A Comparative Evaluation Of Serum Paraoxonase Enzyme In Diabetes Mellitus Type II Patients And Healthy Individuals With And Without Periodontitis

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INTRODUCTION

Periodontitis is characterized by a complex cascade of tissue destructive pathways. Additional factors contributing to this multifaceted local disease process in the oral cavity include a number of systemic diseases, especially Diabetes Mellitus, which can exaggerate the host response to the local microbial factors, resulting in periodontal tissue breakdown. According to Loe et al Periodontitis is considered as the sixth complication of Diabetes Mellitus along with retinopathy, nephropathy, neuropathy, macrovascular diseases and altered wound healing.3

More specifically, a loss of homeostatic balance between reactive oxygen species (ROS) and the antioxidant defence systems that protect and repair vital tissue, cell, and molecular components is believed to be responsible.4 It has been reported that
oxidative stress reduces insulin secretion and increases insulin resistance in some experimental models and may thus play a causal role in the pathogenesis of diabetes. There is also a clearly defined and substantial role for free radicals or reactive oxygen species (ROS) in Periodontitis. Paraoxonase 1 is one such antioxidant. The exact physiologic function of PON-1 has not been explored completely yet, but it seems to be an important factor in lipid metabolism- exposing anti-atherogenic, anti-inflammatory, and antioxidative properties. The association between Periodontitis and various systemic diseases including cardiovascular diseases and Diabetes Mellitus is repeatedly recognised. PON-1 enzyme activity is seen to be reduced in patients with Diabetes Mellitus. The aim of this study is to investigate a possible correlation between Diabetes Mellitus and Paraoxonase enzyme activity in patients with Chronic Periodontitis.

**Aims and Objectives:**

1. To assess Paraoxonase levels in systemically healthy patients with healthy periodontium.
2. To assess Paraoxonase levels in systemically healthy patients with chronic periodontitis.
3. To assess Paraoxonase levels in patients with type II Diabetes Mellitus and Chronic Periodontitis.
4. To compare the serum paraoxonase levels in systemically healthy patients with chronic periodontitis before and after treatment.
5. To compare serum paraoxonase levels in patients with diabetes mellitus and chronic periodontitis before and after treatment.

**MATERIALS AND METHODS:**

**Source of Data:**

A total of 90 patients reporting to Department of Periodontics, A.B Shetty Memorial Institute of Dental Sciences who gave their informed consent to participate in the study were selected. After obtaining ethical clearance from the institution’s ethics
committee, informed consent was taken from the subjects. The subjects were divided into 3 groups:

- **Group I** – 30 Systemically healthy subjects with healthy Periodontium
- **Group II** – 30 subjects who are systemically healthy with Chronic Periodontitis
- **Group III** – 30 subjects with Diabetes mellitus and Chronic Periodontitis

Patients categorized as Type 2 diabetes mellitus with FBS ≥ 126 gm/dl according to WHO criteria were placed in group III.¹⁰

Patients with clinical attachment loss ≥ 4mm in more than 30% of the sites according to AAP 1999 Classification were placed in group II & III. Subjects who have a gingival index score of 0.1-1.0 according to Gingival Index (Loe and Silness, 1963) were placed in group I.¹¹ All the subjects included in the study had a minimum complement of 20 teeth.

Patients with any history of any antibiotic /anti inflammatory therapy for six months prior to study or mouth wash within 3 months prior to study were excluded from the study. Subjects who had undergone any periodontal treatment for at least six months prior to study or Pregnant or lactating women were not included in the study sample. History of any systemic diseases for group I & II and any systemic disease other than Type II Diabetes Mellitus for the group III were excluded from the study. Subjects with a history of smoking and any form of tobacco consumption and subjects with a history of vitamins /minerals or antioxidant supplements intake during the last 6 months were also excluded from the study sample.

A standard proforma consisting of the following data: Name, age, sex, medical and dental history, gingival index (Loe and Silness), clinical attachment and periodontal pocket depth was recorded. Each patient was examined using a mouth mirror and William’s periodontal probe under artificial light. A written informed consent was taken from each patient before examination and drawing of blood.
Method of Collection of Sample:

5 ml. of venous blood sample was drawn from the subject through disposable syringes and was transferred to a centrifuge tube. Blood sample was allowed to stay in centrifuge tube undisturbed for 30 minutes. Then it was centrifuged and serum was separated and sent immediately for biochemical analysis.

Biochemical Analysis:

Quantitative assessment of serum paraoxonase was done using an ELISA kit.

RESULTS:

A total of 90 patients were taken for this study. The aim of this study was to estimate the levels of paraoxonase in serum of diabetic patients with periodontitis and healthy individuals with and without chronic periodontitis. Study group was divided into three subgroups:

- Group I (control grp)- 30 Systemically healthy patients without Chronic Periodontitis
- Grp II: 30 Systemically healthy patients with Chronic Periodontitis
- Group III: 30 Patients with Diabetes Mellitus and Chronic Periodontitis

Quantitative evaluation of paraoxonase in serum was done using Analysis of Variance Test (ANOVA). Results obtained were tabulated and mean values and standard deviations were calculated. The mean values of paraoxonase in these three conditions were correlated and the results were statistically analysed. Paraoxonase levels in serum of systemically healthy patients with and without periodontitis (Group I and II) and patients with diabetes and chronic periodontitis (grp III) were compared before and after treatment. The results showed a statistically significant difference in the serum paraoxonase levels in pre and post treatment samples. (p value <0.001) (Table 1)
### TABLE 1: Comparison of the three groups in pre and post separately using one way anova:

<table>
<thead>
<tr>
<th>GROUPS</th>
<th>N</th>
<th>Mean</th>
<th>Standard Deviation</th>
<th>Statistics/ mean squares</th>
<th>df²(welch) / F(Anova)</th>
<th>P VALUE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre Treatment Paraoxonase level</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>systemically healthy patients without chronic periodontitis</td>
<td>30</td>
<td>150.87</td>
<td>24.354</td>
<td>23084.96</td>
<td>69.151</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>systemically healthy patients with chronic periodontitis</td>
<td>30</td>
<td>88.8</td>
<td>14.948</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>diabetes mellitus patients with chronic periodontitis</td>
<td>30</td>
<td>78.27</td>
<td>13.599</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>90</td>
<td>105.98</td>
<td>36.986</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Post Treatment Paraoxonase level</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>systemically healthy patients without chronic periodontitis</td>
<td>30</td>
<td>150.87</td>
<td>24.354</td>
<td>7250.289</td>
<td>13.025</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>systemically healthy patients with chronic periodontitis</td>
<td>30</td>
<td>130.47</td>
<td>19.401</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>diabetes mellitus patients with chronic periodontitis</td>
<td>30</td>
<td>106.93</td>
<td>26.464</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
In the pre-treatment segment, on comparison between systemically healthy patients without chronic periodontitis and patients with chronic periodontitis a statistically significant decrease in serum paraoxonase levels was observed. (p<0.001) Similar results were observed on comparison between systemically healthy patients without chronic periodontitis and patients with Diabetes mellitus and chronic periodontitis(p<0.001).

**TABLE 2: Posthoc Analysis For Finding The Subgroups Responsible For Significance**

<table>
<thead>
<tr>
<th>Multiple Comparisons</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Tukey HSD</strong></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Dependent Variable</th>
<th>(I) GROUP</th>
<th>(J) GROUP</th>
<th>Mean Difference (I-J)</th>
<th>Std. Error</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>PRE treatment Paraoxonase level</td>
<td>systemically healthy patients without chronic periodontitis</td>
<td>systemically healthy patients with chronic periodontitis</td>
<td>62.067</td>
<td>6.672</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>diabetes mellitus patients with chronic periodontitis</td>
<td>patients with chronic periodontitis</td>
<td>72.600</td>
<td>6.672</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>
However, on examining the serum paraoxonase levels between systemically healthy patients and chronic periodontitis and patients with DM and chronic periodontitis the difference was not statistically significant signifying the effect of inflammatory condition on the levels of paraoxonase. Similar results were observed in the post treatment segment as well. (Table 2)

<table>
<thead>
<tr>
<th></th>
<th>systemically healthy patients with chronic periodontitis</th>
<th>diabetes mellitus patients with chronic periodontitis</th>
<th>10.533</th>
<th>6.672</th>
<th>.266</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>post treatment</strong></td>
<td>systemically healthy patients without chronic periodontitis</td>
<td>systemically healthy patients with chronic periodontitis</td>
<td>20.400</td>
<td>8.615</td>
<td>.057</td>
</tr>
<tr>
<td><strong>paraoxonase level</strong></td>
<td>diabetes mellitus patients with chronic periodontitis</td>
<td></td>
<td>43.933</td>
<td>8.615</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>systemically healthy patients with chronic periodontitis</td>
<td>diabetes mellitus patients with chronic periodontitis</td>
<td>23.533</td>
<td>8.615</td>
<td>.024</td>
</tr>
</tbody>
</table>
TABLE 3: Comparison Of The Pre And Post In The Two Categories With Treatment: Paired t test

<table>
<thead>
<tr>
<th>GROUP</th>
<th>Mean</th>
<th>N</th>
<th>Std. Deviation</th>
<th>Mean</th>
<th>Std. Deviation</th>
<th>t</th>
<th>df</th>
<th>P VALUE</th>
</tr>
</thead>
<tbody>
<tr>
<td>systemically healthy patients with chronic periodontitis</td>
<td>Pre treatment Paraoxonase level</td>
<td>88.8</td>
<td>30</td>
<td>14.948</td>
<td>-41.667</td>
<td>19.819</td>
<td>-8.142</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td>Post treatment paraoxonase level</td>
<td>130.47</td>
<td>30</td>
<td>19.401</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>diabetes mellitus patients with chronic periodontitis</td>
<td>Pre treatment Paraoxonase level</td>
<td>78.27</td>
<td>30</td>
<td>13.599</td>
<td>-28.667</td>
<td>17.332</td>
<td>-6.406</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td>Post treatment paraoxonase level</td>
<td>106.93</td>
<td>30</td>
<td>26.464</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 3 takes into consideration only patients with chronic periodontitis. Statistically significant increase in the serum paraoxonase levels is observed post-treatment in both systemically healthy patients and those with diabetes mellitus and chronic periodontitis. This clearly shows a correlation between serum paraoxonase and inflammatory conditions like chronic periodontitis and diabetes mellitus.
DISCUSSION:

Periodontitis is a disease characterized by a complex cascade of tissue destructive pathways. Additional factors contributing to this multifaceted local disease process in the oral cavity include a number of systemic diseases, especially Diabetes Mellitus, which can exaggerate the host response to the local microbial factors, resulting in periodontal tissue breakdown. An abundance of studies on the complications of diabetes and periodontal disease has revealed that a hyperactive innate immune response may be the antecedent of both diseases, which probably have a synergistic effect when they co-exist in the host.

The results of this study demonstrated a lower level of paraoxonase levels in serum of patients with Type II diabetes mellitus and chronic periodontitis in comparison to systemically healthy patients with chronic periodontitis as well as healthy controls. There are several studies of PON1 activity in diabetic patients; most of them have results confirming the decrements in its activity. Boemi et al. showed that the decrease in the PON1 activity would change its antioxidant capacity and concluded that this would be one of the reasons for high oxidant stress in diabetic patients.

It is thought that high plasma glucose levels inactivate PON1 and increase lipid
peroxidation HDL.\textsuperscript{30, 31} Thus, the results obtained were in concordance with the literature available. The objective of this study was a comparative evaluation of Serum paraoxonase enzyme level in type II Diabetes Mellitus patients and healthy individuals with or without periodontitis before and after treatment. Sample population was divided into three groups and serum was tested for paraoxonase enzyme levels using ELISA test, before and after treatment.

On completion of the study it was observed that the serum paraoxonase levels were increased post treatment in patients with diabetes and chronic periodontitis. This is in accordance with a study conducted by Noack et al which stated that Type 2 Diabetes Mellitus increases the risk of generalized periodontitis.\textsuperscript{40} One of the main features in periodontitis and DM is the proinflammatory state, resulting in an increase of inflammatory mediators and oxidative stress. With respect to substances derived from oxidative damage, there is a correlation of plasma lipid peroxidation and periodontal parameters in individuals with DM.\textsuperscript{41} Thus, impaired PON-1 status in periodontitis patients with DM could be enhanced by an accumulation of oxidized LDL, known to inactivate the enzyme. The increased paraoxonase levels may be due to reduction in oxidative stress after scaling and root planning which is in accordance with a study conducted by Kinane et al that stated that Chronic inflammatory conditions like Diabetes Mellitus and chronic periodontitis are generally associated with increased oxidative stress ie. an increase in ROS particularly with neutrophils being implicated in the pathogenesis because of the generation of oxidative burst during phagocytosis and killing.\textsuperscript{42}

CONCLUSION:

The following Conclusions can be drawn from the present study:

1. Serum Paraoxonase level is decreased in diabetic patients with periodontitis compared to healthy individuals with periodontal disease.

2. Serum Paraoxonase level is decreased in diabetic patients with periodontitis compared to healthy individuals without periodontal disease.

3. Serum Paraoxonase Level increased in patients with chronic periodontitis and
Diabetes Mellitus after periodontal therapy.

To summarise, in the present study it can be concluded that the reduction in the serum levels of Paraoxonase in diabetes patients and healthy patients with periodontitis has led to an imbalance leading to increased ROS and hence periodontal breakdown.

Also, the change in Serum Paraoxonase levels before and after treatment can be taken as an indicator of success of periodontal therapy undertaken.

Further studies need to be carried out on the efficacy of antioxidant therapies that target the free radicals that lead to periodontal tissue breakdown.

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influence on the ability of HDL to protect LDL from oxidation.
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