Original Article

DOI: 10.7860/JCDR/2024/69936.19754

Dentistry Section

Salivary Levels of S100A8, S100A9 and S100A8/9 in Periodontal Health and Disease: A Cross-sectional Study

ANNIE KITTY GEORGE[1], SANKARI MALAIAPPAN[2], VIVEK NARAYAN[3], SELVARAJ JAYARAMAN[4], NIDHITA SURESH[5]

ABSTRACT

Introduction: Damage Associated Molecular Patterns (DAMPs) can initiate and amplify immune responses and can play an important role in the sustenance and progression of periodontal diseases. S100A8, S100A9 and their heterodimer, S100A8/9, are DAMPs or alarmins currently being evaluated for their potential as biomarkers in periodontal diseases.

Aim: To quantify and compare salivary levels of S100A8, S100A9 and S100A8/9 in periodontal health, gingivitis or stage 1 periodontitis and stages 3 or 4 periodontitis.

Materials and Methods: A cross-sectional analytical study was carried out in the Department of Periodontics, Saveetha Dental College and Hospital, Chennai, India, from December 2022 to February 2023. Periodontal examination and saliva sample collection were done for sixty-eight consecutively enrolled subjects who met the inclusion and exclusion criteria. Study subjects were categorised as group 1, which comprised participants with clinical periodontal health (n=20), group 2, subjects with gingivitis or stage 1 periodontitis (n=20), and group 3, which included patients with stages 3 or 4 periodontitis (n=28). Detailed clinical examination and periodontal charting were done in all study subjects. Saliva samples were processed and stored at -80°C, and enzyme-linked immunosorbent assay was done to quantify S100A8, S100A9 and S100A8/9. Data were analysed using International Business Machines (IBM) Statistical Package for the Social Sciences (SPSS) software version 25.0. Salivary levels of S100A8, S100A9 and S100A8/9 were expressed as mean and standard deviation values. Mean values of the three salivary proteins were compared using the non parametric Kruskal-Wallis test. Bonferroni adjusted pairwise comparisons were also done. The p-values less than 0.05 were considered statistically significant.

Results: The mean age of study participants was 38.97±7.62 years. Salivary levels of S100A8 were 41.34±14.34, 141.95±185.25 and 901.37±65.60 pg/mL in groups 1, 2 and 3, respectively. A statistically significant difference was present among the three groups for mean salivary levels of S100A8 (p-value<0.001). Pairwise comparisons showed that salivary S100A8 values in group 3 were significantly higher than the values observed in clinical health (group 1) (p-value<0.001) and those seen in the gingivitis or stage 1 periodontitis group (group 2) (p-value<0.001). Mean salivary levels of S100A9 and S100A8/9 did not show any statistically significant differences between the groups.

Conclusion: There is an altered expression of S100A8, S100A9 and S100A8/9 in periodontal diseases compared to clinical health. Salivary levels of S100A8 are markedly different in cases of advanced periodontal destruction than in periodontal health and early stages of periodontal disease. Salivary S100A8 merits potential as a biomarker for periodontal diseases.

Keywords: Alarmins, Gingivitis, Periodontitis, Proteomics, Saliva

INTRODUCTION

Periodontal diseases reflect dysregulated immune inflammatory responses of the host tissues towards a dysbiotic polymicrobial plaque biofilm. The temporal relationship between inflammation and dysbiosis has yet to be clearly unravelled [1]. A vast array of complex, overlapping cytokine and chemokine networks mediate periodontal tissue damage. Although periodontal diseases are most often initiated by pathobionts and their products, the myriad of underlying mechanisms sustaining this chronic inflammatory process, with both its localised and systemic effects, has not been fully understood. The binding of Pathogen-associated Molecular Patterns (PAMP) or Damage-associated Molecular Patterns (DAMP) with Pathogen Recognition Receptors (PRRs) is the key event in the initiation of periodontal inflammation. Since 2004, the role of host-derived DAMPs or alarmins in inflammatory diseases has been increasingly recognised. Alarmins are chemotactic and activate innate and adaptive immune responses [2,3]. The most studied host-derived DAMP molecules in periodontal inflammation are the S100 proteins, High Mobility Group Box 1 (HMGB1), Neutrophil Extracellular Traps (NETs), and Heat Shock Proteins (HSPs). The binding of S100s with Toll-like Receptor 4 (TLR-4) or Receptor for Advanced Glycation End products (RAGE) amplifies inflammation and leads to the release of proinflammatory cytokines by activating Mitogen-activated Protein Kinase (MAPK) and Nuclear Factor Kappa-B (NF-κB) pathways [4]. The S100 proteins can also bind and activate the Nucleotide-binding domain, Leucine-rich-containing family, Pyrin domain-containing-3 (NLRP3) inflammasome complex, activate caspase 1, and subsequently release proinflammatory Interleukin-1β (IL-1β) and/or IL-18 [5].

The proteins S100A8 and S100A9 are also known as myeloid-related proteins 8 and 14, respectively. They are present in the cytoplasm of neutrophils, macrophages and dendritic cells of myeloid origin and constitute 45% of neutrophilic cytoplasmic proteins. Intracellularly, they are important in maintaining the cytoskeleton. They are released extracellularly in inflammatory environments and form stable hetero or homodimers. These proteins are named S100s because they are soluble in a 100%-saturated solution of ammonium sulfate. The human protein S100A8 is made up of 93 amino acids and S100A9 of 113 amino acids, respectively. These S100 proteins have a unique helix-loop-helix molecular structure. The charged amino acid residues on them have a high binding affinity for calcium and zinc ions. By virtue of their calcium-binding nature, the S100A8 and S100A9 proteins are also called calgranulins A and B [6].

Evidence points to altered expression of both S100A8 and S100A9 in multiple infection-mediated inflammatory conditions

[6]. Salivary proteomic studies have indicated that S100A8 and S100A9 were higher in periodontitis than in health [7]. Emerging evidence points to the potential screening, diagnostic and prognostic potential of S100A8, S100A9 and their heterocomplex-S100A8/9 [8-10].

Calprotectin is the heterocomplex of S100A8 and S100A9 and has been recognised as an antimicrobial peptide by virtue of its metal-binding properties. This protein complex is expressed by granulocytes, endothelial cells and keratinocytes. Serum levels of calprotectin have been extensively studied in gastrointestinal inflammation, rheumatoid arthritis, cystic fibrosis, glomerulonephritis, chronic bronchitis, psoriasis and in association with periodontal diseases. Its potential as a biomarker has been investigated in these inflammatory conditions [6]. Elevated levels of this protein have been associated with dysregulated immune responses [6]. Fecal calprotectin levels are estimated as markers for inflammatory bowel diseases [11]. Evidence points to the biomarker potential of calprotectin in periodontal diseases [11]. However, evidence on its salivary expression in periodontal inflammation is inconclusive [10]. Salivary levels of these proteins have been estimated in different stages of periodontitis [12]. To the best of the authors knowledge, this is the first study to estimate the parallel salivary expression of S100A8, S100A9 and their complex calprotectin, in periodontal health and disease. In the present cross-sectional analytical study, the authors hypothesised that the expression of S100A8, S100A9, and their complex calprotectin (S100A8/9) in saliva is altered in periodontal diseases rather than in health. Therefore, the aim of the current study was to estimate the salivary levels of S100A8, S100A9 and S100A8/9 in clinical periodontal health, gingivitis or stage 1 periodontitis and in stages 3 or 4 periodontitis.

MATERIALS AND METHODS

The present cross-sectional analytical study was conducted in the Department of Periodontics, Saveetha Dental College and Hospital, Chennai, India, from December 2022 to February 2023. The methodology of the study adhered to the Helsinki Declaration regarding ethical principles for medical research involving human subjects. The present study had received approval from the Institutional Review Board of the study Institution, where it was carried out (IHEC/PhD/PERIO-1621/21/230). Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) guidelines were followed in the presentation of the investigation.

Inclusion criteria: Subjects with periodontal and gingival health, Gingivitis, and Stage 1, 3, and 4 periodontitis [13-15] in the age group of 25-55 years, who had a minimum of twenty teeth, with no other self-reported systemic illness and who willingly consented for the study were included in the study.

Exclusion criteria: Patients who reported any systemic illnesses such as diabetes or cardiovascular diseases, or who were under therapy for any acute or chronic inflammatory diseases or lifestyle diseases were excluded from the study. Subjects who were smokers, obese (Body mass index >30 kg/m^2) [16], and those who reported consumption of alcohol, pregnant and lactating females, and those who reported intake of antibiotics or anti-inflammatory agents like non-steroidal anti-inflammatory drugs during the last three months of recruitment were also excluded from the study.

Sample size calculation: The sample size was estimated using G Power. The final sample size was fixed as a minimum of 18 per group using inputs of 0.8 for anticipated effect size, a type 1 error of 5%, and a power of 80%.

Study Procedure

After recording medical and dental history, and conducting a periodontal examination, 68 adults who met the inclusion criteria were enrolled in the study. Body weight in kilograms and height in meters were recorded for each participant to exclude obese individuals. Obesity was defined as per the definition by World Health Organisation (WHO) as Body Mass Index (BMI) equal to or greater than 30 kg/m^2 [16]. Twenty subjects with gingival and periodontal health formed group 1, 20 subjects with gingivitis or stage 1 periodontitis constituted group 2, and 28 patients with stages 3 or 4 periodontitis were taken as group 3.

Periodontal examination and case definitions: Clinical gingival and periodontal health (group 1) were defined as cases with <10% of sites which bled on probing and with probing depth <3 mm according to the Consensus report of workgroup 1 of the 2017 World Workshop on the Classification of Periodontal and Peri-Implant Diseases and Conditions [13].

Gingivitis on an intact periodontium was defined according to the diagnostic criteria proposed by the 2017 World Workshop by a percentage Bleeding on Probing (%BOP) score (yes/no recording) ≥10%, wherein the %BOP was the percentage of sites that bled when a UNC 15 probe was inserted to the base of the sulcus at six sites per tooth on all teeth present except the third molars [14].

Stage 1 periodontitis was defined as clinically detectable attachment loss on two non adjacent teeth equal to 1-2 mm, and stage 3 and 4 periodontitis was defined as interdental Clinical Attachment Loss (CAL) ≥5 mm as per the criteria for staging proposed in the new classification and case definition by the World workshop 2017 [15].

The Gingival Index (GI) (Loe, 1967) was recorded in all study participants. Mean Probing Pocket Depth (PPD), mean CAL, % sites with probing depth >5 mm, and % sites with CAL ≥5 mm were recorded in patients with periodontitis.

Saliva sample collection: Unstimulated whole saliva was collected from participants. All subjects enrolled in the study were at rest for atleast an hour prior to sample collection with no intake of caffeine or alcohol and no tooth brushing or flossing within the last two hours before saliva collection [17]. Study participants were asked to rinse their mouth with normal water. After 10 minutes, they were seated upright on the dental chair, asked to close their eyes, tip their heads forwards and were prompted to imagine their favourite food. Whole saliva was allowed to passively drool into the floor of the mouth for five minutes and was then collected into previously refrigerated collection tubes placed at the corner of the mouth. The sample tubes were labelled with name, age and gender, and immediately transported to the Biochemistry Laboratory, where the samples were centrifuged for 15 minutes at 1500 Rotations Per Minute (rpm) at 4°C. Aliquots were transferred into sterile 2 mL Eppendorf tubes (three tubes per patient) and stored at -80°C prior to analysis.

Enzyme-linked Immunosorbent Assay (ELISA) for S100A8, S100A9, and S100A8/9: S100A8, S100A9 and S100A/9 ELISA kit systems (R&D systems, Minneapolis, MN, USA) were used. The standards were reconstituted as provided in the ELISA kit according to the manufacturer's instructions to establish standard curves for the three proteins. ELISA protocols specified in the manufacturer catalog were followed. The concentration of the three proteins in saliva in picograms/milliliters (pg/mL) was calculated according to the optical density values obtained and read at 450 nm.

STATISTICAL ANALYSIS

The data were entered into spreadsheets and analysed using IBM SPSS software version 25.0. The salivary levels of S100A8, S100A9 and S100A8/9 were expressed using means and standard deviations. The distribution of the outcome variables (S100A8, S100A9 and S100A8/9 in the three groups) was checked for normality using the Shapiro-Wilk test. The non parametric Kruskal-Wallis test was used to compare the mean levels of salivary proteins between the groups as the data did not follow a normal distribution. Bonferroni post-hoc tests were employed for pairwise comparisons. p-value<0.05 were considered statistically significant.

RESULTS

Saliva samples were collected from 68 subjects, of which 20 (29.4%) participants had a clinically healthy periodontium (group 1), 20 (29.4%) had gingivitis or stage 1 periodontitis (group 2) and 28 (41.1%) had stage 3 or 4 periodontitis (group 3). Among the 68 participants, 44 (64.7%) were males and 24 (35.3%) were females. The mean age of the study participants was 38.97±7.62 years. The mean gingival index scores were 0.53±0.38, 2.29±0.45, and 2.57±0.36 in groups 1, 2, and 3, respectively. The mean Probing Pocket Depth (PPD) and Clinical Attachment Level (CAL) in group 3 were 5.15±0.53 and 3.87±0.61, respectively. The mean percentage of sites with PPD >5 mm in group 3 was 47.14±11.8, while the mean percentage of sites with CAL ≥5 mm was 49.24±13.10.

A comparison of the salivary levels of the three proteins in groups 1, 2, and 3 are shown in [Table/Fig-1]. The mean salivary levels of S100A8 were 41.34±14.34 pg/mL in group 1, 141.95±185.25 pg/mL in group 2 and 901.37±65.60 pg/mL in group 3. Comparison of the means by non parametric Kruskal-Wallis test showed that there was a statistically significant difference in salivary levels of S100A8 among the groups (p-value<0.001) [Table/Fig-1]. The mean salivary levels of S100A9 were 94.62±37.69 pg/mL in group 1, 87.07±48.87 pg/mL in group 2 and 136.91±110.01 pg/mL in group 3 and there was no statistically significant difference in salivary levels of S100A9 between the groups [Table/Fig-1].

Group	Salivary protein level in pg/mL mean (SD)					
	S100A8	p-value	S100A9	p-value	S100A8/9	p-value
Group 1 (n=20)	41.34 (14.34)	<0.001*	94.62 (37.69)	0.360	1220.24 (453.35)	0.863
Group 2 (n=20)	141.95 (185.25)		87.07 (48.87)		1265.79 (453.43)	
Group 3 (n=28)	901.37 (65.60)		136.91 (110.01)		1139.57 (600.58)	

[Table/Fig-1]: Comparison of salivary protein levels in periodontal health and disease.
*Kruskal-wallis test; The p-value <0.05 is statistically significant

The Bonferroni post-hoc test showed that salivary levels of S100A8 were significantly different in group 3 than in group 2 (p-value<0.001). Pairwise comparison for Salivary S100A8 levels between group 3 and group 1 also showed a statistically significant difference (p-value<0.001) [Table/Fig-2].

Pairwise comparison	p-value*
Clinical health- Gingivitis or stage 1 periodontitis	0.512
Clinical health- Stage 3 or 4 periodontitis	<0.001
Gingivitis or stage 1 periodontitis- Stage 3 or 4 periodontitis	<0.001

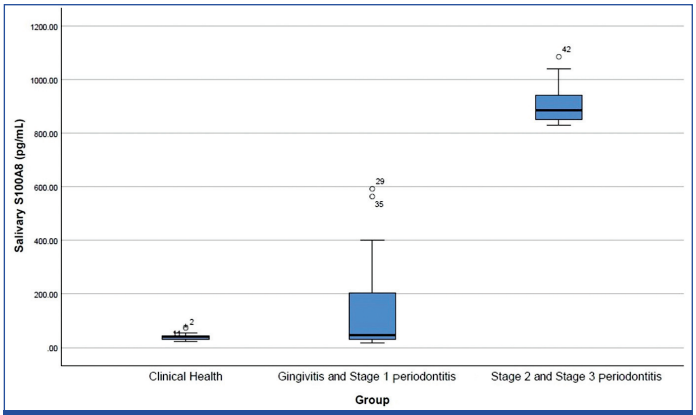
[Table/Fig-2]: Pairwise comparison for salivary S100A8.
*Bonferroni post-hoc adjusted p-value for Kruskal Wallis test

Post-hoc comparisons between the groups also did not show any significant difference in salivary S100A9 between the groups. Salivary S100A8/9 was also not different between or among the groups [Table/Fig-2]. Pairwise comparison for salivary S100A8 is depicted in [Table/Fig-2] depicts. Box plot that graphically represents the salivary levels of S100A8 is depicted in [Table/Fig-3].

DISCUSSION

The present study estimated the salivary expression of calgranulins S100A8, S100A9 and S100A8/9 in periodontal health and disease. The present study results highlighted a marked increase in salivary levels of S100A8 in periodontal diseases than in health.

Saliva is a mirror fluid of oral and systemic health and disease. In addition to salivary glandular secretions, saliva also contains Gingival Crevicular Fluid (GCF) constituents, transudates from the entire oral mucosa, and microbial and host-derived mediators of periodontal tissue destruction. Saliva can be easily and non invasively collected for omics studies. Unstimulated whole saliva



[Table/Fig-3]: Salivary levels of S100A8 in periodontal health and disease.

was used as the diagnostic sample in the present study and was collected by the gold standard passive drool method [17]. Neutrophils, macrophages and keratinocytes of the oral mucosa are the principal sources of S100A8, S100A9 and their complex S100A8/9 in saliva [18].

Study participants were grouped according to case definitions proposed by the World Workshop on the classification of periodontal and peri-implant diseases and conditions of 2017. The classification and case definitions proposed in the new classification point out that, in the absence of validated imaging tools or biomarkers, the clinical distinction of a case of gingivitis may often overlap with stage 1 periodontitis [15]. Therefore, the authors included gingivitis or stage 1 periodontitis as group 2. Clinical staging of advanced periodontal disease as stages 3 and 4 were together included as group 2 in the current study.

The markedly increased salivary expression of S100A8 observed in the present study is well supported by the results of a previous study that estimated the salivary, GCF and serum levels of S100A8, S100A9 and concluded an increased salivary level of S100A8 in incipient and established periodontitis [12]. The increased presence of this protein in saliva compared to GCF may indicate its increased expression from keratinocytes in an inflammatory environment. A salivary shotgun proteomic study reported that the protein of the highest relative level in periodontitis was S100A8. They also confirmed their results by ELISA [7].

In a cross-sectional sample drawn from a Korean population, authors reported that salivary S100A8 was positively (aOR=2.2) and S100A9 negatively correlated {Adjusted Odds Ratio (aOR) =0.5} with stage I-IV periodontitis. The study concluded that these proteins may be used as screening agents in periodontitis [8]. The same group of researchers investigated the diagnostic and prognostic ability of S100A8 and Matrix Metalloproteinase-9 (MMP-9). They observed that salivary S100A8 had higher screening ability than MMP-9 for periodontitis. The study also reported that the salivary levels of S100A8 in patients with periodontitis reverted to values lower than those observed in participants with periodontal health after non-surgical periodontal therapy [9].

In a subsequent study, the same research team reported that salivary S100A8 was higher in patients with stage-I periodontitis (initial periodontitis) and in stage-II-IV periodontitis (established periodontitis). They observed that salivary values of S100A9 were not significantly different in periodontitis groups when compared to values in the participant group with no periodontitis [12].

The 2017 World Workshop on the classification of periodontal and peri-implant diseases and conditions highlights the need for validated biomarkers for more precise case definitions of gingivitis and periodontitis [14,15]. The present study points out the marked increase of S100A8 in periodontal diseases than in health. The protein S100A8 is an important proinflammatory molecule that has been investigated in other chronic inflammatory conditions such

as rheumatoid arthritis, inflammatory bowel disease, and systemic lupus erythematosus [19].

In the present study, although not statistically significant, salivary S100A9 was found to be lower in the gingivitis group than in periodontal health and was seen to be elevated in stage 3 and 4 periodontitis when compared to values in health. The observed decreased level of S100A9 in gingivitis and stage-I periodontitis groups may be attributable to a lowered immune and inflammatory response. Increased S100A9 levels may be suggestive of a dysregulated immune response [6]. A similar trend in serum S100A9 levels has been reported in Chronic Obstructive Pulmonary Disease (COPD), and the levels of S100A9 were seen to be elevated only in advanced disease [20]. The present study observations regarding the increased salivary expression of this protein in stage 3 and 4 periodontitis agree with the findings of a shotgun proteomic study on salivary proteins [7].

In a longitudinal study on ligature-induced periodontitis and therapy in beagle dogs, measures of periodontal inflammation were positively correlated with salivary levels of S100A8 (r=0.822) and S100A9 (r=0.877) but not with S100A8/9. In their study, the authors reported that salivary levels of S1008/9 after therapy returned to values observed in periodontal health, whereas those of S100A8 and S100A9 in periodontal stability remained higher than those in health. The study also discussed that S100A8 and S100A9 are proinflammatory and attributed a more complex function to the heterodimer [21]. Although the homodimers S100A8 and S100A9 have been investigated in oral fluids by only a few researchers, their complex S100A8/9 has been extensively explored for their biomarker potential in periodontal diseases [22]. A recent systematic review that addressed the alterations in salivary protein composition in chronic periodontitis patients compared to subjects with periodontal health has reported the prominent presence of S100s and increased presence of S100A8 and S100A9 in periodontitis. They also suggested that these proteins may be used as supplementary protein tools in the diagnosis of periodontitis [23].

Cross-sectional studies on salivary Myeloid-related Protein 8/14 (MRP8/14) (S100A8/9) using ELISA have previously reported that salivary levels of the protein complex were significantly correlated with plaque index, gingival inflammation and probing depth [24,25]. Systematic reviews also indicate that salivary S100A8/9 is elevated in periodontal diseases than in health [22,23]. A recent study has been based on the premise that salivary calprotectin can be used as a biomarker for periodontal disease [26]. Although calprotectin has been observed to be elevated in the GCF and serum of patients with periodontitis in some studies, the authors did not observed a significantly altered salivary level of this protein in periodontal diseases than in health [27,28]. Serum and GCF calprotectin may be more predictive of systemic inflammatory burden.

The levels of calprotectin S100A8/9 in participants in the present study groups show dual and distinct patterns, and a subset of participants with low salivary calprotectin values can be observed in all the study groups of the current study. Levels of calprotectin may be indicative of distinct host response patterns or susceptibility profiles among individuals, which have also been previously reported [10,29,30].

Gao H et al., investigated the effects of S100A8/9 and its constituents on Human Gingival Fibroblasts (HGF) and reported that the proinflammatory role of this heterodimer is mediated by S100A9. The principal receptors identified for these proteins were TLR-4. S100A8/9 and S100A9 upregulate Reactive Oxygen Species (ROS)-dependent expression of IL-6 from HGF by the NF-κB p38 MAPK and c-Jun Amino-terminal Kinase (JNK) 1/2 pathways. These alarmin molecules also induce the release of IL-8 from HGF involving distinct cell signaling pathways such as NF-κB, p38, Extracellular Signal-regulated Kinase 1/2 (ERK1/2), and JNK 1/2 pathways [31]. The same group of researchers has reported that S100A9 upregulates ROS-dependent expression of IL-6 and IL-8 in human periodontal ligament cells by binding to TLR-4, via distinctive cell signaling pathways for both cytokines, similar to that observed in HGF [32]. S100A9 has also been reported to induce apoptosis in human periodontal ligament cells [33]. Zreiqat H et al., investigated the expression of S100A8 and S100A9 in human and murine osteoblasts and osteoclasts and reported that S100A8 may have an important role in the differentiation of osteoblasts [34]. Evidence gathered from studies on the molecular aspects of calprotectin and its constituent homodimers suggests distinct and diverse roles of the three proteins in periodontal tissues.

The present study is merited by a few strengths. The three calgranulins-S100A8, S100A9 and their complex, S100A8/9-have been simultaneously estimated in each participant. Periodontal health and disease states were defined as per the current classification system. Saliva samples were immediately transferred to the laboratory, and samples were stored at -80 degrees Celsius. Samples were not stored for more than two months prior to analysis.

Limitation(s)

However, the present study is limited by the small sample size. Also, due to the cross-sectional nature of the study, the diagnostic potential of salivary S100A8 for periodontal inflammation cannot be established.

CONCLUSION(S)

There is an altered expression of S100A8 and S100A9 in periodontal diseases than in health, thus accepting the hypothesis. Salivary levels of S100A8 have showed significant and marked increase in periodontal diseases than in health, and have promising potential as a biomarker of periodontal inflammation. The salivary level of this protein may be further explored in large surveys and in pre and post-treatment studies to establish its role as a biomarker. The precise role of S100A8 in periodontal pathogenesis also needs to be elucidate in future studies.

REFERENCES

[1] Van Dyke TE, Bartold PM, Reynolds EC. The Nexus Between Periodontal Inflammation and Dysbiosis. Front Immunol. 2020;11:511. Doi:10.3389/fimmu.2020.00511.
[2] Oppenheim JJ, Yang D. Alarmins: Chemotactic activators of immune responses. Curr Opin Immunol. 2005;17(4):359-65. Doi: 10.1016/j.coi.2005.06.002.
[3] Yang D, Han Z, Oppenheim JJ. Alarmins and immunity. Immunol Rev. 2017;280(1):41-56. Doi:10.1111/imr.12577.
[4] Gong T, Liu L, Jiang W, Zhou R. DAMP-sensing receptors in sterile inflammation and inflammatory diseases. Nat Rev Immunol. 2020;20(2):95-112. Doi:10.1038/s41577-019-0215-7.
[5] Parthasarathy U, Martinelli R, Vollmann EH, Best K, Therien AG. The impact of DAMP-mediated inflammation in severe COVID-19 and related disorders. Biochem Pharmacol. 2022;195:114847. Doi: 10.1016/j.bcp.2021.114847.
[6] Wang S, Song R, Wang Z, Jing Z, Wang S, Ma J. S100A8/A9 in Inflammation. Front Immunol. 2018;9:1298. Doi:10.3389/fimmu.2018.01298.
[7] Shin MS, Kim YG, Shin YJ, Ko BJ, Kim S, Kim HD. Deep sequencing salivary proteins for periodontitis using proteomics. Clin Oral Investig. 2019;23(9):3571-80. Doi:10.1007/s00784-018-2779-1.
[8] Karna S, Shin YJ, Kim S, Kim HD. Salivary S100 proteins screen periodontitis among Korean adults. J Clin Periodontol. 2019;46(2):181-88. Doi:10.1111/jcpe.13059.
[9] Kim HD, Kim S, Jeon S, Kim SJ, Cho HJ, Choi YN. Diagnostic and Prognostic ability of salivary MMP-9 and S100A8 for periodontitis. J Clin Periodontol. 2020;47(10):1191-200. Doi:10.1111/jcpe.13349.
[10] Lira-Junior R, Bissett SM, Preshaw PM, Taylor JJ, Boström EA. Levels of myeloid-related proteins in saliva for screening and monitoring of periodontal disease. J Clin Periodontol. 2021;48(11):1430-40. Doi: 10.1111/jcpe.13534.
[11] Wei L, Liu M, Xiong H. Role of Calprotectin as a biomarker in periodontal disease. Mediators Inflamm. 2019;2019:3515026. Doi:10.1155/2019/3515026
[12] Kim HD, Karna S, Shin Y, Vu H, Cho HJ, Kim S. S100A8 and S100A9 in saliva, blood and gingival crevicular fluid for screening established periodontitis: A cross-sectional study. BMC Oral Health. 2021;21(1):388. Doi: 10.1186/s12903-021-01749-z.

[13] Chapple ILC, Mealey BL, Van Dyke TE, Bartold PM, Dommisch H, Eickholz P, et al. Periodontal health and gingival diseases and conditions on an intact and a reduced periodontium: Consensus report of workgroup 1 of the 2017 World Workshop on the Classification of Periodontal and Peri-Implant Diseases and Conditions. J Periodontol. 2018;89 Suppl 1:S74-S84. Doi:10.1002/JPER.17-0719.
[14] Trombelli L, Farina R, Silva CO, Tatakis DN. Plaque-induced gingivitis: Case definition and diagnostic considerations. J Clin Periodontol. 2018;45(Suppl 20):S44-S67. Doi: 10.1111/jcpe.12939.
[15] Tonetti MS, Greenwell H, Kornman KS. Staging and grading of periodontitis: Framework and proposal of a new classification and case definition (published correction appears in J Periodontol. 2018;89(12):1475). J Periodontol. 2018;89(Suppl 1):S159-S172. Doi: 10.1002/JPER.18-0006.
[16] World Health Organization. Obesity and overweight. WHO website. Available from: https://www.who.int/news-room/fact-sheets/detail/obesity-and-overweight. Accessed May 12, 2024.
[17] Szabo YZ, Slavish DC. Measuring salivary markers of inflammation in health research: A review of methodological considerations and best practices. Psychoneuroendocrinology. 2021;124:105069. Doi: 10.1016/j.psyneuen.2020.105069.
[18] Gorr SU. Antimicrobial peptides of the oral cavity. Periodontol 2000. 2009;51:152-80. Doi:10.1111/j.1600-0757.2009. 00310.x.
[19] Kim JW, Jung JY, Lee SW, Baek WY, Kim HA, Suh CH. S100A8 in serum, urine, and saliva as a potential biomarker for systemic lupus erythematosus. Front Immunol. 2022;13:886209. Doi:10.3389/fimmu.2022.886209.
[20] Pouwels SD, Nawijn MC, Bathoorn E, Riezebos-Brilman A, van Oosterhout AJ, Kerstjens HA, et al. Increased serum levels of LL37, HMGB1 and S100A9 during exacerbation in COPD patients. Eur Respir J. 2015;45(5):1482-85. Doi: 10.1183/09031936.00158414.
[21] Liu M, Won Lee J, Jung S, Ji S, Choi Y. Ability of S100 proteins and matrix metalloproteinase-9 to identify periodontitis in a ligature-induced periodontitis dog model. J Clin Periodontol. 2020;47(2):182-92. Doi:10.1111/jcpe.13215.
[22] George AK, Malaiappan S, Joseph B, Anil S. Calprotectin, S100A8, and S100A9: Potential biomarkers of periodontal inflammation: A scoping review. World J Dent. 2023;14(6):559-67.
[23] Sánchez-Medrano AG, Martinez-Martinez RE, Soria-Guerra R, Portales-Perez D, Bach H, Martinez-Gutierrez F. A systematic review of the protein composition of whole saliva in subjects with healthy periodontium compared with chronic periodontitis. PLoS One. 2023;18(5):e0286079. Doi: 10.1371/journal.pone.0286079.
[24] Holmström SB, Lira-Junior R, Zwicker S, Majster M, Gustafsson A, Åkerman S, et al. MMP-12 and S100s in saliva reflect different aspects of periodontal inflammation. Cytokine. 2019;113:155-61. Doi: 10.1016/j.cyto.2018.06.036.
[25] Haririan H, Andrukhov O, Pablik E, Neuhofer M, Moritz A, Rausch-Fan X. Comparative analysis of calcium-binding myeloid-related protein-8/14 in saliva and serum of patients with periodontitis and healthy individuals. J Periodontol. 2016;87(2):184-92. Doi:10.1902/jop.2015.150254.
[26] Kamatham SA, Chava VK. Comparison of salivary calprotectin levels in periodontitis associated with diabetes mellitus after low-level laser therapy as an adjunct to scaling and root planing: A randomized clinical trial. J Indian Soc Periodontol. 2022;26(2):143-50. Doi: 10.4103/jisp.jisp_149_21.
[27] Kido J, Nakamura T, Kido R, Ohishi K, Yamauchi N, Kataoka M, et al. Calprotectin in gingival crevicular fluid correlates with clinical and biochemical markers of periodontal disease. J Clin Periodontol. 1999;26(10):653-57. Doi: 10.1034/j.1600-051x.1999. 261004.x.
[28] Gao H, Xu J, He L, Meng H, Hou J. Calprotectin levels in gingival crevicular fluid and serum of patients with chronic periodontitis and type 2 diabetes mellitus before and after initial periodontal therapy. J Periodontal Res. 2021;56(1):121-30. Doi:10.1111/jre. 12800.
[29] Lira-Junior R, Öztürk VÖ, Emingil G, Bostanci N, Boström EA. Salivary and serum markers related to innate immunity in generalized aggressive periodontitis. J Periodontol. 2017;88(12):1339-47. Doi:10.1902/jop.2017.170287.
[30] Que ML, Andersen E, Mombelli A. Myeloid-related protein (MRP)8/14 (calprotectin) and its subunits MRP8 and MRP14 in plaque-induced early gingival inflammation. J Clin Periodontol. 2004;31(11):978-84. Doi:10.1111/j.1600-051X.2004. 00594.x .
[31] Gao H, Hou J, Meng H, Zhang X, Zheng Y, Peng L. Proinflammatory effects and mechanisms of calprotectin on human gingival fibroblasts. J Periodontal Res. 2017;52(6):975-83. Doi:10.1111/jre.12465.
[32] Gao H, Zhang X, Zheng Y, Peng L, Hou J, Meng H. S100A9-induced release of Interleukin (IL)-6 and IL-8 through toll-like receptor 4 (TLR4) in human periodontal ligament cells. Mol Immunol. 2015;67(2 Pt B):223-32. Doi: 10.1016/j.molimm.2015.05.014.
[33] Zheng Y, Hou J, Peng L, Zhang X, Jia L, Wang X, et al. The pro-apoptotic and pro-inflammatory effects of calprotectin on human periodontal ligament cells. PLoS One. 2014;9(10):e110421. Doi: 10.1371/journal.pone.0110421.
[34] Zreiqat H, Howlett CR, Gronthos S, Hume D, Geczy CL. S100A8/S100A9 and their association with cartilage and bone. J Mol Histol. 2007;38(5):381-91. Doi: 10.1007/s10735-007-9117-2.

PARTICULARS OF CONTRIBUTORS:
1. Professor, Department of Periodontics, Pushpagiri College of Dental Sciences, Kerala, India; Ph.D. Scholar, Department of Periodontics, Saveetha Dental College and Hospital, Saveetha Institute of Medical and Technical Sciences, Chennai, India.
2. Professor, Department of Periodontics, Saveetha Dental College and Hospital, Saveetha Institute of Medical and Technical Sciences, Chennai, India.
3. Assistant Professor, Department of Public Health Dentistry, Government Dental College, Kottayam, Kerala, India.
4. Professor, Department of Biochemistry, Saveetha Dental College and Hospital, Saveetha Institute of Medical and Technical Sciences, Chennai, India.
5. Assistant Professor, Department of Periodontics, Saveetha Dental College and Hospital, Saveetha Institute of Medical and Technical Sciences, Chennai, India..

NAME, ADDRESS, E-MAIL ID OF THE CORRESPONDING AUTHOR:
Dr. Annie Kitty George,
Professor, Department of Periodontics, Pushpagiri College of Dental Sciences,
Thiruvalla-689107, Kerala, India.
E-mail: dranniekitty121@gmail.com

PLAGIARISM CHECKING METHODS: [Jain H et al.]
- Plagiarism X-checker: Feb 15, 2024
- Manual Googling: Apr 16, 2024
- iThenticate Software: Jun 01, 2024 (12%)

ETYMOLOGY: Author Origin

EMENDATIONS: 7

AUTHOR DECLARATION:
- Financial or Other Competing Interests: None
- Was Ethics Committee Approval obtained for this study? Yes
- Was informed consent obtained from the subjects involved in the study? Yes
- For any images presented appropriate consent has been obtained from the subjects. NA

Date of Submission: **Feb 14, 2024**
Date of Peer Review: **Apr 04, 2024**
Date of Acceptance: **Jun 03, 2024**
Date of Publishing: **Aug 01, 2024**