Original Article

Increased serum interleukin-1β level in chronic periodontal disease as a risk factor for cardio-vascular diseases

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ABSTRACT

Background: Periodontitis, a chronic inflammatory oral infection may have profound effects on systemic health. Studies have suggested that periodontal infection can result in metabolic deregulation of serum lipids causing cardiovascular disease which remain the leading cause of death in industrialized countries. As it is hypothesized that hyperlipidemia in cases of periodontitis could be due to increase in serum IL-1β levels, similarly if a correlation is found between serum IL-1β levels and periodontitis, it will help us in increasing awareness amongst the medical faculty about the role of periodontitis as a risk factor for cardiovascular disease.

Aim: To evaluate and compare the serum Interleukin-1β levels in subjects with healthy periodontium with those suffering from chronic generalized severe periodontitis.

Material & 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 Interleukin-1β (IL-1β) levels after obtaining informed consent. Blood samples were collected and sent for the assessment of serum IL-1β levels and the data was subjected to statistical analysis.

Result: The results of our study show that there is a significantly higher level of serum IL-1β in chronic generalized severe periodontitis as compared to healthy periodontium.

Conclusion: The observation of this study was that there was a statistically highly significant serum IL-1β level in chronic generalized severe periodontitis as compared to healthy subjects suggesting a relationship between periodontitis and cardiovascular disease.

Keywords: Interleukin-1β, Chronic generalized severe periodontitis, Hyperlipidemia Cardiovascular disease.

INTRODUCTION

Periodontitis, a chronic inflammatory oral infection may have profound effects on systemic health. Studies 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. As it is hypothesized that hyperlipidemia in cases of periodontitis could be due to increase in serum IL-1β levels, similarly if a correlation is found between serum IL-1β levels and periodontitis, it will 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 leading to cardiovascular disease. Periodontitis has been traditionally regarded as a chronic inflammatory oral infection. Chronic periodontal disease (periodontitis) represents a primary anaerobic, gram -ve oral infection that leads to gingival inflammation, destruction of periodontal tissues, loss of alveolar bone and eventual exfoliation of teeth in severe cases [1].

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[2,3]. Apart from destruction of local tissues, IL-1β in advanced

periodontitis is dumped systemically, falling within detectable range of biological serum assay. [4,5,6] Recently, there has been great interest in the systemic effects of serum pro-inflammatory cytokine levels potentially elevated by periodontitis. Investigators have suggested that periodontitis-induced elevations of pro-inflammatory cytokines such as IL-1β may play a major role in the development of a variety of systemic diseases such as circulatory, pulmonary and metabolic [7,8,9] . It has been hypothesized that periodontitis can induce profound changes in serum IL-1β concentration which may alter the lipid metabolism resulting in hyperlipidemia [10, 11].

MATERIAL AND METHODS

This study is thus planned, to find out the correlation between the increased levels of IL-1β in subjects with periodontitis which will help us in deciding the role of periodontitis in cardiovascular disease. The subjects for the study were selected from those visiting the Department of Periodontology, Peoples Dental Academy, Bhopal.

Inclusion criteria:

- 30 subjects with healthy periodontium (control group) 30 subjects diagnosed with chronic generalized severe periodontitis (test group) were selected.
- Age ranging from 30-50 years.
- Subjects who had not received any periodontal treatment since last 6 months.
- Systemically healthy.

Exclusion criteria

- Smokers, Alcoholics, Post menopausal, pregnant, lactating females.
- Subjects on high cholesterol diet, Subjects taking drugs for hypercholesterolemia
- Subjects with chronic local & acute systemic infections, Obese subjects

  (BMI≥30kg/m^2)[12].

Study protocol

30 subjects with healthy periodontium (control group) and 30 subjects suffering from chronic generalized severe periodontal disease (test group) were selected for the study. Case history was obtained from each subject using a special proforma. Also indices were noted down. An informed written consent was obtained from each subject.

The following clinical indices were used for assessment

- Simplified oral hygiene index (By Green & Vermilion) [13].
- Ramfjord Periodontal Index (Ramfjord 1959) [14].

Simplified oral hygiene index (By Green & Vermillion 1964) [15].

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

o	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 totaled & 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) [14].

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.
6	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 detail case history and noting down the indices, selected 60 subjects, fulfilling all the criteria's were subjected to laboratory investigations.

Collection of blood sample

Under all aseptic conditions, approximately 5ml of fasting venous blood sample was collected from the anticubital vein of each patient. A sterile disposable syringe and 24 gauge needle was used for this purpose. Collected blood sample was allowed to clot and serum was drawn off.

Estimation of il-1β [15]:

The IL-1β levels were estimated using IMMUNOTECH IM3582 KIT. This is an enzyme immunoassay for the invitro determination of IL-1β in plasma, serum or culture supernatant. The assay was carried out as per manufacturer's direction for use. The components of the kit were allowed to equilibrate at room temperature prior to use.

Kit consisted of

- Microtiter plate, Lyophilized calibrator (Bovine serum of known concentration of analyte) ,Diluent , Biotinylated antibody
- Wash solution (Used to wash unbounded antibodies), Streptavidin-HRP conjugate Substrate
- Stop solution (sulfuric acid solution which stops reaction)

The reagents of the kit were prepared as follows:

- The wash solution was diluted (20x) with 950ml of distilled water and
- The lyophilized calibrator was reconstituted with the volume of distilled water stated on the vial label. At least one-half hour wait is recommended after solubilization before dispensing. Mixing was done gently to avoid foaming. This resulted in a 10 ng/ml IL-1β solution.
- From the 10ng/ml calibrated solution and diluent, a fresh dilution series of known concentration was prepared prior to assay and optical density was checked.

Step 1: 50 μL of calibrator and 50 μL of biotinylated antibody were pipetted into wells of microtiter plate. The microtiter plate was then incubated for 2 hr. at 18-25°C while shaking. The wells were then washed manually. Three cycles were repeated as follows:

A calibrated curve was plotted and the concentrations of IL-1β in the samples were calculated by interpolation from the calibrator curve.

Statistical analysis

Descriptive statistics include mean and standard deviation, which were calculated for each of the study groups.Intergroup comparison of IL-Iβ levels was done by using Z-test.p-value <0.05 was considered statistically significant.

RESULTS

Demographic Parameters

Maximum number of cases were seen in age group of 30-36, of which 14(23.33%) out of 30 patients were from control group (Healthy periodontium and 13(21.67%) out of 30 patients were from test group (Chronic generalized severe periodontitis). 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.

Table 1: Comparison of IL - 1β in Control (Healthy Periodontium) and Test (Chronic generalized severe Periodontitis) group

Particular	Control group Mean ± SD (n=30)	Test group Mean ± SD (n=30)	Z Value	P Value
IL - 1β (pg/ml)	4.11 ± 3.58	9.70 ± 5.41	4.72	<0.0001

Table 1 and Graph 1 depict the comparison between control group (Healthy periodontium) and test group (Chronic generalized severe periodontitis) for the levels of serum IL-1β. The Mean serum IL-1β levels of control group were 4.11 ± 3.58 pg/ml and The Mean serum IL-1β levels of test group were 9.70 ± 5.41pg/ml. Z-value obtained was 4.72 with p-value <0.0001. The results indicated that there was statistically highly significant difference in the mean serum IL-1β levels between healthy periodontium and chronic generalized severe periodontitis group.

Fig.1

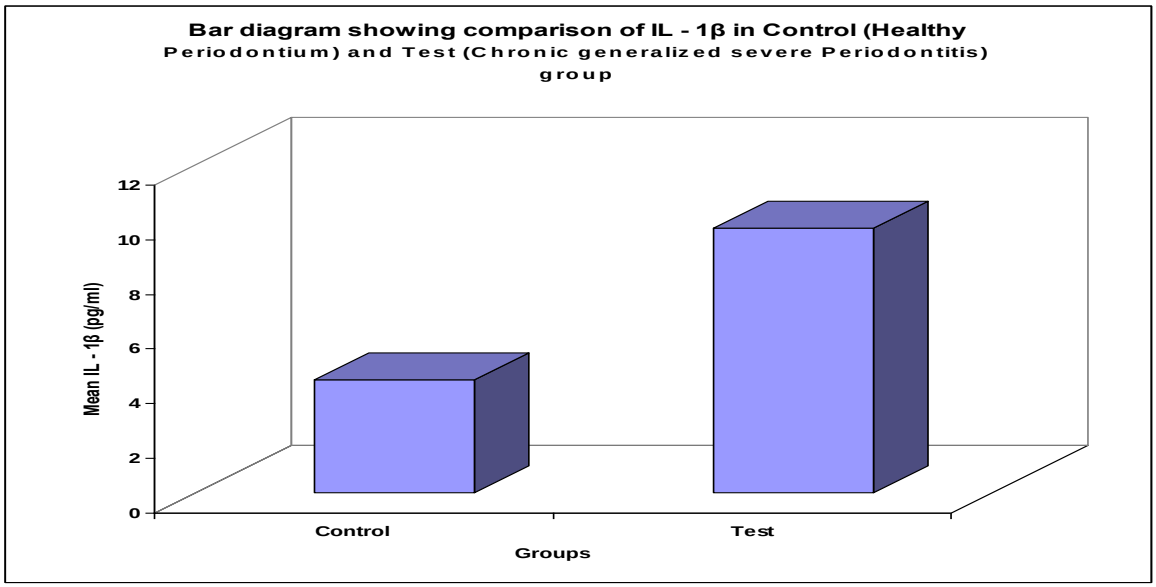


Table -2: Comparison of PDI in study groups

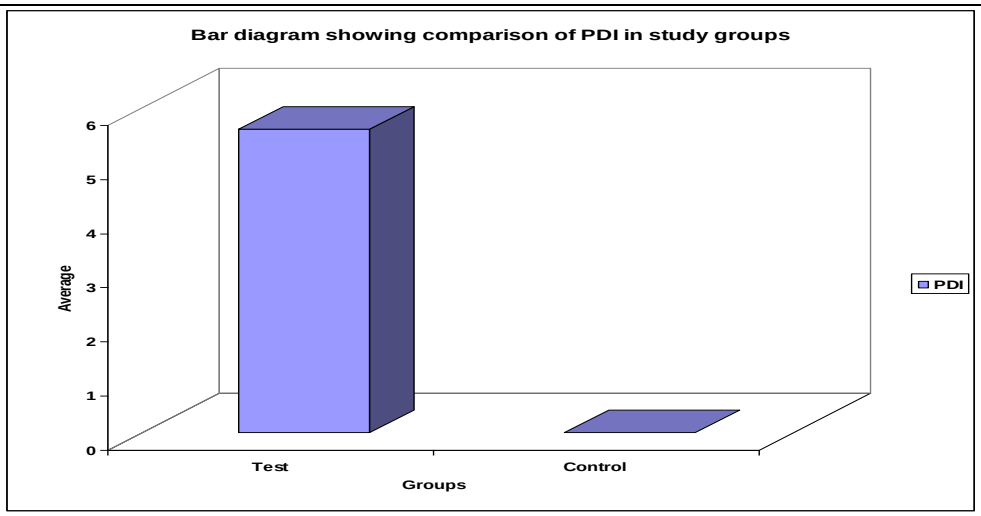
Particular	Test group Mean ± SD (n=30)	Control group Mean ± SD (n=30)	Z Value	P Value
PDI	5.62 ± 0.40	0 ± 0	76.07	<0.0001

DISCUSSION

The results of our study are in agreement with the study carried out by Anthony M Lacopino and Christopher W. Cutler (2000) [16], who suggested that in advanced periodontitis, levels of IL-1β can be elevated in the gingival crevicular fluid to such a degree that they can cross the ulcerated epithelium and enter the circulation. Likewise, Kinane and Lowe (2000) [11], stated that bacteremia associated with periodontitis could increase the levels of circulating lipopolysaccharides (LPS), which in turn cause activation of monocyte and thus cytokine release. A review by D.F.Kinane (1998) [10], suggest that the proinflammatory cytokine IL-1β produced by monocyte can inhibit lipoprotein lipase and thus leads to hyperlipidemia. Kinane and Lowe (2000) [11] also reviewed the same mechanism suggesting the role of IL-1β in hyperlipidemia.

The results of our study are also consistent with the study by Gorska R. et al (2003) [17].They found, significantly higher concentration of IL-1β in serum samples from severe chronic periodontitis patients than healthy controls (p- value was<0.05). The present study was thus designed to estimate and compare the levels of serum IL-1β in subjects with healthy periodontium and chronic generalized severe periodontitis. 30 subjects with healthy periodontium (Control group) and 30 subjects with chronic generalized severe periodontitis (Test group) were subjected to laboratory investigations. Their blood samples were collected and sent for assessment of serum IL-1β levels and subjected to statistical analysis. The observations of our study showed that higher levels of serum IL-1β were found in subjects with chronic generalized severe.

Fig.2



	-0.06	>0.05

periodontitis as compared to healthy periodontium which was statistically highly significant. Thus the result of this study indicates that periodontitis results in an increase in levels of IL-1β which may be responsible for increase in serum lipid levels resulting in cardiovascular disease. Our study supports the hypothesis put forth by Anthony M.Lacopino and Christopher W. Cutler (2000)[16] can be proved. In their review, they stated that there is linkage between periodontal infection and hyperlipidemia, as periodontal infection causes bacteremia, producing release of IL-1β cytokine, which enhances lipogenesis and reduces lipid clearance thus resulting in hyperlipidemia [17,18]. Different studies have demonstrated that there are increased levels of IL-1β locally in response to increased levels of circulating lipopolysaccharides caused by bacteremia due to periodontitis [19,20,21]. It is thus clear from the above discussion that IL-1β levels significantly increase in periodontitis, which can alter lipid metabolism. This could be the probable reason of hyperlipidemia seen in cases of periodontitis. [22,23]

CONCLUSION

The present study was designed to compare the levels of serum IL-1β in periodontally healthy subjects and those diagnosed with chronic generalized severe periodontitis. A total of 60 subjects with age ranging from 30-50 were included in the study after checking for exclusion and inclusion criteria. Subjects underwent an intraoral examination to assess their periodontal status and were divided into two groups. Group I (control group) included 30 subjects with a healthy periodontium and Group II (test group) included 30 subjects suffering from chronic generalized severe periodontitis. Following this, blood samples were collected and sent for the assessment of serum IL-1β levels. The data thus obtained was compiled and arranged in a master chart and subjected to statistical analysis.

The results of this study showed statistically highly significant increased levels of serum IL-1β in chronic generalized severe periodontitis as compared to healthy subjects. Thus this study has proved the hypothesis linking periodontitis and cardiovascular disease through IL-1 β levels. To elucidate the relationship between periodontitis and cardiovascular disease further studies with large sample size and excluding other factors which could influence the IL-1β should be carried out.

REFERENCES

1. Socransky SS, Haffajee AD. The bacterial etiology and progression of destructive periodontal disease:current concepts. Journal of Periodontology, 1992:63:322-31.
2. Liljenberg B, Lindhe J,Dahlen G,Jonsson R. Microbiological histopathological, and immunohistochemical characteristics of progressive periodontal disease. Journal of Clinical Periodontology, 1994:21:720-7.
3. Offenbacher S. Periodontal diseases, pathogenesis. Annal of Periodontology 1996:1:821-78.
4. Offenbacher S,Jared HL,Wells SR et al. Potential pathogenic mechanisms of periodontitis associated pregnancy complications. Annal of Periodontology, 1998:3:233-50.
5. Prabhu A, Michalowicz BS, Mathur A. Detection of local and systemic cytokines in adult periodontitis. Journal of Periodontology, 1996:67:515-22.

6. Salvi GE, Brown CE, Fujihashi K et al. Inflammatory mediators of the terminal dentition in adult and early onset periodontitis. Journal of Periodontal Research, 1998:4:212-25.
7. Syrjanen J, Peltola J, Valtonen V et al. Dental infection is associated with certain infarction in young and middle aged men. Journal of Intern Med 1989:225:179-84.
8. Destefano, Anda RF,Khan HS et al. Dental disease and risk of coronary heart disease and mortality.British Dental Journal 1993:306:688-91.
9. Page RC. The pathobiology of periodontal diseases may affect systemic diseases: inversion of a paradigm. Annal of Periodontology, 1998:3:108-20.
10. Kinane DF. Periodontal diseases' contributions to cardiovascular disease: An overview of potential mechanisms. Annal of Periodontology, 1998:3:142-50.
11. Kinane DF and Lowe. How periodontal disease may contribute to cardiovascular disease. Periodontology 2000:23:121-6.
12. AL-Zahrani MS, Bissada NF, Borawskit EA. Obesity and periodontal disease in young, middle aged and older adults. Journal of Periodontology 2003: 74:610-15.
13. Greene JC, Vermillion JR. The simplified oral hygiene index.Journal of American Dental Association1964:68:7-10.
14. Ramfjord SP. The periodontal disease index. Journal of Periodontology, 1967:38:602-08.
15. Karnoutsos K, Papastergiou P, Stefanidis S, and Vakaloudi A, Periodontitis as a risk factor for cardiovascular disease: The role of anti-phosphorylcholine and anti-cardiolipin antibodies, Hippocratic. 2008 Jul; 12(3): 144–9.
16. Anthony M. Iacopino and Christopher W. Cutler. Pathophysiological Relationships Between Periodontitis and Systemic Disease: Recent Concepts Involving Serum Lipids. Journal of Periodontology, 2000:71:1375-84.
17. Górska R, Gregorek H, Kowalski J, Laskus-Perendyk A, Syczewska M, Madaliński K. Relationship between clinical parameters and cytokine profiles in inflamed gingival tissue and serum samples from patients with chronic periodontitis. Journal of Clinical Periodontology 2003:30:1046-52.
18. Feldner B D, Reinhardt R A, Garbin C P, Seymour G J, Casey J H. Histological evaluation of interleukin-1β and collagen in gingival tissue from untreated adult periodontitis. Journal Periodontal Research,1994:29:54-6
19. Preiss DS, Meyle J. Interleukin-1beta concentration of gingival crevicular fluid.Journal of Periodontology,1994:65:423-8.
20. Lein-Tuan Hou, Cheing-Meei Liu, Edward F. Rossomando. Crevicular interleukin-1β in moderate and severe periodontitis patients and the effect of phase I periodontal treatment.Journal Clinical Periodontology,1995:22:162-7
21. Goutoudi P, Diza E, Arvanitidou M. Effect of periodontal therapy on crevicular fluid interleukin-1beta and interleukin-10 levels in chronic periodontitis. Journal of Dentistry. 2004 :32:511-20.
22. Orozco A, Gemmell E, Bickel M, Seymour GJ. Interleukin-1beta, interleukin-12 and interleukin-18 levels in gingival fluid and serum of patients with gingivitis and periodontitis. : Oral Microbiolog Immunology. 2006 :21:256-60
23. Toker H, Poyraz O, Eren K. Effect of periodontal treatment on IL-1beta, IL-1ra, and IL-10 levels in gingival crevicular fluid in patients with aggressive periodontitis. Journal Clinical Periodontology 2008:35:507-13.

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