MALONDIALDEHYDE- AN END PRODUCT OF TISSUE DESTRUCTION IN PERIODONTAL DISEASE

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INTRODUCTION

Periodontitis is an immuno-inflammatory disease, initiated by the interaction of the subgingival biofilm with the periodontal tissues (Haffajee AD et al, 1994). The primary etiologic agents are the gram negative anaerobic or facultativemicroflora, that are present within the subgingival biofilm but periodontal breakdown occurs due to an inappropriate host response to these pathogens which leads to initiation of an inflammatory and immune response (Lamster IB et al, 1992). The primary defense mediators against bacterial pathogens are the neutrophils and polymorphonuclear leukocytes (PMNs), they generate increased levels of reactive oxygen species (ROS), which can cause tissue damage to the bacterial pathogens directly or to the host microbial interaction pathway (Moseley R et al, 1997). Reactive oxygen species (ROS) reacts with polyunsaturated fatty acids, as a result of which uncontrolled lipid peroxidation (LPO) occurs. These lipid peroxides breaks down to certain cytotoxic aldehydes and also less toxic aldehydes (e.g.malondialdehyde). These aldehydes increase in concentration due to oxidative stress leading to periodontal destruction locally and causing DNA damage systemically (Miller DR et al, 1984).Malondialdehyde is one of the low molecular weight end products of lipid peroxidation and is the most often measured as an index of peroxidation (J Khalili et al, 2014).

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al., 2008). These end products are circulated throughout the system as a result, it can cause damage to lipids, brain cells, DNA and also in several other parts of the body. Factors such as age, gender, smoking, and alcohol consumption can also influence an increase in oxidative levels. Saliva is used as a potential biomarker for diagnosis and management of many systemic and oral diseases, it can also be used as a biomarker to assess MDA levels since it is easy to collect, non-invasive and is a convenient approach towards patients (Miller CS et al., 2006).

In this study, the lipid peroxidation status was sought to be determined by evaluating and correlating the serum and salivary MDA levels, using the level of TBARS in patients diagnosed with chronic gingivitis, chronic periodontitis and periodontally healthy subjects.

**MATERIAL AND METHODS**

90 subjects who were systemically healthy were selected from the Department of Periodontics, AB Shetty Memorial Institute of Dental Sciences, Deralakatte, Mangalore, India. Written informed consent was taken from all the participants before start of the study and they were divided into three groups for assessing the serum and salivary malondialdehyde levels: Group I – 30 subjects with healthy gingiva (Control group), Group II – 30 subjects with gingivitis (Gingivitis group), Group III – 30 subjects with chronic periodontitis (Periodontitis group).

During screening examination, medical and dental history of the subjects was determined. Patients were referred to a physician to determine their systemic condition, and later they were allotted to the three study groups by the single trained investigator as per the inclusion criteria. Patients not fulfilling the various criteria were excluded from the study. Clinical attachment levels were determined with a UNC-15 probe and gingival index was recorded according to the criteria given by Loe and Silness 1963.

Subjects with clinically healthy gingiva formed Group I, those with moderate to severe gingivitis having gingival index score of (>1.1) were allotted to Group II those with moderate form of chronic periodontitis, showing the presence of more than 30% of sites with clinical attachment loss>3mm and probing depth >4mm as measured with a Williams periodontal probe made up Group III. (Offenbacher, S et al, 1996).

Subjects with any known systemic disease or conditions undergone any periodontal treatment in the last 6 months, history of any antibiotic /anti-inflammatory therapy for three months prior to study, history of smoking, tobacco chewing, alcohol consumption, pregnant and lactating women were excluded from the study.

**Biochemical Analysis**

The level of MDA was assayed in the saliva and serum of study subjects by evaluating the levels of thiobarbituric acid reactive substances (Buege method). The instrument used to evaluate TBARS levels was the spectrophotometer. The results were expressed as micromoles per millilitre.

**Statistical Analysis**

Using frequency, percentage, mean, and standard deviation, the collected information was summarized. One-way analysis of variance (ANOVA) was used to compare serum and salivary MDA level between healthy, gingivitis, chronic periodontitis patients.

**RESULTS**

Table I given below shows the comparison of saliva and serum between three groups using One-way analysis of variance (ANOVA).

Results showed that when comparing salivary MDA levels, group 3 (chronic periodontitis) had the highest value of 1.79, group 2 (gingivitis) was 1.197 and group 1 (healthy) had the least value of 0.84. This difference between the 3 groups was statistically significant with a p value of <0.001.

Table II given below shows the pair wise comparison amongst the three groups by Tukey Post Hoc Test.

Results showed that when comparing salivary MDA level between group 1 (healthy) and group 3 (chronic periodontitis), a mean difference of 1.297 was reported, which was statistically significant with a p value of <0.001. Comparing group 2 (gingivitis) and group 3 (chronic periodontitis) showed a mean difference of 1.253, which was also statistically significant with a p value of <0.001. However, while comparing between group 1 (healthy) and group 2 (gingivitis) a mean difference of 0.043 was reported, which was not statistically significant with a p value of 0.885.

Results showed that when comparing serum MDA level between group 1 (healthy) and group 2 (gingivitis) a mean difference of 0.351 and a p value of 0.087 were reported, which was not statistically significant. Comparing group 1 (healthy) and group 3 (chronic periodontitis) showed a mean difference of 0.944, which was statistically significant with a p value of <0.001. Comparing group 2 (gingivitis) and group 3 (chronic periodontitis) showed a mean difference of 0.593, which was also statistically significant with a p value of 0.001.

<p>| TABLE I Comparison of Salivary And Serum Malondialdehyde Levels Among The Three Groups By One Way Anova |
|----------------------------------|--------|----------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|</p>
<table>
<thead>
<tr>
<th></th>
<th>N</th>
<th>MEAN</th>
<th>Std. Deviation</th>
<th>df2(welch) / P(ANOVA)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>SERUM MDA in µM/L</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GROUP 1</td>
<td>30</td>
<td>0.846922</td>
<td>0.092177</td>
<td>14.096</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>GROUP 2</td>
<td>30</td>
<td>1.979326</td>
<td>0.451378</td>
<td>1.790832</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>GROUP 3</td>
<td>30</td>
<td>1.278457</td>
<td>0.888699</td>
<td>0.485338</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>SALIVA MDA in µM/L</strong></td>
<td></td>
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</tbody>
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<p>| TABLE II Pair Wise Comparison Amongst The Groups By Tukey Post Hoc Test |
|----------------------------------|--------|----------|-----------------|-----------------|-----------------|-----------------|-----------------|</p>
<table>
<thead>
<tr>
<th></th>
<th>(I) Group</th>
<th>(J) Group</th>
<th>Mean Difference</th>
<th>Std. Error</th>
<th>P VALUE</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>SERUM MDA in µM/L</strong></td>
<td>GROUP 1</td>
<td>GROUP 2</td>
<td>-0.351034</td>
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<td>0.007</td>
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<td></td>
<td>GROUP 3</td>
<td>GROUP 3</td>
<td>-0.944114</td>
<td>0.163622</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>GROUP 2</td>
<td>GROUP 3</td>
<td>-0.593106</td>
<td>0.163622</td>
<td>0.001</td>
</tr>
<tr>
<td><strong>SALIVA MDA in µM/L</strong></td>
<td>GROUP 1</td>
<td>GROUP 2</td>
<td>-0.04351</td>
<td>0.092177</td>
<td>0.885</td>
</tr>
<tr>
<td></td>
<td>GROUP 3</td>
<td>GROUP 3</td>
<td>-1.297168</td>
<td>0.092177</td>
<td>&lt;0.001</td>
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<tr>
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<td>GROUP 3</td>
<td>-1.253657</td>
<td>0.092177</td>
<td>&lt;0.001</td>
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</tbody>
</table>
Comparison of Serum Malondialdehyde Among Healthy, Gingivitis, And Chronic Periodontitis

Comparison Of Salivary Malondialdehyde Level In Healthy, Gingivitis And Chronic Periodontitis

**DISCUSSION**

The aim of this study was to evaluate and correlate the serum and salivary MDA levels, using the level of TBARS in subjects diagnosed with gingivitis, chronic periodontitis and periodontally healthy control subjects and to determine the lipid peroxidation status.

MDA is one among the many aldehydes, which forms as secondary products during lipid peroxidation. It is an end product of polyunsaturated fatty acid through enzymatic or non-enzymatic processes. It can exist in either free state or bound form.

Malondialdehyde (MDA) levels can be measured by two methods, which are commonly used: by high-pressure liquid chromatography (HPLC) which is more specific and technique sensitive, or by thiobarbituric-acid reactive substance s(TBARS) method. Free MDA and total MDA can be determined by using HPLC and TBARS method. HPLC method is very expensive and difficult to clean, not highly sensitive to certain compounds, which cannot be detected, hence most of the studies use TBARS to estimate the MDA level. However, there are few disadvantages of TBARS method due to its low specificity and sensitivity. Determination of MDA with TBA is usually made at 532 to 535 nm wavelength spectrophotometrically, after MDA-(TBA)2 complex occurred during the process.

During interaction of periodontopathogens or their products and neutrophils within the periodontal pocket, there is an excessive production of superoxide anion. This mechanism of superoxide anion production can lead to an increase in salivary MDA levels (Cenk Fatih et al, 2009)

In this study, unstimulated whole saliva MDA levels were significantly lower in group 2 (gingivitis) compared to group 3 (chronic periodontitis). The difference between group 2 (gingivitis) and group 3 (chronic periodontitis), and group 1 (healthy) and group 3 (chronic periodontitis) is statistically significant, while that between group 1 (healthy) and group 2 (gingivitis) is non significant.

Results of this study are in agreement with the study by J Khalil et al wherein he evaluated salivary MDA levels in generalized chronic periodontitis patients. Subjects were divided into early, moderate and severe periodontitis groups with clinically healthy patients forming the control group. Results showed that there is an increase in the MDA levels according to the severity of periodontal disease. (Lamster IB et al, 1992)

In this study, the level of MDA in serum in all the 3 groups was recorded and compared. The difference between group 2 (gingivitis) and group 3 (chronic periodontitis), and between group 1 (healthy) and group 3 (chronic periodontitis) is statistically significant while group 1 (healthy) and group 2 (gingivitis) is non-significant.

This increase in MDA levels in chronic periodontitis could be due to activation of PMN in the peripheral blood that can activate reactive oxygen species and release them in the circulation with or without exogenous stimulation. ROS generation may be responsible for the bone resorption; degradation of connective tissue and increase in the matrix metalloproteinase activity (Lamster IB et al, 1992). As disease progression in periodontal tissues takes place, there is an increase in number of cytokines, which are more effective in stimulating neutrophil superoxide production than in the control group (Pendyala G et al, 2008)

Madhur Gupta et al 2013 in a case control study evaluated the salivary and serum Malondialdehyde levels in chronic periodontitis patients and matched with healthy control subjects. Results indicated that the salivary MDA levels are elevated in chronic periodontitis patients but there was no change in the serum MDA levels.

Akalin F A et al 2007 conducted a study to evaluate MDA levels and total oxidant status in the gingival crevicular fluid, saliva and serum of chronic periodontitis patients. Results showed that MDA levels were high in the GCF and saliva while the total oxidant status was high in GCF, saliva and serum. The study indicated that increased MDA levels and total oxidant status have an important role in the pathogenesis of periodontal disease.

The results of this study are in variation with the results of other published studies, which indicated, increased MDA levels in GCF and saliva of chronic periodontitis patients and no significant increase in serum MDA levels. The increased serum MDA levels in this study lend credence to the hypothesis that periodontal diseases are not a localized phenomenon but can have systemic manifestations.
The TBARS method used in this study to assess MDA levels has low specificity and is technique sensitive which is a limitation of this study.

**CONCLUSION**

The correlations found in this study not only show similarities between serum and saliva in terms of lipid peroxidation and oxidant status but also suggests an important role for lipid peroxidation in the pathogenesis of periodontal disease. Results suggest that severity of periodontal disease could be due to excessive production of lipid peroxidation products. However as TBARS is considered as less specific and technique sensitive, which is a limitation of this study, the alternative HPLC method can also be used to determine MDA levels.

**References**


**Abbreviations**

1. GCF - Gingival Crevicular Fluid
2. MDA - malondialdehyde
3. PMNs - polymorphonuclear leukocytes
4. ROS - reactive oxygen species
5. LPO- lipid peroxidation
6. TBARS - thiobarbituric acid reactive substances
7. HPLC - high-pressure liquid chromatography

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