INTERNATIONAL JOURNAL OF CURRENT MEDICAL AND PHARMACEUTICAL RESEARCH

IJCMPR

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RESEARCH ARTICLE

EVALUATION OF CYTOKINE INTERLUKIN 1β (IL-1β) IN SERUM IN CHRONIC PERIODONTITIS AS COMPARED TO PERIODONTAL HEALTH

Priyanka Agrawal[1]., Darshana Dalaya[2]., Tejaswini Patil[3]., Amit Thareja[4]., Ankita Goyal[5] and Kashish Singh[6]

[1,2,3,4,6]Department of Periodontology, Bharati Vidyapeeth Deemed University Dental College and Hospital, Pune
Department of Prosthodontics, Bharati Vidyapeeth Deemed University Dental College and Hospital, Pune
[5]Department of Public Health Dentistry, PGIDS, Pt.B D Sharma University, Rohtak

ARTICLE INFO

Article History:
Received 19th June, 2016
Received in revised form 16th July, 2016 Accepted 18th August, 2016 Published online 19th September, 2016

Key words:

Interlukin 1β (IL-1β), Chronic Periodontitis, hyperlipedimeia, cytokine profile, cardiovascular diseases

ABSTRACT

Aim: The aim of our study was to estimate and compare the serum levels of IL-1β in subjects with healthy periodontium with that of chronic periodontitis.
Materials and Methods: 30 subjects diagnosed with healthy periodontium and 30 subjects with chronic periodontitis of age ranging from 20-50 yrs who were systemically healthy were included in the study. All subjects i.e with Healthy periodontium (Control group) and with Chronic periodontitis (Test group) were subjected to laboratory investigations for estimation of IL-1β and comparison of individual levels was done between control and test group by 'Z' test.
Results: Statistically highly significant levels of serum IL-1β were observed in chronic periodontitis as compared to healthy subjects.Statistically highly significant positive correlation was found between IL-1β in chronic generalized severe periodontitis and also in subjects with healthy periodontium.
Conclusion: This study help us to better understand the hypothesis linking periodontitis & cardiovascular disease through increase in levels of IL-1β and therefore increase in serum lipids. It can further help us in increasing awareness amongst the medical faculty about the role of periodontitis as a risk factor for cardiovascular disease.

INTRODUCTION

Periodontitis is a multifactorial and chronic inflammatory disease which may have profound effect on systemic health. Periodontal pathogens produces endotoxins which causes release of IL-1β.[1,2] It's concentration in serum may alter the lipid metabolism resulting in hyperlipidemia. In terms of the potential relationship between periodontitis and systemic disease, it has been demonstrated that periodontitis induce profound changes in serum concentrations of cytokines leading to catabolic state characterized by altered lipid metabolism. Various studies have shown that deregulation of serum lipids can be caused by periodontal infection leading to cardiovascular disease.[3,4] This study was thus planned, to find out the correlation between the increased levels of IL - 1β in serum which may play a major role in the development of a variety of systemic diseases such as circulatory, pulmonary and metabolic.[5]

MATERIALS AND METHODS

Study Protocol

30 subjects with Chronic Periodontitis and 30 subjects with healthy periodontium were considered for clinical examination. Subjects from Out Patient Department of Periodontology of Dr. D. Y. Patil Dental College and Hospital, Pune were selected. Case history was obtained from each subject using a proforma. An informed written consent was obtained from each subject. The following clinical indices were used for assessment:

- Simplified Oral Hygiene Index (By Green & Vermilion)
- Ramfjord Periodontal Index (Ramfjord 1959)

Inclusion criteria

1. 30 subjects with healthy periodontium & 30 subjects with chronic generalized severe periodontitis were selected.
2. Periodontal pocket depth > 7mm
3. Age ranging from 20-50 yrs
4. Subjects who had not received any periodontal treatment since last 6 months.
5. Systemically healthy.

Exclusion criteria

1. Smokers
2. Alcoholics
3. Post menopausal, pregnant, lactating females.
4. Subjects on high cholesterol diet
5. Subjects taking drugs for hypercholesterolemia
6. Subjects with chronic local & acute systemic infections
7. Obese subjects (BMI>27kg/m2)

After taking detailed case history and 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 Severe Chronic Periodontitis were considered as Test group. Selected 60 subjects, fulfilling all the criteria's were subjected to further laboratory investigations.

Collection of blood sample

Under all aseptic conditions, approximately 5ml of fasting venous blood sample was collected from the antecubital vein of each subject. A sterile disposable syringe and 24 gauge needle was used for this purpose. Venous blood was collected in plain bulb of 5ml for estimation of levels of IL-1 β. Collected blood sample was allowed to clot and serum was drawn off. Values of IL-1β in both groups i.e control group and test group were statistically analysed.

Estimation of IL-1β

The IL-1β levels were estimated using IMMUNOTECH IM3582 KIT. This is an enzyme immunoassay for the in vitro 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)
- Stop solution (sulfuric acid solution which stops reaction)
- Streptavidin-HRP conjugate
- Substrate

The reagents of the kit were prepared as follows:

1. The wash solution was diluted (20x) with 950ml of distilled water and
2. 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.
3. 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:
Plate was turned upside- down and shaken vigorously over the sink. Wells were filled with wash solution,(the solution may run over the rim of the wells). Plate was again turned upside – down and shaken vigorously over the sink.

Step 2:100µL of streptavidin-HRP conjugate was added and was incubated for 30 min at 18-25°C while shaking.

Step 3: 100µL of substrate was added and plate was incubated for 30 min. at 18-25°C, while shaking. Then 50µL of stop solution was added.

The absorbance was then read at 450 nm, using 'LILAC' ELISA reader (fig 4).

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

Fig 1 Serum separated from blood sample

Fig 2 Micropipette used for pipetting of blood sample

Fig 3 Microtiter plate

Fig 4 Lilac ELISA reader

Statistical analysis

Descriptive statistics included mean & standard deviation, which were calculated for each of the study groups. Intergroup comparison of all parameters studied were analysed by Z- test. Analysis was done by SPSS software version 10 'p'-value< 0.05 was considered statistically significant.

RESULTS

Table 1 Levels of IL-1β in control and test 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

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 present study showed significant elevated serum IL-1β in periodontitis patients as compared to healthy controls.

DISCUSSION

The results of increase in levels of IL-1β in Chronic Periodontitis in our study is in agreement with many studies in the literature. Study carried out by **Anthony M Lacopino and Christopher W. Cutler (2000)**[6], suggested that in advanced periodontitis, levels of IL-1β can be elevated in the GCF to such a degree that they can cross the ulcerated epithelium and enter the circulation. Likewise**, Kinane and Lowe (2000)**[4] 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. The results of our study are also consistent with the study by **Gorska R. *et al* (2003)**[7]. They found significantly higher concentration of IL-1β in serum samples from severe chronic periodontitis patients than healthy controls (p- value was<0.05).

A review by **D.F.Kinane (1998)**[3] suggest that the proinflammatory cytokine IL-1β produced by monocyte can inhibit lipoprotein lipase and thus leads to hyperlipidemia. **Kinane and Lowe (2000)**[4] again reviewed same mechanism suggesting the role of IL-1β in hyperlipidemia.

Our study suggests that the hypothesis put forth by **Anthony M.Lacopino and Christopher W. Cutler (2000)**[6] 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.

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 for hyperlipidemia seen in cases of periodontitis. Results of our study show that there is significantly higher levels of IL-1β in severe chronic periodontitis as compared to healthy periodontium. It indicates that hyperlipidemia in cases of periodontitis may be due to increased IL-1β levels.

CONCLUSION

This study help us to understand the hypothesis linking periodontitis & cardiovascular disease through increase in IL-1β levels and therefore increase in serum lipids. It can further help us in increasing awareness amongst the medical faculty about the role of periodontitis as a risk factor for cardiovascular disease.

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