Research Article

JRAD Journal of Research and Advancement in Dentistry

J Res Adv Dent 2016;5:1:109-114.

Identification and Comparison of S100 Proteins in the Diagnosis of Aggressive Periodontal Diseases: A pilot study

Vandita Mishra[1*] Mahesh Bajaj[2] Leena Shettar[3] Abhishek Savirmath[4] Srinath L Thakur[5]

[1]Senior Lecturer, Department of Periodontology and Oral Implantology, Sri Sai College of Dental Surgery, Vikarabad, Telangana, India.
[2]PG Student, Department of Periodontology and Oral Implantology, S.D.M. College of Dental Sciences and Hospital, Karnataka, India.
[3]Professor and Head, Department of Periodontology and Oral Implantology, S.D.M. College of Dental Sciences and Hospital, Karnataka, India.
[4]Reader, Department of Prosthodontics, Sri Sai College of Dental Surgery, Vikarabad, Telangana, India.
[5]Principal and Professor, Department of Periodontology and Oral Implantology, S.D.M. College of Dental Sciences and Hospital, Karnataka, India.

ABSTRACT

Aim: Our aim was thus to identify, quantify and compare the levels of S100 proteins in the state of health and in individuals with chronic and aggressive periodontitis.

Methods: This was a single center, cross-sectional study of 4 months duration. A total of 30 subjects were included in the study 10 each with a healthy periodontium and chronic periodontits and 10 with aggressive periodontal disease. 5ml of resting saliva was collected from all the individuals and the samples were immediately sent to the laboratory of the biochemistry department of the institute. The samples were subjected to one dimensional sodium dodecyl sulphate polyacrylamide gel electrophoresis to identify and quantify the total S100 proteins.

Results: Protein bands corresponding to lower molecular weight proteins were seen in the aggressive periodontitis individuals. In both healthy subjects and in the chronic periodontitis group these lower molecular weight bands were not identified.

Conclusion: Lower molecular weight proteins were identified in aggressive periodontitis individuals only which may be correlated to the presence of neutrophilic abnormalities in individuals with this disease.

Keywords: Periodontitis, Saliva, Aggressive periodontitis, S100 protein family.

INTRODUCTION

Periodontitis is a common disease characterized by bacterially induced inflammatory destruction of the supporting tissues of the teeth. The etiology of periodontal inflammation is complex and most likely varies from individual to individual, but in general the disease can be considered to result from an inappropriate balance between proinflammatory and anti-inflammatory signals.

The ultimate aim of any periodontal diagnostic procedure would be not just to identify the disease present but also to recognize the sites of active disease, to monitor quantitatively the response to therapy and to measure the degree of susceptibility of the individual to future disease progression. The traditional clinical criteria used to diagnose any disease such as periodontal pocket depth, attachment level, plaque index, bleeding on probing and radiographic assessment of alveolar bone loss are informative to evaluate disease severity(1) but provide few useful determinants of the disease activity(2,3,4). The presence of bleeding on probing for example though is still the best disease activity predictor available, but is not very specific and reveals many false positives and false

Received: June. 10, 2015: **Accepted**: Sept. 21, 2015
*Correspondence Dr. Vandita Mishra.
Department of Periodontology and Oral Implantology, Sri Sai College of Dental Surgery, Vikarabad, Telangana, India.
Email: vandita5jan@gmail.com

pISSN 2278-0076
eISSN 2321-9270

negatives the latter as in case of smokers. (5) Thus only a historical perspective and not the current appraisal of the disease status can be determined by the conventional diagnostic aids. There is a considerable interest in identifying biomarkers that are associated with periodontal disease activity. Such markers have the potential to assist in the development of new periodontal therapies and in the detection and monitoring of disease(6). A range of candidate host- and pathogen derived markers has been investigated in various body fluids but to date, no single marker has been found which clearly identifies the presence of periodontitis(7,8). Challenge lies more in the identification of aggressive periodontal disease on a simple clinical basis where the clinical condition is often debated by the clinicians which thus affect the treatment approach for the individual(9).

S100 proteins are a group of 25 low molecular weight proteins characterized by the presence of two calcium binding motifs with certain regulatory functions. S100A8 and S100A9 form a heterodimer (calprotectin) which is expressed by cells such as neutrophils and monocytes, which comprises large component granulocytes(10). Also the neutrophils and monocytes, the cells releasing these proteins are more in chronic and defective in the aggressive periodontitis subjects(11) thus a variation in the amounts of these proteins in the saliva of these subjects should also be present. Anticipating the same the aim of this study was to identify, quantify and compare the levels of S100 proteins in the state of health and in individuals with chronic and aggressive periodontitis and find if these can be an add on for existing periodontal diagnostic procedures.

MATERIALS AND METHOD

Thirty subjects visiting the outpatient department, Department of Periodontics and Oral Implantology, S.D.M. College of Dental Sciences and Hospital (SDMCDSH), Dharwad, between the age ranges of 22-55 years were recruited in this cross sectional study. An ethical clearance was obtained from the institutional review board and an informed written consent was obtained from all the subjects before their participation in the study.

Subjects were divided on the basis of clinical and radiographic criteria into periodontally healthy, chronic and aggressive periodontitis individuals with ten subjects in each group as follows:

Group 1(G1): 10 subjects with a clinically healthy periodontium
Group 2 (G2): 10 subjects with chronic periodontitis as per the AAP 1999 definition(12)
Group 3 (G3): 10 subjects with aggressive periodontitis as per the AAP 1999 definition(12)

5ml of unstimulated saliva was collected for all the subjects before any oral intervention was performed in commercially available sterile plastic tubes marked upto 45ml. For collection of saliva patients were demonstrated to drool the saliva into the tubes till 5ml mark was reached. All samples were collected between 9.00am to 12 noon 5minutes after rinse with plain water. All the samples were immediately transported to the department of Biochemistry, SDM Medical College, Dharwad where the samples were subjected to centrifugation at 5000 rpm for 5minutes and stored at -80degrees in separate sterile screw capped vials till further processing was done. At the end of the study all the samples were processed together. Five microliters of the centrifuged sample was collected using microcapillary pipettes and mixed with 2microliters of bromophenol blue dye. Polyacrylamide gel was prepared determining the size of the gel pores so as to enable the differentiation of proteins in the range of low molecular weight of 9-12kDa where the S100 proteins lie. A 13% polyacryl amide gel plate of 7 by 8 centimeters was used. Five microliters of the prepared solution and dye was loaded into the prepared gel and was subjected to electrophoresis by application of 4 milliampere current for one hour which allowed the proteins in the sample to migrate as per their molecular weight. The plate was retrieved after one hour and subjected to silver staining method. Destaining was then carried out using a shaker which lead to the retention of the dye by the proteins and the gel being neutral was destained. Albumin a 69 kDa known protein marker was used and the molecular weight of other proteins that migrated was calculated. The proteins that were obtained in the lower range of 9-12 kDa were characterized as S100 proteins and were compared in the various health and diseased samples.

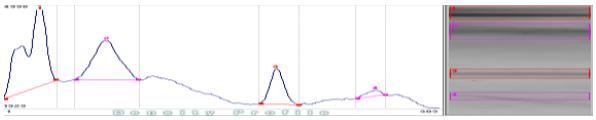


Fig 1: Electrophoretoram in subjects with healthy periodontium.

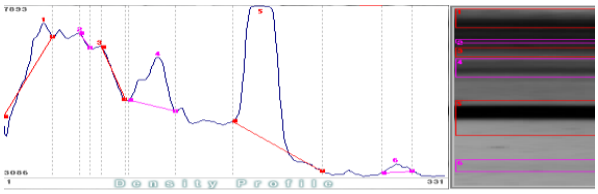


Fig 2: Electrophoretogram in subjects with chronic periodontitis.

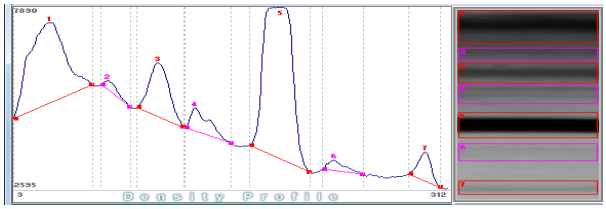
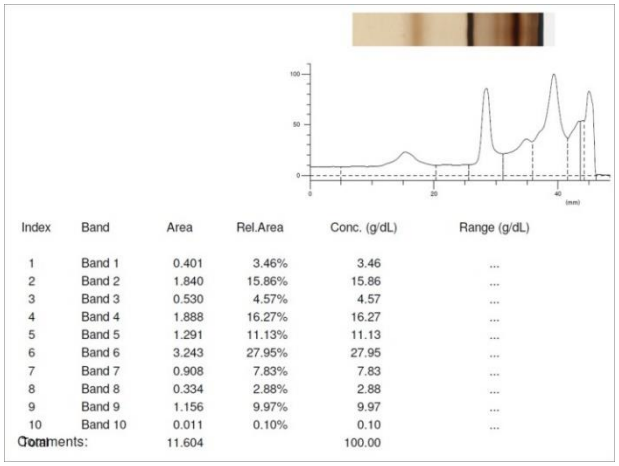


Fig 3: Electrophoretoram in subjects with aggressive periodontitis



Index	Band	Area	Rel.Area	Conc. (g/dL)	Range (g/dL)
1	Band 1	0.401	3.46%	3.46	...
2	Band 2	1.840	15.86%	15.86	...
3	Band 3	0.530	4.57%	4.57	...
4	Band 4	1.888	16.27%	16.27	...
5	Band 5	1.291	11.13%	11.13	...
6	Band 6	3.243	27.95%	27.95	...
7	Band 7	0.908	7.83%	7.83	...
8	Band 8	0.334	2.88%	2.88	...
9	Band 9	1.156	9.97%	9.97	...
10	Band 10	0.011	0.10%	0.10	...
Totals:		11.604		100.00	

Fig 4: A clinical report showing concentration of various protein bands in g/dl for aggressive disease subject.

An electrophoretogram was used so as to characterize the bands obtained in the various groups (figure 1,2,3) and also using the gel electrophoresis software a clinical report was generated for the individual samples which mentioned the relative area and protein content in g/dl in the samples for each protein band that was obtained.

RESULTS

As per the calculation using the 69 kDa albumin marker the migration of proteins in the range of 9-12kDa which we characterize as S100 proteins which are the most important proteins in this range was seen as a dark band in the individuals in aggressive periodontal disease whereas it was not detected in the chronic periodontitis subjects and in periodontally healthy subjects as seen in figure showing migration of proteins in all groups together.

Well characterized bands were seen in the subjects with aggressive disease indicative of the maximum amounts of S100 proteins in the aggressive disease. Density profiles of the migrated proteins were obtained using electrophoretogram in all three groups, the electrophoretograph that was generated showed seven bands generated in aggressive disease which included the lower range proteins, in group 2 with chronic disease 6 bands were generated thereby eliminating some lower molecular weight range proteins which was the S100 proteins thus though some low molecular weight proteins were present the ones in range of S100 proteins were not identifies clearly, while in periodontally healthy subjects only 4 bands were clearly graphed which thus indicated of absence of any low molecular weight range proteins. (Figure 1,2,3)

A biochemical report using the gel electrophoresis software was generated which quantified the amount of proteins present in a particular range of proteins in g/dl. (Figure 4) The range of low molecular weight S100 proteins in aggressive diseased subjects ranged from 6-8g/dl.

DISCUSSION

The S100 proteins are a group of calcium-binding EF-hand (helix E-loop-helix F) protein group. They were first identified by B.W. Moore in 1965(13) and are called S100 because of their solubility in a 100%-saturated solution with ammonium sulphate at neutral pH. At present, at least 25 proteins have been identified as belonging to the S100 protein family, 21 of them having genes clustered at chromosome locus 1q21, known as the epidermal differentiation complex(14). The members of the S100 protein family are

multifunctional proteins expressed in a diverse spectrum of tissues. Through their interaction with several effector proteins within cells they are involved in the regulation of a variety of cellular processes such as contraction, motility, cell growth and differentiation, cell cycle progression, transcription, structural organization of membranes, dynamics of cytoskeleton constituents, protection from oxidative cell damage, protein phosphorylation and secretion. This diversity in function can be associated due to various reasons as different metal-ion binding properties and different spatial distribution in the biological fluids(15). Broadly the diseases associated with the elevated levels of the varieties of S100 proteins can be categorized into four groups; Neurological disorders, neoplastic disorders, cardiac diseases and inflammatory disorders(16).

S100A8, S100A9, and S100Al2, are predominantly expressed in phagocytes and are strongly associated with pro-inflammatory functions. They are secreted especially at sites of inflammation. The serum concentrations of these S100 proteins correlate with inflammatory disease activity; high levels were identified in several inflammatory disorders such as rheumatoid arthritis, chronic bronchitis, and cystic fibrosis(17,18). In neutrophil and macrophage chemotactic activity, the S100A8/A9 molecules have been shown to inhibit macrophage H_2O_2 production, inhibit immunoglobulin synthesis, modulate neutrophil adhesion and exhibit antimicrobial activity(19).

Levels of S100A8/A9 in GCF and saliva have been shown previously to correlate with periodontitis and are likely to result from active secretion by infiltrating neutrophils and gingival keratinocytes, both of which are known to secrete S100A8/ A9 in the presence of bacterial lipopolysaccharides(20,21,22). Haigh *et al* demonstrated significant amounts of these proteins in subjects with chronic periodontitis subjects along with many other proteins in their proteomic study using two dimensional sodium dodecyl sulphate polyacrylamide gel electrophoresis(23). Characterization of these proteins in the aggressive diseased individuals has not been performed till date to our knowledge. Thus in this study we attempted to use these proteins as markers for identification of aggressive disease which becomes clinically challenging many a times.

Saliva was used in this study as opposed to gingival crevicular fluid as it is a simple and non-invasive diagnostic tool that allows rapid screening provides accurate predictive information being the mirror of the oral cavity(24). Highest amounts of these proteins were seen in the aggressive disease subjects which could be attributed to the presence of hyperactive neutrophils in these individuals(11) which are one of the components responsible for increased destruction in these subjects. Since calprotectin a low molecular weight S100A8/A9 dimer is secreted by neutrophilic granule(10) this could explain the elevated levels of these proteins in aggressive disease.

Interestingly, the presence of elevated proteins can also explain via an unexplored pathway the increased destruction in aggressive disease subjects. As S100 proteins are endogenous activators of Toll like receptor-4 by acting as damage associated molecular patterns (DAMPs) (25) . Thus an increase in these could activate TLR-4 in turn resulting in an increased disease activity. We used albumin as a marker in this study to calculate and estimate the molecular weight of other proteins on the basis of which we identified the proteins in the range of 9-12 kDa as S100 proteins, for further characterization of these as S100A1,2 etc mass spectroscopic methods would be needed.

In this study we used electrophoretogram and with the help of software for gel electrophoresis we could also generate a quantified report of the proteins present in g/dl. Using saliva as a simple screening tool in aggressive diseased subjects and identification of these proteins can thus act as a valuable means to rapidly screen the subjects into aggressive disease.

Since it is a simple and non-expensive procedure it can be incorporated as an add on to reduce the diagnostic dilemma that exists for aggressive periodontitis subjects.

CONFLICT OF INTEREST

No potential conflict of interest relevant to this article was reported.

REFERENCES

1. Polson AM, Goodson JM. Periodontal diagnosis: Current status and future needs. J Periodontol 1985;56:25–34.

2. Armitage GC. Periodontal diseases: diagnosis. Ann Periodontol 1996;1:37–215.

3. Greenstein G. Periodontal diseases are curable. J Periodontol 2002;73:950–953.

4. Griffiths GS, Smart GJ, Bulman JS, Weiss G, Shrowder J, Newman HN. Comparison of clinical outcomes following treatment of chronic adult periodontitis with subgingival scaling or subgingival scaling plus metronidazole gel. J Clin Periodontol 2000;27:910–917.

5. Bergstrom J. Cigarette smoking as a risk factor in chronic periodontal disease. Community Dent Oral Epidemiol 1989;17:245.

6. Ebersole, J. L. Humoral immune responses in gingival crevice fluid: local and systemic implications. Perio 2000; 2003 31:135–166.

7. Kaufman, E. & Lamster, I. B. Analysis of saliva for periodontal diagnosis – a review. J Clin Periodontol 2000;27:453–465.

8. Ozmeric, N. Advances in periodontal disease markers. Clinica Chimica Acta 2004;343:1–16.

9. Greenstein G, Caton J. Periodontal disease activity: a critical assessment. J Periodontol 1990; 61:543-552.

10. Gebhardt C, Nemeth J, Angel P & Hess J. S100A8 and S100A9 in inflammation and cancer. Biochem Pharmacol 2006;72, 1622–1631

11. Nibali L, O'Dea M, Bouma G, Parkar M, Thrasher A.J , Burns S et al. Genetic variants associated with neutrophil function in aggressive periodontitis and healthy controls. J Periodontol 2010;81:527-534.

12. Armitage GC. Development of a classification system for periodontal diseases and conditions. Ann Periodontol 1999;4:1.

13. Moore BW. A soluble protein characteristic of the nervous system. Biochem Biophys Res Commun 1965;19:739–744.

14. Marenholz I, Heinzman CW, Fritz G. S100 protein in mouse and man: from evolution to function and pathology. Biochem Biophys Res Commun 2004;322:1111–1122.

15. Santamaria–Kisiel L, Rintala–Dempsey AC, Shaw GS. Calcium dependent and independent interactions of the S100 protein family. Biochem J 2006;396:201–214.

16. F Sedaghat, A Notopoulous. S100 protein family and its application in clinical practice. Hippokratia 2008;12:198-204.

17. Frosch M, Strey A, Vogl T, Wulffraat N, Kuis W, Sunderkotter C et al. Myeloid-related proteins 8 and 14 are specifically secreted during interaction of phagocytes and activated endothelium and are useful markers for monitoring disease activity in pauciarticular-onset juvenile rheumatoid arthritis. Arthritis Rheum 2000;43:628–637.

18. Foell D, Wittkowski H, Hammerschmidt I, Wulffraat N,Schmeling H, Frosch M et al. Monitoring neutrophil activation in juvenile rheumatoid arthritis by S100A12 serum concentrations. Arthritis Rheum 2004;50:1286–1295.

19. Yui S, Nakatani Y & Mikami M. Calprotectin (S100A8/S100A9), an inflammatory protein complex from neutrophils with a broad apoptosis-inducing activity. Biol Pharm Bull 2003;26:753–760.

20. 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;26:653–657.

21. Kojima T, Andersen E, Sanchez JC, Wilkins MR., Hochstrasser DF, Pralong WF et al. Human gingival crevicular fluid contains MRP8 (S100A8) and MRP14 (S100A9), two calcium-binding proteins of the S100 family. J Dent Res 2000;79:740–747.

22. Ramseier CA, Kinney JS, Herr AE, Braun T, Sugai JV, Shelburne CA et al Identification of pathogen and host-response markers correlated with periodontal disease. J Periodontol 2009;80:436–446.

23. Haigh BJ, Stewart KW, Whelan JRK, Barnett MPG, Smolenski GA, Wheeler TT. Alterations in the salivary proteome associated with periodontitis. J Clin Periodontol 2010; 37:241–247.

24. Miller CS, King CP, Langub MC, Kryscio RJ & Thomas MV. Salivary biomarkers of existing periodontal disease: a cross-sectional study. J Am Dent Assoc 2006;137:322–329.

25. Foell D, Wittkowski H, Kessel C, Lüken A, Weinhage T, Varga G et al. Proinflammatory S100A12 can activate human monocytes via toll like receptor 4. Am J Respir Crit Care Med 2013;187:1324-34.