

Original Research Article

Comparative Evaluation of Salivary and Gingival Tissue Glutathione Peroxidase Levels in Subjects with Healthy Periodontium and Chronic Periodontitis

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Abstract: Oxidative stress due to reactive oxygen species (ROS) is one of the etiological factors of periodontal disease. Glutathione peroxidase (GSHPx) has a protective action against ROS and is an established marker of oxidative stress. The present study evaluates the levels of GSHPx in saliva and gingival tissue to further gather information into the role of antioxidants in periodontal disease. A total of 40 subjects participated in the study. The subjects were divided among namely Group A-Total of 20 subjects aged between 30-55 years who have chronic periodontitis. Group B (control) -Total of 20 subjects with healthy periodontium aged between 15-35 years. The values obtained were subjected to students T-test. Saliva and gingival tissue were collected from all subjects to estimate levels of GSHPx. The results showed that the mean salivary and gingival tissue glutathione peroxidase levels were significantly higher in Group A as compared to Group B subjects. From this study it can be concluded that glutathione peroxidase can be used as a diagnostic marker for periodontal disease.

Keywords: Glutathione peroxidase, saliva, gingival tissue, periodontitis, antioxidants, Free radicals

INTRODUCTION

Chronic periodontitis is the most common oral disease. It occurs because of imbalance between pathogenic bacteria and host defence system. Polymorphonuclear leukocytes (PMN) are the cells of first line defense of oral tissues against pathogenic microbes. Interaction between the microbes and leukocytes will initiate the various protective mechanisms that cause the host to combat against microbes, but also leading to damage to local tissues. PMN induced by microbes are characterized by increased consumption of oxygen i.e. increasing the production of free radicals. The free radicals kill the microbes by distorting their cell membrane. However, during this protective defence mechanism if free radicals are released in excess, it can lead to damage of healthy tissues as well [1-3].

Antioxidants are present in all body fluids and tissues, and protect against endogenously-formed free radicals, usually produced by leakage of the electron transport system. Antioxidant enzymes such as superoxide dismutase and glutathione peroxidase provide protection within cell [4]. Glutathione peroxidase, an enzymatic antioxidant dependent on the

micronutrient selenium (Se), plays a critical role in the reduction of lipid and hydrogen peroxides [5]. With this background the present study was conducted with following objectives:

1. To assess the levels of glutathione peroxidase in saliva and inflamed gingival tissues of subjects with chronic periodontitis.
2. To assess the levels of glutathione peroxidase in saliva and healthy gingival tissues of healthy subjects.

METHODOLOGY

Source of data

40 subjects reporting to the outpatient department and Department of Periodontics of A. B. Shetty Memorial Institute of Dental Sciences, were selected –

Method of collection of data

This study was designed as a case-control study comprising of 40 subjects, inclusive of both sexes and were divided into two groups of 20 patients each Group A-Total of 20 subjects aged between 30-55 years who have chronic periodontitis

Group B (control) -Total of 20 subjects with healthy periodontium aged between 15-35 years

Criteria for selection

Clinical Procedure

Screening examination

- Medical history and dental history
- Probing depth
- Gingival index (Loe and Silness)
- Plaque index (Silness and Loe)

Clinical measurement

Clinical measurement was performed immediately after unstimulated whole saliva collection. To determine the periodontal status of the study subjects, the following assessments were recorded: bleeding on probing (BOP) of the marginal gingival tissues was performed by running a probe along the soft tissue wall at the orifice of the pocket. The pocket depth (PD) was the distance in mm from the gingival margin to the base of the probeable crevice; and the clinical attachment loss (CAL) was the distance in mm from the cemento-enamel junction to the base of the probeable crevice [6].

Assessment of gingival inflammation

The Gingival Index (GI) as described by Loe H and Silness P in 1963 was recorded.

The scoring criteria was as follows

- 0- Absence of inflammation/normal gingival,
- 1- Mild inflammation, slight change in colour, slight edema; no bleeding on Probing,
- 2- Moderate inflammation; moderate glazing, redness, edema and hypertrophy, bleeding on probing,
- 3- Severe inflammation; marked redness and hypertrophy ulceration tendency to spontaneous bleeding .

Assessment of plaque

The plaque index as described by Silness P and Loe H was recorded with the scoring criteria as follows. 0 - Gingival area of the tooth surface is free of plaque, 1- A film of plaque adhering to the free gingival margin and adjacent area of the tooth, which can be recognized by passing a probe across the tooth surface, 2- Thin to moderate accumulation of soft deposits within the gingival pocket or on the tooth and gingival margin, which can be seen with naked eye and 4-Abundance of soft matter within the gingival pocket and or on the tooth surface and gingival margin (Løe, 1967).

Inclusion Criteria

1. Subjects who have a pocket of probing depth of ≥ 4 mm and loss of attachment of ≥ 3 mm.(For Group A)

2. Subjects with minimum complement of 20 teeth.(For Group A)
3. Subject's who have given their informed consent to participate in the study.

Exclusion Criteria

1. Subjects with any systemic disorders.
2. Patients who have received periodontal therapy/antibiotics/anti-inflammatory drugs/steroids in the past 6 months.
3. Pregnant women, lactating women
4. Subjects who are tobacco users.

A standard proforma consisting of the following data: name, age, sex, medical and past dental history, plaque index (Silness and Loe), periodontal pocket and clinical attachment for each patient was recorded. Each patient was examined using a mouth mirror and Williams graduated periodontal probe under artificial light.

Method of collection of sample

Collection of saliva

After obtaining the subject's consent to participate in the study unstimulated whole saliva samples was collected by expectoration. Subjects were asked not to eat or drink 1 hour prior to the study. Whole saliva samples was used in the study. Saliva samples was obtained in the morning over 5 min. period with patients seated with instructions to allow saliva to pool in the floor of the mouth. Collected sample was sent immediately for biochemical analysis [7].

Collection of gingival tissue

A gingival tissue sample was obtained under local anesthesia, from the subjects undergoing extraction of periodontally diseased tooth/teeth and the healthy tissue samples was collected from patients undergoing extraction for orthodontic reasons. This sample was stored in phosphate buffer and sent immediately for biochemical analysis [7]. The obtained tissue samples were blotted and kept in an ice bath until needed. The tissue samples were weighed and homogenized using phosphate buffered saline and the resulting extract was used for further biochemical assays[8].

For estimation of Glutathione peroxidase

Modification of coupled assay procedure of Paglia and Valentine

The reaction mixture consisted of 50mM potassium phosphate buffer, 1 mM EDTA, 1mM NaN₃, 0.2 mM NADPH, Glutathione reductase, 1 mM Glutathione, 1.5 mM cumene hydroperoxide in a total volume of 1 ml. Sample(0.1 ml) was added to 0.8 ml to the above mixture and allowed to incubate 5 min. at room temperature and the 0.1 ml peroxide solution was added. Adsorbance was determined at 340 nm using a spectrophotometer.

RESULTS

Data was statistically analysed using unpaired t test. SPSS version 17 & MS Excel was used to analyse

the data. $p < .05$ was considered to be statistically significant.

Table 1: Glutathione peroxidase saliva(mg/dl)

| group | N | Mean | Std. Deviation | Mean diff | p |
|-----------------------|----|--------|----------------|-----------|------------|
| Healthy control | 20 | 1.1494 | .29705 | 2.7536 | <.0005 vhs |
| Chronic Periodontitis | 20 | 3.9030 | .80229 | | |

Table 2: Glutathione peroxidase gingival tissue(μ g/g)

| group | N | Mean | Std. Deviation | Mean diff | p |
|-----------------------|----|-------|----------------|-----------|------------|
| Healthy control | 20 | 30.85 | 7.562 | 12.4 | <.0005 vhs |
| Chronic Periodontitis | 20 | 43.25 | 8.117 | | |

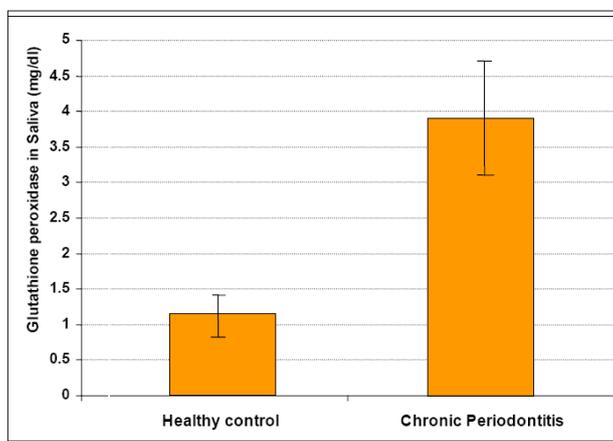


Fig-1: Comparison of mean salivary Glutathione peroxidase between subjects with healthy periodontium and chronic periodontitis

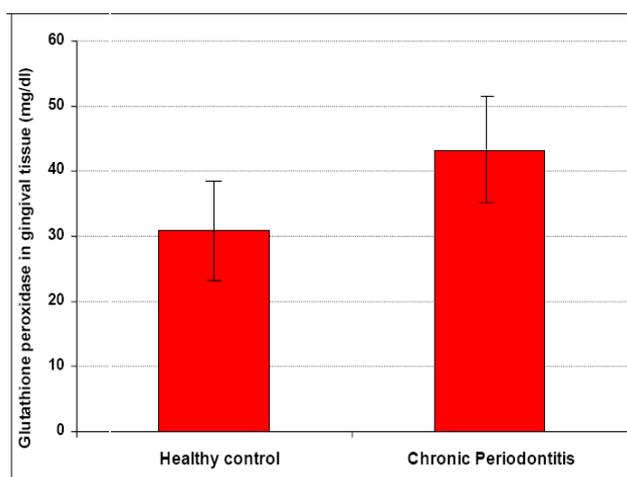


Fig-2: Comparison of mean gingival tissue Glutathione peroxidase between subjects with healthy periodontium and chronic periodontitis

DISCUSSION

The antioxidant mechanisms are the evolutionary designs that avidly react with and annihilate ROS before they inflict oxidative damage to tissues and cells. Glutathione peroxidase, an extracellular antioxidant, which is mainly produced in the kidney and has been found in numerous human fluids [9,10]. Glutathione peroxidase is a selenium-containing enzyme which detoxifies hydrogen peroxide and various hydroperoxides with glutathione as a reducing agent [10]. Glutathione peroxidase has been documented to have regulatory effects on cell proliferation [11]. Mates *et al.* reported that gene expression of glutathione peroxidase is up-regulated by H₂O₂ and other ROS [12]. Wei *et al.*, showed that the total amounts of glutathione peroxidase, was higher in periodontitis sites and gingivitis sites than healthy sites [13]. Whereas Huang *et al.*, and Sobaniec *et al.*, have shown that total amount of Glutathione peroxidase is reduced in periodontitis sites as compared to healthy sites. Panjamurthy *et al* have reported gingival biopsies and plasma of patients with chronic periodontitis have higher levels of Glutathione peroxidase in comparison with healthy subjects [8]. Ivan Borges *et al* also have shown increased levels of GSHPx in saliva and gingiva of patients with chronic periodontitis as compared to healthy subjects [14]. Increased plasma GSHPx activity has been reported as an indirect indicator of oxidative stress [15].

In the present study an attempt was made to evaluate and compare levels of Glutathione peroxidase in saliva and gingival tissues of subjects with and without chronic periodontitis using spectrophotometric quantification. The results of this study demonstrated higher levels of Glutathione peroxidase in saliva and gingival tissue of subjects with chronic periodontitis as compared to healthy control subjects and is consistent with the investigations which reported lower levels of GSHPx in saliva [13,16,17] and gingival tissue [8,14] of chronic periodontitis subjects. In the present study increased GSHPx level in saliva and inflamed gingival tissue may indicate the increase ROS generation at the diseased site. This increase in ROS generation may have led to the occurrence of oxidative stress, which in turn caused an increased need of GSHPx production to establish the ROS-AO balance to protect the tissues. Thus the mean concentration of GSHPx increased progressively from health to periodontitis in saliva and gingival tissue. Increased plasma GSHPx has been reported as an indirect marker of oxidative stress in patients with inflammatory bowel disease. [13] Similarly, increase in salivary and gingival tissue can be considered as a marker of oxidative stress caused by periodontal infection [18].

The observed increase in glutathione peroxidase in the saliva and gingival tissues of patients with periodontitis can therefore be due to the

scavenging of excessive generated lipid peroxidation products at the inflammatory sites [8].

CONCLUSION

Eventhough this study substantiates the fact that salivary and gingival tissue glutathione peroxidase can be used as a periodontal disease marker, further more studies are required with larger sample size for more precise results.

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