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# مجلة ميسان للدراسات الاكاديمية

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# Diagnostic potential of salivary MMP-9 to differentiate between periodontal health and disease in smokers and non-smokers

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**Abstract:**

The drawbacks of the traditional methods for detecting and managing periodontal disease motivated researchers to look for new ways to predict, diagnose, and monitor this condition. Using the biomarkers present in oral fluids could be a groundbreaking alternative to the manual probing/radiographic diagnosis. Several salivary biomarkers can accurately distinguish between periodontal health and disease. The aim of this study was to evaluate the diagnostic accuracy of salivary Matrix Metalloproteinase-9 (MMP-9) for differentiating healthy and diseased periodontal tissues in smokers and non-smokers. The study involved 175 participants who were classified into five equal groups: 1) healthy controls, 2) non-smoker gingivitis, 3) smoker gingivitis, 4) non-smoker periodontitis and 5) smoker periodontitis patients. Periodontal parameters including plaque index (PI), bleeding on probing (BOP), probing pocket depth (PPD), and clinical attachment level (CAL) were recorded for each patient. The salivary MMP-9 concentration was ascertained using an ELISA test. The diagnostic capabilities of MMP-9 were evaluated using the Receiver Operating Characteristic (ROC) curve and the Area Under the Curve (AUC). Salivary MMP-9 was significantly higher in patients with gingivitis and periodontitis compared to healthy individuals. The ROC analysis revealed that salivary MMP-9 had a high sensitivity and specificity to distinguish between periodontal health and both gingivitis and periodontitis regardless of whether the patient smoked or not. salivary MMP-9 demonstrated high sensitivity and specificity in distinguishing between healthy and diseased periodontal conditions. The level of accuracy of MMP-9 was also sufficient regardless of smoking status.

**Keywords:** biomarkers, periodontal diseases, MMP-9, smoking, saliva.

## 1 | INTRODUCTION:

A biomarker is a feature that can be measured to indicate the normal or abnormal functioning of biological systems, or their reactions to external factors or treatments (Group, 2016) Biomarkers have shown great potential in diagnosing and tracking different diseases.(Maher Abdulazeez Nsaif, 2023, Yosuf, 2019, Abdul-Mounther et al., 2017).

Periodontitis is mainly caused by an imbalance in the dental plaque biofilm that favors harmful bacteria.(Papapanou et al., 2018) In the body, the immune system reacts to infection by delivering immune cells to the nearby periodontal tissues.(Tsukasaki, 2021) These cells release various substances that cause inflammation, tissue damage and bone loss.(Hienz et al., 2015) Researchers have found that these substances are much higher in oral fluids during periodontal disease than in health, which makes them useful as biomarkers for predicting, diagnosing, and monitoring the condition.(Gul et al., 2020, Salim and Diajil, 2022, Mohammed et al., 2022).

Matrix Metalloproteinase-9 (MMP-9) which is also called gelatinase B, is one of the key gelatinases that functions as a proteolytic enzyme degrading collagen and gelatin and the extracellular matrix proteins during periodontal disease associated inflammation.(Kim et al., 2014, Chen et al., 2013) This enzyme is responsible for degrading type (IV) collagen, a central constituent of the basal membrane structure. The MMP-9 is secreted as a latent form until its activation by other collagenases (stromelysin-1, Matrix Metalloproteinase-8).(Jotwani et al., 2010) Other activities of MMP-9 besides collagen lysis are angiogenesis, bone and connective tissue formation.(Amălinei et al., 2007) The MMP-9 also plays a vital part in osteoclastogenesis process by helping the movement of osteoclasts and their stem cells to the site of bone destruction.(Xie et al., 2009)

High expression of MMP-9 has been detected in the gingival tissue of periodontitis cases.(Şurlin et al., 2014) Also, MMP-9 was believed to be the key proteinase responsible for bone destructive process because osteoclasts expression of this proteinase was exceedingly high.(Somerville et al., 2003, Delaissé et al., 2003) Smoking has been identified as a risk factor that can trigger an inflammatory response, which in turn can lead to periodontal disease.(Al-Taweel et al., 2019) Consequently, it's important to consider the disruptions smoking can cause when interpreting the results of potential salivary diagnostic tests. It has been reported that salivary MMP-9 levels are primarily impacted in individuals who are currently smoking or have quit smoking within the past year.(Lahdentausta et al., 2019)

The purpose of this research was to find out how accurate and reliable salivary MMP-9 was in distinguishing between healthy and diseased periodontium in people who smoke and who do not smoke.

## 2 | MATERIALS AND METHODS:

### 2.1 | Study design:

an observational (case-control) study, which was carried out at the department of periodontics, Collage of Dentistry, University of Baghdad. This study's methods were all in accordance with the Helsinki Declaration and any later revisions made to it for human research. The protocol has received approval from the Ethics committee in College of Dentistry, University of Baghdad. Before participating in the study, each patient was required to sign an informed consent form that fully explained the nature and goals of the study.

The study divided the participants into five groups based on their periodontal health and smoking status: healthy periodontium (controls), non-smokers with gingivitis, smokers with gingivitis, non-smokers with periodontitis, and smokers with periodontitis. In details, the case definitions were; Gingival health (control group) was <10% bleeding sites, probing depths ≤3 mm with no clinical attachment loss.(Chapple et al., 2018) Gingivitis was ≥10% bleeding sites, with probing depths ≤3 mm with no clinical attachment loss.(Chapple et al., 2018) All cases of gingivitis were generalized gingivitis in which ≥30% bleeding sites.(Chapple et al., 2018) Periodontitis cases were diagnosed when there was detectable interdental clinical attachment loss (CAL) at two or more non-adjacent teeth, or when a buccal or oral CAL of 3 mm or more, along with pocketing greater than 3 mm, is observable at two or more teeth.(Tonetti et al., 2018) periodontitis cases were unstable (PPD ≥ 5mm or PPD at ≥ 4mm and BOP), generalized (more than 30% of teeth included in attachment


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loss).(Tonetti et al., 2018) For the smoking status, as defined by The Centers for Disease Control and Prevention (CDC); non-smokers were subjects who had never smoked, or not currently smoke but may had smoked fewer than 100 cigarettes throughout their life. While, People who have smoked 100 cigarettes or more in their lifetime and at the time of their participation in the study were considered current smokers. All smoker subjects regularly smoke a minimum of 10 cigarettes per day for more than 5 years.

**2.2 | Inclusion and exclusion criteria:**

Participants in this study had to be systemically healthy, have at least 20 teeth, and have not taken any drugs within the last three month. Patient with systemic diseases, patient who had previous or were receiving periodontal treatment at time of participating in the study, patient who had received antibiotics or immunosuppressants within the previous three months of their participation in the study, patient presented with necrotizing ulcerative gingivitis, aphthous ulcer or any lesion not related to the disease, alcoholic patient, patients had received orthodontic, dental implant, crown and bridge or partial dentures, pregnant or lactating mothers and women taking contraceptive pills were excluded.

**2.3 | Periodontal parameters and clinical examination:**

Full mouth plaque index (PI), bleeding on probing (BOP), probing pocket depth (PPD), and clinical attachment loss (CAL) were measured for all of the existing dentition. Examination of full mouth was done using a periodontal probe (Michigan O probe) with marking at (1,2,3,5,7,8,9 and 10) mm at six sites for each tooth (mesiobuccal, buccal, distobuccal, mesiolingual, lingual, distolingual), except for plaque scores where four surfaces were examined (mesial, buccal, distal, lingual). The clinical examination did not include wisdom teeth.

**2.4 | Collection of saliva samples:**

Before salivary sample collection, the participants were instructed to fast for one hour. All participants were instructed to properly rinse their mouths thoroughly with water in order to eliminate any remnants or polluted substances prior to the collection of samples. The whole-unstimulated saliva was then collected and placed in sterile tubes. To separate the salivary supernatants from the cellular debris, the collected samples were centrifuged for 15 minutes at 1000 rpm using an 80-1 Electronic Centrifuge. Using a micropipette, 500 µL of the clear salivary supernatants were transferred into a plastic Eppendorf tube that included 50 µL of a protease inhibitor enzyme solution. In order to preserve it for analysis, the Eppendorf tube was marked and frozen at -20°C.

**2.5 | Enzyme-linked immunosorbent assays (ELISA):**

All samples were defrosted and given some time to reach to room temperature. Protein levels in salivary MMP-9 were assessed Using commercially available enzyme-linked immunosorbent assay (ELISA) kits (SunLong Biotech Co., China). The analysis was carried out in accordance with each kit's manufacturer's instructions. Optical density (OD) was measured with a Microtiter plate reader.

**2.6 | Statistical analysis:**

For categorical variables, frequency and percentage were utilized, while descriptive statistics for continuous data included mean, standard deviation, and median. Using the Shapiro-Wilk test, the distribution of the data was examined. An analysis of variance test was employed for continuous parametric variables, followed by post hoc analysis. To find out how the levels of salivary biomarkers were related, the Pearson’s correlation test was applied. Using the receiver operating characteristic (ROC) curve and area under the curve (AUC), the biomarkers' sensitivity and specificity were examined. GraphPad Prism software (version 9.0) was used for all analyses. To determine if there were significant differences between groups, a p-value of less than 0.05 was required.


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**3 | RESULTS:**

The study included 175 participants who were assigned to five groups based on their periodontal status and smoking habits. These groups were healthy periodontium (control, n = 35), gingivitis without smoking (n = 35), gingivitis with smoking (n = 35), periodontitis without smoking (n = 35), and periodontitis with smoking (n = 35). The sex and mean age distribution of the participants are shown in figure (1), and the clinical periodontal parameters for each group are shown in figure (2).

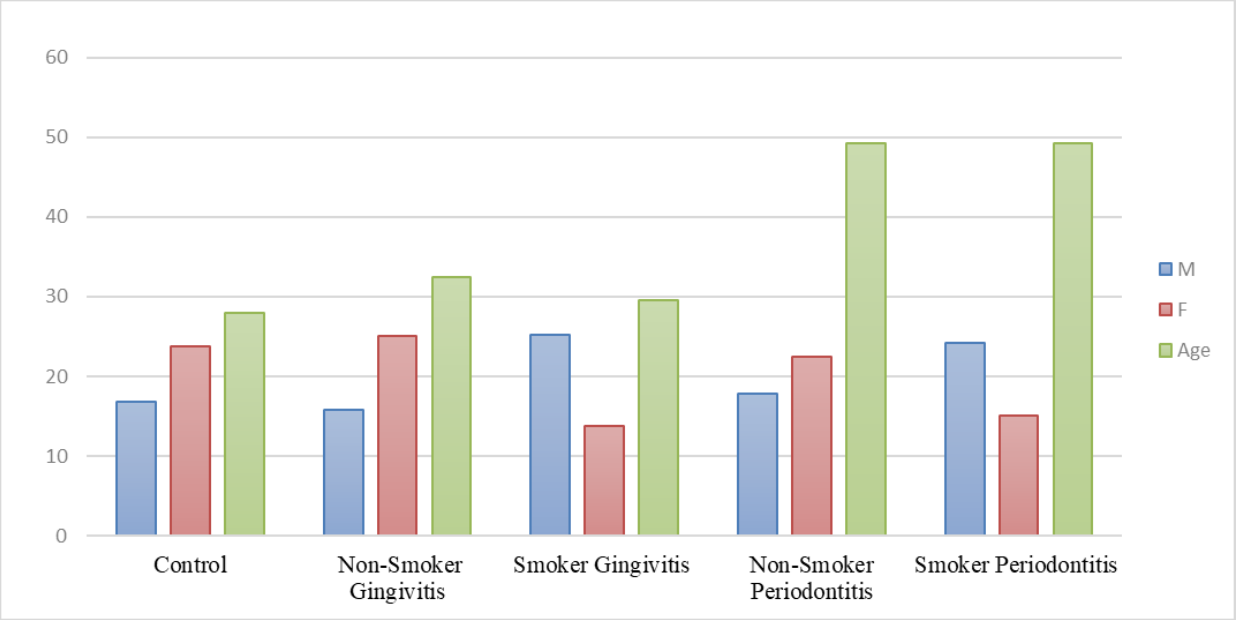


FIGURE 1: Demographic data of the study groups.

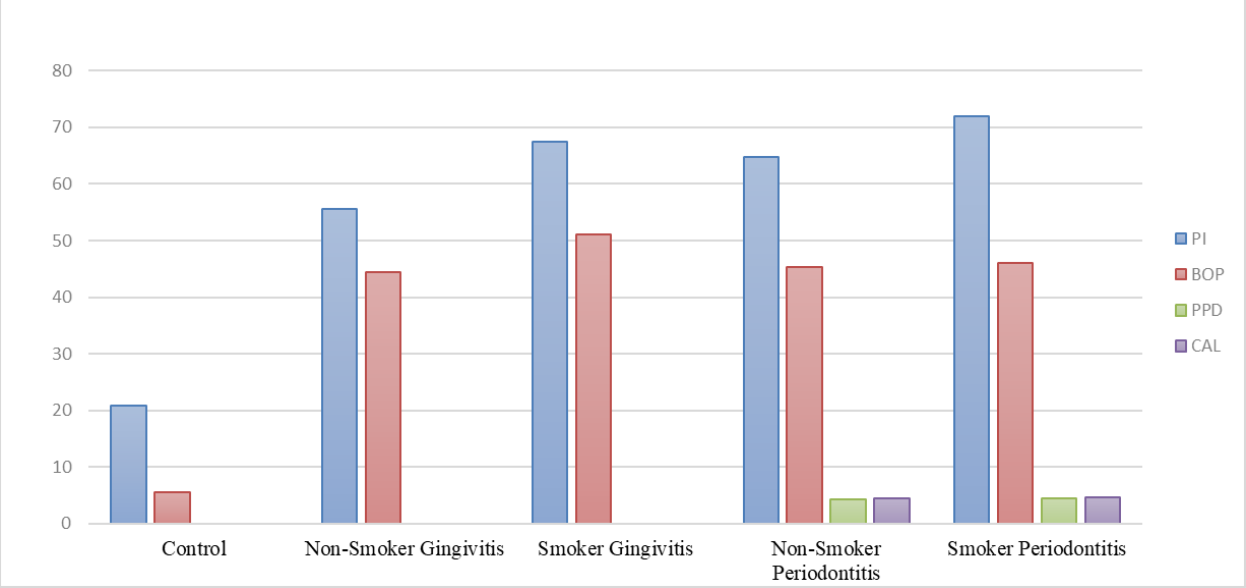


FIGURE : Clinical periodontal parameters of the study groups.


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The results of the biochemical tests showed that salivary MMP-9 was significantly higher in the groups with gingivitis and periodontitis than in the control group. Smokers with periodontitis had significantly higher MMP-9 in their saliva than non-smokers with the same condition (Figure 3).

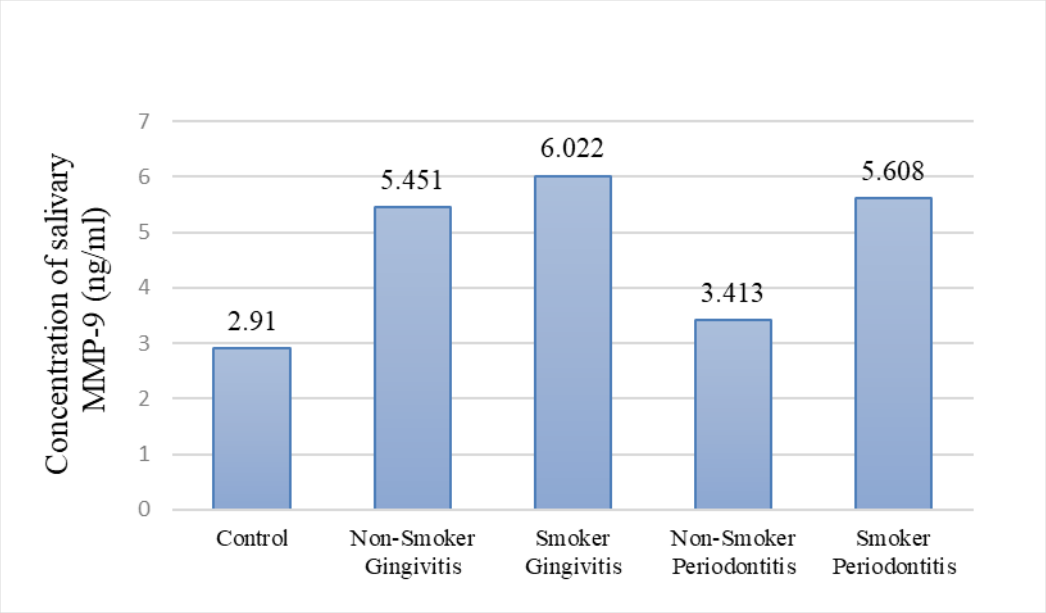


FIGURE 3: Salivary concentrations of Matrix Metalloproteinase-9 (MMP-9).

The diagnostic accuracy of the chosen salivary biomarker was assessed by ROC curve and AUC (Figure 4). MMP-9 and its ratio had high sensitivity and specificity to distinguish healthy periodontal tissues from those with gingivitis and periodontitis. The same was true for separating healthy periodontal tissues from smokers with gingivitis and periodontitis, as shown in Table 1.

Correlation analyses showed a positive and significant relation between periodontal parameters (PL, BOP and CAL) and MMP-9. (Table 2).

TABLE 1 Area under the curve (AUC), sensitivity, specificity, and cut-off values for all groups.

| | AUC | P value | Optimal cut | % Sensitivity | % Specificity |
|---|---|---|---|---|---|
| Control vs Non-Smoker Gingivitis | 1.000 | > **0.001** | 1.915 | 100 | 97.1 |
| Control vs Smoker Gingivitis | 0.980 | > **0.001** | 2.372 | 100 | 94.3 |
| Control vs Non-Smoker Periodontitis | 0.803 | > **0.001** | 2.599 | 91.4 | 85.7 |
| Control vs Smoker Periodontitis | 1.000 | > **0.001** | 1.915 | 100 | 97.1 |


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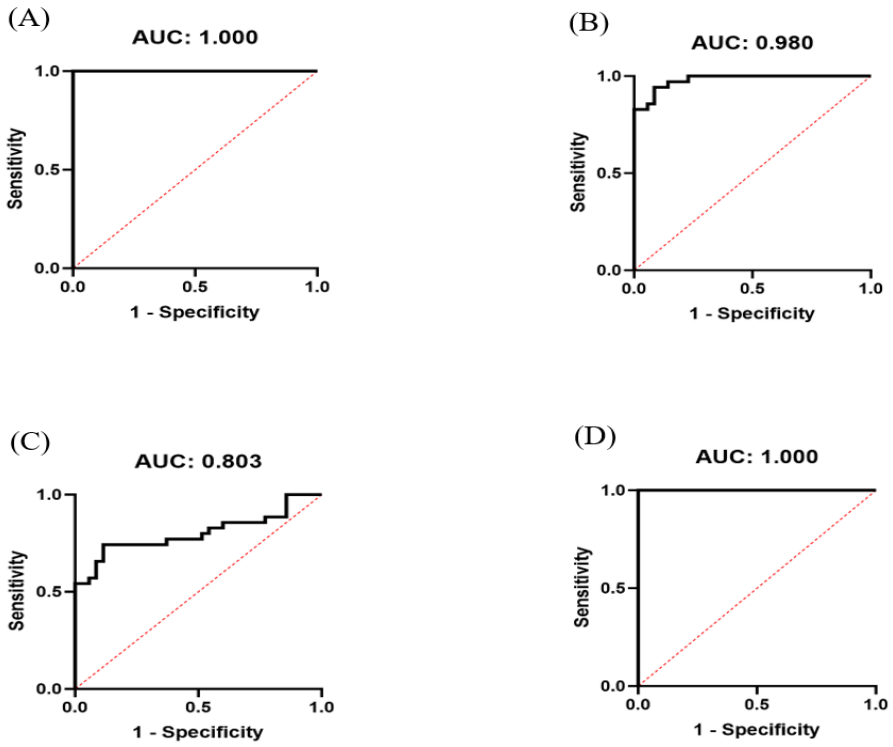


FIGURE 4: Receiver operating characteristic (ROC) of salivary MMP-9. (A) Control vs non-smoker gingivitis, (B) Control vs smoker gingivitis, (C) Control vs non-smoker periodontitis and (D) Control vs smoker periodontitis.

Table 2: Correlation between periodontal parameters and MMP-9.

| | MMP-9 | |
|---|---|---|
| | r | p |
| PI | 0.443 | > **0.001** |
| BOP | 0.486 | > **0.001** |
| PPD | 0.167 | 0.168 |
| CAL | 0.313 | **0.008** |

r: Correlation coefficient. Significant difference at p <0.05 by Pearson's correlation assay.


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**4 | DISCUSSION:**

Salivary MMP-9 showed high sensitivity and specificity in identifying healthy and diseased periodontal tissues. A similar pattern was observed in discriminating healthy periodontium from smokers with gingivitis or periodontitis cases.

Manual probing is a technique that measures periodontal parameters, but it can have errors such as causing bleeding by applying too much force or recording deeper pocket depth than the actual one. These drawbacks can happen for various reasons.(Laugisch et al., 2021, Elashiry et al., 2019) Therefore, it is important to have an accurate diagnosis to design the right treatment plan. Using biomarkers in oral fluids has shown promising results in the last decades to tell apart healthy and diseased periodontal tissues.(Kc et al., 2020)

Saliva was the oral fluid of choice because it is easy to collect without making the patient uncomfortable, it can be obtained in enough amounts, and it has many biomarkers that accurately reflect various local and systemic conditions.(Malamud, 2011, Bhattarai et al., 2018) However, salivary biomarkers are more suitable for screening purposes than for giving site-specific information, which are better for monitoring disease activity and prognosis.(Wei and Wong, 2012, Farah et al., 2018)

The MMP-9 is a biomarker that has been studied extensively. In the current study, MMP-9 was able to distinguish periodontal health from gingivitis and periodontitis with high sensitivity. The ability to distinguish between health and periodontitis was consistent with most of the published studies.(Bostanci et al., 2021, Kim et al., 2020a, Kim et al., 2020b) However, other authors have suggested that MMP-9 is able to distinguish only health from periodontitis but not rise to a significant level during gingivitis.(Bostanci et al., 2021, Lazăr et al., 2015) MMP-9 is more efficient in degrading collagen type IV, a major component of the basement membrane structure. In addition, MMP-9 is also responsible for degrading gelatins (hence the name gelatinase B); collagens that forms the structure of extracellular matrix proteins, type V, VII. X. X, plus elastin. MMP-9 causes tissue degradation physiologically as a part of the normal remodeling process or pathologically during the destructive phase of periodontal disease.(Reynolds, 1996)

This enzyme is produced as a pro type by a variety of cells, mainly by Polymorphonuclear leukocytes (PMNs) and also by the connective soft tissue cells of the gingiva and osteoclasts, activated by the inflammatory process and would degrade extracellular collagen structure.(Vandooren et al., 2013) Subsequently causing both soft tissue and bone loss that has been associated to the destructive stage of periodontitis.(Escalona et al., 2016) As it was explained, MMP-9 level rises in subjects suffering from recurrent loss of attachment level and its concentration decreases after successful periodontal therapy. This explains why MMP-9 level rises during periodontitis which could be attributed to the breach of the basal membrane structure and the loss of the hard and soft tissues. This is reflected by the deepening of periodontal pockets, soft tissue damage, and bone loss that is caused by the imbalance in the execration of the host derived proteinases including MMP-9 that happens during the active stage of periodontitis. MMP-9 is excreted in both health and disease process but the difference in the concentration during the inflammatory process with the activation of the pro type into active MMP-9.(Teng et al., 1992)

The impact of smoking on salivary MMP-9 has been a topic of debate in various studies.(Nishida et al., 2008, Gupta et al., 2016, Mäntylä et al., 2006) This could be due to the fact that smoking has a direct and multifaceted influence on inflammatory pathways and, as a result, on biomarkers derived from the host.(Johannsen et al., 2014) It has been observed that smoking is linked to an increased count of total white blood cells, with current smoking habits having a more pronounced effect than the number of pack years.(Smith et al., 2003) The most significant effect was seen in granulocytes, with current smokers having the highest count. A noticeable downward trend in granulocyte count was observed with time since quitting, with non-smokers having the lowest count. Smoking can also stimulate


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lymphocytes and elevate CRP levels. These smoking-induced changes in inflammatory cells can further affect the production and secretion of MMPs.(Yanbaeva et al., 2007)

The study suggests that measuring the salivary biomarkers in GCF and checking their levels after periodontal treatment could help overcome the study's limitations. Although the salivary biomarkers were showed potential as diagnostic tools, more clinical studies are needed to confirm the recent findings.

**5 | CONCLUSION:**

Salivary MMP-9 is a potential candidate for differentiating periodontal health from both gingivitis and periodontitis regardless of smoking status.

CONFLICT OF INTEREST

None declared.

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