International Journal of Current Advanced Research
ISSN: O: 2319-6475, ISSN: P: 2319-6505, Impact Factor: 6.614
Available Online at www.journalijcar.org
Volume 8; Issue 04 (F); April 2019; Page No.18423-18427
DOI: http://dx.doi.org/10.24327/ijcar.2019.18427.3521

Research Article

EVALUATION OF SALIVARY PROTEIN THIOL IN HEALTHY AND DIABETIC PATIENTS WITH AND WITHOUT CHRONIC PERIODONTITIS

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A R T I C L E I N F O

Article History:
Received 6th January, 2019
Received in revised form 15th February, 2019
Accepted 12th March, 2019
Published online 28th April, 2019

Key words:

Antioxidants, Saliva, Periodontitis, Protein thiol, Type 2 diabetes mellitus.

A B S T R A C T

Introduction: Oxidative Stress (OS) is implicated in the pathogenesis of many systemic and oral diseases such as periodontal disease. Periodontitis and diabetes are common chronic diseases with bidirectional relationship having increased oxidative stress as primary etiological feature resulting in release of reactive oxygen species (ROS). Antioxidants such as thiol which modulate the ROS production are early products of protein oxidation during oxidative stress. As a result, assessment of thiol during oxidative stress is one of the best measures of primary effects of oxygen radicals.

Aim and Objectives: To evaluate and compare levels of salivary protein thiol in healthy and diabetic patients with and without chronic periodontitis and to correlate it with clinical parameters.

Methodology: A total of 90 subjects were randomly selected and divided into three groups i,e; healthy, type2 diabetes mellitus (TY2DM) patients with and without chronic periodontitis. Unstimulated whole saliva samples of subjects were collected after obtaining consent and analyzed for protein thiols.

Result: Protein thiol levels were found to be significantly decreased in TY2DM patients with chronic periodontitis compared to TY2DM patients without chronic periodontitis and healthy subjects ($p<0.001$).

Conclusion: Salivary protein thiols can be a appropriate marker of the antioxidant status in chronic periodontitis patients with TY2DM and can be used to predict the prognosis of periodontal disease in diabetic patient.

INTRODUCTION

Oxidative stress(OS) is known to be a component of molecular and cellular tissue damage mechanism in a wide spectrum of human diseases.[1] In recent years, there has been a tremendous expansion in medical and dental research concerned with OS, free radicals, reactive oxygen species (ROS) and anti-oxidant defense mechanisms as a possible link between oral and systemic diseases.[2] There is enough evidence that periodontitis and diabetes mellitus are common chronic diseases with bidirectional relationship having increased OS as primary etiological feature resulting in release of ROS. [3, 4] Therefore diabetes mellitus is by now recognized as a risk factor for periodontal disease**.** [5, 6, 7] which was coined as the 6th complication of diabetes mellitus. Majority of researches have focused on TY2DM as type 2 diabetes accounts for 90 to 95 percent of all diabetes cases. [8]

In normal physiology, there is a dynamic equilibrium between ROS activity and antioxidant defense capacity and when that equilibrium shifts in favour of ROS, oxidative stress (OS) results either by reduction in anti-oxidant defenses or an increase in ROS production or activity. ROS are a family of highly reactive species, skilled in extracting electrons and thereby oxidizing a variety of bio molecules vital to cell and tissue function. Excessive accumulation of ROS can harm bio molecules, including lipids, proteins and nucleic acids. Therefore the body contains a number of protective anti-oxidant (AO) mechanisms, whose definite role is to remove damaging oxidants (ROS), as soon as they form, or to repair the damage caused by ROS. [9] Antioxidants are present in all body fluids and tissues. Saliva, being one of those body fluids contains a wide range of antioxidants such as uric acid, vitamin C, reduced glutathione, oxidized glutathione, protein thiols and others. Biochemical analysis of saliva is of importance, since whole saliva is a combination of gingival crevicular fluid, which has a composition similar to serum, and fluid released from salivary glands due to its non-invasive, easy collection and cost-effective move towards screening of huge population**.** [10] The thiol groups are most essential components of the antioxidant team that are present both intracellularly and extracellularly either in free form (reduced

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glutathione) or bound to proteins (protein bound thiols) prevent the irreversible unfolding of the protein structure and have been shown to destroy ROS and other free radicals by enzymatic and non-enzymatic mechanisms play a key role in maintaining the antioxidant status of the body during OS**.** [11] Thiol groups which are therefore, responsible for their antioxidant response, serve as a sensitive marker of OS. [12]

To the best of our knowledge there is no enough scientific data available on protein thiol levels in chronic periodontitis subjects with type 2 diabetes mellitus. So the present study is aimed to evaluate the salivary protein thiol levels in healthy and type 2 diabetes mellitus with and without chronic periodontitis subjects and to correlate the protein thiol levels with the clinical parameters.

MATERIALS AND METHODS

The cross sectional study was designed considering the difference in group means to be 20%, power of the study as 80%, at 95% confidence interval, a ratio of sample size (group1/ group 2/ group 3) as 1 and with the significance level set at 5%, a sample size of 90 was derived (i.e; 30 in each group). 90 samples aged 18-65years were randomly selected from the outpatient department of Department of Periodontics, P.M. Nadagouda Memorial Dental College, Bagalkot, Karnataka, India. The study was conducted for a period of five months. The protocol for this study was approved by the Institutional Ethical Committee. Prior to enrolment in the study, a written consent was obtained from the subjects who fulfilled the inclusion criteria. After clinical and radiographic examination, the subjects were divided in three groups. Group 1 consisted of 30 healthy subjects showing absence of clinical and radiographic manifestations of periodontal disease, at least 20 teeth present. Group 2 consisted of 30 TY2DM subjects with healthy periodontium. Group 3 consisted of 30 TY2DM subjects with chronic periodontitis with the presence of bleeding on probing, probing pocket depth (PPD) of ≥5 mm along with attachment loss ≥ 3 mm at more than 30% of all sites in the mouth. [13]

Subjects suffering from systemic conditions like rheumatic fever, heart diseases, hypertension, liver and kidney disease etc, or any infection requiring prophylactic antibiotic therapy, pregnant females, lactating women, subjects on contraceptives, hormone replacement therapy, steroids, NSAIDs, vitamin supplements, alcoholics and have undergone scaling and root planing in past six months were excluded from the study as they are shown to affect the levels of protein thiols. The diagnosis of patients with TY2DM was based on the criteria of the World Health Organization. The glycemic status of patients formerly diagnosed with TY2DM was confirmed by their glycosylated hemoglobin (HbA1c >7%), a pre prandial glycemia of >130 mg/dl and postprandial >180 mg/dl. All diabetic subjects included in the present study had records of at least 4 to 5 years of diagnosed TY2DM, were on medication with oral anti diabetic drugs, and all had well-controlled TY2DM and none used any antioxidant agent. After proper grouping of the subjects, a full mouth periodontal examination was performed by a single examiner. The clinical parameters such as bleeding on probing, pocket probing depth, clinical attachment level and gingival index (Loe and Silness 1963) were assessed.

Method of Saliva Collection

All the participants of the study were instructed not to eat, drink or smoke for 1 hour before sampling. Unstimulated whole saliva [14] was collected in sterile containers after allowing saliva to pool in the floor of the mouth for 5 min by tilting head slightly forward and letting saliva drain into a sterile container. Since the salivary composition show diurnal variations [15] all the samples were collected between9.00 to 12.00 am to reduce bias. Immediately after collection, the saliva samples were centrifuged for 10 min at 8000 rpm in cold centrifuge. The supernatant, free of debris was extracted and transferred to eppendorf tubes and were stored at –80°C until analysed. [16]

Biochemical Analysis

Salivary protein thiol was measured by spectrophotometric method using dithionitrobenzene (DTNB)-Ellman's reagent**.** [17, 18, 19] Ellman's reagent or 5,5'-dithiobis 2-nitrobenzoate, (DTNB) is a symmetrical aryl disulfide which readily undergoes the thiol-disulfide interchange reaction in the presence of a free thiol**.** [20] The TNB dianion has a moderately intense absorbance at 412nm compared to both disulfides. The protein thiol concentration in saliva was determined by using the molar extinction coefficient of the TNB complex in the assay mixture at 412nm obtained after using known standard concentrations and their absorbance values**.** [21]

Statistical Analysis

The obtained data was statistically analysed using SPSS software. Statistical analysis for comparison of means among the groups was done by ANOVA, followed by post-hoc Tukey's test for group wise comparison.Pearson correlation was applied to correlate between the parameters. Data were expressed as mean±standard deviation (SD) and $p<0.001$ was considered to be statistically significant.

RESULTS

The study included 90 individuals of whom, 30 were healthy controls , 30 were TY2DM patients and 30 were TY2DM patients with chronic periodontitis . Subjects were recruited in the study, when they came to the college outpatient department for a dental examination. Age and sex matching was not possible for the three groups. The mean age of TY2DM with chronic periodontitis subjects is higher than the healthy group. [Table/Fig-I]

Protein thiol levels showed a statistically significant difference in TY2DM patients with chronic periodontitis group compared to TY2DM patients with healthy periodontium and the healthy. Protein thiol levels were significantly lower in TY2DM patients with chronic periodontitis ($p < 0.001$) compared to healthy. Protein thiol levels were lower in TY2DM patients with chronic periodontitis ($p < 0.001$) compared to TY2DM patients with healthy periodontium. Protein thiol levels were lower in TY2DM patients with healthy periodontium compared to healthy . [Table/Fig-II]
The mean gingival index, pocket probing depth, clinical attachment level and bleeding on probing was found to be highest in TY2DM patients with chronic periodontitis i.e; group 3. [Table/Fig-III, IV, V] TY2DM patients with chronic periodontitis showed a statistically significant difference in the loss of clinical attachment level than the other two

groups.[Table/Fig-IV]. All the three parameters show positive correlation with the protein thiol levels in the study groups.

Table I Comparison of age in terms of {Mean (SD)} among all the 3 groups using ANOVA test

Group	N	Mean	Std. Deviation	F value	P value
Group 1	30	27.00	7.125	**109.391**	**<0.001****
Group 2	30	50.30	9.132		
Group 3	30	54.87	7.045		
Total	90	44.06	14.508		

(p< 0.05 - Significant*, p < 0.001 - Highly significant**)

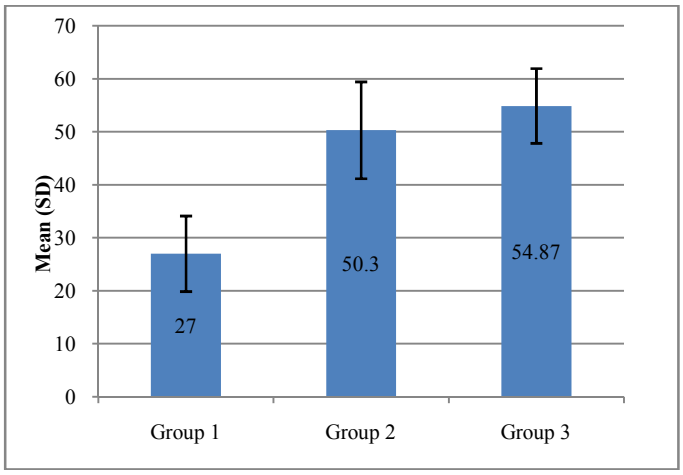

Figure I

Table II Comparison of Thiol level values in terms of {Mean (SD)} among all the 3 groups using ANOVA test

Group	N	Mean	Std. Deviation	F value	P value
Group 1	30	0.00776	0.00236	**215.856**	**<0.001****
Group 2	30	0.00200	0.00050		
Group 3	30	0.00057	0.00042		
Total	90	0.00344	0.00342		

(p< 0.05 - Significant*, p < 0.001 - Highly significant**)

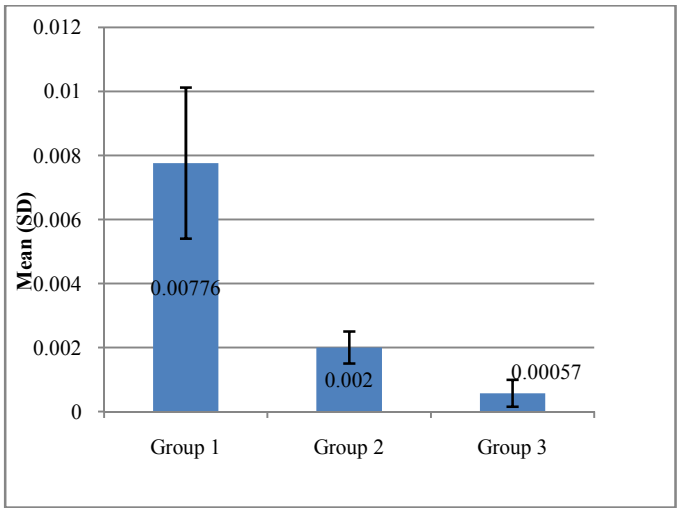

Figure II

Table III Comparison of Gingival index values in terms of {Mean (SD)} among all the 3 groups using ANOVA test

Group	N	Mean	Std. Deviation	F value	P value
Group 1	30	0.490	0.1954	**632.854**	**<0.001****
Group 2	30	0.447	0.1889		
Group 3	30	2.263	0.2810		
Total	90	1.067	0.8799		

(p< 0.05 - Significant*, p < 0.001 - Highly significant**)

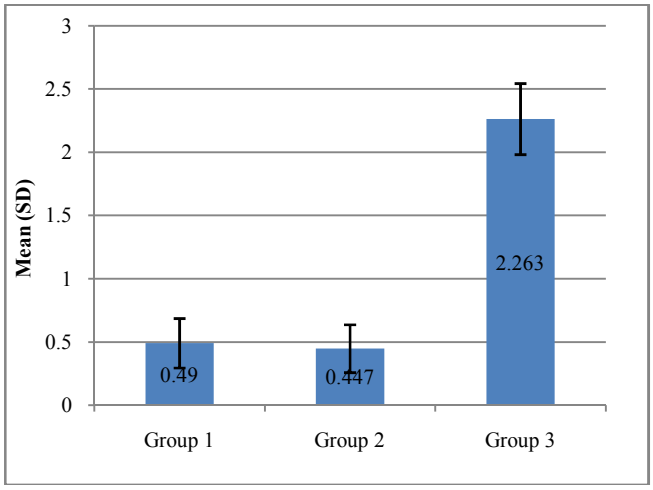

Figure III

Group	N	Mean	Std. Deviation
Group 1	30	0.00	0.000
Group 2	30	0.00	0.000
Group 3	30	6.63	0.850

Group	N	Mean	Std. Deviation
Group 1	30	0.00	0.000
Group 2	30	0.00	0.000
Group 3	30	6.33	0.922

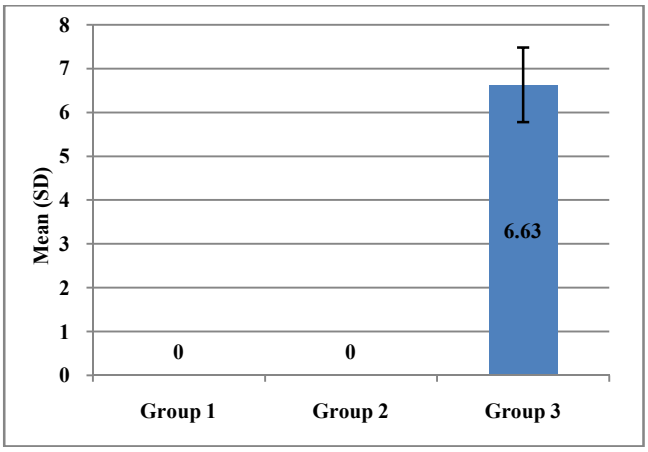

Figure IV

Table V Comparison of Bleeding on probing values in terms of {Mean (SD)} among all the 3 groups using ANOVA test

Group	N	Mean	Std. Deviation	F value	P value
Group 1	30	0.947	0.4447	234.023	<0.001**
Group 2	30	0.907	0.4828		
Group 3	30	3.023	0.3645		
Total	90	1.626	1.0825		

(p< 0.05 - Significant*, p < 0.001 - Highly significant**)

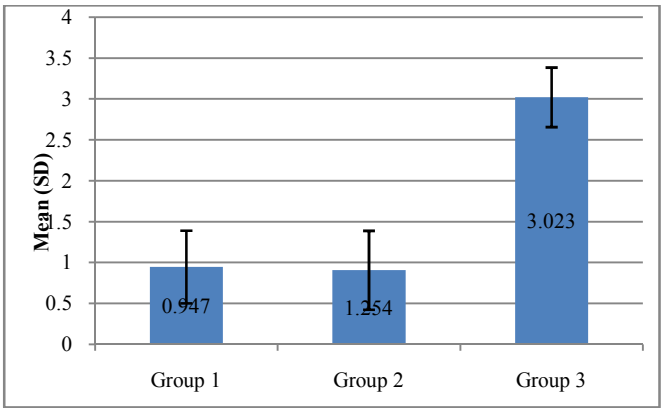

Figure V

DISCUSSIONS

The present clinical trial was carried out with the objective of analyzing the activity of thiol antioxidants in the saliva. Thiols, major antioxidants in body fluids are very early products of protein oxidation during OS, ensuing within few seconds after generation of oxygen radicals thus protecting the biomolecules. The thiol groups exist both intracellularly and extracellularly either in free form (reduced glutathione) or bound to proteins (protein bound thiols) play a major role in maintaining the antioxidant status of the body. [11] Due to their high nucleophilicity, thiols in peptides and proteins are particularly susceptible to direct oxidation by ROS and oxidation of thiols results in alterations in protein structure and function. Thus, thiols not only represent the most weak targets of ROS and related oxidants, but also represent a resourceful and robust defense system against biochemical perturbations caused by oxidative stress. In addition, some protein thiols are selectively oxidized by low, physiological levels of oxidants, and such oxidative modifications play an important role in signal transduction, metabolism, as well as proliferation and cell death. [22] Therefore, assessment of the extent and specificity of protein thiols role during OS is one of the best trial of primary effects of oxygen radicals.

Many studies have observed altered protein thiols in various disease conditions with oxidative stress like study by Prakash *et al* in patients with uremia and study by Karthikeyan *et al* in pediatric patients with nephritic syndrome [11], [23] Similar reports were available in studies on salivary protein thiols and total antioxidant power of saliva in brain tumor patients. [21] Thiol status has also been determined in both type 1 and type 2 diabetes mellitus patients and the levels were found to be decreased. [24, 25] In studies on salivary and serum antioxidants levels (peroxidase, superoxide dismutase, salivary total antioxidant status) in type 1 diabetes mellitus patients where altered antioxidant levels were observed indicating OS. [26] In this study, protein thiol levels were significantly ($p < 0.001$) lower in TY2DM patients with chronic periodontitis compared to healthy controls. Protein thiol levels were lower in TY2DM patients with chronic periodontitis compared to TY2DM patients with healthy periodontium. We speculate that the decreased thiols could be because of increased oxidation of thiol groups due to already existing oxidative stress. Existence of oxidative stress in diabetes mellitus is well proved. [27] Many biochemical pathways related to hyperglycemia, such as glucose auto oxidation, polyol pathway, prostanoid synthesis, protein glycation, and endothelial cell exposure can increase ROS production. Hence this might be one of the reasons why alterations of periodontal tissues occur in TY2DM, with least presence of dental plaque and calculus which are the most important etiologic factors of periodontal disease**.** [4]

Hence, with this observation it can be concluded that salivary protein thiol levels are appropriate marker of oxidative stress in TY2DM patients with chronic periodontitis and can predict the progression of periodontal disease in subjects with TY2DM.

Future Directions

The modulation of key mitochondrial thiol proteins, which participate in redox signaling, OS responses, and cell death programming, provides a pivotal direction in developing new therapies towards the prevention and treatment of several diseases.

The results of this research lay emphasis on the need for studies with large sample size, pre and post therapy analysis to authenticate the impact of periodontal disease with T2DM on protein thiol levels. Gender distribution was not possible as patients were randomly selected from the outpatient department. These could be the possible limitations of the study.

CONCLUSION

Based on the results, there is increasing evidence that OS is a causative factor in the pathogenesis of diabetes and periodontal disease. The co-existence of both diabetes and periodontal disease augment the pathological effects of OS. The data reported here emphasize the need for diagnosis and treatment of periodontitis in TY2DM patients to minimize the cumulative pathogenic effects of oxidative stress upon B-cell function and glycemic control.Measurement of salivary protein thiol being an easy, reliable and inexpensive test, it can be put to use as a diagnostic chair side test for early detection of OS and can serve as markers of redox status and as an attractive therapeutic target in TY2DM patients with periodontitis. Researchers have confirmed that periodontal diseases are not just confined to the oral cavity but are also known to have an effect on systemic health. Hence, protein thiols can be a promising option to establish both a good oral and systemic health.

Conflict of Interest: No conflict of interest was declared by the authors.

Acknowledgements

Authors wish to thank Dr Chandrashekhar.V.M, Department of Pharmacology, H. S. K. College of Pharmacy, Bagalkot., for his kind support to carry out the study.

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How to cite this article:

Shivaraj B Warad *et al* (2019) 'Evaluation of Salivary Protein thiol in Healthy and Diabetic Patients with and without Chronic periodontitis', *International Journal of Current Advanced Research,* 08(04), pp. 18423-18427.
DOI: http://dx.doi.org/10.24327/ijcar.2019.18427.3521

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