration was a contributing factor for resorption, with the absorption spectrum of DSPP rising in subjects within 3, 6, and 12 months of treatment. Studies have also evaluated changes in biomarkers in resorption associated with two different force levels (100 g and 150 g). Wahab et al. showed a statistically significant increase in TRAP levels from baseline to 3–5 weeks for 100 g force and in AST at 5 weeks for 150 g force, with the ALP group only showing a slight increase in both force levels.

**DISCUSSION**

The variation in multiple biomarkers in EARR based on the outcome measurements of severity, physiologic resorption, and orthodontic treatment versus controls, different time intervals, and methods of detection is presented in Table 3. Figure 3 presents a pictorial compilation of all biomarkers studied in this review.

Wide heterogeneity was noticed in the reviewed studies with regard to tooth selection for resorption, study settings, biomarker selection, collection, and evaluation. However, the majority of studies took measures to alleviate confounding bias in terms of inflammation caused by coexistent periodontal or gingival inflammation. Several studies ensured good oral hygiene and gingival periodontal condition by measuring probing depth, bleeding on probing, and the gingival index, since inflammation may alter the biomarker levels in biofluids. Furthermore, to rule out confounding variables for biomarker levels, many of the studies excluded patients with smoking, pregnancy, previous orthodontic treatment or systemic illness, and craniofacial disorders. A few studies also mentioned discouraging the use of antibiotics and anti-inflammatories or mouthwashes like chlorhexidine, but this was not a standard practice across all the studies.
### Table 3. Evidence-based Compilation of Biomarkers in External Apical Root Resorption.

| Biomarker Category                      | Biomarkers Studied | References to Related Studies | Specified Relative Risk Characteristics                                                                 | Outcomes Related to Characteristics                                                                                                                                                                                                   |
|-----------------------------------------|--------------------|-------------------------------|----------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Dentinal proteins                       | DSPP, DSP, DPP, DMP1 | 2, 5, 7, 13, 16, 23, 25, 29, 30 | RR severity                                                                                                                                                            | PP and DSP in severe RR (≥2 mm) greater than mild root resorption (≤2 mm)\(^7\)                                                                                                                                                    |
|                                         |                    |                               |                                                                                                                                                                        | DSP in coronal RR greater than apical RR (NS)\(^5\)                                                                                                                                                                                      |
| Physiologic relative risk               |                    |                               |                                                                                                                                                                        | DPP in primary resorbing molar greater than orthodontically treated tooth\(^13\)                                                                                                                                                         |
|                                         |                    |                               |                                                                                                                                                                        | DSP levels in physiologic RR greater than non-resorbing teeth\(^6\)                                                                                                                                                                     |
| Comparison with controls undergoing no orthodontic Tx or no relative risk               |                    |                               |                                                                                                                                                                        | DPP in orthodontically treated teeth (1-3 mm of resorption) greater than in controls (Sig. diff)\(^13\)                                                                                                                                     |
|                                         |                    |                               |                                                                                                                                                                        | DMP1, PP, DSP in RR greater than in control groups (Sig. diff)\(^7\)                                                                                                                                                                   |
| Time-related changes                    |                    |                               |                                                                                                                                                                        | DSP levels increased in GCF in 12 months of orthodontic treatment\(^5\)                                                                                                                                                                 |
|                                         |                    |                               |                                                                                                                                                                        | DSP levels increased significantly in GCF in 2 months of intrusion compared to baseline\(^5\)                                                                                                                                          |
| ELISA method for detection              |                    |                               |                                                                                                                                                                        | DSPP detection with ELISA using spectrophotometry and electrochemistry possible but NS. Lower end of detection is 10 times greater in spectrophotometry (5 pg/mL) than in electrochemical detection (0.5 pg/mL), hence latter method more sensitive\(^6\) |
|                                         |                    |                               |                                                                                                                                                                        | Modified micro-bead approach is more reliable than standard ELISA for DSP\(^23\)                                                                                                                                                           |
| Cytokines and growth factors            | IL (1β, 2, 4, 5, 6, 8, 10, 12, 13), TNF-α, IL-1RA, IFN-γ, OPG, OPN, RANKL, GM-CSF, salivary cytokine profile | 9, 12, 16, 19, 26, 29 | Severity of relative risk                                                                                                                                              | RANKL/OPG ratio in severe (≥2 mm) RR greater than in controls (Sig. diff)\(^19\)                                                                                                                                                         |
|                                         |                    |                               |                                                                                                                                                                        | Higher GM-CSF levels in low vs high RR\(^26\)                                                                                                                                                                                          |
|                                         |                    |                               |                                                                                                                                                                        | DSPP levels lower in controls vs orthodontic patients, and least in pediatric patients; Sig. diff between IL-1RA and DSPP, IL-1RA cut-off for OIRR \(≤432.6\) pg/mL, and DSPP \(≥7.33\) pg/mL; reliability of DSPP \((100%)\) vs IL-1RA \((80%)\) |                                                                                                                                                                                                                                    |
|                                         |                    |                               |                                                                                                                                                                        | TNF-α in GCF significantly increased in teeth receiving 225 g of controlled buccal tipping force, as early as 3 h and at 28 days when compared with contralateral control teeth\(^26\)                                                                 |
| Variation with orthodontic force levels |                    |                               |                                                                                                                                                                        | OPN (66 kDa), OPG (30 kDa) detected in RR; proteins detected in controls\(^19\)                                                                                                                                                         |
|                                         |                    |                               |                                                                                                                                                                        | RANKL in root resorption greater than controls\(^19\)                                                                                                                                                                                  |
|                                         |                    |                               |                                                                                                                                                                        | GCF IL-6 levels in female subjects with severe RR (>½ root) higher than without RR\(^9\)                                                                                                                                                 |
|                                         |                    |                               |                                                                                                                                                                        | Moderate to severe RR shows significantly increased IL-7, IL-10, IL-12p70, and IFN-γ vs no resorption\(^12\)                                                                         |
|                                         |                    |                               |                                                                                                                                                                        | Moderate to severe RR shows significantly decreased IL-4 vs no RR\(^12\)                                                                                                                                                               |
|                                         |                    |                               |                                                                                                                                                                        | In blood, RR has higher osteocalcin and P1NP vs no RR\(^13\)                                                                                                                                                                             |
| Comparison to controls with no orthodontic Tx or no relative risk                       |                    |                               |                                                                                                                                                                        | IL-1RA significantly downregulated in physiologic RR vs no RR\(^16\)                                                                                                                                                                  |
|                                         |                    |                               |                                                                                                                                                                        | IL-1RA levels greater in controls than in physiologic RR group\(^29\)                                                                                                           |

*Continued on next page.*
| Biomarker Category | Biomarkers Studied | References to Related Studies | Specified Relative Risk Characteristics | Outcomes Related to Characteristics |
|--------------------|--------------------|-----------------------------|----------------------------------------|-----------------------------------|
| Early detection of biomarkers | ALP, AST, TRAP, MMP-8 | 15, 16, 31 | Detection of TNF-α as early as 3 h in GCF in RR | 
| Enzymes | Variation with orthodontic force levels/type | ALP shows higher levels in 1 week upon application of continuous 150 g force compared to 100 g force and faster canine movement with no RR | Significant increase in TRAP from baseline to 3-5 weeks in 100 g force while AST increased in 5 weeks upon application of 150 g force; 100 g force as effective as 150 g force |
| Resorption proteins and metabolites | Protein profile in GCF and saliva | 20, 21, 24, 27, 28 | Comparison to controls with no orthodontic Tx or no relative risk | Higher protein bands in mild to moderate RR as compared to controls | 187 salivary metabolites identified in female RR patients compared to controls |
| Physiologic resorption | 37 RR proteins upregulated and 59 RR proteins downregulated in primary molar physiologic RR compared to teeth with no RR | In moderate-to-severe young RR group, 244 salivary proteins significantly increased and 97 decreased |
| Influence of age on relative risk and protein levels | In moderate-to-severe adult RR group, 58 salivary proteins significantly increased and 198 significantly decreased | In young mild RR group, 318 salivary proteins significantly increased and 78 decreased | In adult mild RR group, 102 salivary proteins increased, and 153 significantly decreased |
| Potential biomarker candidates | Fetuin-A and p21-ARC | Early detection of 16 proteins in GCF in mild RR patients after 1 month of orthodontic force application |

ALP, alkaline phosphatase; AST, aspartate aminotransferase; DMP1, dentin matrix protein 1; DPP, dentin phosphophoryn; DSP, dentin sialoprotein; DSPP, dentin sialophosphoprotein; ELISA, enzyme-linked immunosorbent assay; GCF, gingival crevicular fluid; GM-CSF, granulocyte-macrophage colony-stimulating factor; h, hours; IFN-γ, interferon gamma; IL, interleukin; IL-1RA, interleukin-1 receptor antagonist; MMP-8, matrix metalloproteinase-8; NS, not significant; OIIRR, orthodontically induced root resorption; OPG, osteoprotegerin; OPN, osteopontin; P1NP, procollagen type I N-terminal propeptide; p21-ARC, cyclin-dependent kinase inhibitor p21; PP, dentin phosphophoryn (alternate abbreviation in the literature); RANKL, receptor activator of nuclear kappa B ligand; RR, root resorption; Sig. diff, significant difference; Tx, treatment; TNF-α, tumor necrosis factor-α; TRAP, tartrate-resistant acid phosphatase.
Various biomarkers in the GCF were identified by this review, including dentinal proteins (DPP, DSP), cytokines (IL-6, OPG, OPN), RANKL, and enzymes (ALP, AST). A few of these were identified in the 2019 systematic review by Tarallo et al., who evaluated EARR biomarkers in GCF from seven studies after quality assessment. However, this scoping review identified additional biomarkers, including DSPP, DPP, DMP1, cytokines, and their receptor antagonists (IL-1β, 2, 4, 5, 6, 7, 8, 10, 12, 13, TNF-α, OPG, OPN, RANKL, and IL-1RA), along with resorption proteins in both the GCF and saliva. A recent review by Mona et al. evaluated protein–protein interactions of EARR biomarkers in variable study designs of human and animal studies, including case-control studies, reviews, and physiologic resorption. However, it has limited applicability in studying resorption in clinical orthodontic practice, unlike this scoping review. In light of these data, future research should include a bioinformatics analysis for the biomarkers identified by this scoping review, to ascertain the protein interactions responsible for clinical resorption overlapping other periodontal and pathological problems.

The majority of studies in the current review identified dentin-specific proteins in EARR, espe-
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especially DSPP, DSP, DPP/PP, and DMP1. Of these, the DSP and DPP proteins are the most abundant non-collagenous proteolytic cleavage products of DSPP found in dentin (5%–8% and 50%, respectively). This review also identified DSP as a potent resorption marker, both in orthodontic and physiologic resorption. It is more dentin-specific than DPP and is found in odontoblasts and the extracellular matrix of pre-dentin, dentin, and dental pulp, but is not prevalent in bone, cartilage, ameloblasts, or other oral tissue components. However, the presence of DSP and DPP in control subjects with no resorption also indicates the release of dentinal matrix proteins in the GCF from pulpal cells during root mineralization in young permanent teeth with patent apices. These dentinal matrix proteins may not be exclusively present in dentin, since both are products of a larger precursor protein, DSPP, which is also present in osteoblast cells. Osteopontin is another glycosylated protein of the dentin matrix and bone, produced by odontoblasts along with other bone precursors such as cementum and macrophages. The current review shows the presence of degraded fragments (54 kDa and 66 kDa) of OPN in the GCF of mild and severe resorption. This occurs as a result of the enzymatic activity of cysteine proteases, causing degradation of bone and the dentin extracellular matrix, which is also seen in periodontal disease. In addition, this review found that different cytokines, including pro-resorptive IL-6, show higher GCF levels in severe compared to no resorption, which is supported by rat studies showing an association of IL-6 with induction and further progress of mechanically induced root resorption. Furthermore, IL-6 has an established role in osteoclastogenesis and bone remodeling associated with orthodontic force application by inducing RANKL and osteoclasts formation. Additionally, osteoclastogenesis is governed by the RANKL/OPG ratio, as seen in the current review, where this ratio was significantly higher in severe resorption than in controls. Other clastogenic mediators (TNF-α and IL-7) also augment resorption in GCF with previous literature supporting their role in bone resorption in orthodontic tooth movement.

The orthodontic force levels, 150 g force versus 100 g force, seem to have no effect on tooth resorption. Nevertheless, 150 g force application causes a significant increase in ALP on the mesial side within one week compared to 100 g force. Alkaline phosphatase (ALP) is known to support osteoblastic activity. A similar rise in ALP was seen in previous studies at 1 to 3 weeks and at 2 weeks after orthodontic force application. The TRAP and AST enzymes also vary with the level of force. The TRAP levels showed a significant rise from baseline with 100 g force but not with 150 g force. The AST on the other hand showed a significant rise with 150 g force within 5 weeks, but not with 100 g force. Previous literature also supports a rise in TRAP proportionally with the orthodontic force magnitude, and higher AST levels at compression versus tension sites, thus favoring the resorptive activity.

This review found that salivary metabolome was associated with specific clusters of metabolites in EARR using partial least squares discriminant analysis, which may be further explored for diagnosis of resorption. These clusters include purine and arachidonic acid metabolites, known for chemotaxis of inflammatory cells as well as periodontal damage propagation/resorption. This further produces reactive oxygen species causing a shortage of local oxygen concentrations, and triggering the RANKL pathway. Thus, these metabolites may indicate resorption as well as periodontal damage, further confirming the need to ascertain periodontal health when performing such biomarker studies or examining the reciprocal effect of periodontal inflammation on these biomarkers and on resorption.

Best practices for biomarkers isolation and detection have also been highlighted by this review. While several of the reviewed studies primarily mentioned conventional ELISA, two comparative studies established the increased sensitivity of electrochemical over spectrophotometric ELISA, as well as micro-beads over conventional ELISA. These conventional and microbead assays offer several advantages: they are sensitive, non-invasive, include no radiation exposure, provide stage-wise monitoring and at-risk assessment, and can be used to diagnose and predict the clinical course of therapy. This review also found a newer non-invasive approach for non-targeted metabolomics using high-resolution nuclear magnetic resonance spectroscopy. This method can identify newer mediators or varied human disease pathways in the EARR domain, offering significant benefits by providing multi-component information simultaneously.

Hence, the current review answers our primary research question by examining the variation in levels of all biomarkers in EARR which can be isolated in the oral fluids. The resorption markers
have been studied in orthodontic treatment as well as in comparison with physiologic resorption. In addition, this review also highlights the best methods for biomarker isolation. It also mentions the study design drawbacks for consideration in future evaluations and proposes further bioinformatic analysis of identified cellular markers.

LIMITATIONS
Although the reviewed studies met all inclusion and exclusion criteria, there was an extensive heterogeneity of biomarkers, including a wide range of cytokines, dentinal proteins, receptors, and colony-stimulating factors, as well as resorptive proteins and metabolites. The study designs were also varied, mostly cross-sectional using single observation samples, although a few studies evaluated resorption longitudinally with variation in mediator levels at different time points. None of the reviewed studies performed randomization to examine the effects of variable orthodontic forces or treatments on resorption. The sample size was generally small and unequal between the experimental and control groups in the majority of studies. Other confounders were unequal male-to-female ratio, no standardization of study prerequisites related to inflammatory conditions or history of smoking, and antibiotics or anti-inflammatories, all of which may have a bearing on biomarker levels.

CONCLUSIONS
The conclusions of this scoping review may be summarized as follows:

- Several biological markers have been identified in external apical root resorption in various oral body fluids (GCF, saliva, and blood). These include dentinal proteins, cytokines, enzymes, and protein metabolites.
- Dentinal proteins (DSP, DMP1, DPP, DSPP and DPP/PP) and cytokines (IL-6, IL-1β, IL-4, TNF-α, IFN-γ, RANKL, and RANKL/OPG ratio) show significant increase, and granulocyte-macrophage colony-stimulating factor levels decrease in resorption compared to no resorption. The opposite is true for IL-1RA which is higher in controls.
- Physiologically resorbing teeth show higher DSP, DPP/PP, and DSPP and lower IL-1RA levels when compared with non-resorbing permanent teeth.
- Higher severity of resorption showed increased DSP, DPP, and RANKL/OPG ratio and higher resorption protein concentration compared to mild resorption, although the evidence is scanty.
- Salivary biomarkers show significant increase in IL-7, IL-10, IL-12p70, IFN-γ, resorption proteins, and metabolites and significant decrease in IL-4 in resorption.
- Cut-off values of biomarkers for root resorption were mentioned with IL-1RA (≤432.6 pg/mL) and DSPP (>7.33 pg/mL), but this evidence requires further validation.
- Detection of DSPP by electrochemical ELISA (limit, 0.5 pg/mL) is more sensitive than spectrophotometric ELISA (limit, 5.0 pg/mL). Furthermore, DSP detection in the GCF by modified micro-bead approach proved better than standard ELISA.

Several points for further investigation are suggested based on the findings of the current review:

- Next steps include identifying the most sensitive and specific biomarkers (dental proteins/inflammatory cytokines/metabolites) in the GCF or saliva for early-stage EARR detection, and evaluating them repeatedly during the progress of treatment. A biosensor point-of-care screening device based on the most potent biomarker to detect root resorption is also suggested.
- Cut-off levels for biomarkers need to be established, and a non-invasive clinical test developed for early diagnosis of iatrogenic resorption.
- Study designs should be standardized to generate unbiased high-quality evidence.
- Bioinformatic analysis is needed to identify the protein interactions, which may also overlap with other oral inflammatory conditions including external cervical resorption in chronic periodontitis.

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