Keywords
Tobacco smoking; Myeloid related protein 8/14; Periodontitis; Inflammatory response.

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# Association between Salivary Myeloid-Related Protein-8/14 Levels and Tobacco Use in Patients with Periodontitis

**Abstract**
**Background:** Tobacco use is the main contributor factor for periodontitis (PD), smoking is still a serious global health concern. Human neutrophils and monocytes constinousely generate myeloid related protein 8/14 (MRP 8/14), which release following stimulation.
**Aim of study:** The purpose of the study is to determine how tobacco use affects patients' salivary levels of MRP 8/14, and the role of this biomarker in pathogenesis of PD.
**Materials and Methods:** Of the 64 males in this study, 20 were in good periodontal health and 44 (22 smokers and 22 non-smokers) had PD. Their ages varied from 25 to 59. Unstimulated saliva was taken from both groups, and periodontal clinical data were used to assess the patients' periodontal conditions. The (ELISA) was utilized to measure the concentration of MRP 8/14 in the saliva.
**Results:** Both smokers and non-smokers with PD had salivary MRP 8/14 levels that were statistically greater than those of healthy controls ($p<0.0001$). Additionally, smokers had substantially greater MRP 8/14 levels than non-smokers ($p<0.05$). By using ROC assessment, the results demonstrated that this protein could distinguish between PD patients (smokers and non-smokers) and healthy controls (area under the curve values were 0.947 and 0.833).
**Conclusion:** The findings pointed out that smoking-induced elevation of MRP 8/14 may have clinical implications for PD patients. MRP levels 8/14 have shown excellent clinical accuracy in differentiating between the control group and the patient groups under study (smokers and non-smokers).

## Introduction

An infectious oral illness called periodontitis (PD) results in tooth loss by inflaming and degrading periodontal tissues. It is one of the most prevalent long-term inflammatory conditions. The host's cells are constantly being irritated by periodontal microbes, which triggers a defense mechanism and generates inflammatory mediators that may lead to tissue and bone degradation (1, 2). Among the modifiable risk factors for periodontal illness, cigarette smoking is the most powerful and well-established. Research indicates that smokers are more prone than non-smokers to experience problems like receding gingival tissue, tooth and loss of bone, and the development of periodontal pockets, which raise the risk of developing more severe gum disease (3, 4). In addition to increasing protein citrullination, cigarette smoke recruits neutrophils and macrophages to the lungs. Myeloid related proteins 8 and 14 (MRP 8/14) or calprotectin, a heterodimeric peptide mostly made by neutrophils, make up 60% of the cytosolic protein of a neutrophil (5). The MRPs 8/14, also known as S100A8 and S100A9, as well as calgranulin A and B, belong to the S100-protein family. They appear in myeloid cells, including neutrophils, and are mostly secreted as the heterodimer MRP 8/14 (6). It is essential in the migration of neutrophils within organs. An essential cell in inflammation is the neutrophil. Neutrophil measurement and count provide information regarding the level of inflammation (7). Dental disorders have been linked to high levels of MRPs (8). MRP 8/14 levels in serum, GCF, and salivary fluid were observed more

prevalent in patients than in normal individuals controls; however, these levels can be lowered with early periodontal therapy (9). To better comprehend the pathophysiology of periodontal diseases, this study compared the levels of MRP 8/14 in the saliva of smokers, non-smokers, and a control group.

**Materials and Methods**

*Research design:* It is a case-control study, this research was conducted out at the University of Baghdad, College of Dentistry, between September 2025, and January 2026. The World Medical Association's Helsinki Declaration served as the study's primary source of ethical guidance, and the Dentistry College/University of Baghdad's ethical committee granted ethical permission.

*Study population:* 64 male participants, ages 25 to 59, participated in this study. The PD patients were diagnosed using Tonetti's diagnostic guidelines for periodontal diseases (10). The selected individuals were divided into two groups: 20 healthy control individuals with an undamaged periodontium (BOP less than 10% and PPD less than 3) who had proper dental hygiene and no evidence of gum illness (11). On the other hand, 44 individuals had generalized unstable stage periodontal disease (PPD more than 4 mm, CAL more than 4 mm, encompassing at least 30 percentage of teeth). There were twenty-two smokers and twenty-two non-smokers.

*Inclusion and Exclusion criteria:*Patients with generalized unstable PD who were between the ages of 25 and 59, had at least 20 natural teeth, with PPD more than 4 mm and CAL more than 4 mm, comprising a minimum of thirty percent of teeth,, were eligible for inclusion. Patients with systemic illnesses, those who were given dental treatment during the preceding six-month period, those who had taken antibacterial or medications for inflammation within the last three months, those who had previously consumed alcohol, and those who smoked vapes or electric cigarettes being disqualified.

*Quantity of samples:* The G power 3.1.9.7 system, created by Franz-Faull at Kiel University in Germany, was used to determine the number of samples. The sample size is roughly 64 people with three groups, a significant effect size of F at 0.4, a power of 90%, and an alpha error of 0.05 on both sides. The effect size, denoted by F, is small (0.1), medium (0.25), and large (0.4).

*Saliva collection:* Two milliliters of whole, unstimulated saliva were taken from each study group and placed in a sterile container. One hour before to saliva donation, participants were instructed to abstain from food and liquids. Saliva was collected within an hour, centrifuged for ten minutes at 3000 rpm, and resulting supernatants were promptly aspirated, split into 3 parts, and stored at -40°C till needed (12).

*Clinical periodontal data:* William's periodontal probe was used to measure clinical indicators (PLI, BOP, PPD, and CAL) after saliva samples were obtained. To measure BOP, PPD, and CAL, each tooth was carefully checked in six separate regions: Lingual, Distolingual, Buccal, Mesiobuccal, and Distobuccal. In the meanwhile, just four surfaces (mesial, distal, labial/buccal, lingual/palatal) were examined in order to elegantly establish PLI scores. Except for PLI, the third molar was excluded from the evaluation of other parameters. To determine whether dental plaque was present or not, PLI was carefully evaluated using revealing agents (13), and BOP percentage was recorded as 1 present 0 absence (14). PPD is the measurement between the bottom of the pocket and the unconstrained gingival border. On the other hand, in the event of gingival regression, CAL is the measurement from the bottom of the pocket to the CEJ. If the gum boundary is situated over the CEJ, the CAL is computed by subtracting the distance between the gingival border and the CEJ from the PPD (10).

*Detection salivary concentrations of MRP 8/14:* MRP 8/14 salivary levels were detected using a sandwich ELISA in compliance with the guidelines provided by the supplier (Shanghai, China). In short, MRP 8/14-specific capture antibodies were mounted on a strip plate's wells. Biotinylated secondary antibodies were added after the standards and samples had been added and incubated. Following washing, HRP-conjugate was added, and a chromogenic substrate was used to create color. Sulfuric acid was used to halt the process, and quantify absorbance at 450 nm. Standard curves were used to calculate MRP 8/14 concentrations.

*Analysis of Statistical:* The computational statistical tools for social research (SPSS) software (version 25, the IBM, America) and Prism from GraphPad software (version 9.0) were used for every statistical assessment of the data. When $p<0.05$, statistical variance was regarded as significant. Descriptive statistics using the average and SD were utilized. The normality test was used to determine the distribution of clinical and MRP 8/14 data. The following statistical hypotheses were either accepted or rejected using inferential statistics: The ANOVA parametric test and chi-square test were used to document the differences between at least three different groups. The variance between two groups was examine using the post hoc test. The MRP 8/14 level and clinical parameters were correlated using the Pearson correlation coefficient test. Additionally, ROC curve was created to demonstrate this protein's potential for diagnosis.

**Results**

**Participants' clinical and demographic characteristics**

The clinical and demographic picturesof the 64 trial participants are mentioned in Table 1. The control group's mean age was 39.25±9.29 years, while the mean age of smokers and non-smokers with PD was 41.43±7.66 and 43.35±7.81 years, respectively. The age distribution of the groups did not change significantly ($P>0.05$). When the mean values of the clinical parameters (PLI, BOP, PPD, and CAL) were compared between the study groups, there was a substantial difference ($p<0.05$); smokers with PD had higher levels of PLI, while nonsmokers had higher levels of BOP, and both parameters (PLI and BOP)

were higher in PD than controls (Table 2). However, in both patient groups, mean levels of PPD and CAL were not different considerably (P>0.05).

**Table 1: Case-control differences in age and periodontal parameters among studied groups.**

| Characteristics | | Study groups | | | ANOVA (*P*-value) |
|---|---|---|---|---|---|
| | | Smokers PD group No.=22 | Non-smokers PD group No.=22 | Healthy control group No.=20 | |
| Age | Range | 30-58 | 30-59 | 25-55 | 0.279$^{NS}$ |
| | Mean | 41.43 | 43.35 | 39.25 | |
| | SD | 7.66 | 7.81 | 9.29 | |
| Periodontal parameters | | | | | |
| PLI | Mean | 59.77 | 50.07 | 21.29 | 0.0001$^{*}$ |
| | SD | 27.26 | 24.57 | 13.35 | |
| BOP | Mean | 33.42 | 45.28 | 5.28 | 0.0001$^{*}$ |
| | SD | 17.34 | 18.87 | 2.50 | |
| PPD | Mean | 4.43 | 4.58 | - | 0.163$^{NS}$ |
| | SD | 0.57 | 0.56 | - | |
| CAL | Mean | 4.92 | 5.19 | - | 0.237$^{NS}$ |
| | SD | 1.29 | 1.21 | - | |

NS: non-significant; *: significant; SD: standard deviation.

**Table 2: Mean PLI and BOP values for each pair of groups are compared across groups..**

| Groups | Mean of difference | Tukey's HSD (*P*-value) |
|---|---|---|
| PLI | | |
| Smokers PD vs. Non-smokers PD | 9.70 | 0.353 $^{NS}$ |
| Smokers PD vs. control | 38.48 | 0.00001$^{*}$ |
| Non- smokers PD vs. control | 28.78 | 0.0003$^{*}$ |
| BOP | | |
| Smokers PD vs. Non-smokers PD | 11.83 | 0.034$^{*}$ |
| Smokers PD vs. control | 28.14 | 0.00001$^{*}$ |
| Non- smokers PD vs. control | 39.97 | 0.00001$^{*}$ |

**The relationship between salivary MRP 8–14 levels and clinical parameters**

The findings we obtained showed that the mean salivary MRP 8\14 levels were considerably greater (p<0.01) in the PD groups (smokers and nonsmokers) than in the controls. Additionally, the smokers group had the greatest level in Table 3, and the multiple pairwise comparison was of statistical importance (p<0.01) in Table 4. As seen in Table 5, the present analyses demonstrated a high correlation between the mean level of BOP and the MRP 8\14 level in smokers with PD.

**Table 3: The difference in mean values of salivary MRP 8\14 among study groups.**

| Salivary MRP 8\14 | Study groups | | | ANOVA (*P*-value) |
|---|---|---|---|---|
| | Smokers PD group No.=22 | Non-smokers PD group No.=22 | Healthy control group No.=20 | |
| Range | 550-271 | 455-149 | 427-70 | 0.0001$^{*}$ |
| Mean | 386.21 | 303.15 | 159.6 | |
| SD | 93.03 | 94.6 | 69.54 | |

**Table 4: Intergroup comparisons of mean values of salivary MRP 8\14between groups.**

| Groups | Mean of difference | Tukey's HSD(*P*-value) |
|---|---|---|
| Salivary MRP 8\14 | | |
| Smokers PD vs. Non- smokers PD | 83.06 | 0.0169 $^{*}$ |
| Smokers PD vs. control | 226.58 | 0.00001$^{*}$ |
| Non- smokers PD vs. control | 143.52 | 0.00002$^{*}$ |

**Table 5: Correlation between MRP 8\14 level and clinical periodontal parameters in Periodontitis patients**

| Salivary MRP 8\14 | R-value | *P*-value |
|---|---|---|
| Smokers PD | | |
| PLI | 0.118 | $0.598^{NS}$ |
| BOP | 0.526 | $0.012^{*}$ |
| PPD | 0.126 | $0.573^{NS}$ |
| CAL | 0.295 | $0.182^{NS}$ |
| Non- smokers PD | | |
| PLI | 0.208 | $0.351^{NS}$ |
| BOP | 0.390 | $0.072^{NS}$ |
| PPD | 0.004 | $0.985^{NS}$ |
| CAL | 0.119 | $0.593^{NS}$ |

**Salivary MRP 8–14 diagnostic accuracy**

ROC analysis was used to differentiate smokers from nonsmokers and PD patients from healthy controls in order to assess the sensitivity and specificity of salivary MRP 8\14. Excellent discriminatory power was established by MRP 8\14, as Table 6 and Figures (1, 2, 3) reveal.

**Table 6: Comparison of the diagnostic properties of salivary MRP 8\14 among all pairs of groups.**

| Comparison | AUC | P-value | Accuracy | Sensitivity | Specificity |
|---|---|---|---|---|---|
| Smokers PD vs. Non-smokers PD | 0.694 | 0.018 | 0.704 | 100% | 40% |
| Smokers PD vs. control | 0.947 | 0.001 | 0.952 | 100% | 90% |
| Non- smokers PD vs. control | 0.884 | 0.004 | 0.833 | 90% | 76% |

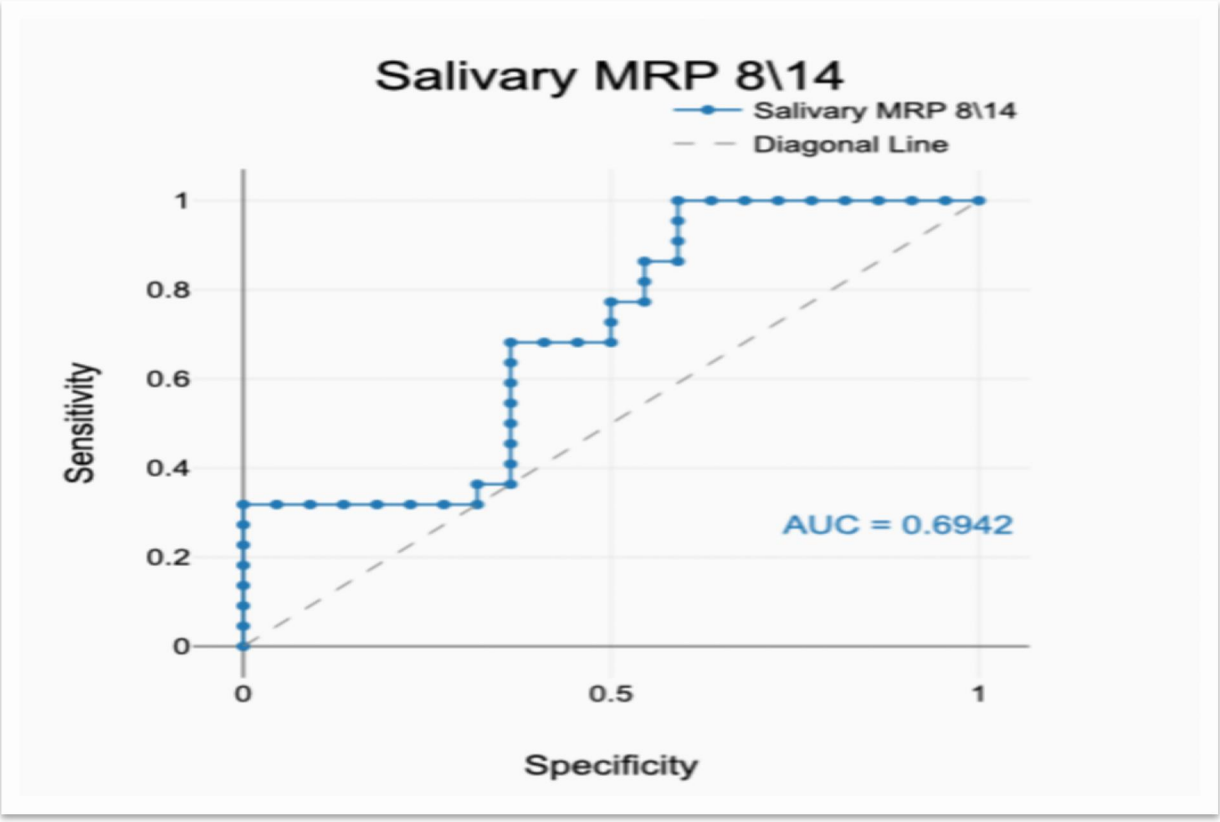


**Figure 1: Receiver operating curves for salivary MRP 8\14 in Smokers PD vs. Non-smokers PD.**

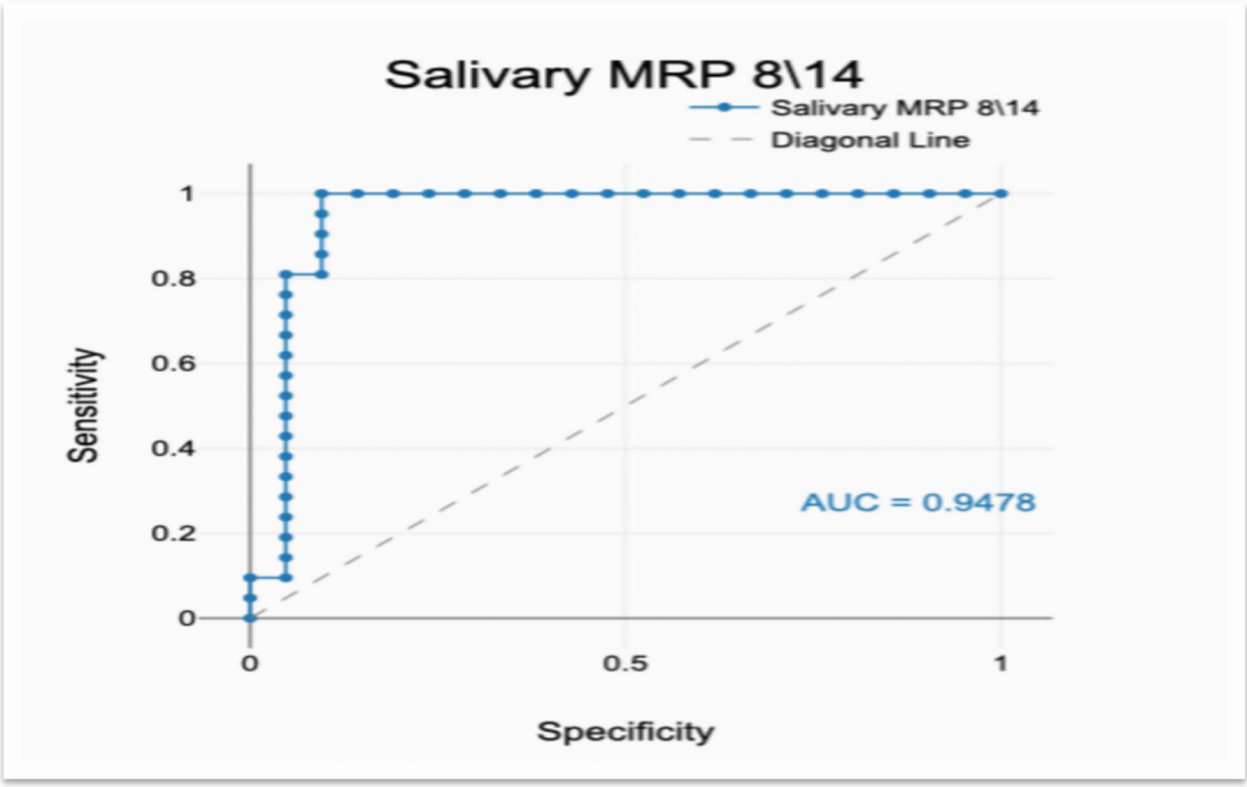


**Figure 2: Receiver operating curves for salivary MRP 8\14 in Smokers PD vs. control**

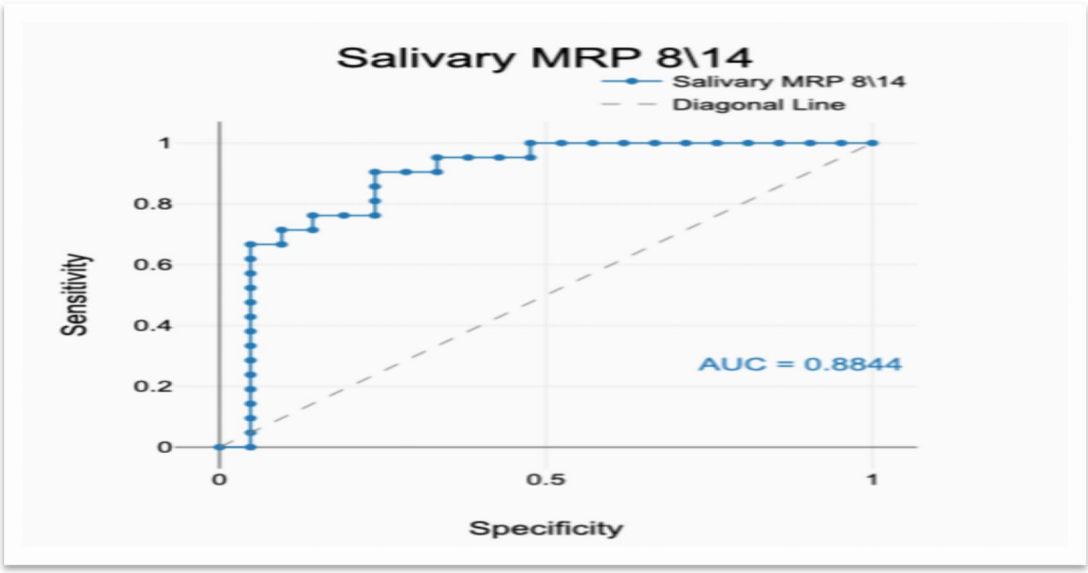


**Figure 3: ROC curves for salivary MRP 8\14 in Non-smokers PD vs. control.**

**Discussion**

Nicotine was thought to be among the most harmful of the numerous chemicals found in tobacco that are known to be detrimental to periodontal tissues (15). In PD, inflammation-induced tissue damage is largely caused by myeloid cells (16). As a result, biomarkers that reflect their functions are potential candidates for periodontal inflammation screening and monitoring as well as for providing hints about the pathophysiology of the disease. Potential salivary indicators for periodontal inflammation screening and monitoring include MRP 8–14. The mean level of MRP 8\14 is importantly greater among study groups as compared to healthy individuals, according to our results. In agreement with the findings of another result by Lira-Junior *et al.* (17), that found that the concentration of MRP 8\14 in whole saliva was greater in PD than in healthy, our results demonstrated an important elevation of MRP 8\14 in the study groups compared to the control group.

PMN cell infiltration, which serves as an inherent immune system barrier in the inflamed PD site, and the utilization of this protein as an antimicrobial agent produced PMN as a reaction to a periodontal problem could be the cause of the elevation of MRP 8–14 (18). Furthermore, the current result was consistent with a study by Brembach et al. (19), which demonstrated that salivary MRP 8\14 were greater in

smokers with PD than in non-smokers. The study concluded that smoking has a significant impact on host response, can create an oral environment that is more favorable to oral dysbiosis, and that nicotine is a potentially harmful substance that negatively affects periodontal tissue either directly or indirectly (16). However, a prior investigation found that PD patients had lower salivary levels of MRP 8\14 or calprotectin (20). Furthermore, these findings demonstrated a high link between the mean BOP level and the MRP 8\14 level in smokers with PD. Similarly, a recent study revealed a significant correlation between BOP and calprotectin in smokers with PD (21). Other research, however, revealed that GCF MRP 8\14 levels linked with clinical measures including PPD and GI score, and that MRP 8\14 in GCF within periodontal pockets are importantly greater as compared from healthy locations without. These results demonstrate the usefulness of GCF calprotectin at local periodontal sites as a biomarker of periodontal disorders (22). The diagnostic value of MRP 8\14 in distinguishing between periodontal health and disease was assessed using ROC curve analysis. Salivary MRP 8\14 performed exceptionally well in differentiating between sick and healthy individuals. Kaner and colleagues note that MRP8/14 can differentiate between states of periodontal health and disease, which is comparable to our findings. There is evidence that the crevicular neutrophil count may be reflected in MRP8/14 levels (23). The number of samples and the examination of a single inflammatory biomarker are two of the study's shortcomings because the pathophysiology of periodontitis involves numerous biomarkers.

## Conclusion

The findings pointed out that smoking-induced elevation of MRP 8/14 may have clinical implications for PD patients. MRP levels 8/14 have shown excellent clinical accuracy in differentiating between the control group and the patient groups under study (smokers and non-smokers).

## Conflicting interests

The authors can attest that they don't have any conflicting interests.

## Funding

No financing obtained.

## Authors' contributions

A combination of BHAG and AAA were engaged in the study's conception and design, literature search, clinical analysis, data analysis, statistical analysis, manuscript preparation, and manuscript review. The concept and design of the study, data analysis, manuscript writing, and review were all contributed to by each author. All writers have reviewed and approved the final version.

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