SALIVARY BIOMARKERS IN PATIENTS UNDERGOING FIXED ORTHODONTIC TREATMENT: A SYSTEMATIC REVIEW

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ABSTRACT
Background: Saliva serves as a non-invasive, cost-effective, and convenient medium for the assessment of biochemical markers. These biomarkers have the potential to facilitate early disease detection, monitor physiological changes, and provide insights into orthodontic tooth movement, potentially preceding clinically observable changes. This systematic review aims to analyze the current literatures on salivary biomarkers in individuals undergoing fixed orthodontic treatment. Materials and Methods: A systematic literature search was conducted across PubMed, ScienceDirect, and Scopus databases. Only peer-reviewed studies involving human saliva samples from patients undergoing fixed orthodontic treatment were included. Results: From a total of 407 articles, five studies were systematically selected for this review. Sample sizes in the selected studies ranged from three to thirty participants. Thirteen salivary biomarkers were identified, primarily through mass spectrometry-based proteomic analyses and enzyme-linked immunosorbent assay (ELISA). Conclusion: Salivary proteomics represents a promising approach for monitoring therapeutic responses in patients undergoing orthodontic treatment.
Keywords: Salivary Biomarkers, Fixed Orthodontic Treatment, Orthodontic Tooth Movement

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INTRODUCTION

Orthodontic tooth movement is a complex, biologically mediated process that plays a crucial role in maintaining homeostasis within the dentofacial complex by enabling structural adaptation in response to externally applied forces. This process involves a series of cellular and molecular events that facilitate the remodeling of bone and periodontal structures, ultimately leading to the desired repositioning of teeth within the oral cavity (Li et al., 2018). The evaluation of orthodontic treatment outcomes has traditionally been centered on assessing the degree of tooth alignment and the quality of occlusion achieved following intervention. However, determining the overall effectiveness of orthodontic treatment remains a challenging task due to the multifaceted nature of tooth movement and the variability in individual patient responses (Sfondrini et al., 2020). One of the primary difficulties lies in the fact that treatment success is not always immediately quantifiable, as measurable clinical improvements may take considerable time to become evident (Allen et al., 2019). Moreover, factors such as patient compliance, biological variability, and treatment mechanics further contribute to the complexity of outcome assessment. From a theoretical perspective, orthodontic tooth movement is associated with a cascade of biochemical changes within the periodontal ligament and surrounding alveolar bone (Wise & King, 2008). Consequently, the identification and analysis of such molecular changes could provide valuable insights into the biological mechanisms underlying orthodontic treatment and may enhance the ability to predict treatment outcomes with greater accuracy.

The discovery of salivary biomarkers and their utilization as a diagnostic tool offers numerous benefits. Recent advancements in mass spectrometry techniques, such as surface-enhanced laser desorption/ionization time-of-flight MS and matrix-assisted laser desorption/ionization time-of-flight MS, have led to the identification of saliva biomarkers. This method is highly suitable due to its potential for high sensitivity (Zhang et al., 2012). Saliva is the collection of secretions from salivary glands, a proteomic approach to identify salivary biomarkers is a non-invasive, low-cost, convenient and effective approach to measure the effectiveness of orthodontic tooth movement. The simple and non-invasive nature of saliva collections allow for multiple and repetitive collections which can potentially aid in early diagnosis, measure disease progression and treatment response such as orthodontic tooth movement (Al-Tarawneh et al., 2011). As of now, there is no widely agreed upon set of salivary biomarkers for assessing orthodontic tooth movement (Allen et al., 2019).

The objective of this systematic review was to critically analyze and synthesize the updated literature on salivary biomarkers in patients undergoing fixed orthodontic treatment. Additionally, this review aimed to identify salivary biomarkers with potential applications in monitoring orthodontic tooth movement. Furthermore, it sought to examine the differential regulation of various biomarkers across distinct phases of orthodontic treatment, providing a deeper understanding of their role in the biological mechanisms underlying orthodontic tooth movement.

MATERIALS AND METHODS

The search strategy was conducted independently by two investigators to ensure methodological rigor and minimize bias. The systematic review utilized three major academic databases: PubMed, ScienceDirect, and Scopus. A comprehensive search was performed using a combination of relevant keywords, including “salivary biomarkers,” “salivary proteins,” “salivary analysis,” and “saliva proteomics,” in conjunction with terms related to orthodontics such as “orthodontic treatment,” “tooth movement,” and “orthodontic tooth movement.” Following the initial search, the abstracts of all identified articles were screened based on predefined inclusion and exclusion criteria. Studies categorized as review articles, expert opinions, case reports, letters to the editor, news articles, or those that merely described a

technique were excluded from consideration. Only studies that involved human saliva samples collected from individuals undergoing fixed orthodontic treatment, with saliva analysis conducted at multiple time points, were deemed eligible for full-text review. Furthermore, only articles published in English were included in the final selection process.
The final selection of studies was made through mutual agreement between the two primary reviewers. In cases where discrepancies arose, a third reviewer was consulted to resolve any disagreements and ensure a consensus was reached. Once selected, each article was systematically abstracted, with key information extracted, including details on the study population, methods of salivary sample collection and processing, and the techniques used for detecting salivary biomarkers.

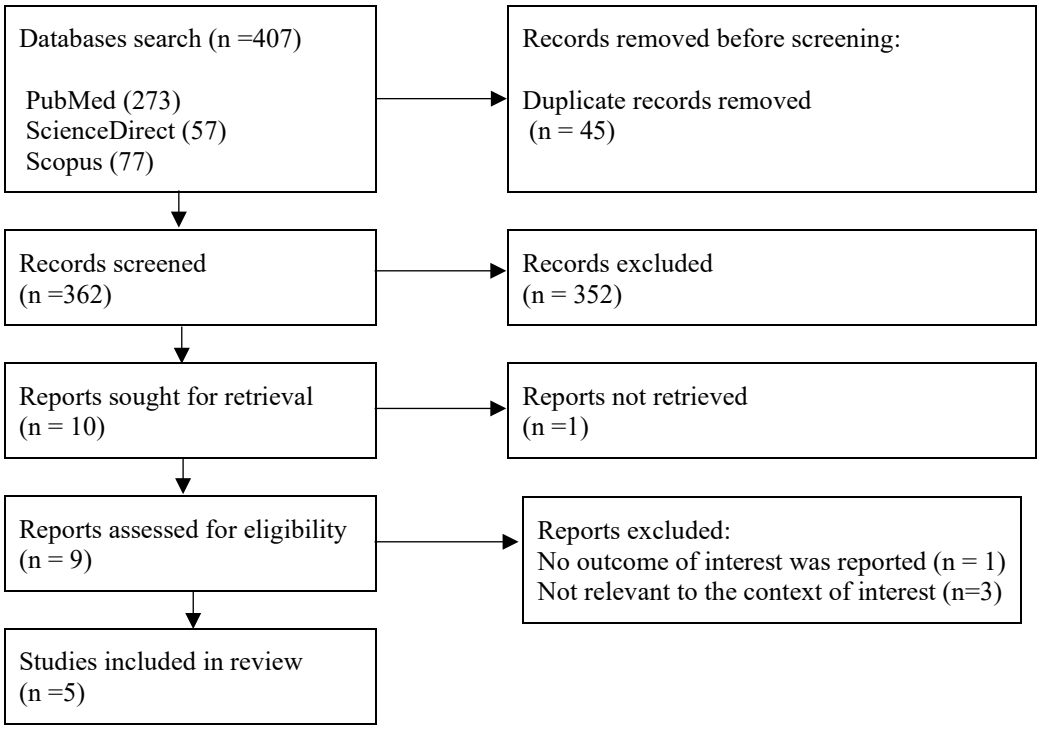

Figure 1 PRISMA Flowchart

RESULTS

A comprehensive database search initially identified 407 articles. Following the removal of duplicate records, the remaining studies were systematically screened based on predefined eligibility criteria. The primary reasons for exclusion included the absence of orthodontic treatment, non-human or in vitro study designs, lack of saliva sample collection, classification as review or opinion articles, and publications in languages other than English (Figure 1). After applying these exclusion criteria, a total of five studies met the inclusion requirements and were selected for full-text review. Key data were subsequently extracted from these studies, including sample size and demographic characteristics, saliva collection protocols, control group specifications, analytical methodologies, and the salivary biomarkers identified (Table 1, 2, 3).
The timeframe in which the five studies were undertaken spanned from 2012 to 2022. The sample size ranged from 3 to 30 participants. The age of the participant was quite vary ranging from 11 years old to 57 years old. In most studies, saliva was collected at baseline, such as prior to treatment initiation and following force activation at multiple time points. Some studies commenced saliva collection before the placement of fixed orthodontic appliances, while others began the process prior to tooth extraction. Two studies exclusively recruited female

participants, whereas the remaining three included both male and female participants. The majority of the studies analyzed collected unstimulated whole saliva, with only one study obtaining both stimulated and unstimulated saliva. The timing of saliva collection varied across the studies. Approximately three studies instructed participants to abstain from eating, drinking, or performing oral hygiene procedures prior to sample collection. In one study, saliva was collected at 2:00 PM following a 90-minute period of non-oral activity, whereas another study collected saliva at 8:30 AM, requiring participants to refrain from consuming food or beverages after dinner the previous evening and to avoid brushing their teeth on the morning of collection. Four studies specified collecting approximately 5 mL of saliva, instructing patients with fixed orthodontic appliances to expectorate into a 50-mL sterile plastic centrifuge tube, while one study did not report the volume of saliva collected. Furthermore, only one study did not employ centrifugation, whereas three studies subjected the collected saliva to immediate centrifugation. All studies preserved the saliva samples at temperatures between -80°C and -75°C for further analysis.

In this systematic review, methods for identifying salivary biomarkers were primarily categorized into two approaches. The first method, enzyme-linked immunosorbent assay (ELISA), was utilized to detect known biomarkers such as interleukin-1 beta (IL-1β), vascular endothelial growth factor (VEGF), soluble receptor activator of nuclear factor kappa B ligand (sRANKL), osteoprotegerin (OPG), deoxypyridinoline (DPD), and bone-specific alkaline phosphatase (BAP). The second method involved mass spectrometry, specifically matrix-assisted laser desorption/ionization time-of-flight tandem mass spectrometry (MALDI-TOF/TOF), which was employed in two of the studies. The trend of the salivary biomarker regulation was listed in table 3.

Table 1 Summary of studies sites, settings, sample sizes, age and gender of the participants.

Study/reference	Year	Study site	Study setting	Sample size	Age	Gender
Cevik-Aras	2021	Sweden	Orthodontic specialist clinic	N = 9	15-20	F = 9
Ellias	2012	Malaysia	Orthodontic specialist clinic	N = 3	20-25	F = 3
Flórez-Moreno	2013	Colombia	Postgraduate Orthodontic Clinics	N = 20	M (11-19) F (11-57)	M = 9 F = 11
Flórez-Moreno	2013	Colombia	Postgraduate Orthodontic Clinics	N = 22	M (11-52) F (11-57)	M = 9 F = 13
Zhang	2020	China	Orthodontic department of Peking University School	N = 30	mean age 16.07	M = 14 F = 16

Table 2 Summary of saliva types, collection time points, collection process and processing

Study/reference	Type of saliva	Saliva collection time points	Saliva collection process	Saliva processing
Cevik-Aras	Stimulated and unstimulated whole saliva	1) Before tooth extraction (Baseline) 2) Every 6-8 weeks for 2 years	No eating, drinking and brushing 1 hours before 5 ml of both stimulated of unstimulated saliva was collected	Protease inhibitor and EDTA were immediately added to saliva and stored at -80 °C No centrifugation
Ellias	Unstimulated whole saliva	1) Before fixed appliance 2) 14 days after fixed appliances application	At 2:00 pm after 90 minutes of nonoral activities expectorate for 10 mins	Protease inhibitor mix was added to the saliva. Centrifugation at 10,000 rpm for 10 min at 4 °C and stored at -80 °C
Flórez-Moreno	Unstimulated whole saliva	1) Before fixed appliance 2) 24 to 48 hours 3) 2 weeks 4) 5 weeks 5) 8 weeks	5 mL of saliva was collected before breakfast and any dental hygiene procedure.	Centrifugation for 5 min at 800 x g and stored at-75 °C
Flórez-Moreno	Unstimulated whole saliva	1) Before fixed appliance 2) 24-28 hours 3) 2 weeks 4) 5 weeks	5 mL of saliva was collected before breakfast and any dental hygiene procedure.	Centrifugation for 5 min at 800 x g and stored at-75 °C
Zhang	Unstimulated whole saliva	1) 2 months after applied fixed appliance 2) 12 months	At 8:30 am, and not to eat or drink after dinner the previous evening or to brush their teeth on the collection day morning.	Centrifugation at 9000g for 7 min at 4 °C to remove insoluble materials, at -80 °C

DISCUSSION & CONCLUSION

This systematic review encompasses more recent literature on salivary biomarkers associated with orthodontic tooth movement, distinguishing it from prior work. Furthermore, the inclusion criteria of the current review specifically exclude studies pertaining to orthognathic surgery and apical root resorption and solely focus on salivary biomarker and patient undergoing fixed orthodontic treatment.

The sample sizes in most studies were relatively small and varied. However, the collection of saliva at multiple time points enabled researchers to obtain a substantial dataset despite the limited number of participants. For example, in the study conducted by Cevik-Aras et al. although the sample size consisted of only nine participants, the saliva collection spanned a two-year period. As a result, a significant number of samples were obtained, including 134 unstimulated and 134 stimulated saliva samples (Çevik-Aras et al., 2021). This extensive data collection increased the study's reliability and validity, mitigating the limitations associated with a small sample size.

The majority of studies collected unstimulated whole saliva, with the exception of one study that analyzed both stimulated and unstimulated saliva. In terms of saliva processing protocols, most studies incorporated centrifugation, though there were variations in duration. Only one

study opted not to use centrifugation, citing concerns about protein loss (Çevik-Aras et al., 2021). However, centrifugation plays a crucial role in saliva processing, as it helps remove cellular debris and reduces turbidity, which can otherwise compromise the accuracy of saliva analysis (Mohamed et al., 2012).

All studies agreed on storing collected saliva at temperatures between -75°C and -80°C for future analysis. Notably, the saliva collection time frame varied considerably across studies. While some studies required participants to collect saliva in the morning before consuming food or beverages, one study instructed participants to expectorate in the evening (Ellias et al., 2012). According to Flink et al. unstimulated saliva flow rate follows a circadian rhythm, with lower rates observed in the morning compared to the afternoon (Flink et al., 2005). Similarly, Dawes reported that unstimulated whole saliva exhibits significant circadian variations in flow rate and in sodium and chloride concentrations, though no such patterns were observed for protein, potassium, calcium, phosphate, or urea (Dawes, 1972).

The objective of this systematic review was to analyze and identify salivary biomarkers with potential applications in monitoring orthodontic tooth movement. The enzyme-linked immunosorbent assay (ELISA) was the primary method used to detect key biomarkers, including interleukin-1 beta (IL-1β), vascular endothelial growth factor (VEGF), soluble receptor activator of nuclear factor kappa B ligand (sRANKL), osteoprotegerin (OPG), deoxypyridinoline (DPD), and bone-specific alkaline phosphatase (BAP).The findings indicated that at the initial stage of orthodontic treatment, both IL-1β and VEGF levels gradually increased, peaking significantly during the space closure phase before declining in the finishing phase without returning to baseline levels. VEGF plays a critical role in bone repair, and its deficiency may lead to impaired healing. Additionally, IL-1β, a proinflammatory cytokine, increases in response to inflammation, as bone remodeling during orthodontic tooth movement is considered a form of sterile inflammation (Çevik-Aras et al., 2021). However, elevated IL-1β levels may also result from gingivitis, emphasizing the importance of maintaining proper oral hygiene to prevent misleading results.

The study by Flórez-Moreno et al. utilized ELISA to detect sRANKL and OPG, reporting a significant increase in median sRANKL levels, while OPG levels demonstrated a notable downward trend. The sRANKL/OPG ratio showed a notable increase over time following activation visits. However, variations in salivary concentrations of sRANKL and OPG, as well as their ratio, were observed, potentially due to differences in the phases of orthodontic tooth movement (Flórez-Moreno, Isaza-Guzmán, et al., 2013). Additionally, a study using ELISA to assess DPD and BAP found that DPD levels increased following force application, whereas BAP levels showed a decreasing trend. Interestingly, DPD appeared to dominate in the early phase of orthodontic tooth movement, suggesting its potential role as a biomarker for initial bone remodeling (Flórez-Moreno, Marín-Restrepo, et al., 2013).

An alternative approach for detecting non-targeted salivary biomarkers is mass spectrometry. Two studies utilized matrix-assisted laser desorption/ionization time-of-flight tandem mass spectrometry (MALDI-TOF/TOF) for this purpose. The study conducted by Ellias et al. identified an upregulation of cysteine-rich secretory protein 3 precursor (CRISP-3). However, the relatively short 14-day interval between baseline and follow-up saliva collection posed limitations in determining its applicability across different phases of orthodontic tooth movement (Ellias et al., 2012). Notably, Zhang et al. reported an upregulation of apolipoprotein E (Apo E), a protein traditionally associated with lipid metabolism. Recent evidence suggests a potential relationship between Apo E and bone metabolism, indicating its possible relevance in the context of orthodontic research (Zhang et al., 2022).

Orthodontic tooth movement is theorized to induce distinct biochemical alterations, including changes in protein and metabolite levels, which may be detectable prior to the emergence of observable clinical signs. The identification of salivary biomarkers with the potential to

monitor these changes holds significant clinical value, as it may pave the way for personalized orthodontic treatment strategies. Such biomarkers could enable the early prediction of tooth movement progression, enhancing treatment efficiency and individualization before clinical manifestations are evident (Allen et al., 2019).

The limitation of this systematic review lies in the small number of studies included, which may constrain the generalizability of the conclusions. This limited inclusion is primarily due to the application of strict eligibility criteria, coupled with the current scarcity of research exploring the association between salivary biomarkers and orthodontic tooth movement.

For future research direction in this field, the inclusion of larger sample sizes and the adoption of more standardized protocols are recommended. As the body of literature expands, studies should aim to further investigate the relationship between salivary biomarkers and specific clinical outcomes. Ultimately, these efforts may lead to the identification of universally accepted salivary biomarkers for the prediction and monitoring of orthodontic tooth movement.

This systematic review encompassed a diverse range of studies, which introduces heterogeneity that may pose challenges in terms of practical application. Given that protein expression can fluctuate at different time points, variability among studies could impact the consistency and generalizability of the findings.

Table 3 Summary of biomarker detection methods and regulatory trends for salivary biomarkers

Study/reference	**Methods of biomarker detection**	**Biomarkers Upregulations**	**Biomarkers Downregulations**
Cevik-Aras	Sandwich ELISA	IL-1β, VEGF	
Ellias	Second-Dimension SDS-PAGE and MALDI-TOF/TOF	Cysteine-rich secretory protein 3 precursor (CRISP-3) is presented at only day 14	At day 14 1) Protein S100-A9 (S100 calcium-binding protein A9) (Calgranulin-B) 2) Serum albumin precursor 3) Immunoglobulin J chain 4) Ig alpha-1 chain C region Presented only at day 0 1) Hemoglobin subunit beta (Hemoglobin beta chain) (Beta-globin) 2) 14-3-3 protein σ (Stratifin) (Epithelial cell marker protein 1)
Flórez-Moreno	ELISA	sRANKL/OPG ratio increase significantly over time after the activation visit.	
Flórez-Moreno	ELISA	DPD	BAP
Zhang	MALDI-TOF/TOF	Apo E	

Through a proteomic approach, researchers have identified a diverse array of proteins in human saliva, offering new opportunities for the development of rapid, cost-effective, and non-

invasive diagnostic techniques. Further research is essential to substantiate the conclusions drawn from this systematic review. Furthermore, saliva serves as a valuable medium for personalized medicine. By analyzing salivary biomarkers in patients undergoing different treatments with varying outcomes, saliva proteomics presents a promising tool for monitoring therapeutic responses.

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