Research Article

Periodontal disease as a risk factor for coronary artery disease

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ABSTRACT

Background: To evaluate and compare the serum levels of total cholesterol, triglycerides and LDL in subjects with healthy periodontal disease with that of chronic generalized severe periodontitis.

Methods: 30 subjects with a healthy periodontium (control group) and 30 subjects suffering from chronic generalized severe periodontitis (test group) were selected for the study to compare their serum levels of total cholesterol, triglycerides and LDL after obtaining informed consent. Blood samples were collected and sent for the assessment of lipid (total cholesterol, triglyceride, LDL) levels and the data was subjected to statistical analysis.

Results: The results of the our study show that there is significantly higher levels of total cholesterol, triglycerides and LDL in chronic generalized severe periodontitis as compared to healthy periodontium.

Conclusion: The observation of this study was that there was a statistically highly significant level of total cholesterol, triglyceride and LDL in chronic generalized severe periodontitis as compared to healthy subjects suggesting a relationship between periodontitis and cardiovascular disease.

Keywords: Cholesterol, Triglyceride, LDL, Generalized severe periodontitis, Coronary artery disease

INTRODUCTION

There are numerous studies which have suggested that periodontal infection can result in metabolic dysregulation of serum lipids causing cardiovascular disease which remain the leading cause of death in industrialized countries. Understanding the correlation between periodontitis and total cholesterol triglycerides and LDL will help us to better understand the link between periodontitis and cardiovascular diseases and further help us in increasing awareness amongst the medical faculty about the role of periodontitis as a risk factor for cardiovascular disease. Also if periodontal conditions are diagnosed and treated at an early stage, it may prevent the systemic involvement i.e. hyperlipidemia and cardiovascular disease. Periodontitis has been traditionally regarded as a chronic inflammatory oral infection. Chronic periodontal disease represents a primary anaerobic, gram -ve infection that leads to gingival inflammation, destruction of periodontal tissues, loss of alveolar bone and eventual exfoliation of teeth in severe cases. It is generally accepted that certain organisms within microbial flora of dental plaque are the major etiologic agents of periodontitis. These microorganisms particularly Porphyromonas Gingivalis (P. Gingivalis), produce endotoxins in the form of lipopolysaccharides. Lipopolysaccharides and other microbial substances gain access to the gingival tissues, initiate and perpetuate inflammation, resulting in production of high levels of cytokines (IL-1β, TNF-α), which leads to the destruction of the periodontal ligament and alveolar bone. Several studies have shown an association between periodontal disease and hyperlipidemia which may play an important role in atherosclerotic plaque formation leading to cardiovascular diseases. Thus indicating that there are...
increased chances of cardiovascular problems due to periodontitis.\textsuperscript{4,5} However molecular /cellular basis for the interaction between periodontitis and hyperlipidemia leading to cardiovascular disease has been a subject of intense study. It has been hypothesized that periodontitis can induce profound changes in serum IL-1β concentration which may alter the lipid metabolism resulting in hyperlipidemia.\textsuperscript{6,7} This study is thus planned, to find out the correlation between the increased serum levels of lipids and presence of periodontitis. Depending upon the correlation found it will help us in deciding the role of periodontitis in cardiovascular disease.

**METHODS**

The subjects for the study were selected from the patients visiting the Department of Periodontology, College of Dentistry, Jizan.

**Inclusion criteria**

- 30 subjects with healthy periodontium & 30 subjects of chronic generalized severe periodontitis were selected.
- Age ranging from 20-50 yrs
- Subjects who had not received any periodontal treatment since last 6 months and are systemically healthy.

**Exclusion criteria**

- Smokers, alcoholics, obese subjects, post-menopausal, pregnant or lactating females.
- Subjects on high cholesterol diet or subjects taking drugs for hypercholesterolemia
- Subjects with other chronic local & acute systemic infections

**Study protocol**

30 subjects of periodontitis and 30 subjects with healthy periodontium were considered for clinical examination. An informed written consent was obtained from each subject. Case history was obtained from each subject using a special proforma and the following clinical indices were used for using a special proforma and the following clinical indices were used for assessment.

- Simplified oral hygiene index (By Green & Vermillion)\textsuperscript{8}
- Ramfjord Periodontal Index (Ramfjord 1959)\textsuperscript{9}

Simplified oral hygiene index (By Green & Vermillion 1964)\textsuperscript{8}

Teeth considered for the index were

- 11, 16, 26, 31 facial aspect.
- 36, 46 lingual aspect.

**Criteria**

**Oral Debris Index (DI-S)**

0  No debris or stain present
1  Soft debris covering not more than one third of the tooth surface or the presence of extrinsic stains without other debris, regardless of surface area covered
2  Soft debris covering more than one third but not more than two third of the exposed tooth surface
3  Soft debris covering more than two third of the exposed tooth surface.

**Calculus index**

0  No calculus present
1  Supragingival calculus covering not more than 1/3rd of the exposed tooth surface.
2  Supragingival calculus covering not more than 2/3rd of the exposed tooth surface or the presence of individual flecks of subgingival calculus around the cervical portion of the tooth or both
3  Supragingival calculus covering more than 2/3rd of exposed tooth surface or a continuous heavy band of subgingival calculus around the cervical portion of the tooth or both

For each Individual, the debris & calculus scores were totalled & divided by number of tooth surfaces scored. Once the DI-S & CI-S were calculated separately, then they were combined or added together for the OHI-S.

OHI- score -Good - 0.0-1.2, Fair - 1.3 – 3.0, Poor - 3.1-6.0

Ramfjord Periodontal Index (Ramfjord 1959)\textsuperscript{9}

All teeth present in the mouth were examined in each subject. The periodontal status of each tooth was assessed visually and with the help of periodontal probe. Crevicular measurements were made on four surfaces of each tooth – mesial, distal, buccal and lingual using William’s graduated probe. The scoring criteria used were:

**Criteria**

0  Absence of inflammation
1  Mild to moderate inflammatory gingival changes not extending all around the tooth.
2  Mild to moderately severe gingivitis extending all around the tooth.
3  Severe gingivitis, characterized by marked redness, tendency to bleed, and ulceration.
4  Gingival crevice in any of the four measured areas (mesial, distal, buccal, lingual), extending apically to the cemento-enamel junction but not more than 3 mm.
5  Gingival crevice in any of the four measured areas (mesial, distal, buccal, lingual) extending apically to the cemento-enamel junction between 3-6mm.
Gingival crevice in any of the four measured areas (mesial, distal, buccal, lingual), extending apically more than 6 mm from the cemento-enamel junction

Periodontal disease index score was calculated by dividing the total score of all teeth examined by the number of teeth examined. After taking detailed case history and noting down the indices, subjects were selected depending on their periodontal status. 30 subjects diagnosed with healthy periodontium were considered as Control group and 30 subjects with chronic generalized severe periodontitis were considered as Test group. All subjects were subjected to laboratory investigations.

**Armamentarium and Materials**

- Mouth mirror, Straight probe, Explorer, Tweezer, William’s graduated probe
- Mouth mask, Surgical drape, Surgical gloves, Needle, Syringe, Plane bulbs
- Test tube stand, Micropipette with disposable tips, Bayer autopak kit
- Trivitron automatic analyser, Immunotech IM 3582 kit, Blot shaker
- Remi R&С laboratory centrifuge, ELISA reader (Lilac)

Periodontitis is an infectious disease of gram –ve species resulting in bacteremia and leading to systemic involvement. Several mechanisms have been proposed in an attempt to link the cardiovascular disease with periodontitis.\(^{11,12}\) Recently it has been hypothesized that serum proinflammatory cytokine (IL-1β) levels elevated by periodontitis, can alter the lipid metabolism resulting in hyperlipidemia which is an important risk factor for CVD.\(^6\)

Subjects with age ranging from 20-50 years of both sexes were selected for clinical examination. 30 subjects diagnosed with healthy periodontium (Control group) and 30 subjects diagnosed as chronic generalized severe periodontitis (Test group) were evaluated for serum lipid levels (Total Cholesterol, Triglycerides, LDL).

**Collection of blood sample**

Under all aseptic conditions, approximately 4ml of fasting venous blood sample was collected from the antecubital vein of each patient for estimation of levels of serum lipids. Collected blood sample was allowed to clot and serum was drawn off.

**Estimation of Serum Lipid Levels: 10**

The Serum lipid estimation of each sample was performed by using ‘BAYER AUTOPAK KIT’.

Kit consisted of
- Reagent 1
- Reagent 1A
- Standard

Reagents were allowed to attain room temperature. Then reagents were reconstituted manually by mixing reagent 1 (required for the reaction with serum) with reagent 1A and working reagent was prepared.

‘Trivitron’ automatic analyzer was programmed as per the manufacturers’ instructions.

Serum samples were added to the reagent and color change (red colored complex) was observed.

Optical density of red colored complex (Red quinine) was measured at 300C flowcell temperature.

The concentration of cholesterol in the serum sample was directly proportional to the intensity of the red complex which was measured at 500 nm.

**Estimation of Triglycerides**

The Serum triglyceride estimation was also performed by using same kit but with different reagents.

Composition of reagents of kit:

Reagent 1 (Enzymes/Chromogen):
- Cholesterol Esterase > 200U/L
- Cholesterol Oxidase > 250 U/L
- Peroxidase > 1000U/L
- 4-Aminoantipyrine 0.5 mmol/L
- Reagent 1 A (Buffer):
  - Pipes buffer, pH 6.90 50 mmol/L
  - Phenol 24 mmol/L
  - Sodium Cholate 0.5 mmol/L
- Standard (Cholesterol 200 mg/dl):
  - Cholesterol 2 g/L

Reagents were allowed to attain room temperature. Then reagents were reconstituted manually by mixing reagent 1 (required for the reaction with serum) with reagent 1A and working reagent was prepared. ‘Trivitron’ automatic analyzer was programmed as per the manufacturers’ instructions.
instructions. Serum samples were added to the reagent and color change (purple colored complex) was observed. Optical density of purple coloured complex was measured at 300°C flowcell temperature. The concentration of triglyceride in the serum sample was directly proportional to the intensity of the purple colour complex which was measured at 546 nm.

**Estimation of HDL-Cholesterol**

VLDL (Very Low Density Lipoproteins) and LDL fractions in serum or plasma was separated from HDL by precipitating with Phosphotungstic Acid and Magnesium Chloride. After centrifugation, the cholesterol in the HDL fraction, which remains in the supernatant is assayed with enzymatic cholesterol method, using Cholesterol Esterase, Cholesterol Oxidase, Peroxidase and the chromogen 4-Aminoantipyrine/Phenol.

**Estimation of LDL-Cholesterol**

LDL cholesterol is calculated is calculated by friedwald’s equation:

\[
\text{LDL cholesterol (mg/dl)} = \text{total cholesterol} - \text{triglycerides} - \text{HDL cholesterol}\\
\]

\[\text{5 cholesterol}\]

**RESULTS**

**Statistical Analysis**

Descriptive statistics include mean and standard deviation, which were calculated for each of the study groups. The intergroup comparison of Total Cholesterol, Triglycerides and Low Density Lipoprotein in healthy and periodontitis group was done using Z- test. ‘p’-value <0.05 was considered statistically significant. Formulae used

Analysis was done by SPSS software version 10 using Z-test and correlation test

**Observed difference**

\[
\text{SE} = X_1 - X_2\\
\]

\[\text{SE} - \text{standard error}\]

\[a) \ \bar{X}_i = \frac{\sum X_i}{N}\\
\]

Where \( X_i \) is the sum of all values.

\[N \text{ is the number of all values.}\]

\[b) (x – \bar{x})_2 \\\n\]

Where \( \bar{x} \) is the Mean of all values.

\[N-1 \]

\[\text{N is the number of the values.}\]

\[c) \ \text{SE} X_1 - X_2 = \frac{Sd_{12}}{n_1} - \frac{Sd_{22}}{n_2}\\
\]

**Table 1: Comparison of age in Control (Healthy Periodontium) and Test (Chronic generalized severe Periodontitis) group.**

<table>
<thead>
<tr>
<th>Particular</th>
<th>Control group</th>
<th>Test group</th>
<th>Z Value</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (Yrs)</td>
<td>33.93 ± 5.77</td>
<td>36.17 ± 5.91</td>
<td>1.48</td>
<td>&gt;0.05</td>
</tr>
</tbody>
</table>

There was no statistically significant difference between the mean age of control (Healthy periodontium) and test(Chronic generalized severe periodontitis) groups. In control group (Healthy periodontium)out of 30 subjects, 13(21.67%) were males and 17(28.33%) were females, whereas in test group (Chronic generalized severe periodontitis) out of 30 subjects 16(26.67%) were males and 14(23.33%) were females.

**Serum Lipid Levels**

A. Comparison of Total Cholesterol in control and test groups:

**Table 2: Comparison of Total Cholesterol in Control (Healthy Periodontium) and Test (Chronic generalized severe Periodontitis) group.**

<table>
<thead>
<tr>
<th>Particular</th>
<th>Control group</th>
<th>Test group</th>
<th>Z Value</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Cholesterol</td>
<td>146.47 ± 19.00</td>
<td>221.33 ± 18.72</td>
<td>15.30</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>
Figure 1: Bar diagram showing comparison of Total Cholesterol in Control (Healthy Periodontium) and Test (Chronic generated severe Periodontitis) group.

Table 2 and Figure 1 shows the comparison of mean value of total cholesterol between control (Healthy periodontium) and test (Chronic generalized severe periodontitis) group. Mean total cholesterol level were 146.47 ± 19 in control group (Healthy periodontium) and 221.33 ± 18.72 in test group (Chronic generalized severe periodontitis). Z- value obtained was 15.30 with p value <0.0001. The results indicated that there was statistically highly significant difference in the mean Total Cholesterol levels between healthy periodontium and chronic generalized severe periodontitis group. The levels of serum Total Cholesterol was found to be higher in subjects with chronic generalized severe periodontitis than that of healthy periodontium.

Comparison of Triglycerides in control and test group:

Table 3 and Figure 2 depicts the comparison of mean triglyceride level between control (Healthy periodontium) and test (Chronic generalized severe periodontitis) group. Mean triglyceride levels were 89.27 ± 10.55 in control group (Healthy periodontium) and 156.8 ± 14.39 in test group (Chronic generalized severe periodontitis). Z- value obtained was 20.72 with p value <0.0001. The results indicated that there was statistically highly significant difference in the mean triglyceride levels between healthy periodontium and chronic generalized severe periodontitis group. The levels of serum triglycerides was found to be higher in subjects with chronic generalized severe periodontitis than that of healthy periodontium.

B. Comparison of LDL in control and test group:

Table 4: Comparison of LDL in Control (Healthy Periodontium) and Test (Chronic generalized severe Periodontitis) group

Table 4 and Figure 3 depicts the comparison of mean LDL levels between control (Healthy periodontium) and test (Chronic generalized severe periodontitis) group. Mean LDL levels were 86.96 ± 14.32 in control group (Healthy periodontium) and 135.37 ± 15.63 in test group (Chronic generalized severe periodontitis). Z-value obtained was 12.51 with p value <0.0001. The results indicated that there was statistically highly significant difference in the mean LDL levels between healthy periodontium and chronic generalized severe periodontitis group. The level of serum triglycerides was found to be higher in subjects with chronic generalized severe periodontitis than that of healthy periodontium.
The present study shows highly significant elevated serum lipid levels (i.e. Total Cholesterol, Triglycerides and LDL) in periodontitis patients as compared to healthy controls. Results of the our study show that there is significantly higher levels of total cholesterol, triglycerides and LDL in chronic generalized severe periodontitis as compared to healthy periodontium.

**DISCUSSION**

Periodontal disease is an infectious disease caused by a small group of predominantly anaerobic gram -ve bacteria. Lipopolysaccharide and other microbial substances gain access to gingival tissues, initiate and perpetuate inflammation, resulting in production of high levels of proinflammatory cytokine (IL-1β). Different studies demonstrated increased levels of IL-1β locally in cases of periodontitis. It has also been suggested that these locally produced IL-1β can cross the epithelium and enter the circulation, which have profound effect on lipid metabolism resulting in hyperlipidemia, a major risk factor for cardiovascular disease. The present study was designed to estimate and correlate the levels of lipid (total cholesterol, triglyceride, LDL) in chronic generalized severe periodontitis and healthy subjects. A total of 60 subjects with age ranging from 20-50 were included in the study after checking for exclusion criterias. Subjects were underwent an intraoral examination to assess their periodontal status and divided into two groups. Group I included 30 subjects with chronic generalized severe periodontitis and Group II included 30 healthy subjects. Following this blood samples were collected and and send for the assessment of lipid (total cholesterol, triglyceride, LDL) levels. The data thus obtained was compiled and subjected to statistical analysis. The observations of our study were as follows:

Statistically highly significant levels of total cholesterol, triglyceride and LDL in chronic generalized severe periodontitis as compared to healthy subjects. Thus this study has proved the hypothesis linking periodontitis and cardiovascular disease through lipid levels. To elucidate the relationship between periodontitis and cardiovascular disease (due to hyperlipidemia) further studies with large sample size and excluding other factors which could influence the lipid levels should be carried out.

- Higher levels of total cholesterol was found in subjects with chronic generalized severe periodontitis as compared to healthy periodontium which was statistically highly significant.
- Higher levels of triglycerides was found in subjects with chronic generalized severe periodontitis as compared to healthy periodontium which was statistically highly significant.
- Higher levels of LDL was found in subjects with chronic generalized severe periodontitis as compared to healthy periodontium which was statistically highly significant.

From the above observation, it can be concluded that there is an increase in levels of serum lipid (i.e. Total Cholesterol, Triglycerides and LDL) in subjects with chronic generalized severe periodontitis as compared to subjects with healthy periodontium suggesting that subjects with periodontitis may be more prone for cardiovascular disease. Present study is in full accordance with that of Christopher W Cutler et al (1999)\(^3\), who conducted a study to investigate the association between periodontitis and hyperlipidemia.

Ebersole et al (1999)\(^4\) carried out an animal study using 47 non-human primates. They investigated the effects of periodontitis on serum lipid profile, a known risk factor for cardiovascular disease. The findings indicated positive correlation between severity of periodontitis and the levels of Total Cholesterol, Triglycerides and LDL. Losche et al (2000)\(^5\) studied an association between destructive periodontal disease and plasma lipid levels. The study comprised of 39 subjects with periodontitis and 40 healthy subjects. They found, significant elevation of total cholesterol in periodontitis patients as compared to controls and also significant elevation of triglycerides in periodontitis patients as compared to controls. Similarly LDL was also significantly elevated in periodontitis patients as compared to controls.

Katz et al (2002)\(^6\) designed a cross sectional study on 10,590 israeli military service men and women to correlate periodontal pockets with elevated blood lipid levels. In their study, plasma levels of total cholesterol and LDL were significantly higher in 46 patients with periodontal disease as compared to age and sex matched controls. Authors also found that CPITN score was strongly associated with total cholesterol and LDL levels and minor significance was found with triglycerides. Craig et al (2003)\(^7\) examined the relationship between destructive periodontal disease and lipid levels. They found significantly increased levels of total cholesterol, triglycerides and LDL in periodontitis cases as compared to healthy controls. Our results are also similar with the studies carried out by Joshipura K J et al (2004)\(^8\) and Katz J, Chaushu G, Sharabi Y (2001)\(^9\). The periodontitis and CVD are chronic diseases with common risk factors especially with tobacco smoking as this is significantly associated with both the diseases. Only studies with inappropriate adjustment to tobacco have found significant associations between periodontal disease and CVD18.

**CONCLUSION**

The present study was designed to compare the levels of lipids (total cholesterol, triglyceride, LDL) in chronic generalized severe periodontitis and healthy subjects. A total of 60 subjects with age ranging from 20-50 were included in the study after checking for exclusion criteria. Subjects underwent an intraoral examination to assess their periodontal status and divided into two groups. Group I included 30 subjects with chronic generalized...
severe periodontitis and Group II included 30 healthy subjects. Following this, blood samples were collected and sent for the assessment of lipid (total cholesterol, triglyceride, LDL) levels. The data thus obtained was subjected to statistical analysis. The observation of this study was that there was a statistically highly significant level of total cholesterol, triglyceride and LDL in chronic generalized severe periodontitis as compared to healthy subjects suggesting a relationship between periodontitis and cardiovascular disease. To elucidate the exact relationship between periodontitis and cardiovascular disease (due to hyperlipidemia), further studies with larger sample size should be carried out. Also there may be some unknown systemic factors which could influence serum lipid levels and modify the results, they need to be excluded.

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