

SALIVARY GLYCOCONJUGATES AS BIOMARKERS OF CHRONIC PERIODONTITISRoopa Sebastian¹, Shivarajashankara Y. M², Manjula Shantaram³, Shivashankar A. R⁴, Dayakar M. M⁵**HOW TO CITE THIS ARTICLE:**

Roopa Sebastian, Shivarajashankara Y. M, Manjula Shantaram, Shivashankar A. R, Dayakar M. M. "Salivary Glycoconjugates as Biomarkers of Chronic Periodontitis". Journal of Evolution of Medical and Dental Sciences 2014; Vol. 3, Issue 42, September 08; Page: 10542-10550, DOI: 10.14260/jemds/2014/3374

ABSTRACT: BACKGROUND: Periodontitis is the major cause of tooth loss and is significantly associated with an increased risk of stroke, type 2 diabetes and heart diseases. Glycoconjugates are expressed on the cell surface as components of glycoproteins, glycosphingo lipids and proteoglycans and plays a vital role in infection and disease. The objective of the study was to assess the changes in salivary glycoconjugates levels in chronic periodontitis (CP) and in healthy individuals without periodontitis. This study was undertaken as a preliminary step towards the use of salivary glycoconjugates as reliable markers of CP. **MATERIALS AND METHODS:** A total of 150 subjects took part in the study. 75 patients with CP (aged 30-55) and 75 age and sex matched healthy controls. Salivary glycoconjugates – sialic acid both – free (FSA) and protein bound (PBSA), L- Fucose (Fuc) and Total protein (Tp) were assessed in both cases and in controls by standard methods. **RESULTS:** The salivary glycoconjugate levels were significantly higher in CP compared to normal healthy controls. Salivary FSA, Fuc and Tp in CP were elevated with mean \pm SD values being 25.9 \pm 4.02, 46.9 \pm 5.65 and 178.4 \pm 23.1 mg/L respectively when compared to healthy controls 19.69 \pm 4.53, 42.1 \pm 3.2, 90.62 \pm 15.8 mg/ L respectively which was found to be statistically significant (p<.001). The values of salivary PBSA were significantly lowered when compared to healthy controls. (p <.01) **CONCLUSIONS:** The results of our study indicate that salivary glycoconjugates could be sensitive biochemical markers of chronic periodontitis.

KEYWORDS: Glycoconjugates, chronic periodontitis, saliva, fucose, sialic acid.

INTRODUCTION: 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, glycosphingo lipids 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 signaling pathways¹. Several studies have revealed that glycoconjugates play key roles in infection and disease.^{2,3}

Chronic periodontitis is an inflammatory disease that affects the supporting tissues of the teeth. It is multifactorial, influenced by genetics as well as by the environment. It is initiated by specific bacteria within the plaque biofilm and progresses due to an abnormal inflammatory- immune response to those bacteria.⁴ Periodontal diseases are second most common oral diseases next to dental caries.⁵

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Most prominent feature in periodontal diseases is connective tissue breakdown, more specifically degradation of extracellular matrix.⁶ Because of increasing prevalence and co morbidities, screening and diagnostic modalities for the early identification of oral disease initiation and progression as well as objective measures for response to therapy, are being sought.⁵

Saliva is an underused diagnostic tool. Collection of whole saliva is non-invasive, does not need skilled technical staff, and is suitable for repeated sampling without any compliance problems. For the past two decades, saliva has been increasingly evaluated as a diagnostic fluid for detecting breast cancer, oral cancer, caries risk, salivary gland diseases, periodontitis, and systemic disorders such as hepatitis C and the presence of human immunodeficiency virus (HIV)⁷⁻¹².

It may reflect levels of therapeutic, hormonal, and immunologic molecules and can yield diagnostic markers for infectious and neoplastic diseases. Various mediators of chronic inflammation and tissue destruction have detected in whole saliva of patient with oral diseases. Salivary biomarkers have also been used to examine the effect of lifestyle factors including smoking, on periodontal health. Also, for some diagnostic purposes, salivary biomarkers proved more useful than serum analysis.⁵

The purpose of the study is to evaluate changes in salivary glycoconjugates – both free sialic acid (FSA) and protein bound sialic acid (PBSA), L-fucose (Fuc) and total proteins (Tp) levels in chronic periodontitis and to compare the values with that of normal healthy controls so as to assess the feasibility of using salivary glycoconjugates as useful marker in chronic periodontitis (CP).

MATERIALS AND METHODS: A total of hundred and fifty subjects, seventy five patients with chronic generalized periodontitis (CP), aged between 30- 62 yrs and equal number of age and sex matched periodontally healthy controls participated in the study. Patients referred to the Dept. of Periodontics of our institution for diagnosis and treatment were recruited for the study.

Control group consisted of student and staff volunteers with no history of periodontal disease with a probing depth of <3mm, with no sign of gingival inflammation and overall good oral hygiene.¹³ Patients recruited for the study were diagnosed as having chronic generalized periodontitis with at least two non-adjacent sites per quadrant with probing depth of ≥ 5 mm, and clinical attachment loss of ≥ 4 mm. All patients had a minimum of 20 teeth in the mouth.

The subjects included in the study were nonsmokers, non- alcoholics, had no history of systemic illnesses, had not received periodontal treatment or antibiotic or anti-inflammatory therapy at least six months prior to participation in the study. Participants who were pregnant and on vitamin supplementation were also excluded from the study.

Informed consents were obtained from all the participants and the study was approved by the institutional ethical committee.

CLINICAL MEASUREMENTS: The periodontal status of the subjects was identified by the measuring bleeding on probing (BOP), probing depth (PD) and clinical attachment level (CAL). PD and CAL were measured on six sites of teeth (mesial, median and distal points at buccal and palatal aspects) using Williams periodontal probe (Hu Freidy™, Chicago, IL). All clinical examinations were carried out by a single examiner from the Periodontics Department.

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COLLECTION OF SALIVA SAMPLES: Unstimulated whole saliva samples were collected between 9AM and 11AM. This was to ensure that the variability in salivary flow and compositions due to diurnal variations were minimized. Subjects were asked to rinse their mouth thoroughly with distilled water, to remove any food debris and then to spit into sterile disposable plastic container.¹⁴ The subjects were instructed not to spit forcefully so as to avoid blood contamination. The collected saliva was placed in ice carrier box transferred to laboratory for biochemical analysis. About 2ml of unstimulated whole saliva collected was centrifuged immediately to remove cell debris (1000 x for g for 10 min). The supernatant was used for analysis.

Biochemical assays of saliva:

1. Sialic acid - was estimated by the method of Yao et al. Saliva samples were treated with ethanol to precipitate proteins. Sialic acid contents of both the precipitate and supernatant were estimated on the basis of reaction of sialic acid with ninhydrin reagent. Sialic acid concentration obtained from the precipitate was 'protein bound sialic acid (PBSA) and sialic acid concentration in the supernatant was 'free sialic acid' (FSA). The absorbance of the blue colored complex was measured at 470nm.¹⁵
2. L-Fucose was estimated according to the method of Winzler using cysteine hydrochloride. L-Fucose was assayed by dissolving ethanol-precipitated proteins of serum in alkali, heating with sulphuric acid and determining the color after addition of cysteine. Standard L-Fucose was procured from Sigma Chemical Company, MO, US. The color produced by hexoses under these conditions was corrected by determining absorbance at 400 nm and 430 nm¹⁶.
3. Total protein -was estimated by Lowry's method¹⁷.

STATISTICAL ANALYSIS: 'Z' test was employed to analyze the differences in clinical measurements, age and biochemical parameters between healthy and chronic periodontitis (CP) group. A value of P <0.05 was considered to be significant. All values were expressed as mean \pm standard deviation (SD).

RESULTS: The values of clinical measurements are listed in Table 1. Scores of all clinical parameters (mean \pm SD) were found to be significant in CP when compared to healthy controls. The values of glycoconjugate parameters are listed in Table 2. Salivary FSA and Fuc and total protein levels were significantly elevated in CP compared to healthy controls (p<0.001). Values of salivary PBSA were found to be lowered and it was statistically significant (p<0.01). Total protein levels in saliva showed significant increase in CP in comparison to healthy controls (p<0.001).

Parameter	CP group (n=75)	Controls (n=75)
Age (in years)	47.8 \pm 6.8	48.4 \pm 5.6
PD (in mm)	6.54 \pm 2.2*	2.21 \pm 0.43
CAL (in mm)	4.7 \pm 2.1*	0.0 \pm 0.0

Table 1: Comparison of age and clinical measurements in case and control group

PD-Probing Depth, CAL-Clinical Attachment Loss

*-Indicate p<0.05

Glycoconjugates	Normal healthy controls (n=60) [Mean +SD]	CP (n=60) [Mean +SD]	P value
FSA (mg/L)	19.69 ±4.53	25.9± 4.02	<.001
PBSA(mg/ L)	15.2±2.14	13.8 ±2.1	<.01
L-Fucose (mg/ L)	42.1 ± 3.2	46.9 ± 5.65	<.001
Tp (mg/ dL)	90.62 ± 15.8	178.4 ± 23.1	<.001

Table 2: Comparison of glycoconjugate parameters in CP and in healthy controls

DISCUSSION: Alterations in glycoconjugate levels have been observed in various diseases which make them useful indicators of different pathological conditions. Saliva contains glycoproteins, which consist of short carbohydrate chains covalently attached to either serine/threonine or asparagine residues in the protein core.

The carbohydrate part of glycoprotein consists nearly of nine monosaccharides, these are: D-Glu, D-Gal, D-Man, GlcNAc, GalNAc, α -L-fucose, sialic acid, L-Arabinose and D-xylose¹⁸. They are important components of the connective tissue and extracellular matrix. The microorganisms either can release hydrolytic enzymes or agents that may cause direct damage to periodontal tissue by stimulating host mediated responses.⁶

Defensive responses to periodontal disease include the production of different enzymes, which may be produced or released through stroma, epithelium or inflammatory cells, which in turn causes degradation of the tissue surrounding the teeth.¹⁸ Shinohara and coworkers studied the relationship between salivary sialic acid in rats with naturally occurring gingivitis. They suggested elevation of sialic acid can be a useful index of severity of periodontal disease.¹⁹

Oktay et al found a significant and positive correlation between serum and saliva total sialic acid levels in periodontitis patients with and without CVD and suggested that serum total sialic acid and saliva salivary total sialic acid may be used as effective markers for low-grade inflammation in periodontitis as well as in patients with CVD.²⁰

In the present study, we found significantly higher levels of salivary free sialic acid in chronic periodontitis compared to healthy controls. This may be due to the release of different lysosomal exoglycosidases during the progression of periodontal disease.

The glycosidases could be of bacterial origin since adhesion of materials containing microorganisms at the tooth surfaces is considered to be a primary factor in the development of periodontal diseases. Exoglycosidases, for the preferred anomeric links of the principal monosaccharide units of salivary glycoproteins are produced by the oral bacteria in vitro and in dental plaque in vivo.

There is indirect evidence to indicate that oral bacteria utilize the released carbohydrates as metabolic fuel. Lysosomal exoglycosidases act on the external non-reducing part of oligosaccharide chain of glycoconjugates, thus starting the chain reaction, where the product of the previous reaction becomes a substrate for the next one. The exoglycosidases degrade, in consequence, glycoproteins, proteoglycans, glycolipids that form the cellular membrane and intercellular matrix.

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Among exoglycosidases there are: N-acetyl- β -hexosaminidase (HEX), β -glucuronidase (GLU), β -galactosidase (GAL), α -mannosidase(MAN) and α -fucosidase (FUC).^{6,21} Pattabhraman reported increased levels of free sialic acid in periodontitis which was consistent with the findings of this study.⁶

Among the nine sugars present in the structure of glycoproteins, fucose can be used reliably and conveniently for the investigations of glycoprotein synthesis and secretion. The increase in L-Fucose levels in saliva seen in the current study was in agreement with the previous study.²² This increase in salivary fucose in periodontitis might represent the breakdown of plasma and tissue glycoproteins which may occur as a result of inflammation. There is indirect evidence to indicate that, increased salivary glycoprotein level is offset by increased fucosidase activity that causes the breakdown of saliva and tissue glycoproteins ²³. Thus periodontitis may cause an increase in fucosidase activity.

Elevated salivary total protein seen in the study was in accordance with results of previous studies.^{6, 24} The increase in salivary total protein concentration in periodontitis patients may be related to the salivary glands, which had responded to inflammatory disease (periodontitis) by enhancing synthesis of acinar proteins. The synthesis of protein or glycoprotein increased with progression and severity of periodontal disease.²³ 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.²⁵

To conclude, the results of the current study suggest that salivary glycoconjugates hold promise as reliable biochemical markers of extent of tissue destruction in periodontitis. They may also be used in diagnosis, treatment and prognosis of the disease. Despite the many benefits of saliva, further studies need to be undertaken to validate saliva as a useful diagnostic fluid in future for monitoring diseases.

ACKNOWLEDGEMENTS: The authors are extremely indebted to the Principal and management of K.V.G Medical College, Sullia, D.K for the infrastructure, support and encouragement which they provided to carry out this study. We are also thankful to our Medical Laboratory Technology Students for their valuable services.

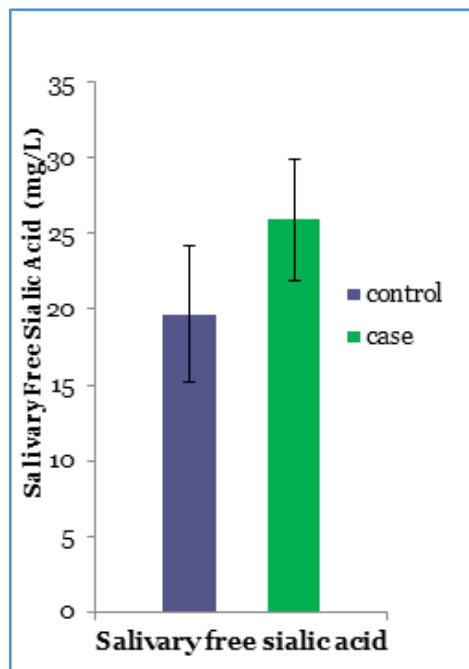
REFERENCES:

1. Sasaki N, Toyoda M. Glycoconjugates and Related Molecules in Human Vascular Endothelial Cells. *Int J Vasc Med* 2013; Article ID 963596, 10 pages.
2. Kamerling J. Structural studies on glycoprotein glycans. *Pure & Appl. Chem.* 1994; 66(10/11): 2235-2238.
3. Karlsson E K, Kwiatkowski DP, Sabeti PC. Natural selection and infectious disease in human populations. *Nature Reviews Genetics* 2014; 15: 379-393.
4. Jawzaly JW, Hasan HG, Ahmed B M. Relationships of salivary sialic acid and its fraction in periodontitis with demographic properties and medical history. *International Journal of Dental Science and Research Development* 2013; 3(2); 17-26.
5. Khashu H, Baiju CS, Bansal SR, Chhillar A. Salivary biomarkers: a periodontal overview. *J Oral Health Comm Dent* 2012; 6(1) 28-33.

6. Shetty PK, Pattabiraman TN. Salivary glycoproteins as indicators of oral diseases. *Indian J Clin Biochem.* 2004; 19(1): 97-101
7. Streckfus C, Bigler L, Tucci M, Thigpen JT. A preliminary study of CA15-3, c-erbB-2, epidermal growth factor receptor, cathepsin-D, and p53 in saliva among women with breast carcinoma. *Cancer Invest.* 2000; 18: 101-10.
8. Li Y, St John MAR, Zhou X, et al. Salivary transcriptome diagnostics for oral cancer detection. *Clin Cancer Res.* 2004; 10: 8442-8450.
9. Bratthall D, Hänsel Petersson G. Cariogram-a multifactorial risk assessment model for a multifactorial disease. *Community Dent Oral Epidemiol* 2005; 33: 256-64.
10. Hu S, Zhou M, Jiang J, Wang J, Elashoff D, Gorr S, et al. Systems biology analysis of Sjogren's syndrome and mucosa associated lymphoid tissue lymphoma in parotid glands. *Arthritis and Rheumat* 2009; 60: 81-92.
11. Balwant R, Sammi K, Rajnish J, Suresh CA. Biomarkers of periodontitis in oral fluid. *J Oral Sci* 2008; 50(1): 53-56.
12. Hodinka R L, Nagashunmugam T, Malamud D. Detection of human immune deficiency virus antibodies in oral fluids. *Clin Diagn Lab Immunol* 1998; 5: 419-426.
13. Armitage GC. Development of a classification system for periodontal diseases and conditions. *Annals of Periodontology* 1999; 4: 1-7.
14. Navazesh M. Methods for collecting saliva. *Ann NY Acad Sci.* 1993; 694:72-77.
15. Yao K, Ubuka T, Masuoka N, Kinuta M, Ikeda T. Direct determination of bound sialic acids in sialoglycoproteins by acidic ninhydrin reaction. *Anal Biochem* 1989; 179: 332-335.
16. Winzler RJ. Determination of serum glycoproteins. *Methods Biochem Anal.* 1955; 2: 279-311.
17. Lowry OH, Rosebrough NJ, Farr AL, Rabdall RJ. Protein measurement with Folin's phenol reagent. *J Biol Chem* 1951; 193: 265-275.
18. Paknjad M, Rezaei A. Salivary biochemical markers of periodontitis. *Rom J Biochem* 2013; 50 (2): 129-146.
19. Shinohara M, Ohura A, Ogata K, Inour H, Miyata T, Yoshioka M., *Jpn J Pharma.*; 1994, 64, 61-65.
20. Oktay S, Basar I, Emekli-Alturfan E, Malai E, Elemek E, Ayan F, Koldas L, Noyan U, Emekli N. Serum and saliva sialic acid in periodontitis patients with or without cardiovascular disease. *Pathophysiol Haemost Thromb* 2010; 37(2-4) 67-71
21. Bierc M, Minarowski L, Woźniak L , Chojnowska S, Knas M, Szajda S, Zwierz K. The activity of selected glycosidases in salivary gland tumors. *Folia Histochemica Et Cytobiologica* 201; 48(3): 471-474.
22. Musa MAW, Ahmed BM. Evaluation of salivary α -L-Fucose and its related parameters in periodontitis. *Zanco J Med. Sci* 2013; 17(3): 563-569.
23. Park D, Ryu KS, Choi JK, Park C. Characterization and role of fucose mutarotase in mammalian cells. *Glycobiology.* 2007; 17(9): 955-62.
24. Koss MA, Castro CE, Salum K M, Lopez M E. Changes in saliva protein composition in patients with periodontal disease. *Acta Odontol Lati-noam.* 2009; 22(2): 105-112.
25. Henskens YM, van der Velden, U Veerman, EC Nieuw, Amerongen AV. Protein, albumin and cystatin concentrations in saliva of healthy subjects and of patients with gingivitis or periodontitis. *J Periodontal Res.* 1993; 28: 43-8.

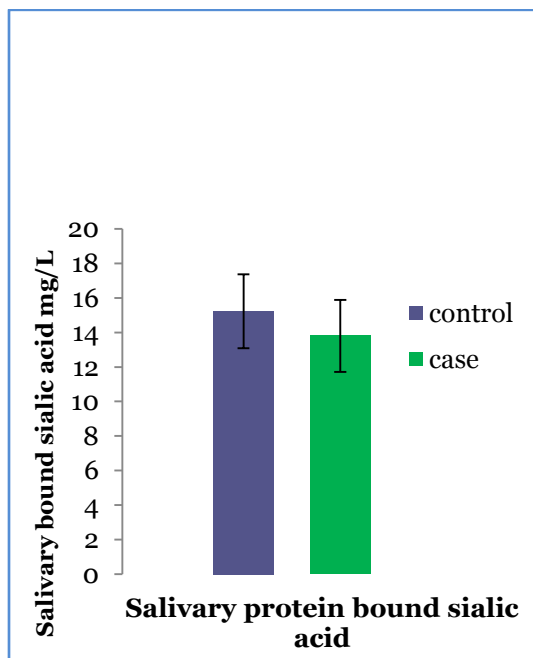
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Comparison of salivary free sialic acid (FSA) in CP and in healthy controls



Graph 1

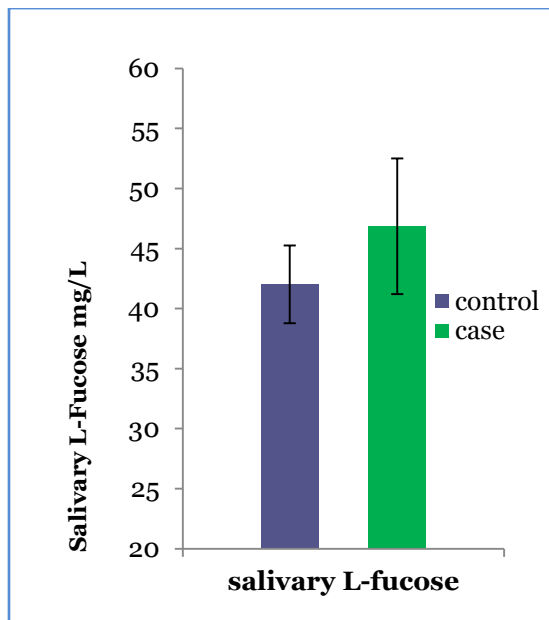
Comparison of salivary bound sialic acid (PBSA) in CP and healthy controls



Graph 2

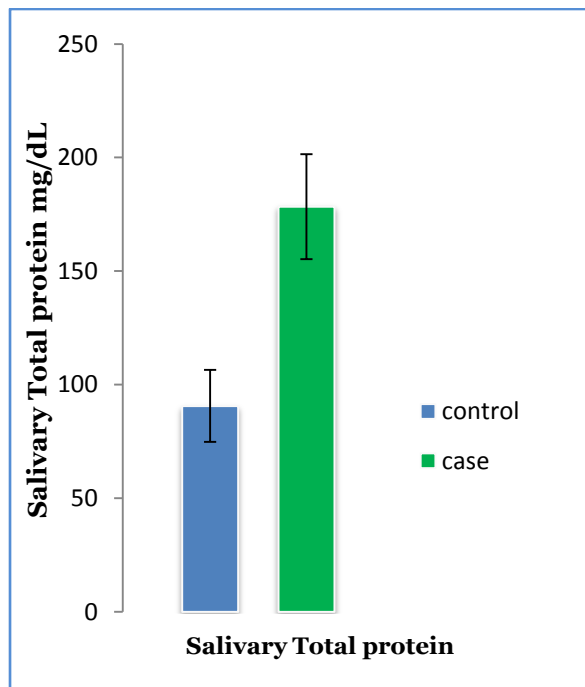
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Comparison of salivary L-Fucose in CP and healthy controls



Graph 3

Comparison of salivary Total protein (Tp) in CP and healthy controls



Graph 4

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Date of Submission: 20/08/2014.

Date of Peer Review: 21/08/2014.

Date of Acceptance: 01/09/2014.

Date of Publishing: 08/09/2014.