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Evaluation of Malondialdehyde, Glutathione Peroxidase and Defensin Levels in Patients with and without Periodontitis

J. Hemashree[1], Sreedevi Dharman[2], Selvaraj[3]

[1]Graduate Student, [2]Reader, Department of Oral Medicine and Radiology, [3]Assistant Professor, Department of Biochemistry,, Saveetha Dental College & Hospital, Saveetha Institute of Medical and Technical Sciences, Chennai, India

Abstract

Aim: Aim of the study is to estimate the levels of Malondialdehyde, glutathione peroxidase and Defensin levels on patients with and without periodontitis.

Materials and Method: The levels of Malondialdehyde and Glutathione peroxidase was estimated using colorimetric estimation and Defensin was estimated using ELISA

Results: Levels of Defensin is increased in patients with periodontitis 9.18+-1.72 when compared to healthy subjects 6.22+-2.73. The mean levels of MDA is increased in patients with periodontitis 2.35+-0.32 when compared to normal patients 3.58+-0.36. The levels of Gpx is lower in patients with periodontitis 2.52+-0.58 when compared to normal patients 3.58+-0.36. The p – value was found to be significant (0.001)

Conclusion: Thus, this study reveals that Malondialdehyde, Defensin and glutathione peroxidase can be considered as biomarkers in the periodontal diseases.

Keywords: *Periodontitis, oxidative stress, inflammation, saliva, tooth.*

Introduction

Periodontitis is an inflammatory disease of supporting tissues of the teeth that are caused by specific microorganisms or groups of microorganisms, which eventually results in progressive destruction of the supporting tissues of the teeth. It is also combined with periodontal pocket formation, gingival recession or both.[1] Periodontitis is a complexive disease resulting from interaction of bacterial infections and host response to such bacterial infections. Environment, acquired risk factors and genetic susceptibility are certain modification factors predisposed to this disease.[2] Periodontitis is one of the most common causes of tooth loss in adults.[3] However this can be prevented by preventing plaque and calculus formation which harbours the periodontal pathogens . The periodontal pathogens in the causal of the disease include a vast list of organisms among which Porphyromonas gingivalis, and Aggregatibacter actinomycetemcomitans, which are commonly seen in the biofilm. The colonization and subsequent invasion of these Gram-positive and Gram-negative microorganisms into the gingival epithelium leads to progression of the disease.

The severity of periodontitis could be diagnosed on the basis of its typical clinical parameters. These include periodontal probing depth, pocket depth, clinical attachment loss and amount of gingival bleeding. Moreover,analysis of saliva as an important laboratory test for the evaluation of many salivary conditions, including periodontitis, has gained attention during the last few decades.[4] Using saliva as a diagnostic marker

Corresponding Author:
Dr. Sreedevi Dharman
Reader, Department of Oral medicine and Radiology, Saveetha Dental College & Hospital, Saveetha Institute of Medical and Technical Sciences, 162, Poonamalle High Road, Chennai -600077 Tamil Nadu, India

for monitoring various biological alternations in human is an emerging trend worldwide. Human saliva is an easily collectable biochemical fluid, which is similar to blood in various biological aspects.It possesses a simple and non-invasive collection with low-cost storage and easily storage nature. Saliva contains both locally and systemically derived biochemical molecules with relatively important diagnostic value, which could be used for detecting periodontal diseases.[5] It can be used to assess both the severity of the disease as well as monitoring the patient's responses towards the treatment. Detection of salivary biomarkers is a non-invasive laboratory examination for early diagnosis of periodontitis.[6]

Glutathione peroxidaseis a marker of oxidative stress and dependent on the micro nutrient selenium (Se).[7] Periodontal diseases are associated with disturbances in the balance between the oxidants and antioxidants. This causes an increase in Reactive oxygen species(ROS) as well as decreased antioxidant activity in saliva.[8] Lipid peroxidation is a outcome of periodontitis. This is caused by free radicals which leads to the production of toxic and reactive aldehyde metabolites such as MDA formed by the peroxidation of poly unsaturated fatty acids.[9] Defensin is an anti microbial peptide of human flora. In case of infection or inflammation, it gets expressed on the surface of neutrophils. They also directly stimulate antigen-presenting dendritic cells and memory T-cell, and thus can link innate and adaptive immune responses.[10,11] So, this study aims at evaluating the salivary bio markers which get expressed in the periodontal diseases. The markers are Glutathione Peroxidase (Gpx), Malondialdehyde (MDA) and Defensin (beta-2) .

Materials and Method

This was a case control study performed in the Department of Biochemistry, Saveetha Dental College and Hospitals, Chennai. It was carried out in 20 subjects among which 10 patients had chronic periodontitis aged 50-70 years (group I) and 10 patients were healthy subjects (group II). Informed consent was obtained from each patient before the study.

Saliva Collection: Unstimulated whole saliva (Resting Saliva) from each participant was expectorated into sterile tubes prior to any kind of treatment.

Inclusion Criteria:

1. Presence of plaque
2. Presence of calculus
3. Generalised clinical attachment loss ≥ 4 mm
4. Probing depth more than 5mm
5. Generalised Gingival recession

Exclusion Criteria:

1. No history of systemic diseases
2. Patients who have undergone periodontal therapy
3. No history of diabetes
4. No history of hypertension

Estimation of levels of MDA and glutathione peroxidase: The levels of MDA and Gpx was determined by the use OF chemical reagents and subjecting it to colorimetric analysis with the help of a colorimeter. Colorimetric analysis is a method of determining the concentration of a chemical element or chemical compound in a solution with the aid of a color reagent

Estimation of Defensin: This was estimated using the Enzyme Linked Immunosorbant Assay(ELISA).

Results

The data was statistically analysed. The table given below shows the statistical data of the bio markers estimated in the study.

It is seen that the levels of Defensin is increased in patients with periodontitis 9.18+-1.72 when compared to healthy subjects 6.22+-2.73(Table 1).The mean levels of MDA is increased in patients with periodontitis 2.35+-0.32 when compared to normal patients 3.58+-0.36 (Table 2). The levels of Gpx is lower in patients with periodontitis 2.52+-0.58 when compared to normal patients 3.58+-0.36 (Table 3). The p – value was found to be significant (0.001). Bar Graph 1,2,3 shows the levels of Defensin, Gpx, MDA respectively among patients with periodontitis and normal patients.

Table 1: Levels of Defensin among patients with periodontitis and normal patients

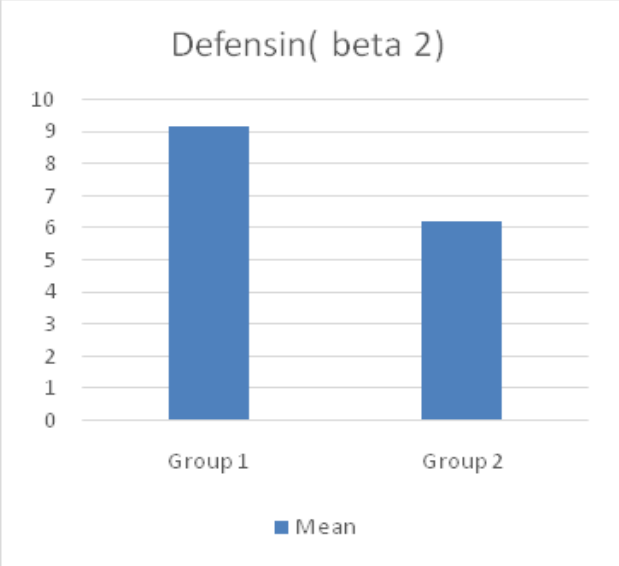
Subject	Mean	Standard Deviation	p value
Group I	9.18	1.72	0.001
Group II	6.22	2.73	

Table 2:Levels of Gpx among patients with periodontitis and normal patients

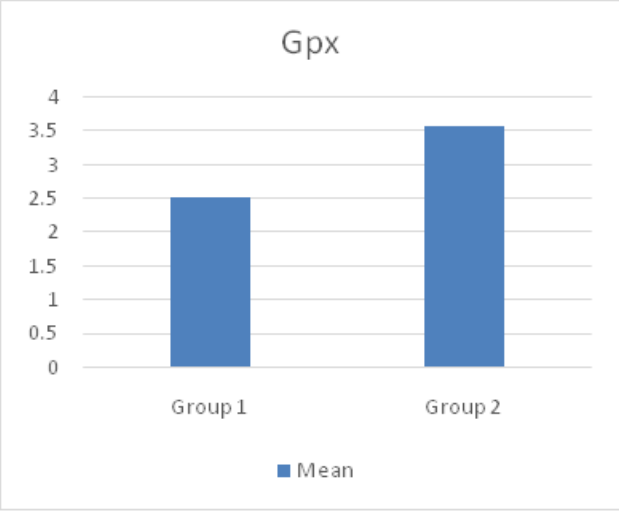
Subject	Mean	Standard Deviation	p value
Group I	2.52	0.58	0.001
Group II	3.58	0.36	

Table 3: Levels of MDA among patients with periodontitis and normal patients

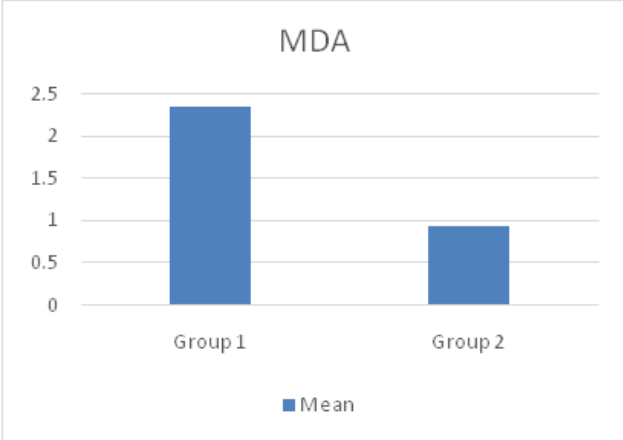
Subject	Mean	Standard Deviation	p value
Group I	2.35	0.32	0.001
Group II	0.94	0.58	



Graph 1: Graph showing comparison of comparison of levels of Defensin (beta 2). among patients with periodontitis and normal patients.



Graph 2: Graph showing Comparison levels of Gpx among patients with periodontitis and normal Patients.



Graph 3: Graph showing comparison of levels of MDA among patients with and without periodontitis.

Discussion

From the above results it is seen that the levels of Defensin beta -2 and Malondialdehyde levels are increased in case of periodontitis patients whereas the level of Glutathione peroxidase is decreased when compared to healthy subjects. The reason for the increased levels of MDA is because, in case of periodontitis, there is an imbalance between the oxidants and antioxidants present in the oral cavity which ultimately leads to the production of ROS. Therefore lipid peroxidation occurs and there is increased MDA. For patients with periodontitis there is increased oxidative stress occurs which causes reduced antioxidant activity causing the decrease of Gpx. Human defensin beta- 2 gets expressed on the surface of neutrophils which involves in innate immunity causing phagocytosis of the bacterial flora. Moreover it protects the undifferentiated stem cells of the periodontium.

Several studies have shown similar results. In a study done by Akalin et al,[12] the levels of MDA was elevated in saliva in patients with periodontitis. Lipid peroxidation causes release of ROS which causes the suitable host environment for resorption of bone, degradation of connective tissue and increase in the matrix metalloproteinase activity.[13] Dhotre et al conducted a study to find of any significant relation between the serum and salivary levels of MDA. It was reported that there was a significant increase in the levels of serum MDA in patients with periodontitis. They even concluded that this increase in the levels of MDA can be referred to the possibility of an association between periodontitis and cardiovascular disease. [14]

In accordance with the studies done to evaluate the levels of glutathione peroxidase there was varying results. In contrary to our study, A study done by Wei et al[15,] showed the levels of Gpx was increased in the gingival crevicular fluid in patients with periodontitis. Not all patients demonstrated the increased level of Gpx in periodontal diseases . For eg. A study by Brock et al[16] reported the levels of GpX was reduced when compared to healthy subjects. This indicates that there is hampered antioxidant capacity in patients with periodontitis. This study is in accordance with the our study. The levels of Gpx might have a positive correlation between the progression of disease.[17]

Human Defensin beta -2 is profoundly indicative of the antimicrobial activity occurring in the oral cavity. They play a major role in the adaptive and innate Immune responses. They provoke efficient epithelial barrier repair to limit entry of invading bacteria.[18] Related to the severity of disease, some study conducted by Ertugrul et al,[19] found that significantly higher level of HBD-2 from chronic periodontitis than gingivitis subjects ($p<0.05$) Another study by Sulijaya et al.[20] reported that HBD-2 protein level was higher in severe chronic periodontitis than other patients.

Conclusion

Thus to conclude the role of biomarkers in periodontal diseases is to prevent the onset as well as the progression of the diseases. Antimicrobial peptides provide multiple benefits as frontline defense molecules and antioxidants can provide better integration of the tissues. Nevertheless, therapeutic considerations for the adjunctive use of glutathione, defensin and the elimination of MDA in management of periodontitis can limit the tissue damage and enhance the wound healing.

Ethical Clearance: Taken from Instituitional Ethical Committee.

Source of Funding: Self

Conflict of Interest: Nil

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