International Journal of Research in Medical Sciences
Roy JS et al. Int J Res Med Sci. 2017 Aug;5(8):3578-3583
www.msjonline.org
pISSN 2320-6071 | eISSN 2320-6012
DOI: http://dx.doi.org/10.18203/2320-6012.ijrms20173566

Original Research Article

Estimation of salivary glycoconjugates and salivary ros levels in chronic periodontitis: a clinico-biochemical study

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Received: 06 June 2017
Accepted: 29 June 2017

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ABSTRACT

Background: Periodontitis is a chronic inflammatory disease of periodontal tissue, characterized by persistent inflammation of the connective tissue and alveolar bone destruction. Patients with periodontal disease show the differences in the composition of saliva. Newer diagnostic tools based on analysis of body fluids such as saliva, GCF and serum are found to be useful for diagnosis as well as monitoring the disease activity. Thus, aim of the study was to estimate the concentration of salivary glycoconjugates (sialic acid, total protein) and salivary ROS in unstimulated whole saliva of subjects with chronic periodontitis and to compare the concentration with healthy/gingivitis subjects.
Methods: The study sample consisted of 60 subjects (33 males and 27 females) with age ranging from 30-60 years. A detailed case history was taken from all the subjects and periodontal disease parameters (bleeding on probing, probing pocket depth and clinical attachment levels) were recorded at baseline and subjects were divided into 2 groups. Group I- control group (healthy/gingivitis subjects) and Group II -test group (chronic periodontitis). Saliva samples were collected from the subjects and stored at -700 ºC. Periodate Resorcinol Assay was done to estimate the levels of sialic acid, Biuret test was done to assess the levels of total protein and d-ROMs test was done to assess the level of ROS. Statistical analysis was done using students unpaired 't' test and Pearsons correlation test.
Results: It was found that the levels of salivary glycoconjugates and ROS are increased in subjects with chronic periodontitis when compared to healthy/gingivitis subjects. Thus it can reflect the clinical status of gingival and periodontal tissues.
Conclusions: Estimation of the levels of glycoconjugates and ROS may be used as one of the reliable biomarkers to assess the severity of periodontal disease and to monitor the disease progression.

Keywords: Chronic periodontitis, Glycoconjugates, Reactive oxygen species

INTRODUCTION

Periodontitis is a term used to describe an inflammatory process, initiated by plaque biofilm, that lead to loss of periodontal attachment to the root surface and adjacent alveolar bone loss which ultimately results in tooth loss. Primary etiological agent of periodontitis is specific, predominantly gram negative anaerobic or facultative bacteria within subgingival biofilm.[1]

The ability to monitor the periodontal disease onset, progression and treatment outcome through noninvasive means is a highly desirable goal in health care management. Saliva is increasingly used and well

validated in diagnosing, monitoring health and disease status.

Glycoconjugates are biologically important molecules with diverse functions. They consist of oligosaccharides of varying size and complexity, attached to a non-sugar moiety as a lipid or a protein. Glycoconjugate structures are often very complex and have intricate biosynthetic pathways. Carbohydrate antigens are expressed on the cell surface as components of glycoproteins, glycosphingolipids and proteoglycans; these carbohydrate antigens constitute significantly to fundamental biological functions, such as cell differentiation, cell adhesion, cell-cell interactions, pathogen-host recognition, toxin-receptor interactions, cancer metastasis, immune responses and regulation of signalling pathways.[2-4] Sialic acid is a 9-carbon monosaccharide. An important function of host Sialic acid is to regulate innate immunity. Removal of terminal Sialic acid (either by neuraminidase enzyme of virulent bacteria or by inherited disorder of host endogenous neuraminidase) from sialylated glycoprotein, could incorporate onto the surface of developing plaque which may play a role in plaque formation and cause destruction of host tissue.[5] Sialic acid is present in several acute phase proteins which are known to be associated with periodontitis.[6] Salivary total protein is a vital component of saliva with salivary proteins predominately comprising of proline rich proteins ,mucins, amylase, immunoglobins, statherin and antibacterial factors and these are responsible for most of the functions of saliva. Total protein is one of the routine test performed in patients suspected of any pathology and therefore any significant changes, if proved, can aid in early diagnosis.

PMNs possess two main pathways for controlling microorganisms (i.e. oxidative and non -oxidative) which kill bacteria, influence bacterial growth or modify bacterial colonization in relation to periodontium.[7] Upon recognition of phagocytic or soluble stimulus, both neutrophils and macrophage experience a 'respiratory burst', which is characterized by an increase in oxygen consumption, activation of hexose monophosphate (HMP) shunt and generation of free radicals (FR), reactive species and their metabolic products.[8] At sites of chronic inflammation, there is considerable over production of free radicals (FR) and reactive species.[9] In normal physiology there is a dynamic equilibrium between ROS activity and antioxidant defense capacity and when that equilibrium shifts in favour of ROS, either by a reduction in antioxidant defences or an increase in ROS production or activity, oxidative stress results. The reactive oxygen species are produced in large quantities by activated neutrophils which are capable of radical formation in extracellular and intracellular environment. Biology of ROS-mediated damage is highly complex. Overproduction of reactive oxygen species is thought to contribute in the severity of the disease. Hence the aim of the study was to assess the changes in salivary glycoconjugates (sialic acid and total protein) and salivary ROS levels in chronic periodontitis and to compare the values with that of individuals without periodontitis.

METHODS

The present study was conducted in the outpatient department of periodontics, AJ Institute of Dental Sciences, Manglore, India. 60 adult subjects belonging to both sexes and with the age ranging from 30-60 years (33 males and 27 females) were randomly selected and divided into 2 groups according to the periodontal status.

- Group 1: Healthy subjects (however we could not find individuals with healthy periodontium so patients with marginal gingivitis with isolated gingival bleeding were also included in the first group)
- Group 2: Chronic periodontitis.

Inclusion criteria

- Individuals with clinically healthy periodontium and isolated bleeding on probing are considered as a control group
- Patients who exhibited generalized form of periodontal destruction i.e. more than 30% of the sites are considered as chronic periodontitis and these patients with chronic periodontitis having clinical evidence of blood forming confluent line on margin or heavy/ profuse bleeding on probing and periodontal pockets measuring ≥5 mm.

Exclusion criteria

- Patients who have undergone any periodontal, surgical or non-surgical therapy for past 6 months
- Patients who have received any chemotherapeutic mouth rinse or oral irrigation during the past 6 months
- Patients who have received antibiotics in the past 4-6 weeks
- Patients with a history of underlying systemic diseases
- Patients with habit of smoking and tobacco chewing
- Pregnant and lactating women and those using hormonal contraceptives.

Informed consent was obtained from all the patients and a proforma was used to collect demographic data of the patient regarding age, sex, address, oral hygiene habits. The proforma also included modified sulcus bleeding Index (by Mombelli et al) 9 along with probing pocket depth, clinical attachment loss and other significant intraoral findings. 5 ml of whole saliva was collected from the subjects by simply drooling into a sterilized vial with the forward tilted head or by allowing the saliva to accumulate in the mouth and then expectorate into a vial. After centrifugation, the collected saliva was samples were analysed for estimation of salivary glycoconjugates

(sialic acid and total protein) and ROS. Estimation of Sialic Acid was done by Periodate Resorcinol Assay. Total protein was determined by biuret method and ROS was assessed by d ROM test.

Statistical analysis

The data obtained was entered into Microsoft excel sheet and mean and standard deviation were used as descriptive statistics. The statistical package for social sciences (SPSS version 17.0) was used for statistical analysis. The results were tabulated in the form of tables. Data was presented as mean and standard deviations. Differences between the means were proved using students unpaired 't' test. The p value of 0.05 or less was considered for statistical significance and comparison of the values was done by Pearson correlation test.

RESULTS

The study was performed in 60 subjects – 30 subjects in Group I (Control group) –Healthy subjects/gingivitis and 30 subjects in Group II (Test group) - Chronic Periodontitis. Statistical analysis was carried out using students unpaired 't'test for estimating the mean values and standard deviation of salivary glycoconjugates (sialic acid and total protein) and reactive oxygen species levels in both the groups and the statistical significance was defined as p value <0.05.

The correlation between the salivary glycoconjugates and salivary reactive oxygen species levels with respect to the periodontal findings and with respect to each other was performed using Pearsons Correlation test.

Table 1: Correlation between the parameters by Pearsons correlation test.

Correlations							
		BOP	**PPD**	**CAL**	**Total P**	**ROS**	**Sialic acid**
BOP	Pearson correlation	1					
	N	60					
PPD	Pearson correlation	0.760	1				
	Sig. (2-tailed)	<0.001					
	N	60	60				
CAL	Pearson correlation	0.153	0.829	1			
	Sig. (2-tailed)	0.419	<0.001				
	N	30	30	30			
Total P	Pearson correlation	0.246	0.534	0.321	1		
	Sig. (2-tailed)	0.058	<0.001	0.084			
	N	60	60	30	60		
ROS	Pearson correlation	0.447	0.507	0.280	0.426	1	
	Sig. (2-tailed)	<0.001	<0.001	0.135	0.001		
	N	60	60	30	60	60	
Sialic acid	Pearson correlation	0.641	0.600	-0.150	0.164	0.274	1
	Sig. (2-tailed)	<0.001	<0.001	0.429	0.212	0.034	
	N	60	60	30	60	60	60

The mean bleeding on probing and probing pocket depth of healthy subjects/gingivitis was 1.07 and 2.97 and in chronic periodontitis is 2.67 and 6.77.

On comparison of the bleeding on probing (BOP) between the groups it was noted that BOP is higher in Periodontitis group with a t value of -11.664 and is statistically significant with a p value of <0.001.

On Comparison of the Probing Pocket Depth (PPD) between the two groups results showed that Probing Pocket Depth is higher in periodontitis group with a t value of -15.494 and it is statistically significant with a p value of <0.001. This signifies that in periodontitis, there occurs an increase in the depth of the pockets, increased loss of attachment of the involved teeth, increased severity in plaque formation and increased inflammation of the gingiva, when compared to patients in control group.

Comparison of the Sialic Acid between the two groups showed that Sialic Acid levels are higher in Periodontitis group with a t value of -8.285 and is statistically significant with a p value of <0.001 .

The mean Sialic acid concentrations in healthy/gingivitis subjects is 10.86 mg/L and in chronic periodontitis subjects is 27.40 mg/L (Figure 1). This indicated that the sialic acid values were raised significantly in chronic periodontitis patients.

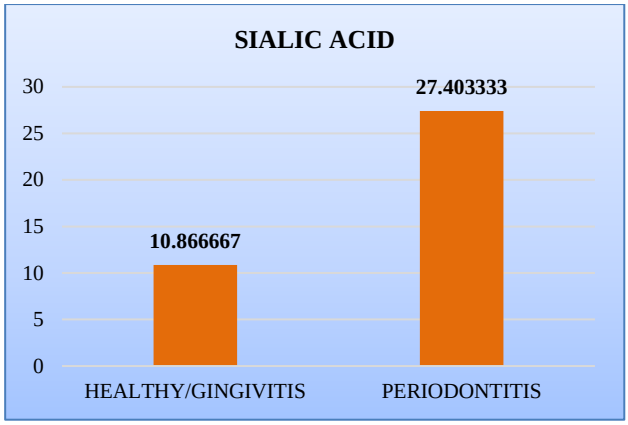

Figure 1: Mean values of sialic acid between the two groups.

On Comparison of the total protein values between the two groups it was noted that Total Protein is higher in Periodontitis group with a t value of -3.797 and is statistically significant with a p value of <0.001.

The mean values of total protein in healthy /gingivitis subjects were 178.73 mg/dl and in chronic periodontitis was 230mg/dl (Figure 2). This signifies that the total protein values were higher in chronic periodontitis group. Thus this revealed that the glycoconjugates levels were higher in chronic periodontitis patients.

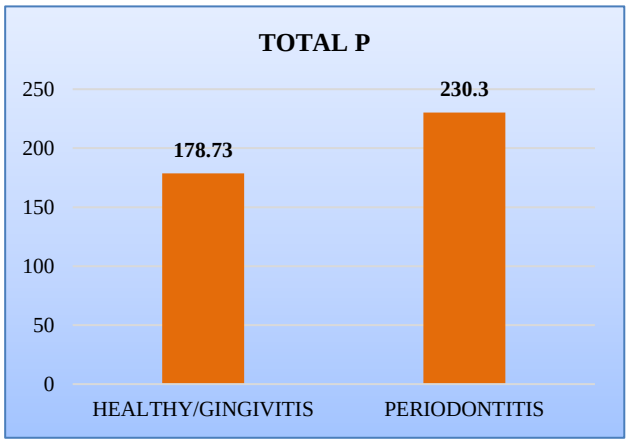

Figure 2: The mean values of total protein between 2 groups.

The mean Reactive Oxygen species (ROS) in healthy/gingivitis subjects is 281 Carratelli units and in chronic periodontitis patients is 486.73 Carratelli units. On Comparison of the ROS between the two groups shows that ROS is higher in Periodontitis group with a t value of -4.217 and is statistically significant with a p value of <0.001 (Figure 3).

The mean value of ROS in control group is 281.3 and in case group is 486.73. This signifies that reactive oxygen species levels increased significantly in chronic periodontitis subjects.

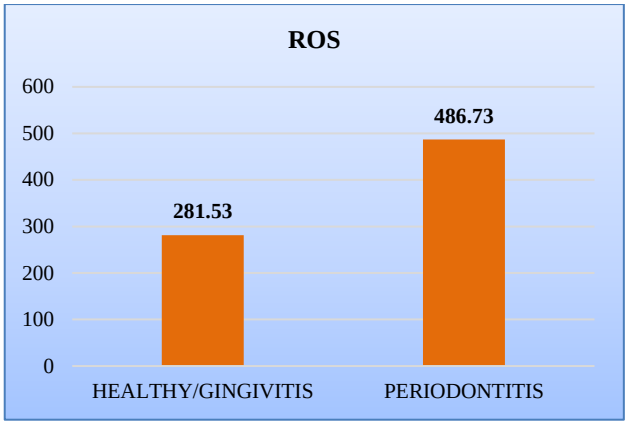

Figure 3: Mean values of reactive oxygen species between the two groups.

The sialic acid concentrations when correlated with bleeding on probing and probing pocket depth showed a correlation of 0.641 and 0.600 which showed that it has a very good positive correlation which was statically significant. The total protein concentrations when correlated with bleeding on probing are 0.246 which showed it has a fair positive correlation and it is significant. The protein concentrations with probing pocket depth are 0.534 which was interrupted as good positive correlation. When ROS was correlated with bleeding on probing it was noted that it had a correlation of 0.447 which states that it is in good positive correlation and an when analyzing with probing pocket depth it showed a correlation of 0.600 which suggests that it is in Very good positive correlation (Table 1). These finding are inferred that there occurs corresponding rise of salivary glycoconjugates and reactive oxygen species levels as the bleeding on probing, probing pocket depth and attachment loss increases. Therefore the levels of salivary glycoconjugates and Reactive oxygen species can be considered as directly proportional to periodontal disease. As, periodontal health deteriorates, the glycoconjugates and ROS increases.

DISCUSSION

Salivary biomarkers could predict the presence and severity of periodontitis prior to the clinical evidences. A biomarker, or biological marker, is in general a substance used as an indicator of a biological state. In oral diagnostics, it has been a great challenge to determine biomarkers for screening, prognosis and evaluating the disease activity and the efficacy of therapy. Researchers involved in periodontal disease diagnostics are currently investigating the possible use of oral fluids, such as saliva, for disease assessment. Alterations in glycoconjugate levels have been observed in various diseases which make them useful indicators of different pathological conditions. In recent years, more attention has been focused on the role of reactive oxygen species,

lipid peroxidation products and antioxidant systems in the pathology of periodontitis.

Elevated levels of Sialic Acid in saliva have protective role in periodontitis as the elevated levels of total sialic acid might be considered as a defense molecule against the increased oxidative stress in inflammatory diseases. This may be due to the release of different lysosomal exoglycosidases during the progression of periodontal disease. Sialic acid levels are markedly increased in those with periodontal diseases, confirming that these play an important role in the immune system. In at the present study, Sialic acids levels had a significant association with periodontal health and disease and the levels of sialic acid were elevated in chronic periodontitis subjects. This agreed with studies conducted by Roopa et al, Shiny Inasu et al, Surekha et al and Oktay et al.[10-13]

The synthesis of protein or glycoprotein increased with progression and severity of periodontal disease. In addition, the rise in salivary albumin also plays a role in the rise in the total proteins. High salivary albumin levels that found in subjects with gingivitis and periodontitis may be due to the leakage of plasma proteins as a result of the inflammation. In present study, we noticed elevated levels of total protein in periodontitis subjects, this increase in concentration may be attributed to modulation by extent of plasma protein exudation. Present study was in accordance with study conducted by Amita Ahire et al, Arati et al, Swati et al and Nada et al.[14-17]

Elevated levels of ROS levels were noted in the saliva of chronic periodontitis subjects. These elevated levels of ROS in saliva can result in imbalance between oxidants and antioxidants causing destruction of the periodontium leading to periodontitis. ROS can cause tissue destruction by degrading the ground substance or by release of collagenase and by oxidizing DNA, proteins, lipids and by stimulating proinflammatory cytokines.

Present study is in accordance with some previous studies conducted by Aiuto et al, Sohini et al, Sudhakar U and Sivasankari et al which have shown that the reactive oxygen metabolites levels increased with periodontitis.[18-21] This study was of the short duration needs evaluation on a long-term basis. The reason for Small sample size selected for the study and for quantitative research design because of lack of use of probability sampling techniques and limits the ability to make broader generalization from present results.

CONCLUSION

Thus, salivary glycoconjugates and ROS may be considered as reliable biochemical markers to detect the extent of tissue destruction in periodontitis. It can be used as an adjunct to diagnose, monitor response to therapy, to determine current periodontal disease status and to assess the treatment outcomes.

Funding: No funding sources
Conflict of interest: None declared
Ethical approval: The study was approved by the Institutional Ethics Committee

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Cite this article as: Roy JS, Manjunath N, Bhat K, George AM, Nishana F, Mathew L. Estimation of salivary glycoconjugates and salivary ros levels in chronic periodontitis: a clinico-biochemical study. Int J Res Med Sci 2017;5:3578-83.