ORIGINAL RESEARCH

Gene expression of S100A8 in periodontitis: A pilot real time quantitative polymerase chain reaction study and in-silico analysis of potential gene targets

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Abstract

Introduction: S100A8 is an endogenous 'alarmin' that is expressed from immune cells during their activation or damage. This damage associated molecular pattern can sustain and amplify inflammation.The objective of our study was to estimate and compare the mRNA expression of S100A8 in gingival biopsies drawn from diseased and healthy periodontium. An in-silico analysis of S100A8 gene targets and their biologic and functional relevance in periodontal pathogenesis was also carried out.

Methodology: In this pilot cross-sectional study, gingival tissue samples were collected, one sample each, from 20 participants of which ten participants had clinically healthy periodontium(H) and ten patients were diagnosed with stages III or IV periodontitis. (P) Samples were immediately processed by real-time quantitative polymerase chain reaction (RT-qPCR) and gene expression of S100A8 was estimated and compared between H and P groups

In silico protein-protein Interaction (PPI) network was constructed using differentially expressed genes (DEGs) that were identified through the Search Tool for the Retrieval of Interacting Genes (STRING) database. ENRICHR database was used to perform functional and pathway enrichment analysis to understand the biological significance of the identified DEGs and gene clusters. Kyoto Encyclopedia of Genes and Genomes (KEGG) and Reactome pathway analysis was performed to identify pathways significantly enriched with the DEGs.

Results: Mean gingival index (GI) in H and P groups were 0.52±0.40 and 1.52±0.48 respectively. ($p< 0.001$) Mean fold change in expression of mRNA of S100A8 was 1.1577 in group H and 2.240 in group P. ($p=0.03$). $p<0.05$was considered to be statistically significant.

The ten gene targets of S100A8 that were identified by in silico analysis were TP53, GSK3B, HSP90AA1, HSP90AB1, UBC, UBA52, RPS27A, UBB, SQSTM1 and PTEN.

Conclusion: The expression of S100A8 mRNA was observed to be higher in diseased gingival tissue samples when compared to healthy gingival biopsies. The potential gene targets of S100A8 identified by in-silico analysis indicate that this protein may have a prominent role in periodontal pathogenesis.

Key words: Alarmins, Transcriptome,Gingiva,Bioinformatics

Introduction

Introduction: The periodontium supports and maintains the teeth in their normal position and function. Periodontal diseases lead to destruction of the gingiva, cementum, periodontal ligament and alveolar bone encasing the teeth and can progressively lead to loss of teeth. In addition to localized tissue destruction, periodontal diseases can also contribute to low grade systemic inflammation. Inflammation in the periodontal tissues is initiated by dysbiosis in the plaque biofilm. The temporal relationship between inflammation and dysbiosis is yet to be fully unravelled.[1] The progression of this inflammatory process is however largely determined by host susceptibility factors.

Periodontal inflammation is underlined by a vast array of complex interconnected cell signalling pathways involving cytokine and chemokine networks. Pathogen associated molecular pattern (PAMP) binding to pathogen recognition receptors (PRR's) initiates these immunoinflammatory pathways. The cellular and tissue destruction which ensues will lead to the expression of a variety of damage associated molecular patterns (DAMP's) such as S100 proteins, neutro-

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phil extracellular traps (NETs) and high mobility group box 1 (HMGB1)proteins which can sustain and augment inflammation.[2]

DAMP's are intracellular proteins released from immune cell during their activation, damage or necrosis. Continuous activation of the innate immune system is mediated by PAMP's and DAMP's leading to sustained and augmented host responses. DAMP's bind to toll-like receptors (TLR's)or receptors for advanced glycation end products (RAGE) to activate endothelial and immune cells. DAMP's can also cause inflammasome activation.[3] The role of DAMP's is now being increasingly recognized in the etiopathogenesis of periodontal diseases.

S100A8 belongs to the S100 family of 25 proteins which are named S100's due to their solubility in a 100% saturated neutral ammonium sulphate solution. Each of the S100 proteins are encoded by individual genes and 19 of these are located as a cluster on chromosome 1q21. These are low molecular weight proteins with high affinity for calcium. Of the S100's, S100A8, S100A9 and S100A12 have been recognized as innate immune modulators. They have been recognized as 'alarmins' as they initiate innate immune responses. S100A8 is also named as Calgranulin A[4] or myeloid related protein-8(MRP8).[5] This protein is an important component of the neutrophilic cytosol and are chiefly expressed by neutrophils, macrophages and dendritic cells. In an inflammatory environment, these proteins are also expressed by endothelial cells, epithelial cells, osteoclasts and fibroblasts.[6,7]

It has been reported that the S100 proteins have regulatory roles in cellular functions such as proliferation, migration and invasion, differentiation, phosphorylation of amino acids, apoptosis, calcium homeostasis and energy utilization.[7] Extracellular functions of S100A8 includes chemotaxis of neutrophils, stimulation of expression of IL-1 beta and TNF-α from myeloid cells and protection from oxidative tissue damage in sites of inflammation. It can induce differentiation of keratinocytes and also has anti-inflammatory effects.[6] S100A8 can bind to metal ions such as Ca^{2+} or Zn^{2+},deprieve microbes of their essential nutrients thus exert an antimicrobial effect. With its pro and anti-inflammatory roles in inflammation and its antimicrobial functions, S100A8 qualifies as an immunomodulator in microbe induced inflammation such as periodontitis.

Presence of S100A8 in gingiva crevicular fluid of periodontitis patients by high-performance liquid chromatography was reported in by Kojima et al[8] and Lundy et al.[9] Lundy et al quantified S1008 in GCF using microbore high-performance liquid chromatography.[10] A shotgun proteomic study reported that this protein could be a candidate biomarker for periodontitis.[11] It has been reported that salivary S100A8 could be used to screen for periodontal diseases.[12] Further, recent studies have reported the diagnostic[13] and prognostic potential of this protein.[14] In a previous cross-sectional study, we have also identified the potential of salivary S100A8 as a candidate biomarker for periodontal diseases.[15]

Although there is unequivocal evidence regarding the prominent presence of S100A8 in oral fluids of periodontitis patients, to the best of our knowledge, gene expression of this protein in gingival tissues of periodontally diseased versus healthy subjects have not been previously reported. This study hypothesized that mRNA expression of S100A8 would be higher in periodontally diseased gingival tissue than in healthy gingival tissue samples. The objective of this study was to investigate and compare mRNA expression of S100A8 in gingival tissue samples drawn from periodontally diseased and healthy individuals. In-silico investigations also sought to identify and investigate the probable gene targets associated with S100A8 in periodontal pathogenesis.

Methodology:

In this pilot study, a cross-sectional design was employed. Patients in the age group of 25-60 years reporting for periodontal therapy to the Periodontology clinics of Saveetha Dental College and hospital constituted the sampling frame. The study duration was 6 months from January 2025 up to June 2025. Consecutive samples were drawn from participants who met inclusion and exclusion criteria. Aims of the study were explained to each patient and written informed consent was taken from all participants and this study was carried out after clearance from the Institutional ethics and scientific review board of the dental college. (IHEC/PhD/PERIO-1621/21/230) This study was carried out according to the guidelines regarding research in human subjects laid down in the declaration of Helsinki of 1975, and revised in 2024. Those patients who gave a self-reported prior personal history of diabetes, smoking or any cardiovascular disease were excluded. Obese subjects (BMI>30), pregnant or lactating females and those who had antibiotic or steroid therapy in the preceding three months were also excluded. Periodontal examination was done after seating the patient comfortably on the dental chair and examined using a mouth mirror and University of North Carolina-15(UNC-15) periodontal probe. Patients with stages III or IV periodontitis[16] with periodontal pockets > 10mm,and who had periodontally involved mobile teeth with hopeless prognosis, indicated for extraction constituted the Periodontitis (P) group and subjects with clinical periodontal health[17] who were referred to the periodontology clinic for crown lengthening procedures constituted the healthy (H) group . Stages III or IV periodontitis and clinical periodontal health were defined according to the criteria laid down by the American Academy of Periodontology and European Federation of Periodontology World Work shop on the classification of periodontal and peri implant health and diseases. Gingival health was defined as <10% bleeding sites with probing depths ≤ 3mm.[18] Stages III or IV periodontitis was defined based on staging and complexity criteria.[16] Stages III or IV periodontitis cases had ≥ 5mm interdental clinical attachment loss(CAL) at site of greatest loss and probing depths (PD) of ≥ 6mm. Gingival index (GI) was recorded in all participants. Percentage of sites with PD≥ 5mm and CAL≥ 5mm was also recorded. One gingival tissue sample from each participant was collected using a universal curette during tooth extraction for the periodontitis group and during gingivectomy in the healthy.

As there were no previous studies available, we estimated the sample size for this pilot study using G Power Software. The input used for power calculation is an anticipated effect

size of 1.4, Type 1 error rate of 5% and Type 2 error rate of 20%. Based on these inputs, a sample size of 10 patients per arm was finalized. A total of 20 gingival tissue samples were collected from the 20 participants, ten each per group and gene expression of S100A8 was estimated by quantitative real time polymerase chain reaction. (RT-qPCR)

Gene expression analysis:

RNA extraction and cDNA synthesis:

Total RNA was isolated from the gingival tissues using TRIzol reagent (Thermo Fisher Scientific, USA) according to the manufacturer's instructions. Nanodrop One (Thermo Scientific, USA) was used to analyse the quality and amount of the RNA. Following the manufacturer's instructions, the first-strand complementary DNA (cDNA) was synthesized using the Takara First-Strand cDNA Synthesis Kit (Takara, Tokyo, Japan).

RT-qPCR analysis:

The primer sequence for S100A8 was designed using Primer3web, version 4.1.0 (https://primer3.ut.ee/) and synthesized from Eurofins, Bangalore, India. The assay for S100A8 mRNA expression analysis was performed according to the reaction setup and conditions given below. qPCR runs were performed in duplicates. The reaction mixture consists of 20 microliters of PCR reaction, comprising two microliters of cDNA template, 50 mM forward and reverse primers, 10 microliters of 2X SYBR master solution, and ddH2O. The Bio-Rad CFX Opus 96 (Bio-Rad, Hercules, CA, USA) was used to quantify S100A8 gene expression in all the samples. The GAPDH was used as a reference gene, and the cycling protocol was standardized based on the conditions. Specificity of amplification was ensured through proper primer design using reference sequences and in silico validation. (NCBI BLAST) The gene expression analysis results were analysed using Bio-Rad CFX Maestro 1.0 software (version 4.0.2325.0418).

Primer	Forward (5' to 3')	Reverse (5' to 3')
S100A8	CCGAGTGTCCTCAGTATATCAGGA	GCCCATCTTTATCACCAGAATGA
GAPDH	TTGGCTACAGCAACAGGGTG	GGGGAGATTCAGTGTGGTGG

In silico assessment of potential gene targets of S100A8 in periodontal pathogenesis:

Networking analysis strategy of S100A8 associated with periodontitis using the Search Tool for the Retrieval of Interacting Genes (STRING) database: To identify and validate potential therapeutic targets associated with S100A8 in periodontitis, an integrated bioinformatics approach using Comparative Toxicogenomic Database (CTD: http://ctdbase.org/) and STRING databases [online tool for investigating drug-protein interactions (http://stitch.embl.de/)] was employed. Protein-Protein Interaction (PPI) network was constructed using Differentially Expressed Genes (DEGs). In the PPI network, STRING database enabled us to visualize and map the regulatory connections between S100A8, and periodontitis associated gene targets. In the PPI network, the edge widths between proteins were adjusted according to the combined scores, and reflected the strength of the interactions. The Cytoscape software (version 3.5.1; http://www.cytoscape.org) was also used to build and visualize the PPI network. Visualization was further enhanced using the CytoHubba plugin in Cytoscape, which helped highlight key elements within the network. CytoHubba allowed us to rank the targets based on key parameters, including betweenness, bottleneck, Closeness, Maximal Clique Centrality, (MCC) and radiality which helped prioritize the most influential genes in the network.

Functional annotations: An in-depth functional and pathway enrichment analysis was done for understanding the biological significance of the identified differentially expressed genes (DEGs) and gene clusters. A comprehensive exploration of the biological functions and potential pathways enriched among the up-regulated biomolecules was also carried out. The ENRICHR (Tool for enrichment analysis of genes) database was utilized for this analysis, and provided systematic and consistent annotations that categorized genes into those involved with molecular functions (MF), cellular components (CC), and biological processes (BP). A Kyoto Encyclopedia of Genes and Genomes (KEGG) and Reactome pathway analysis was also carried out for S100A8 associated gene targets.

Statistical analysis: All data were entered into spread sheets. Periodontal parameters such as GI, Percentage of sites with PD ≥ 5mm and CAL ≥ 5mm was expressed in terms of mean and standard deviation. GI was compared between groups by unpaired t-test.

Gene expression was calculated using the $2^{-\Delta\Delta Ct}$ method, with GAPDH used as an internal control. The statistical analysis was performed using IBM SPSS Statistics version 23 (IBM Corp., Armonk, NY, USA). Unpaired t-test was used to compare the two groups. $p<0.05$ was considered to be statistically significant.

Results of RT-qPCR

This pilot study enrolled 20 participants from whom 20 gingival tissue samples were collected, with ten healthy gingival samples drawn from participants with clinical periodontal health (Group H) and ten samples from periodontally diseased sites of patients with stages III or IV periodontitis (Group P). The mean GI of H group was 0.52±0.40 and that of the P group was 1.52 ± 0.48 and the difference between the groups is statistically significant. ($p< 0.001$). There were no sites with PPD≥ 5mm and CAL ≥ 5mm in the H group. The mean percentage of sites with PPD ≥ 5 mm was 74.10±24.80 and CAL ≥ 5mm was 59.75 ±30.84 in the P group. (Table 1) The mean fold change in mRNA expression in H group was 1.1577 and was 2.240 in the P group. ($p=0.03$, Unpaired t-test). (Table 2) The relative S100A8 mRNA expression is described graphically in Figure 1.

***In-silico* identification of S100A8 associated targets in periodontitis:** Potential gene targets of S100A8 identified using the STRING data base are shown in Figure 2. CytoHubba analysis identified the top 10 significant targets with high scores. The 10 gene targets identified were TP53, GSK3B, HSP90AA1, HSP90AB1, UBC, UBA52, RPS27A, UBB, SQSTM1 and PTEN. (Fig. 3) The results of GO and pathway enrichment analysis,

visualized in a bubble chart (Fig.4), highlight the top five highly enriched GO terms within each category, offering insights into the biological roles of these gene targets for periodontitis. Within the biological processes (BP) category, the enriched GO terms revealed that these genes are involved in essential functions such as regulation of cell cycle, cellular response to hypoxia, positive regulation of gene expression, cell proliferation, and cellular response to interleukin-9. Additionally, the cellular component (CC) analysis pointed to significant subcellular localizations of these targets, particularly within the nucleolus, nuclear lumen, and nucleus. In terms of molecular functions (MF), the targets were found to be associated with crucial activities, including protease binding, DNA binding, and protein phosphatase binding. (Figure 4) KEGG and Reactome pathway

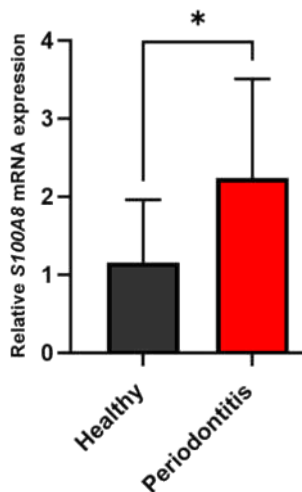

Fig. 1: Relative mRNA expression in periodontally diseased and healthy gingival tissue samples

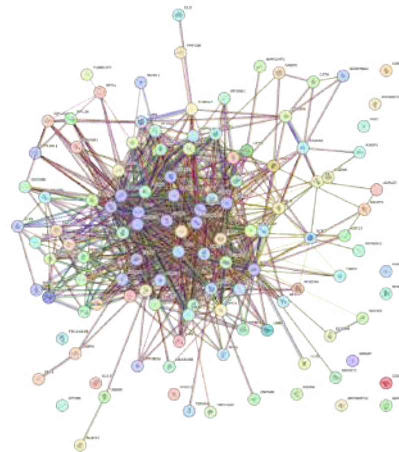

Fig. 2. S100A8 associated targets with periodontitis were identified using STRING database.

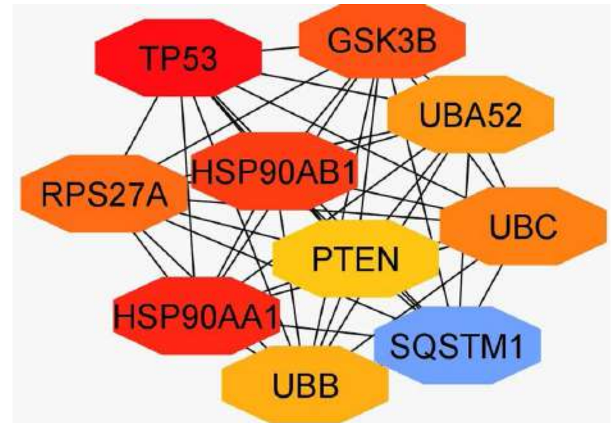

Fig. 3: Target genes of S100A8 in cytohubba

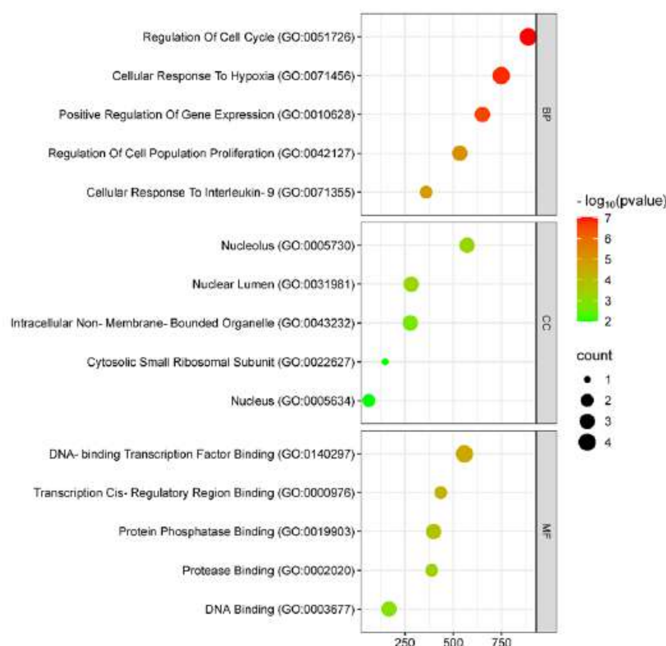

Fig. 4. Gene ontologies -Biologic Processes, Cellular Components, and Molecular Functions

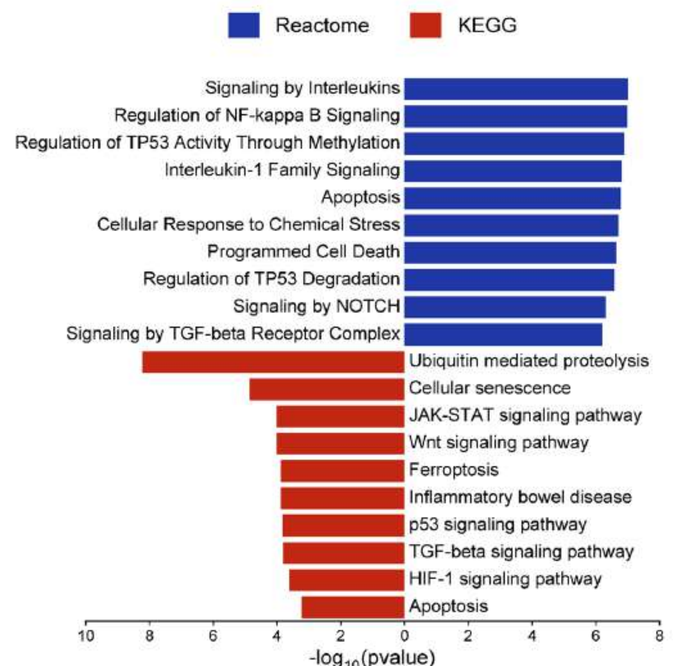

Fig. 5. Pathway prediction analysis (KEGG and Reactome pathways).

Table 1: Clinical periodontal parameters in periodontal health and disease

Unpaired t-test, p<0.05 is statistically significant
H-Health, P-Periodontitis, PD-Probing pocket depth, CAL-Clinical attachment loss

Periodontal parameters	Group H n=10 Mean (SD)	Group P n= 10 Mean (SD)	P value*
Gingival Index	0.52(0.40)	1.52(0.48)	< 0.001
% sites with PD >5mm	–	74.10 (24.80)	
% sites with CAL >5mm	–	59.75 (30.84)	

Table 2: Relative gene expression of S100A8 in Groups H and P and result of statistical analysis

S100A8 mRNA expression	Group H	Group P
Mean fold change	1.1577	2.240
P value		0.03
t		2.249

Unpaired t-test, p<0.05 is statistically significant

analysis highlighted significant enrichment in pathways like signalling by interleukins, regulation of Nfkβ signalling and interleukin-1 family signalling. (Figure 5)

Discussion

This study estimated and compared the expression of mRNA of S100A8 in gingival tissues drawn from periodontally diseased and healthy participants. mRNA expression of S100A8 was quantified by quantitative reverse transcription real time PCR. This pilot investigation observed higher expression levels of S100A8-mRNA in tissue samples drawn from diseased periodontal tissues when compared to healthy gingival tissues. Samples of diseased periodontal tissues subjacent to teeth indicated for extraction and healthy samples from gingival tissues discarded during gingivectomy were used for the qt-PCR assay.

Saliva is a largely non-specific oral fluid and the source of a protein expressed in saliva may be from other sources such as the salivary glands. Although GCF is in close proximity with the gingival sulcus/periodontal pocket, its composition may reflect serum components. Proteomic studies in oral fluids have to be validated by omics studies in the periodontal tissues to confirm the periodontal source of the target protein. Gene and protein expression from the periodontal tissues substantiates that the periodontium is the source of specific proteins that have been identified in saliva and GCF. Prominent presence and higher levels S100A8 in oral fluids have been identified by few researchers.[19] This transcriptomic study was done to underline that the periodontium was the source of S100A8 and that the inflamed periodontium is the source of higher levels of the protein observed in oral fluids. Gingival tissue samples comprises both epithelium and connective tissue. The gingival epithelium present in the samples may be the outer oral epithelium, sulcular epithelium and or the junctional epithelium. The gingival connective tissue is composed of a cellular component dominated by gingival fibroblasts and an extracellular component chiefly made up of collagen fibers. Gingival tissue samples may also house resident and recruited immune cells. The source of S100A8mRNA may be from any of these structural elements of the gingiva.

Gene expression of a specific protein in tissue at a particular time point reflects the tissue's capacity for the synthesis of the target protein. In this study we used the quantitative real-time PCR (qRT-PCR) Real-time PCR is a quantitative method for determining copy number of PCR templates, such as complimentary DNA or cDNA. In this advanced molecular biology technique, RNA is first converted into cDNA. This is followed by real time PCR amplification. Fluorescent dyes are used for the detection of cDNA. It is an extremely sensitive and specific tool to determine the expression level of target genes.[20] By confirming the higher expression levels of S100A8,we can deduce that the gingiva is the source of elevated levels of this protein previously reported[10,11,14] in oral fluids. Expression of S100A8 mRNA in junctional epithelium of mice has been previously reported.[21] Gao et al have reported expression of S100A8 from human gingival fibroblasts and periodontal ligament cells.[22]

Periodontal disease diagnosis is based on parameters of past periodontal destruction such as probing pocket depth, clinical attachment loss and bone loss. Clinical parameters can distinguish health and disease states, but cannot address individual patient 'susceptibility' factor. The new AAP-EFP classification system on periodontal and peri implant diseases and conditions emphasises the need for the incorporation of adequately validated biomarkers for the diagnosis and classification of periodontal diseases.[23,16] Recent studies have underlined the potential of salivary S100A8 as a biomarker for periodontal diseases.[24,25] Results of this study points out that the diseased gingival tissues may be the source of the observed higher salivary levels of this protein in periodontitis.

In this study, the probable target gene interactions of S100A8 were explored by in-silico analysis. Protein-protein interactions were studied using the STRING data base.[26] The STRING database collects and integrates both physical and functional associations between proteins. The probable gene targets of S100A8 were identified and their potential biologic and molecular functions were identified. These genes were prioritized due to their established roles in periodontitis progression, where they are known to influence critical cellular processes such as apoptosis, cell survival, and inflammatory regulations. The TP53 gene which was identified as an important gene target of S100A8 in this study was recently identified as an immune modulator in periodontitis with its effects on macrophage differentiation.[27] GSK3B gene has also been implicated in the generation of proinflammatory cytokines and alveolar bone loss

in periodontitis.[28] HSP90AA1 gene was reported to augment inflammatory response in human gingival fibroblasts induced by Porphyromaonas Gingivalis by mediating autophagy.[29] The Ubiquitin-Protease system has been strongly implicated in periodontal pathogenesis.[30] RPS27A was reported to be an important regulator in the molecular mechanisms in periodontal therapy.[31] PTEN gene was also reported to have crucial roles in alveolar bone remodelling in periodontitis.[32] The high enrichment of these gene targets within pivotal signalling pathways involved in regulation of Nuclear factor kappa-light-chain-enhancer of activated B cells (Nfk-β) and interleukin-1 family of cytokines, suggests that S100A8-associated targets may influence pivotal signalling routes that regulate periodontal pathogenesis. This pilot study is unique because the mRNA expression of S100A8 in diseased gingiva has been much less explored. Sites of sample collection was very close to the molecular biology laboratory and this allowed rapid processing of samples.

However, this study is limited by its small sample size. Moreover, the cross-sectional design of the study does not establish temporal relationship between the observed higher gene expression of S100A8 and periodontitis. Future studies should investigate gene expression of S100A8 in combination with other well-established cytokines in periodontal inflammation such as IL-1β.

Conclusion

This study observed that mRNA expression of S100A8 was significantly higher in periodontally diseased gingival tissue samples when compared to healthy gingival tissues. Diagnostic accuracy studies may further substantiate the potential role of S100A8 as a biomarker candidate of periodontal inflammation. In-silico analysis points out that this protein may be important in multiple biologic processes in the pathogenesis of periodontal diseases.

References

1. Van Dyke TE, Bartold PM, Reynolds EC. The Nexus Between Periodontal Inflammation and Dysbiosis. Front Immunol. 2020;11. doi:10.3389/fimmu.2020.00511
2. Zhu X, Huang H, Zhao L. PAMPs and DAMPs as the Bridge Between Periodontitis and Atherosclerosis: The Potential Therapeutic Targets. Front Cell Dev Biol. 2022;10. doi:10.3389/fcell.2022.856118
3. Sharma H, Sharma A, Tripathi U. Deciphering Inflammasome Mechanisms in Periodontal Diseases: A Review. Arch Med Heal Sci. 2025;13(1):100-109. doi:10.4103/amhs.amhs_90_24
4. Lundy FT, Chalk R, Lamey PJ, Shaw C, Linden GJ. Identification of MRP-8 (calgranulin A) as a major responsive protein in chronic periodontitis. J Pathol. 2000;192(4):540-544. doi:10.1002/1096-9896(2000)9999:9999<::AID-PATH740>3.0.CO;2-1
5. Kaner D, Bernimoulin JP, Dietrich T, Kleber BM, Friedmann A. Calprotectin levels in gingival crevicular fluid predict disease activity in patients treated for generalized aggressive periodontitis. J Periodontal Res. 2011;46(4):417-426. doi:10.1111/j.1600-0765.2011.01355.x
6. Xia P, Ji X, Yan L, Lian S, Chen Z, Luo Y. Roles of S100A8, S100A9 and S100A12 in infection, inflammation and immunity. Immunology. 2024;171(3):365-376. doi:10.1111/imm.13722
7. Gonzalez LL, Garrie K, Turner MD. Role of S100 proteins in health and disease. Biochim Biophys Acta - Mol Cell Res. 2020;1867(6):118677. doi:10.1016/j.bbamcr.2020.118677
8. Kojima T, Andersen E, Sanchez JC, et al. Human gingival crevicular fluid contains mrp8 (s100a8) and mrp 14 (s100a9), two calcium-binding proteins of the s100 family. J Dent Res. 2000;79(2):740-747. doi:10.1177/00220345000790020701
9. Lundy FT, Chalk R, Lamey PJ, Shaw C, Linden GJ. Identification of MRP-8 (calgranulin A) as a major responsive protein in chronic periodontitis. J Pathol. 2000;192(4):540-544. doi:10.1002/1096-9896(2000)9999:9999<::AID-PATH740>3.0.CO;2-1
10. Lundy FT, Chalk R, Lamey PJ, Shaw C, Linden GJ. Quantitative analysis of MRP-8 in gingival crevicular fluid in periodontal health and disease using microbore HPLC. J Clin Periodontol. 2001;28(12):1172-1177. doi:10.1034/j.1600-051x.2001.281213.x
11. 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):3571-3580. doi:10.1007/s00784-018-2779-1
12. Karna S, Shin YJ, Kim S, Kim HD. Salivary S100 proteins screen periodontitis among Korean adults. J Clin Periodontol. 2019;46(2):181-188. doi:10.1111/jcpe.13059
13. Kim HD, Karna S, Shin YJ, 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):1-10. doi:10.1186/s12903-021-01749-z
14. 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):1191-1200. doi:10.1111/jcpe.13349
15. George AK, Narayan SMV, Jayaraman S, Suresh N. Salivary Levels of S100A8, S100A9 and S100A8/9 in Periodontal Health and Disease: A Cross-sectional Study. J Clin DIAGNOSTIC Res. Published online 2024. doi:10.7860/JCDR/2024/69936.19754
16. Tonetti MS, Greenwell H KK. Staging and grading of periodontitis: Framework and proposal of a new classification and case definition. J Clin Periodontol. 2018;45(Suppl 2:S149–S161. doi:/10.1111/jcpe.12945
17. Lang NP, Bartold PM. Periodontal health. J Periodontol. 2018;89(S1). doi:10.1002/JPER.16-0517
18. Chapple ILC, Mealey BL, Van Dyke TE, 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 Clin Periodontol. 2018;45(S20). doi:10.1111/jcpe.12940
19. George AK, Malaiappan S JB& AS. Calprotectin, S100A8, and S100A9: Potential Biomarkers of Periodontal Inflammation: A Scoping Review. World J Dent. 2023;14(6):559-567. doi:10.5005/jp-journals-10015-2244
20. Mo Y, Wan R, Zhang Q. Application of Reverse Transcription-PCR and Real-Time PCR in Nanotoxicity Research. In: ; 2012:99-112. doi:10.1007/978-1-62703-002-1_7
21. Nishii K, Usui M, Yamamoto G, et al. The distribution and expression of S100A8 and S100A 9 in gingival epithelium of mice. J Periodontal Res. 2013;48(2):235-242. doi:10.1111/jre.12000
22. Gao HY, Huang BX, Hou JX MHZKQYXZZ. Preliminary study on the expression and distribution of S100A8 and S100A9 in healthy and experimental periodontitis tissues. doi:10.3760/cma.j.cn112144-20191219-00456
23. Trombelli L, Farina R, Silva CO, Tatakis DN. Plaque-induced gingivitis: Case definition and diagnostic considerations. J Clin Periodontol. 2018;45(S20). doi:10.1111/jcpe.12939
24. Blanco-Pintos T, Regueira-Iglesias A, Relvas M, et al. Diagnostic Accuracy of Novel Protein Biomarkers in Saliva to Detect Periodontitis Using Untargeted 'SWATH' Mass Spectrometry. J Clin Periodontol. Published online December 27, 2024. doi:10.1111/jcpe.14103
25. Blanco-Pintos T, Regueira-Iglesias A, Suárez-Rodríguez B, et al. Characterisation of the periodontal proteome in gingival crevicular fluid and saliva using SWATH-MS. Front Cell Infect Microbiol. 2025;15. doi:10.3389/fcimb.2025.1576906
26. Szklarczyk D, Kirsch R, Koutrouli M, et al. The STRING database in 2023: protein–protein association networks and

functional enrichment analyses for any sequenced genome of interest. Nucleic Acids Res. 2023;51(D1):D638-D646. doi:10.1093/nar/gkac1000

27. Liu T, Chen D, Tang S, et al. P53 Alleviates the Progression of Periodontitis by Reducing M1-type Macrophage Differentiation. Inflammation. 2024;47(4):1170-1184. doi:10.1007/s10753-024-01968-w
28. Adamowicz K, Wang H, Jotwani R, Zeller I, Potempa J, Scott DA. Inhibition of GSK3 Abolishes Bacterial-Induced Periodontal Bone Loss in Mice. Mol Med. 2012;18(8):1190-1196. doi:10.2119/molmed.2012.00180
29. Zhang H, Huang J, Fan X, Miao R, Wang Y. HSP90AA1 promotes the inflammation in human gingival fibroblasts induced by Porphyromonas gingivalis lipopolysaccharide via regulating of autophagy. BMC Oral Health. 2022;22(1):366. doi:10.1186/s12903-022-02304-0
30. Ma Y, Jia R, Chen S, et al. Ubiquitin-Proteasome System in Periodontitis: Mechanisms and Clinical Implications. Cell Prolif. 2025;58(3). doi:10.1111/cpr.13781
31. Ma JJ, Liu HM, Xu XH, Guo LX, Lin Q. Study on gene expression patterns and functional pathways of peripheral blood monocytes reveals potential molecular mechanism of surgical treatment for periodontitis. World J Clin Cases. 2019;7(12):1383-1392. doi:10.12998/wjcc.v7.i12.1383
32. Fu C, Wei Z, Zhang D. PTEN Inhibits Inflammatory Bone Loss in Ligature-Induced Periodontitis via IL1 and TNF- α. Biomed Res Int. 2019;2019:1-9. doi:10.1155/2019/6712591