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EVALUATION OF MATRIX METALLOPROTEINASE-9(MMP -9) IN GINGIVAL CREVICULAR FLUID AND SALIVA IN RELATION TO ORTHODONTIC TOOTH MOVEMENT: A SYSTEMATIC REVIEW.

Orthodontics

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ABSTRACT

Background: Matrix metalloproteinase-9 (MMP-9) plays a crucial role in extracellular matrix remodeling and periodontal changes during orthodontic tooth movement (OTM). Understanding its expression in gingival crevicular fluid (GCF) and saliva can provide insights into the biological response to orthodontic forces. **Objective:** This systematic review aims to evaluate the levels of MMP-9 in GCF and saliva during orthodontic treatment, examining its correlation with different phases of tooth movement and treatment modalities. **Methods:** A comprehensive search was conducted in PubMed, Scopus, and Google Scholar following the PRISMA guidelines. Studies assessing MMP-9 levels in saliva and GCF during orthodontic treatment were included. Quality assessment was performed using the ROB-2 tool for RCTs. **Results:** The included studies demonstrated a significant increase in MMP- 9 levels following the application of orthodontic force, with peak concentrations observed at different time points. Some studies highlighted variations in MMP-9 expression between extraction and non-extraction groups, as well as differences based on age andtreatment phase. The use of low-intensity laser therapy (LILT) was found to modulate MMP-9 expression, potentially enhancing the rate of tooth movement. **Conclusion:** MMP-9 serves as a potential biomarker for monitoring biological responses during orthodontic treatment. Its level in GCF and saliva reflect periodontal remodelling and may guide personalized orthodontic approaches. Future research should focus on standardizing sampling techniques and exploring therapeutic interventions to optimize treatment outcomes.

KEYWORDS

Orthodontic tooth movement, gingival crevicular fluid, saliva, PRISMA, RCT, low-intensity laser therapy.

INTRODUCTION

Orthodontic tooth movement (OTM) involves the coordinated response of the periodontium, including the gingiva, periodontal ligament (PDL), cementum, and alveolar bone. The PDL, a vascular connective tissue, is the first to respond to orthodontic forces, initiating extracellular matrix (ECM) remodeling and alveolar bone adaptation[1]. Within hours of force application, PDL thickness changes, and inflammatory responses activate matrix metalloproteinases (MMPs), leading to collagen degradation and ECM reorganization[2] .The compression–tension theory, the most widely accepted explanation of orthodontic tooth movement(OTM), suggests that cellular responses in the periodontal ligament and alveolar bone are modulated by chemical messengers released from blood flow or cells in situ in response to mechanical stress[3].Orthodontic forces induce strain in the periodontium, creating compression and tension zones in the PDL. These zones lead to bone resorption in compression areas and bone formation in tension areas, ultimately resulting in tooth movement[4].Gingival fibroblasts also contribute to bone remodeling by regulating collagen synthesis and MMP activity [5].In the alveolar bone, osteoclasts appear within 30–40 hours at compression sites, while osteoblasts facilitate bone deposition at tension sites, ensuring balanced remodeling[6].

The desire to accelerate OTM has long been a goal for orthodontists and patients, as it could reduce treatment duration and minimize adverse effects such as pain, discomfort, periodontal diseases, dental caries, and iatrogenic damage like root resorption[7]. One of the key factors modulating OTM is the activity of MMPs, particularly MMP-9, in the gingival crevicular fluid (GCF) at the pressure side of the PDL. MMP-9, also known as gelatinase B, plays a critical role in ECM degradation, specifically in the breakdown of gelatin and type IV collagen, which are major components of basement membranes[8–10].MMP-9 is involved in various physiological and pathological processes, including tissue remodeling, wound healing, inflammation, and cancer progression[3, 11] . Itis primarily produced by polymorphonuclear leukocytes (PMNs), epithelial cells, and oral keratinocytes[12] and its expression is induced by inflammatory stimuli, cytokines, and growth factors [13]. MMP-9 activity is regulated at multiple levels, including geneexpression, secretion as an inactive proenzyme, and activation by proteolytic cleavage[3]. Tissue inhibitors of metalloproteinases (TIMPs), particularly TIMP- 1, play a crucial role in controlling MMP-9 activity by binding to both active and pro-MMP-9 [10].

In normal conditions, MMP levels are low, but their expression is elevated in inflamed tissues or those undergoing remodeling, such as during orthodontic treatment [13,14].Orthodontic forces trigger an aseptic inflammatory response characterized by vascular changes and inflammatory cell infiltration [15]. MMPs are key regulators of extracellular matrix remodeling during orthodontic treatment, with their expression being influenced by mechanical stress, biological mediators, and inhibitors. Several studies have investigated the effect of orthodontic forces on MMP production. The objective of this current systematic review is to evaluate the role of MMP-9 in gingivalcrevicular fluid (GCF) and saliva during orthodontic treatment, focusing on their regulation, site-specific expression, and potential therapeutic modulation to better understand their impact on orthodontic tooth movement (OTM).

MATERIALAND METHODS:

Protocol And Registration

This systematic review was conducted and performed according to the preferred reporting items for systematic review and meta-analysis (PRISMA) statement[16]After searching for previously registered similar studies, the Review protocol of this Systematic review was registeredregistered in Prospective Registration of Systematic Review (PROSPERO)(CRD42024609046).

Literature Search

A comprehensive electronic search was performed till December 2024for the studies published within the last 34 years (from 1990 to 2024) using the following databases: PubMed, Google Scholar and Scopus to retrieve articles in the English language. The searches in the clinical trials database, cross- referencing and grey literature were conducted using Google Scholar, Greylist and OpenGrey.

Initial search in PubMed ws done using the search strategy: "orthodontic*"[All Fields] AND ("saliva"[MeSH Terms] OR "saliva"[All Fields] OR "salivas"[All Fields] OR "saliva s"[All Fields] OR "GCF"[All Fields] OR "Gingival crevicular fluid"[All Fields]) AND ("MATRIX METALLO PROTEINASE 9"[All Fields] OR "mmp 9"[All Fields]). In addition to the electronic search, a hand search was also made, and reference lists of the selected articles werescreened. The reference lists of identified studies and relevant reviews on the subject were also scanned for possible additional studies.

Study Selection And Eligibility Criteria:

Does the level of Matrix Metalloproteinase 9 (MMP-9) in gingival crevicular fluid (GCF) and saliva change in response to orthodontic tooth movement, and how does it correlate with different phases of orthodontic treatment. Inclusion and exclusion criteria were determined (Table1). After the removal of duplicates, articles were screened based on title, abstract, and full text. When the decision on the basis of title and abstract was inconclusive, the full-text article was acquired. When an identical study was published in more than one language, the English publication was considered.

Eligibility Criteria

Inclusion Criteria

- **Population:** Human participants undergoing orthodontic treatment with fixed or removable appliances.
- **Intervention :** Assessment of MMP-9 levels in gingival crevicular fluid (GCF) and/or saliva in response to orthodontic tooth movement.
- **Comparator:** Studies comparing MMP-9 levels before, during, or after orthodontic treatment or between different phases of tooth movement.
- **Outcome:** Studies reporting quantitative changes in MMP-9 levels in GCF and/or saliva during orthodontic tooth movement.

Exclusion Criteria

Animal or in vitro studies., Individuals not undergoing orthodontic treatment. Studies assessing biomarkers other thanMMP-9. Studies evaluating MMP-9 in conditions unrelated to orthodontictooth movement. Studies without a comparative analysis of MMP-9 levels in different treatment phases.

Studies withoutspecific data on MMP-9 levels.Studies withincomplete orinsufficient dataregarding MMP-9 andorthodontic treatment. Systematic reviews, meta-analyses, editorials, and casereports. Studies published in languages other than English.

Screening Process

Following the electronic search, all citations found were compiled and uploaded to Covidence, and duplicates were removed. Both titles and abstracts were evaluated by two investigators. In the event of ambiguity, the full text was read for a joint decision. The full texts of the abstracts that were screened were obtained and evaluated for eligibility. Any disagreement about whether a study should be included was discussed between the two reviewers until a mutual agreement was reached or a third reviewer was approached.Quality assessment of included studies

The quality of included studies was assessed using ROBINS-I (Risk Of Bias In Non-randomized Studies -of Interventions) It examines seven domains: bias due to confounding, bias in selection of participants, bias in classification of interventions, bias due to deviations from intended interventions, bias due to missing data, bias in measurement of outcomes, andbias in selection of the reported result. Each domain is rated as low, moderate, serious, or critical risk, guiding the overall judgment of a study's internal validity.

Data Collection Process And Dataitems

The PRISMA flow diagram outlines the stepwise process of study selection in a systematic review. It begins with the identification phase, where records were retrieved from database searches (162 studies) and additional sources (2 studies). After removing duplicates, 127 records remain for screening. In the screening phase, titles and abstracts were reviewed, and 97 records were excluded for not being relevant to the research question. The remaining 30 full- text articles were assessed for eligibility based on predefined inclusion and exclusion criteria.

During the eligibility phase, 21 articles were excluded as they did not meet the inclusion criteria, leaving 9 studies for inclusion in the qualitative synthesis.

These final selected studies contribute to the systematic review by providingdata for further analysis. The PRISMA diagram ensures transparency in the study selection process, helping researchers maintain a standardized approach while minimizing bias in systematic reviews and meta-analyses. (Figure 1.PRISMA flow diagram).

Assessment Of Methodological Quality Of Included Studies

The risk of bias assessment using the ROBINS-I tool (Figure 2) evaluates several domains across nine studies. Most studies demonstrated a low risk of biasacross multiple domains, particularly in D3 (bias in classification of interventions), D4 (bias due to deviations from intended interventions), and D7 (bias in selection of the reported result). However, moderate riskswere noted frequently in domains such as D1 (bias due to confounding), D2 (selection of participants), D5 (missing data), and D6 (measurement of outcomes). Notably, the studies byRody et al. (2013), Bildt et al. (2009), Capelli et al. (2011), Jivrajani et al., and Alikhani et al. (2017) showed consistently low risk across almost all domains, suggesting higher methodological rigor. In contrast, Suriln et al. (2014), Xu et al. (2020), and Lin et al. (2021) exhibited moderate risk in several areas, particularly in confounding and measurement domains, leading to an overall moderate risk of bias.

The bar graph (Figure 3) provides a summary of the distribution of risk of bias across the seven domains assessed using the ROBINS-I tool, along with theoverall risk of bias. It is evident that bias due to missing data and bias in measurement of outcomes show the highest proportion of moderate risk among the domains, indicating these as the most common sources of bias across the included studies. Bias due to confounding, selection of participants, and deviations from intended interventions also exhibit a notable proportion of moderate risk, though to a slightly lesser extent. In contrast, bias in classification of interventions and bias in selection of the reported resultare predominantly rated as low risk. [33]

RESULTS:

Study Selection

The search strategy resulted in 164 articles. A total of 134 records were excluded on the basis of screening of title and abstract. The full texts of 30 articles were obtained and assessed for eligibility. Respecting all selection phases based on the eligibility criteria, 9 articles qualified for final analysis. Figure 1 displays the different steps of the selection process entered in PRISMA flow chart.Study Characteristics

From Table 2 we can conclude that the participants in the studies were generally healthy individuals with no history of periodontal disease or systemic conditions that could affect orthodontic outcomes. Age groups varied across studies, with some including adolescents [17] and others focusing on both adolescents and adults (e.g., Alikhani et al.,) [26]. Exclusion criteria commonly included the use of medications such as antibiotics, anti-inflammatory drugs, and corticosteroids, as well as smoking and pregnancy.

Intervention

Orthodontic movement was the primary intervention across all studies, with different methodologies such as canine retraction, fixed appliance placement, and comparisons between extraction and non-extraction groups. Some studies, like Jivrajani et al.[19], investigated additional interventions such as LILT to assess its effect on MMP-9 expression

and the rate of tooth movement.

Total Sample
Sample sizes varied across studies, ranging from small cohorts of 8-20 participants to larger groups of over 100 participants. Most studies had a balanced distribution of male and female participants.Mean Age Group

The mean age of participants ranged from early adolescence to adulthood. Studies such as Xu et al[19] had an average age of 21-22 years, while others like Lin et al. [21] [26]focused on younger subjects aged 12-18 years. Alikhani et al. included both adolescents (mean age 13.3 years) and adults (mean age 31 years), allowing for comparisons based on age-related differences in orthodontic responses.

Sample Collected
Of the 9 studies 7 studies collected gingival crevicular fluid (GCF) and in 2 studies saliva was collected at various time points before, during, and after orthodontic force application. Sampling schedules varied, with some studies[21] collecting GCF at multiple intervals ranging from baseline to 12 weeks post- treatment. Xu et al[19] collected saliva at four time points, including pre- and post- orthodontic appliance placement, to analyze MMP concentrations.

The results from the included studies consistently demonstrated the impact of orthodontic tooth movement on matrix metalloproteinase-9 (MMP-9) levels in gingival crevicular fluid (GCF) and saliva. Several studies confirmed a significant increase in MMP-9 levels following the application of orthodontic force, with peak concentrations varying across different time points. This indicates that MMP-9 coul d serve as a valuable biomarker for assessing the biological response to orthodontic treatment. Additionally, studies comparingextraction and non-extraction groups (Xu et al.,)[19] found that orthodontic forces modulated salivary MMP levels, reinforcing the role of these enzymes in tooth movement.

Age-related differences were another key finding across multiple studies. Alikhani et al. [26] found that adults exhibited higher levels of cytokines and osteoclastic activity in response to orthodontic forces compared to adolescents. However, despite the increased biological response, adults experienced slower tooth movement, suggesting that age influences the rate and intensity of the orthodontic response. Similarly, Rody et al. [22] observed no significant changes in MMP-9 levels over time, indicating variability in individual responses to orthodontic forces.

The effect of external interventions was also explored in some studies. Jivrajani et al. [25]investigated the impact of Low-Intensity Laser Therapy (LILT) and found that it increased MMP-9 concentrations in GCF, suggesting a potential role for LILT in enhancing orthodontic treatment outcomes.

Additionally, Capelli et al. [24] demonstrated that MMP levels were significantly modulated at the compression side of the tooth, further confirming the role of MMPs in periodontal remodeling during orthodontic movement.

The methods of sample collection varied across studies, with GCF and salivabeing the primary sources for biomarker analysis. Studies utilizing GCF sampling (Lin et al., ; Bildt et al.,) [21, 24] provided more localized insights into periodontal responses, while saliva-based studies (Xu et al.,) [18] offered a non-invasive method for monitoring systemic changes. Both methods demonstrated that MMP- 9 levels fluctuated in response to orthodontic forces, suggesting their potential use in clinical assessments.

Overall, the studies confirm that orthodontic forces induce significant biochemical changes, particularly in MMP-9 expression, which plays a critical role in periodontal remodeling. The findings underscore the importance of considering patient-specific factors such as age, intervention strategies, and biomarker sampling techniques in optimizing orthodontic treatment. Future research should focus on longitudinal studies with larger sample sizes to establish standardized protocols for monitoring orthodontic responses using MMP biomarkers.

Reason For Not Conducting A Meta-analysis
A meta-analysis was not performed due to the substantial heterogeneity across the included studies. The variations in study design, sample collection methods (gingival crevicular fluid vs. saliva), time points for MMP-9 measurement, orthodontic treatment protocols (fixed appliances, extractions, and non- extractions), and analytical techniques made it challenging to pool the datameaningfully. Additionally, the differences in statistical reporting, outcome measures, and study populations further limited the ability to generate a standardized effect estimate. Given these inconsistencies, a qualitative synthesis was deemed more appropriate to summarize the findings and provide a comprehensive overview of the role of MMP-9 in orthodontic tooth movement.

DISCUSSION:
The findings from the reviewed studies indicate that matrix metalloproteinase- 9 (MMP-9) plays a crucial role in orthodontic tooth movement (OTM) and periodontal remodeling. Several studies have shown a significant upregulation of MMP-9 in gingival crevicular fluid (GCF) and saliva following the application of orthodontic forces, suggesting its involvement in the extracellular matrix degradation necessary for tooth movement.

MMP-9 and Orthodontic Tooth Movement
The pattern of MMP-9 expression varies across studies. For instance, Surlin et al [17] observed an increase in MMP-9 levels, peaking at 8 hours and remaining elevated for up to 72 hours post-activation. Similarly, Lin et al [3] found that MMP-9 levels were significantly upregulated between 24 hours and 4 weeks after the application of orthodontic force, returning to baseline levels by 12 weeks. These findings suggest that MMP-9 is an early biomarker of orthodontic-induced tissue remodeling, potentially influencing the rate andefficiency of tooth movement.

Moreover, Xu et al[19] reported that salivary MMP-9 levels were significantly elevated in the extraction group at one hour post-orthodontic appliance fitting, highlighting a possible correlation between the magnitude of force application and MMP-9 expression. This was further supported by Sioustis et al [20], who noted a medium positive correlation between MMP-9 levels and bleeding on probing (BOP) before and after orthodontic treatment, emphasizing its role in the inflammatory response associated with orthodontic therapy.

Variability in MMP-9 Expression Across Different Study Designs
The studies included in this review exhibit variability in study design, sample collection methods, and participant characteristics. While most studies used GCF as the primary biological sample, some utilized saliva, which might have contributed to variations in reported MMP-9 concentrations. Additionally, differences in sample collection time points (e.g., immediate post-force application vs. several weeks later) further complicate direct comparisons between studies.

Rody et al [22]found no significant changes in MMP-9 levels over time, which may be attributed to differences in sample size, orthodontic force magnitude, or analytical techniques used for MMP-9 quantification.Age and MMP-9

Age-related differences emerged as a key finding in multiple studies. Alikhani et al.[26] also studied on adults and adolescents and reported that adults showed higher cytokine levels and osteoclastic activity in response to orthodontic forces than adolescents. Despite this heightened biological response, tooth movement was slower in adults, suggesting that age affects both the intensity and rate of orthodontic adaptation. Similarly, Rody et al. [22] found no significant temporal changes in MMP-9 levels, highlighting individual variability in responses to orthodontic force

Effect of external intervention and MMP-9
Interventions such as Low-Intensity Laser Therapy (LILT), as studied by Jivrajani et al., [25] have shown potential in modulating MMP-9 levels, offering a novel approach to enhancing orthodontic treatment outcomes. According to the study LILT increased the rate of orthodontic tooth movement by 38% and application of LILT increased the concentration of MMP-9 in GCF, in the initial period of orthodontic tooth movement .The changes in the application schedule for LILT were desirable as it was more effective in the earlier part of tooth movement as compared to the later part.

Site specific expression of MMP-9
According to study by Lin et al[21] The levels of MMP-9 of both tension

andpressure side of the study teeth were significantly up-regulated during first 4 weeks compared and decreased to baseline level after that and for the control teeth MMP-9 at pressure and tension side .In Another study by capelli et al [24] For MMP 9 changes over time were only statistically significant at the pressure side. There was an increase in MMP-9 levels 1 hour after activation of the orthodontic appliance, followed by a sharp decrease after 1 day of tooth movement. Thereafter, there was a steady increase in GCF content of MMP 9 until the end of the observation period.

Clinical Implications

The findings suggest that MMP-9 could serve as a potential biomarker for monitoring orthodontic treatment progression and assessing individual variations in biological response. Understanding MMP-9 dynamics may also aid in optimizing orthodontic force application to minimize treatment duration and adverse periodontal effects.

Clinical, Research, And Policy Implications

Clinical Practice:

- Clinicians can leverage MMP-9 as a potential biomarker to monitor patient- specific responses to orthodontic forces, potentially allowing for personalized treatment approaches.
- Optimizing orthodontic force magnitude based on MMP-9 expression levels may help minimize adverse effects such as excessive inflammation and periodontal breakdown.

Research:

- Future studies should focus on longitudinal research with larger sample sizes to validate MMP-9 as a reliable biomarker for orthodontic treatment monitoring.
- Standardization of sampling methods (GCF vs. saliva) and analytical techniques is necessary to ensure consistency across studies.
- Investigations into the interactions between MMP-9 and other inflammatory mediators may provide a more comprehensive understanding of its role in OTM.

Policy: Funding for research on molecular biomarkers in orthodontics should be prioritized to advance precision orthodontics and improve treatment efficiency.

CONCLUSION

This systematic review examined the expression and modulation of matrix metalloproteinase-9 (MMP-9) in gingival crevicular fluid (GCF) and saliva during orthodontic treatment. Findings showed that MMP-9 levels rise significantly in response to orthodontic force, especially in the early stages of tooth movement, highlighting its role in periodontal remodeling. Higher levels were noted on the pressure side of moving teeth, indicating site-specific expression.

Therapeutic interventions like low-intensity laser therapy enhanced MMP-9 activity, and age-related differences suggested greater biological response but slower tooth movement in adults. Despite some variability across studies, MMP-9 appears to be a promising biomarker for monitoring and potentially enhancing orthodontic outcomes. Future studies should focus on standardizing methods and exploring MMP-9–based therapeutic strategies.

The reviewed studies have several limitations. There is notable heterogeneity in methodologies, including variations in sample collection, participant selection, and MMP-9 measurement, leading to inconsistent findings. Many studies also suffer from small sample sizes, limiting the generalizability of results. Additionally, short follow-up durations hinder the understanding of long-term changes in MMP-9 levels and their influence on treatment outcomes.

Legends To Illustrations And Tables –

A) Illustrations

Figure 1: Prisma Flow Diagram For The Selection Of Studies
Figure 2: Risk of Bias of the included studies
Figure 3: Overall summarative risk of bias of the studies included in the present systematic review

B) Tables

Table 1: Search Strategy Of Different Databases
Table 2: Demographic Data Of The Nine Included Studies In The Current Systematic Review

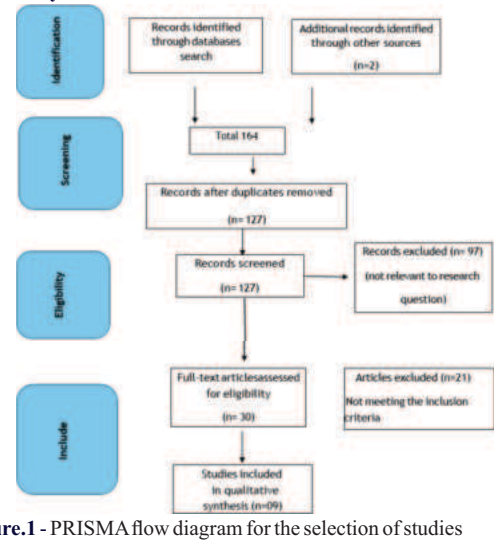

Figure.1 - PRISMA flow diagram for the selection of studies

Table 1: Search Strategy Of Different Databases

Database	Search strategy	Results
Pubmed	"orthodontic*"[All Fields] AND ("saliva"[MeSH Terms] OR "saliva"[All Fields] OR "salivas"[All Fields] OR "saliva s"[All Fields] OR "GCF"[All Fields] OR "Gingival crevicular fluid"[All Fields]) AND ("MATRIX METALLO PROTEINASE 9"[All Fields] OR "mmp 9"[All Fields])	87
Scopus	TITLE-ABS-KEY (orthodontic*) AND (saliva OR salivas OR "saliva s" OR GCF OR "gingival crevicular fluid") AND ("matrix metalloproteinase 9" OR "mmp 9")	22
Google scolar	"orthodontic" OR "orthodontics" AND ("saliva" OR "salivas" OR "gingival crevicular fluid" OR "GCF") AND ("matrix metalloproteinase 9" OR "MMP-9")	53

Table 2– Demographic Data Of The Nine Included Studies In The Current Systematic Review

Author, Year	Study design	Characteristics of the patient	Intervention	Total sample	Mean age group	Sample collected	Conclusion
Bildt et la, 2009[23]	Controlled trials in which MMP- 9 level is estimated in gcf collected from resorption side and apposition side side of teeth which were orthodontically moved and around control teeth ..	Eight orthodontic patients .The following inclusion criteria were applied: no use of medication, good periodontal health .	All 8 patients had fixed appliances with superelastic nickel-titanium coil springs (GAC international Inc., Bohemia, New York, USA) that gave light continuous forces of approximately 150 cN.	Eight orthodontic patients [two males, six females]	mean 13.6 ± 2.6 years)	GCF was collected at the apposit ion and resorption sides of teeth that were orthodontically moved and around control teeth. The type and number of teeth differed between the patie nts depen ding on the treatment plan. In most cases, bo th upper first premolars were distalized.	More active MMP- 9 was present at the resorption side of orthodontically moved teeth compared with the control (*P* = 0.003)

capelli et al, 2011(24)	Controlled trial in which MMP 9 level was estimated from gcf collected from pressure and tension side of maxillary canin es during retraction after first premolar extraction.	Fourteen subjects (three males and 11 females, 18.8 ± 4.8 years of age; range from 12 to 28 years) had their maxillary canines retracted Participants requiring maxillary first premolar extractions as part of their orthodontic treatment were enrolled in this study. Exclusion criteria were autoimmune diseases, pregnancy, lactation, and use of any medication that could interfere with orthodontic tooth movement.	Retraction of canines after the premolar extraction using niti springs in archwire with 150 g force	Fourteen subjec ts (three males and 11 females)	18.8 ± 4.8	Samples of GCF were collected from the mesial (tension) and distal (pressure) sides of the maxillary canines of each subject at seven time points. The first collec tion took place 7 days before the orthodo ntic force was first applied (−7 days), the second was undertaken on the day of force application, denominated time 0, and third, 1 hour, the fourth, 24 hours, the fifth, two weeks, the sixth, three weeks (21 days), and the Seventh,80 days after orthodonticforce was first applied	For MMP 9 changes over time were only statistical ly significant at the pressure side $P <$ 0.01). There was an increase in Mmp9 levels 1 hour after activation of the orthodont ic appliance, followed by a sharp decrease after 1 day of tooth movement.Thereafter, there was a steady increase in GCF content of MMP 9 until the end of the observation period.
Rody et al, 2013(22)	Controlled trial in which MMP-9 levels were estimated from GCF collected from the upper incisors (experimental sites) and lower incisors (control sites) of ten adolescents and 10 adults at five time points. .	Ten adolescents (14.4 6 1.43) and 10 adults (28.5 6 7.83) with Class I malocclusions and minor upper incisor crowding were allocated to two different age groups.	Orthodontic movement(Brac kets were bonded only in the upper arch over the 20-week period of the experiment.)	20 patients	Ten adolescents (14.4 6 1.43) and 10 adults (28.56 7.83)	Samples of GCF were collected from the upper incisors (experimental sites) and lower incis ors (control sites) of each subject at five time points. G CF samplin g was per formed acco rding to the follow ing sched ule: T0 (baselin e), immedia tely before bonding;T1, 3 weeks after applian ce placemen t and inserti on of the first alignin g archwire: T2, 6 weeks after inserti on of the first archwir e: T3, 6 weeks after inserti on of the third arch wire: T4, 2 weeks after the last aligning archwire	There were no statistical ly signifi cant chan ges over time in the conce ntration of MMP-9between experimental and control teeth (P . .05).Also, there were no statistical ly signific ant differences in the concentration of MMP-9 collected from experimental teeth between adult and adolescents
Surlin et al, 2014(17)	Split mouth study in which canine distalisation was done on experimental side using laceback appliance and no force applied on contra lateral side. MMP- 9 level was checked at different intervals from both sides	The pati ents were enro lled according to the foll owing criteria: (1) patien ts in gene ral good health, (2) with a good oral hygiene, (3) with clinically and radio logical healthy periodontal tissues, (4) no therapy with antib iotics in the last 3 months, and (5) no use of anti- infla mmatory drugs in the previo us 30 days.	GCF samples were collected from 16 orthodontic young patients requiring upper canine distalization done with laceback appliance placed on experimental side and no force was applied on control side .	16 (10 females an d 6 males)	age range 13 to 17 years, me an age 14 ± 1.67 years	, GCF samples were collected from distal side of an upp er cani ne requiring distalization on which was lace dalace back app liance, to assess total MMP9 in GCF 1h befo re and after 1, 4, 8, 24, and 72 hours, 1 and 2 weeks from the tooth which is consid ered as test tooth (TT) and also from the contrala teral canine considered as control tooth (CT) on which no force was applied.	The results obtained for the Test teeth showed an increase of GCF levels of MMP9 from 1 hour before the orthodontic appliance(−1 h as baseline level) t o a maximum at 8 hours (approximately 1.6- fold compared to the baseline level), followed by a decrease reaching the value of baseline at 72 hours and stabilization starting with first week at levels higher than baseline. For control teeth levels of mmp9 was similar to the base line level of test teeth
Alikhani et al, 2017(26)	Controlled trials in which MMP- 9 level was estimated from Gcf collected from canine at differe nt intervals which were retracted using Niti closed coil spring of	18 health y subject s, adoles cents(11-14 years) and adult s (21-45 years), with Clas s II Divis ion 1 ma locclusi on requir ing 4 first premol ar extrac tions. Inc lusion criteria: Age ran ge, 11-14	Canines were retracted with a constant force of 50 cN. One canine was rando mly selected from each subject.	18 healt hy subjects	Adolescents: 13.3 6 0.9 Adults: 31 6 5.5	Gingival crevicular fluid was collected before orthodontic treatment and at days 1, 7, 14, and 28 after the canine retraction.	When compared with day 0, MMP- 9 concentrations increased significantly (P\0.05) 1, 7, and 14 days after canine retraction in both adults (6.6- , 5.5-, and 4.8- fold, respectively) and adolescents (3.6-,2.9-,

	force 50 Cn from adolesclents and adults.	or 21-45 years Ex clusion criteria: Long-term use (6 month before study enr ollment) of antib iotics, phenytoi n, cyclo sporin, anti-infla mmatory drugs, systemic corticosteroids, and calci um chan nel block ers.					and 2.7-fold, respectively). The increase at all 3 time points were significantly higher in adults than in adolescents (P\0.05). At day 28,MMP-9 Concentrations decreased to day 0 levels in both age groups (p 0.05)
Xu et al, [2020] (19)	Controlled trial In which MMP- 9 levels of saliva in extraction and non extraction group were compared at different time points of orthodontic tooth movement	In total, 11 patients who needed tooth extraction and 11 patients who didn't before being subjected to fixed orthodon tic appliance therapy were assigned into two groups: The extraction group and the non-extraction group. The subjects were required to be in good general health and had no periodontal disease or a history of treatment mune diseases, pregnancy, lactation, or use of any medication that could interfere with orthodont ic tooth move ment (e.g. antibiotics, antihistamines, cortisone, and hormones) within a month preceding the beginning of the study were excluded.	Two groups, extraction and non extraction groups	22 patients	Non-extraction grou p: 21.0±3.3 Extraction grou p: 22.7±2.8	The saliva samples were collected from each patient at four indicated time points, including the first visit time (T1), time before fitting the orthodontic appliance (T2), one hour after (T3) and eight weeks after fitting the appliance (T4). Salivary MMPs concentration was determined using multiplexed bead immunoassay.	In the non-extraction group, the concentration of MMP-9 were significantly increased at T3. In the extraction group, the concentration of MMP-9 increased during the orthodontic tooth movement process, reaching a peak at T3
Sagar J. Jivrajani, Wasundh ara A. Bh ad(Patil) (2020)	A rando mized controlled study in which Split mouth study was done in patients who req uired first premol ar extraction to estimate MMP - 9 level in gcf which was collected from canine of experimental side and control side at different points of canine retraction using coil spring using 150 gm force.	Ten patients (3males and 7 females) who req uired ma xillary first pre molar ex traction for rout ine orth odontic treatment were recruited. Patien ts with systemic illness, those under systemic medications, pati ents wit h impact ed canin es or can ines hav ing dilac erated roots were excluded from the study	The individual canine retraction was studied, and the side of the experimental canine was randomly selected. The laser regimen was followed on the 1st, 3rd, 5th, 7th, 14th and then 15th days consecutively. GCF was collected at baseline, 14th day, 3 months and at the end of canine retraction on experimental side	Ten patients (3 males and 7 females)	(14–24 years).	GCF was collected at basel ine, 14th day, 3 months and at the end of canine retraction on experimental side and MMP-9 was estimated quantitatively using a standa rd ELISA kit.	After three months of canine retraction and until the completion of treatment (T1-T2) the change in marker expres-sion on experimental side was 0.013 ng/ml while on control side it was 0.51 ng/ml. The difference was statistically insignif- icant. The mean change in MMP-9 throughout the canine retraction on experimental side was 11.09 ng/mL while on control side it was 10.13 ng/ml and the difference was statistically insignificant
Sioustis et al [2021](20)	Clinical trial in which saliva ry MMP 9 level changes was assessed at different points on patients who received fixed orthodontic treatment .	The inclusion criteria: 111 patient s who were about to receive fixed ortho dontic treatment .patients in generally good healt h who wer e about to receive fixed-appli ance treatm ent and had a healthy periodontal status. The exclusion criteria were diagn osis of peri odontal disease or a history of treatment, immune disease, systemic disease, smoking, pregnancy, lactation, and use of any medication that could interfere with OTM (antihistamines, cortis one, and hormones)	Orthodontic treatment was performed with a Roth prescription 0.022 bracket slot appliance .	111 patients aged between 18 and 39 years	25.5 _ 5.4 years	Saliva sampling. Unstimulated saliva was collected before placement of orthodontic appliances (T1), after the placement of orthodontic appliances but before periodontal therapy (T2), and during orthodontic treatment, one month after applying the periodontal treatment (T3).	For salivary MMP-9 levels, the highest values were observed at T2, with a mean of 1.89 ± 1.82 ng/mL, and the lowest value was observed at T1, with a mean of 0.45±0.48 ng/mL . The mean MMP-9 level at T3 was significantly lower than that at T2 (p- value < 0.01),	
Lin et al, 2021[21]	Clinical trial in which MMP- 9 level was estimated from Gcf was collected from	This study recruited 18 healthy subjects (12–18 years old, 8 males, 10 females) undergoing OTM. The subjects had full	Orthodontic brackets (0.022×0.028-inch slot) were used. Then, an archwire (0.014-	18 healt hy subjects undergoing OTM	(12– 18 years old, 8 males, 10 females)	Gingival crevicular fluid (GCF) sampling. The collection time points were as	The levels of MMP-9 of both tension and pressure side of the study teeth at T2-T4 were	None

	both pressure and tension side of both study teeth (lower incisors) and control teeth (upper incisors)	permanent dentition and their Little Irreg ularity ind ex was bet ween 3 and 5 mm in the anterior segment of the lower arch,which allowed for continuous archwire for alignm ent and leveling. The exclus ion criteria were autoi mmune diseases, deep overb ite, caries or restorat ions in the anterior teeth and the use of any drugs (such as antibiotics, antihistamines, cortiso ne and hormones) that might interfere with OTM in the first six months of the study	inch Nickel-Titanium) was inserted into the brackets of the lower teeth and bonded with modul ar elastics. For the upper arch, only modul ar elastics were placed. The lower incisors were the selected study teeth and the upper incisors were selected as control teeth.			follows: the day of application (T0), one hour (T1), 24 hours (T2), one week (T3), 4 weeks (T4) and 12 weeks (T5) after the application of orthodontic force.	significantly up-regulated compared with that of T0 and T1 and decreased to baseline level at T5.for the control teeth cytokines at pressure and tension side were not significantly different at different times .	

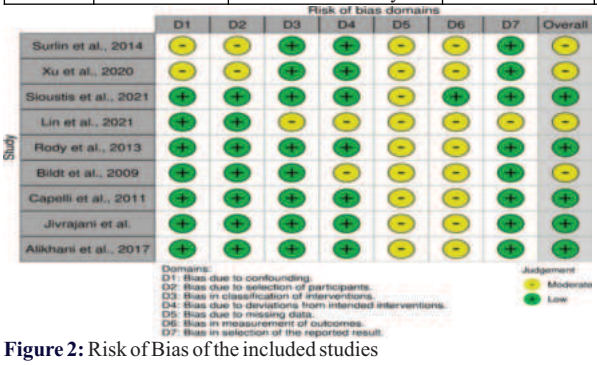

Figure 2: Risk of Bias of the included studies

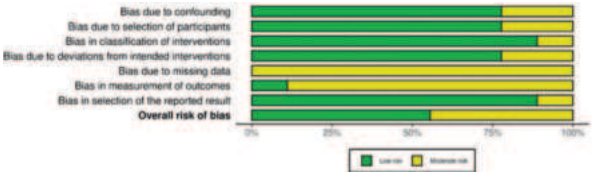

Figure 3: Overall summarative risk of bias of the studies included in the present systematic review

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