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## RESEARCH ARTICLE

### A COMPARATIVE BIOCHEMICAL STUDY OF SALIVARY MALONDIALDEHYDE LEVELS IN HEALTHY INDIVIDUALS AND PATIENTS WITH CHRONIC PERIODONTITIS

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#### ABSTRACT

**Aim:** Periodontitis is an acute or chronic inflammatory process, initiated by the plaque biofilm, that causes loss of periodontal adherence to the root surface and adjacent alveolar bone and which ultimately results in tooth loss. Our objective was to evaluate malondialdehyde levels in healthy individuals and patients with chronic periodontitis.

**Materials and Methods:** 50 subjects aged between 17-50 years attending the Out Patient Department of Periodontics in the A.B Shetty Memorial Institute of Dental Sciences were divided into 2 groups. 25 patients served as the control group and the rest 25 patients served as the test group 3 ml of unstimulated whole saliva was collected from subjects using disposable spittoons and were centrifuged and frozen at  $-20^{\circ}\text{C}$  until analysis. Lipid peroxidation products malondialdehyde were analyzed by Thiobarbituric acid (TBA) reaction.

**Results:** The results obtained were subjected to statistical analysis (Test for Normality and Mann Whitney U Test).

**Conclusion:** Our study reveals that malondialdehyde levels, a lipid peroxidation product in increased in Chronic Periodontitis cases compared to Control cases with a median of 2.98 and the control has a median of 0.94 which is statistically significant with a p value of  $<0.001$ .

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## INTRODUCTION

Periodontitis is an acute or chronic inflammatory process, initiated by the plaque biofilm, which causes loss of attachment periodontal tissue to the root surface and adjacent alveolar bone and which ultimately results in tooth loss (Jan C Chapple and Mathews, 2000). Periodontal disease affects between 10 and 15% of the world's population, representing the greatest cause of tooth loss (Baelum and Lopez, 2004). Even though mild to moderate forms of chronic periodontitis are rather common, but a severe variety of periodontitis with advanced tissue destruction are rare worldwide (Sheiham, 1997). The destruction of periodontal tissue is primarily due to increased gram negative anaerobic or facultative bacteria present in the subgingival biofilm. These pathogens have the ability to invade gingival tissues and interact with the host immune response which is accompanied by an increase in number of cytokine expression and possible immunological activity in the gingival tissue. This complex interaction between the

polymorphonuclear leukocytes and the pathogenic bacteria will result in large amounts of reactive oxygen species that will cause oxidative damage to the gingival tissue, periodontal ligament and alveolar bone (Canakci et al., 2009). Polymorphonuclear leukocytes which are the first mediators of host response against these pathogenic bacteria, migrate to the site of infection and engulf the bacteria to release microbial peptides and oxygen metabolites. Studies have suggested that there is an increase in the number of neutrophils with the severity of periodontal disease (Miller et al., 1984). Free radicals are molecules or molecular fragments with an unpaired electron impart certain characteristics to the free radicals, such as reactivity. Reactive free radicals are capable of producing biochemical modifications and damage to proteins, lipids, carbohydrates and nucleotides in the tissues (B rai, 2007). It has been noted that the generation of reactive oxygen species is an integral feature of normal cellular metabolism (Luqman and Rizvi, 2006). Reactive oxygen species interacts with polyunsaturated fatty acid present in the cell membrane leading to the process of uncontrolled lipid peroxidation. Uncontrolled lipid peroxidation will cause oxidative stress and substantial damage to cell integrity. As lipid peroxidation is an outcome of

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oxidative stress, many markers have been used to screen this process. Malondialdehyde is usually the principal and most established product of PUFA peroxidation showed to increase following oxidative stress (Del Rio *et al.*, 2005). Malondialdehyde is one of the many end product with low molecular weight due to lipid peroxidation and is most often measured as the index of peroxidation (de Zwart *et al.*, 1999). Saliva has always been an important physiologic fluid for experiments and research. This reason for this is because Saliva as whole contains a high mixture of substances with complex nature. It also has certain amounts of blood and its products, serum, serum products, gingival fluid, electrolytes, epithelial and also immune cells, few micro organisms, some bronchial products and other foreign substances (Schenkels *et al.*, 1995). So, it widely is used in the diagnosis of several systemic diseases, oral diseases and assessment of the severity of some illness. Saliva has also been used as a diagnostic tool for periodontal diseases. We can assess the extent of tissue damage in a diseased patient by measuring the concentration of lipid peroxidation products (Streckfus and Bigler, 2002; Bardow *et al.*, 2001; Ozmeric, 2004). In this study, we have tried to determine the levels of malondialdehyde, by analyzing the status of lipid peroxidation as the level of (thiobarbituric acid reactive substances) in the unstimulated saliva of patients with Chronic Periodontitis and Healthy subjects volunteers. The aim of this study was to evaluate the level of malondialdehyde in the saliva of patients with Chronic Periodontitis by comparing with normal healthy individuals.

## MATERIALS AND METHODS

Ethical clearance was obtained from the Institutional Ethics Committee, A.B Shetty Institute of dental Science, Mangalore (Ref.No. ABSM/EC/4/2012), Mangalore, Karnataka, India and informed consent was obtained from each research participant. 50 subjects aged between 17-50 years attending the Out Patient Department of Periodontics in the A.B Shetty Memorial Institute of Dental Sciences were divided into 2 groups. Among them, about 25 patients served as the control group and the rest 25 patients served as the test group from whom about 3 ml of unstimulated whole saliva was collected using disposable spittoons and were centrifuged and frozen at  $-20^{\circ}\text{C}$  until analysis.

### Inclusion Criteria

- Subject should have at least 20 or more teeth.
- Subjects who have attachment loss of  $>4\text{mm}$  in at least 30% sites.
- Radiographic evidence of bone loss in more than 30% of sites involved.
- Gingival index  $<1$  and plaque index  $<1$  for healthy individuals

### Exclusion Criteria

- Use of NSAIDs or Anti Microbial Drugs or Mouthwash within a 3 month period before the study commences.
- Periodontal Therapy in the previous 6 months.
- Pregnant and lactating women.
- Smokers and tobacco chewers

### Screening examination

- Medical history and dental history
- Probing depth

- Gingival index (Loe and Silness)
- Plaque index (Silness Loe and Silness)

### Clinical measurement

The periodontal status of the subjects was determined by measuring the probing depth, and recording gingival index (Loe and Silness), plaque index (Silness and Loe and).

### Determining pocket depth

Probing pocket depth is the distance between the base of the pocket and the gingival margin. Williams graduated periodontal probe is inserted parallel to the vertical axis of the tooth and walked circumferentially around surfaces of each tooth to detect deepest areas of penetration (Madianos *et al.*, 2005; Page R kornman, 2000; Chapple *et al.*, 2007; Panjamurthy *et al.*, 2005; Cenk Faith Canakci *et al.*, 2009; Akalin *et al.*, 2007; Tsai *et al.*, 2005; Panjamurthy *et al.*, 2005; Garg *et al.*, 2006; Taghreed *et al.*, 2009; Khalili JBiloklytska, 2008; Newman *et al.*, 2006).

### 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 (Løe, 1967).

### Assessment of plaque

The plaque index as described by Silness P and Loe H was recorded with the scoring criteria as follows. "O"- 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 3- Abundance of soft matter within the gingival pocket and or on the tooth surface and gingival margin (Løe, 1967).

### Collection of saliva

Subjects will be advised not to eat or drink 1 hour prior to the sample collection and 3ml of unstimulated whole saliva was collected. Collected sample was sent immediately for biochemical analysis.

### Biochemical analysis

It was done to detect the levels of malondialdehyde by using following reagents

- Tri chloro acetic acid (TCA)-( $\text{CH}_3\text{COOCl}_3$ )
- 2-Thiobarbituric acid (TBA)-( $\text{C}_4\text{H}_4\text{N}_2\text{O}_2\text{S}$ )
- Hydrochloric acid (HCl)
- Malonaldehyde bis (dimethyl acetal) – ( $\text{C}_7\text{H}_{16}\text{O}_4$ )

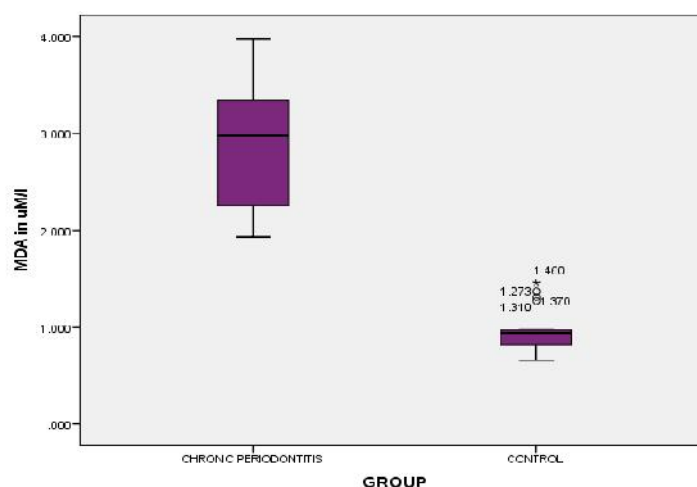
**Table 1. Test for Normality**

Groups	Statistic	Shapiro-Wilk (Df)	Significance
Control	.855	25	.002
Chronic periodontitis	.924	25	.064

**Table 2. Comparison of the MDA Levels in control and cases of Chronic Periodontitis**

	N	Percentiles					Mea-n Ran-k	Mann- Whitney U Test	Wilcoxo-n Test	Z Test	Exact Sig. (2- tailed value)
		Minimu-m	Maximu-m	25	media-n	75					
Control	25	0.66	1.46	0.82	0.94	0.975	13				<0.001
Chronic Periodontitis	25	1.93	3.98	2.225	2.98	3.4	38	0.000	325.000	-6.065	

n- Number of Samples, Mann Whitney U Test. p<0.001- Statistical significant

**Fig 1. Comparison of the MDA Levels in control and cases of Chronic Periodontitis, Mann Whitney U Test**

## Sample Preparation

Saliva is diluted to 500µL with distilled water. To the diluted sample 1mL of TCA-TBA-HCl reagent is added. The samples are kept in boiling water bath for 15 minutes. The reaction mixture is cooled and centrifuged. The supernatant is taken and the optical density of the pink colour formed is read at 535nm. The concentration of malondialdehyde in the sample is got by plotting the obtained absorbance against the standard graph. The optical density of the pink colour formed is directly proportional to the concentration of malondialdehyde in the given sample (Buege and Aust, 1978).

## Statistics

The results obtained were subjected to statistical analysis by "Test for Normality" and "Mann Whitney U Test"

## RESULTS

Mann Whitney U Test which clearly indicates that there is a significant higher level of malondialdehyde in generalized chronic periodontitis patients with a median of 2.98 (p<0.001) when compared to control with a median of median of 0.94 which is very significant.

## DISCUSSION

Inflammation represents the response of living mammalian tissue to a noxious stimulus. Inflammation is dependent upon the humoral and cellular activity against the stimulus. The cellular inflammation is characterized by increase in the tissue infiltration of polymorphonuclear leukocytes and monocytes. These polymorphonuclear leukocytes and monocytes transmigrate from the endothelium in the extra cellular matrix and subsequently help in the phagocytosis either by O<sub>2</sub> dependants or O<sub>2</sub> independent mechanism. Periodontal disease is an inflammatory condition caused by infectious stimuli which colonizes in the sub gingival area. It is well understood from the models of pathogens of periodontitis that the gram negative bacteria causes tissue destruction either directly by the toxic products or indirectly through molecular species like reactive oxygen species. Reactive oxygen species interacts with the polyunsaturated fatty acids leading to the process of Lipid Peroxidation. One such product of this process is Malondialdehyde, which causes significant damage to the cell integrity (Battino *et al.*, 1999). The results of the study showed that controls does not follow a normal distribution (Table 1). There were significantly higher levels of malondialdehyde in the saliva of subjects with generalised Chronic periodontitis when compared to the saliva of the healthy control subjects (Fig 1 & Table 2). This is supported by the findings of the

study conducted by Akalin *et al.* 2007 which showed significant high levels of malondialdehyde in saliva of patients with periodontitis in comparison with the healthy control groups. Their study indicated the lipid peroxidation product concentration was co-related with the gingival index, pocket depth and probing attachment level. Although a different classification was used our findings were in agreement with their report and indicate that an elevated malondialdehyde level is markedly related to the clinical status of patients. Tsai *et al.* stated that the whole saliva of periodontitis subjects had a significantly higher mean lipid peroxidation than the healthy ones. Findings suggest that increased lipid peroxidation may play an important role in the pathology of periodontitis. It is in accordance with Faith *et al.* 2009, where he demonstrated that malondialdehyde level in gingival tissue around the teeth with chronic periodontitis was higher compared to healthy gingival (Canakci *et al.*, 2009). Our results are in agreement with the above studies stating that lipid peroxidation concentration is markedly increased in periodontal destruction.

## Conclusion

The result of this study highlights the possible clinical value of unstimulated whole saliva as a valid and convenient diagnostic biofluid. This novel approach to harness the potential of salivary malondialdehyde level may prove to be useful in identifying patients with chronic inflammatory periodontal disease and may provide additional advantages in elucidating the role of oxidative stress in the pathogenesis of periodontal disease. However, further studies on a larger scale should be performed to clarify the exact role of malondialdehyde in generalized chronic periodontitis.

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