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ALTERATION IN SALIVARY PROTEINS FOLLOWING NON SURGICAL PERIODONTAL THERAPY IN GENERALIZED CHRONIC PERIODONTITIS SUBJECTS.

[1*]DR ANAMIKA SHARMA BDS, MDS , [2]DR S NAGTILAK, M.Sc, Ph.D, [3]BRIG (DR.) B.P. KHATTAK, VSM, BDS, MDS, [4]DR GANESH SINGH, Ph.D, STATISTICS AND [5]DR TANVEER BANO, MBBS, M.D

[1]Professor & Head, Dept. Periodontology, Subharti Dental College and Hospital, Subharti Puram. – Meerut.
[2]Professor, Department of Biochemistry, Subharti Medical College and Hospital, , Subharti Puram. – Meerut.
[3]Professor Department of Periodontology, Subharti Dental College and Hospital, Subharti Puram. – Meerut.
[4]Assistant Prof, Department of Social and Preventive Medicine LLRM Medical College Meerut.
[5]Associate Professor, Department of Social and Preventive Medicine LLRM Medical College Meerut.

ABSTRACT

The purpose of the present study was to evaluate and compare the effect of nonsurgical periodontal therapy on salivary proteins in subjects with periodontitis and compare it with healthy subjects. A total of 30 subjects in age group between 30 and 45 years were enrolled, of which 15 had generalized severe Chronic Periodontitis (CP), and 15 periodontally healthy as a control (C). The clinical parameters included Plaque index (PI), Gingival index (GI), Pocket probing depth (PPD) Bleeding on probing (BOP) and Clinical attachment loss (CAL). Paraffin wax stimulated whole saliva was collected and immediately centrifuged at -4^{0}C, assayed for salivary protein content using the Biuret Bicinchonic Acid protein assay reagent (BCA Kit)., both at baseline and at 4 week following scaling and root planing. Salivary proteins were significantly higher in Generalized Chronic Periodontitis subjects ($p<0.05$) as compared to control. After one month of scaling and root planing the salivary protein concentration between pre and post-periodontal therapy improved significantly ($p<0.05$).Subjects with established periodontitis exhibited elevated concentrations of salivary protein, suggesting that monitoring for change in salivary composition may be useful tool to establish favorable response to periodontal therapy.

KEYWORDS: **Periodontitis, Salivary protein, Plaque index, Gingival index, Host response.**

DR ANAMIKA SHARMA BDS, MDS
Professor & Head, Dept. Periodontology, Subharti Dental College and Hospital, Subharti Puram. – Meerut

***corresponding author**

INTRODUCTION

Periodontitis is a destructive inflammatory disease of the supporting tissues of the teeth. This disease is primarily related to chronic plaque accumulation. Putative periodontopathic bacteria, such as Porphyromonas gingivalis, Prevotella intermedia or Actinobacillus actinomycetemcomitans are suspected to play a role in the periodontal disease process. These bacteria release proteolytic enzymes that degrade salivary proteins, immunoglobulins and collagen type I[1]. Furthermore, these pathogenic bacteria provoke an immune response that results in the release of cytokines, which trigger polymorphonuclear leukocytes (PMNs), macrophages, fibroblasts, keratinocytes and osteoclasts. These host cells release proteinases which may degrade extracellular matrix components, e.g. the highly resistant collagen fibers [2]. Bacterial endotoxins cause the release of acute phase proteins and activates the inflammatory cascade. Saliva plays a vital role in dental health as patients strive to maintain a healthy dentition throughout their lives. It is a primary growth environment for flora of the oral cavity. As the physicochemical properties are changed, it affects the microorganisms which grow in the mouth[3]**.** Recently, the entire human salivary proteome was reported by a consortium of three research groups, and this revealed that 1166 proteins are present in human saliva. The term Protein was coined by Berzelius in 1838, and is the most important cell constituent. There are approximately 300 amino acids present in various animal, plant and microbial systems, but only 20 amino acids are involved in the formation of protein. These identified proteins are involved in molecular processes ranging from structural functions of enzymatic／catalytic activities and identification of these proteins provide an informative salivary proteome knowledge base for translational and clinical evaluations[4]. Salivary total protein is a vital component of saliva, with salivary proteins, predominantly comprising proline rich proteins, mucin, amylase, immunoglobulins, statherin and antibacterial factors[5]. However, it has been difficult to demonstrate that persons with various salivary levels of particular proteins show corresponding differences in oral health. That conflict makes it difficult to establish the role of salivary proteins in oral ecology. Four major types of salivary protein-microbe interaction can be observed in vitro. These include aggregation, adherence, inhibition/cell-killing, and nutrition [6]. Majority of protein molecules in saliva thus may be capable of interacting with oral microbes. Many clinical studies have investigated the role of salivary proteins in oral disease. These studies address the question of salivary protein interactions with microbes to the extent that diseases such as caries, gingivitis, or periodontitis are mediated by oral bacteria. In most cases, the primary hypothesis has been that individual variations in the amount of particular salivary proteins are related to the variation in the extent or severity of clinical disease. There is general consensus in the field of clinical periodontology of the need for accurate and sensitive markers of destructive periodontitis in order to place the diagnosis and management of disease on a more rational and less empirical basis [7-9].

MATERIALS AND METHODS

Subjects

The present interventional study was approved by the Institutional Review Board of Swami Vivekanand Subharti University Meerut U.P (India). Subjects were recruited in the age group between 30 and 45 years with moderate to severe Chronic Periodontitis (CP) from out-patient department of Periodontology at Subharti Dental College and Hospital Meerut U.P (India). A written informed consent obtained from all patients participating in the study. Each subject had at least 20 standing teeth, with at least five teeth with sites probing 5mm or deeper, and radiographic evidence of alveolar bone loss. Subjects had received no previous periodontal treatment, antimicrobial therapy or periodontal surgery in the preceding 6 months, and had no periapical lesions, or restorations. Subjects with systemic diseases, pregnancy, lactating mothers, or smokers were excluded

from the study. Chronic periodontitis was diagnosed according to the criteria of the American Academy of Periodontology (AAP) [10].

Sample collection

Patients were appointed during early hours between 8-10 AM. Paraffin -Stimulated saliva was collected for 5 minutes (~5-10 ml) from each subject sitting in an upright posture, into sterile tubes as per the procedure described by Navazesh[11]. The subjects refrained from eating, drinking, and oral hygiene for 2 hours prior to saliva collection. Saliva was immediately centrifuged at 3500 rpm at - 4 □C for 10 minutes, and assayed for salivary protein content using Bicinchonic Acid reagent (BCA Kit) for quantitation of salivary proteins at baseline and at 4 weeks following scaling and root planing. The Novagen® BCA Protein Assay Kit based on a biuret reaction, which reduces Cu^{2+} to Cu^{+} by proteins in an alkaline solution with concentration-dependent detection of the monovalent copper ion. Bicinchonic acid is a chromogenic reagent that chelates the reduced copper, producing a purple complex with strong absorbance at 562 nm [12]. BCA Protein Assay Kit can be used to determine protein concentration in the range of 20-2000 µg/ml in either a standard assay or micro assay configuration. A Bovine Serum Albumin (BSA) standard (2 mg/ml) albumin was used for convenient and reliable quantification of proteins.

Clinical Parameters

Clinical and radiographic examinations were performed by a single, experienced examiner (First Author) after collection of saliva. Plaque index (PI)[13,] gingival index (GI)[14], pocket probing depth (PPD) bleeding on probing (BOP) and clinical attachment loss (CAL) using UNC 15 periodontal probe were measured at six sites per tooth.

Treatment

Following collection of saliva, subjects received single sitting of full mouth scaling and root planing. No antibiotic, anti-inflammatory or any form of chemical plaque control was prescribed as treatment. The patients were re-evaluated one month after completion of phase I therapy and saliva was re sampled as per the procedure described earlier [11].

Statistical Analysis

Data analysis was performed by software Package SPSS version-10. Continuous variables of baseline clinical and biochemical results were analyzed using Student's t-test for study groups. Values of $p<0.05$ were considered as statistically significant.

RESULTS

The healthy group consisted of 11 male and 4 female. They displayed low BOP scores, with mean ± SD (3.20 ± 2.76), PI scores with mean ± SD (0.11 ± 0.12). Mean ± SD for GI (0.08 ±0.15) and for PPD mean ± SD was 1.83 ± 0.29. Mean ± SD values for total Protein were 107.07±34.84 mg% .The periodontitis group consisted of 15 subjects, (8 male and 7 female). These subjects had significant higher scores for all the clinical parameters. The values remained at a higher level post treatment when compared to healthy individuals. Mean± SD for BOP scores were 87.26 ±13.20% and 33.05 ±14.25 % ($P<0.05$) pre and post treatment respectively, Mean± SD for PI scores were between 1.69 ± 2.04 and 0.93 ± 0.34 ($P<0.05$), GI scores between1.77 ± 0.26 and 1.15 ±.54 ($P<0.05$), PPD ranged between 3.34 ± 0.74 mm and 2.28 ± 0. 37 mm ($P<0.05$).However CAL showed no significant difference between pre and post treatment group (4.10 ±1.18 mm) and (3.36 ±1.80 mm) respectively. ($P>0.05$). Protein levels also did not show significant difference in pre (164 ± 31.13mg/100ml) and post treatment values (144.8 ± 36.14mg/100ml) ($P>0.05$) respectively.(Table-1) (Table-3).All the clinical parameters and the total protein content were statistically significant ($P<0.05$) in healthy and periodontitis group.(Table-2).Statistical significant difference between BOP scores, PI scores ,GI scores, PPD and total protein content were seen when healthy group was compared with the difference of pre and post values of periodontitis group, however no difference was seen when comparing the clinical attachment levels between the above

two groups (Table 4).There is no correlation seen between any of the clinical parameters and the total protein content in any of the above groups (p>.05) (Table-5)

TABLE NO.1

Comparison of clinical and biochemical parameters between healthy and subjects with periodontitis

S.NO.	PARAMETERS	MEAN ± S.D.		
		HEALTHY GROUP	PERIODONTITIS GROUP (n=15)	
			PRE TREATMENT (n=15	POST TREATMENT (n=15
1	Gingival Index	0.08 ± 0.15	1.77 ± 0.26	1.15 ± 0.54
2	Plaque Index	0.11 ± 0.12	1.69 ± 0.24	0.93 ± 0.34
3	Bleeding on Probing (%)	3.20 ± 2.76	90.15 ± 9.62	33.05 ± 14.25
4	Probing Depth	1.83 ± 0.29	3.46 ± 0.71	2.48 ± 0.37
5	Clinical Attachment Level	0 ± 0	4.10 ± 1.18	3.36 ± 1.80
6	PROTEIN (mg/100 ml)	107.07 ± 34.84	164 ± 31.13	144.80 ± 36.14

Values: Mean ± S.D. (Standard Deviation)

TABLE NO. 2

Test of significance between healthy and subjects with periodontitis

S.NO.	PARAMETERS	PROBABILITY OF UNPAIRED "t" TEST B/W HEALTHY AND PERIODONTITIS GROUP	P-VALUE/ SIGNIFICANCE
1	Gingival Index	0.0000	P<0.05
2	Plaque Index	0.0000	P<0.05
3	Bleeding on Probing (%)	0.0000	P<0.05
4	Probing Depth	0.0000	P<0.05
5	Clinical Attachment Level	0.0000	P<0.05
6	PROTEIN (mg/100 ml)	0.0000	P<0.05

P<0.05 = Significant,
P>0.05 = Not Significant

TABLE NO. 3

Test of significance between pre and post treatment clinical and biochemical Parameters in subjects with periodontitis

S.NO.	PARAMETERS	MEAN ± S.D., (n=15) (DIFFERENCES)	PROBABILITY OF PAIRED "t" TEST	P-VALUE/ SIGNIFICANCE
1	Gingival Index	0.63 ± 0.45	0.0001	P>0.05
2	Plaque Index	0.79 ± 0.37	0.0000	P>0.05
3	Bleeding on Probing (%)	57.10 ± 19.35	0.0000	P>0.05
4	Probing Depth	0.93 ± 0.61	0.0000	P>0.05
5	Clinical Attachment Level	0.78 ± 2.11	0.1738	P>0.05
6	PROTEIN (mg/100 ml)	19.40 ± 44.80	0.1340	P>0.05

P<0.05 = Significant,
P>0.05 = Not Significant

TABLE NO. 4

Test of significance between healthy and difference of values between pre and post group

S.NO.	PARAMETERS	PROBABILITY OF UNPAIRED "t" TEST BETWEEN HEALTHY & DIFFERENCE (PRE & POST) GROUP	P-VALUE/ SIGNIFICANCE
1	Gingival Index	0.0004	P<0.05
2	Plaque Index	0.0000	P<0.05
3	Bleeding on Probing (%)	0.0000	P<0.05
4	Probing Depth	0.0000	P<0.05
5	Clinical Attachment Level	0.1738	P>0.05
6	PROTEIN (mg/100 ml)	0.0000	P<0.05

P<0.05 = Significant,
P>0.05 = Not Significant

TABLE NO. 5

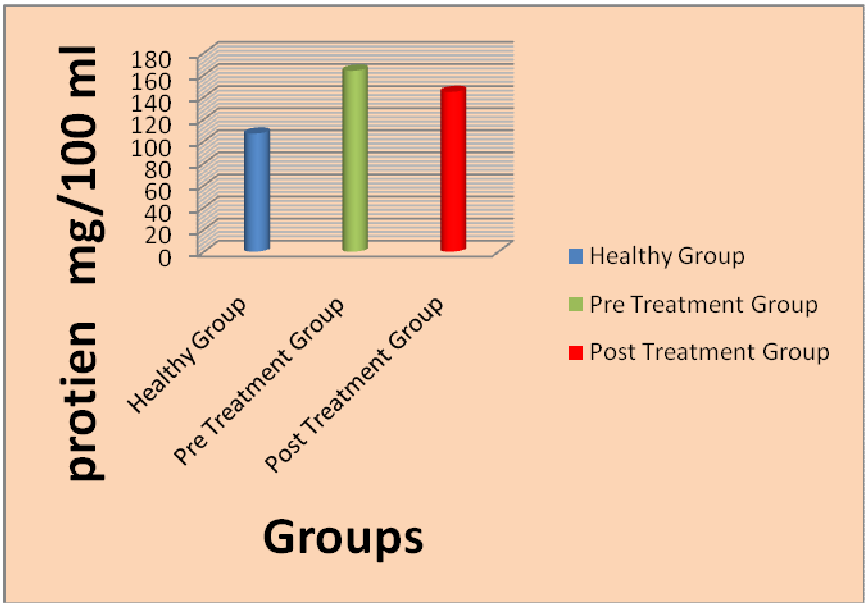
Test of significance between total protein content and various clinical parameters of all the groups.

PARAMETERS	Healthy Group Total Protein		Periodontitis Group Total Protein			
			Pre Operative		Post Operative	
	r^2	P-Value	r^2	P-Value	r^2	P-Value
Gingival Index	0.008	0.96	0.04	0.85	0.01	0.95
Plaque Index	-0.195	0.35	0.137	0.51	-0.277	0.17
Bleeding on Probing (%)	0.038	0.85	-0.324	0.11	0.11	0.58
Probing Depth	0.157	0.45	0.154	0.46	0.000	0.99
Clinical Attachment Level	-	-	0.19	0.35	-0.01	0.94

r^2= spearman rank correlation coefficient.

FIGURE -1

Comparison of total protein concentration between healthy group and subjects with periodontitis pre and post treatment



DISCUSSION

Saliva plays a principal role in the protection of the oral mucosa and teeth. It controls the metabolism and growth of microbes by several mechanical, immunologic, and non immunologic mechanisms[15]. Oral bacteria cause inflammatory responses, which results in tissue destruction in various ways[16]. Bacteria can either directly contribute to periodontal disease by releasing proteolytic enzymes, which can damage the oral tissues or may induce tissue destruction indirectly by activating host defense cells, e.g. polymorphonuclear leukocytes (PMNs), which can release their lysosomal proteolytic enzymes at the inflamed sites. Ideally, diagnostic tests should demonstrate high specificity and sensitivity. Given the complex nature of periodontal disease, it is unlikely that a single marker will prove to be both sensitive and specific. A combination of two or more markers may provide a more accurate assessment of the periodontal patient. Because of the simple and non-invasive method of collection, salivary diagnostic tests appear to hold promise for the future. Furthermore, a salivary diagnostic test can aid in screening large populations, and in the context of tests that demonstrate either high sensitivity or high specificity may prove valuable i.e., excluding healthy patients and referring patients at increased risk for a thorough periodontal evaluation [17].Salivary protein interactions with oral microbes in vitro include aggregation, adherence, cell-killing, inhibition of metabolism, and nutrition. Such interactions might be expected to influence oral ecology. However, inconsistent results have been obtained from in vivo tests of the hypothesis that quantitative variation in salivary protein concentrations will affect oral disease prevalence[18].Salivary protein concentrations also may be subject to circadian variations. Values for saliva collected at the same time of day tend to remain consistent within subjects, but events such as stress, inflammation, infection, menstruation, or pregnancy may induce short-term changes[19].Salivary proteins may form heterotypic complexes with unique effects, and different proteins may exert redundant effects. However, it has been difficult to demonstrate that persons with various salivary levels of particular proteins show corresponding differences in oral health. The conflict makes it difficult to establish the role of salivary proteins in oral ecology.The literature reviewed suggests that in comparison with healthy subjects, higher concentrations of salivary enzymes exists in-patients with periodontitis. This is due to the contributions of PMNs, bacteria, and the presence of connective tissue destruction seen in association with periodontal disease. Clinical studies have investigated the role of salivary proteins in oral disease. In most of the cases, the primary hypothesis has been that individual variation in amount of particular salivary proteins is related to variations in the extent or severity of clinical disease. In general, the major factors affecting the protein concentration and composition of whole saliva are the salivary flow rate, the protein concentration of the contributing glandular salivas and the contribution of crevicular fluid proteins[17].Recent information on salivary protein interactions with oral microbes can be summarized in terms of three concepts: multifunctionality, amphifunctionality, and redundancy respectively[20].Contradictory results were found, however, when levels of salivary immunoglobulins were studied in relation to periodontal status and the response to treatment. Some studies reported an increase in levels of immunoglobulins with more pronounced periodontal disease [21-23],while few demonstrated decreased levels of salivary immunoglobulins [24,25].Henskens et al.[26] investigated Protein, albumin and cystatin concentrations in saliva of healthy subjects and of patients with gingivitis or periodontitis and found that all the periodontally healthy subjects had salivary proteins concentration between 0.5 and 1.5 mg/ml with mean value of 1.06±0.25 mg/ml. On the contrary gingivitis and periodontitis showed a mean value of 1.49±0.58 mg/ml and 2.21±1.00 mg/ml respectively. The salivary proteins were significantly higher in a diseased state than in healthy subjects ($p<0.01$). The Gingivitis and periodontitis group

showed significant differences (p<0.01).These are in accordance with the findings of the present study.Results of the present study are in accordance with study of Alvesr et al [27] who evaluated the influence of periodontal disease on the biochemical parameters of the saliva, and found alterations in the total protein in the subjects of the test group compared to the control group (P<0.05). However, there was no correlation between the clinical periodontal parameters and the salivary parameters (P>0.05). Few studies[28,29] have reported total protein content higher in periodontitis group as compared to healthy individuals which correlates with the present study. Sudhir Shetty [30] conducted a study to estimate the role of salivary factors such as calcium, phosphorus, protein and pH in progression of periodontal disease. Mean levels of salivary protein increased with severity of disease. Protein levels in Group 1 (healthy) Group 2 (Chronic marginal gingivitis.) Group III (localized chronic periodontitis.) Group IV (chronic generalized Periodontitis) were 263.05+183.25 mg/100ml, 281.32+187.35 mg/100ml, 367.12+256.02 mg/100ml, and406.80+308.12mg/100ml respectively.The literature reports available on several studies have found the levels of free amino acids in saliva in relation to periodontal status. It appears that in some patients, elevated levels of certain amino acids, especially proline, may be detected[31, 32]. These amino acids probably appear in whole saliva as a result of bacterial metabolism or degradation of salivary proteins rich in proline. In another study by the same investigators [33] no differences in amino acid concentrations in saliva were found between patients with progressive periodontitis and controls. The authors concluded that levels of amino acids in oral fluids (including GCF) have no diagnostic significance of periodontal disease.Ellis et al [34] assessed the total protein concentration in biopsies of three groups of gingival tissues: those adjacent to a) ≤ 3mm (normal),b) 4-6mm and c) ≥ 6mm gingival sulcus and found 35.6±3.8 mg/ml,65.5±10.1 mg/ml and109.2±16.2mg/ml respectively.

CONCLUSION

Salivary protein concentrations in affected persons never fall below the lower range of "normal" levels in the general population—the importance of such change may lie in its potential to shift the balance between host factors and the indigenous flora within an individual. Careful longitudinal studies of persons likely to exhibit specific changes in salivary protein could provide a useful model for investigating in vivo interactions of those proteins with oral microbes. Long-term factors such as aging, systemic disease, or medication may influence salivary protein concentrations. Such sources of variation may increase the sample size needed to find statistically significant differences. Clinical studies must consider factors such as human population variation, strain and species differences in protein-microbe interactions, protein polymorphism, and synergistic or antagonistic interaction between proteins.In conclusion the findings of the present study suggest that salivary protein, serve as a marker of inflammation of the periodontium. Future investigations are needed to find the cause and origin of the increased salivary protein concentrations in subjects with varying degrees of periodontal disease.

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