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# Quantitative Changes of Immune Complexes and Leukocyte Count Due to Periodontitis and their Effect on Diabetes Mellitus

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Abstract

The formation of circulating immune complexes (CIC) is a normal human response but persistence elevated level of CIC is a feature of many diseased conditions. These immune complexes produced due to Periodontitis may give rise to a variety of systemic diseases such as type 2 diabetes mellitus (DA) and rheumatoid arthritis (RA). The aim of this study was to investigate the level of CIC in serum of patients with severe chronic Periodontitis and their effect on type 2 diabetes mellitus. Polyethylene glycol (6000) method was used to isolate immune complexes from sera. The concentration of immunoglobulins was determined by radial immunodiffusion plates.

The level of neutrophils($p<=0.001$) and circulating immune complexes (IgG, IgM) in the sera of diabetic patients with Periodontitis were found to be significantly higher compared to control, Periodontitis and diabetic patients. This may suggest a bidirectional causal association between severe chronic Periodontitis and type 2 diabetes mellitus.

Key Words: Circulating immune complexes (CIC), Periodontitis, Diabetes mellitus, PEG

## Introduction

Periodontitis is defined as an inflammatory disease of the supporting tissue of the teeth caused by specific microorganisms (bacteria) resulting in progressive destruction of periodontal ligament and alveolar bone with pocket formation, recession or both. The gram negative anaerobic bacteria possess various antigens [1,2] that provoke a host mediated immune response to the offending species [2,3]. This complex immunopathogenic process involves interaction between T and B-lymphocytes, neutrophils, monocytes and phagocytes and the subsequent production of cytokines and prostaglandins. The humoral immune response, in which immunoglobin G (IgG) and IgA antibodies are produced, is considered to have a protective role in the pathogenesis of periodontal disease [2,4].

Diabetes mellitus is a clinical syndrome characterized by hyperglycemia due to the deficiency of insulin. Periodontitis is the sixth complication of diabetes mellitus [5]. Hyperglycemia in diabetic patients causes dehydration, reduce salivary flow and affects immune function that leads to periodontal destruction and infection [6,7]. The relationship between diabetes and Periodontitis has been investigated in this study and a bidirectional causal association between severe chronic Periodontitis and type 2 diabetes mellitus has been suggested.

## Material and Methods

The blood samples from 240 subjects were collected from People's Dental College and Hospital Bhopal M.P. These subjects were divided into four groups as 60 healthy control, 60 periodontitis patients (patients had at least 24 teeth and clinical attachment loss is 5mm or greater, but no history of diabetes and other systemic diseases), 60 patients with type 2 diabetes mellitus (disease duration 1-2 years, but no clinical attachment loss) and 60 diabetic patients (disease duration 2-3 years) with severe chronic Periodontitis.

Table I. Age and sex distribution of subjects

| Subjects | Male(n) | Age(mean ± SD) | Female(n) | Age(mean ± SD) |
|---|---|---|---|---|
| Control (C) | 35 | 43.4 ± 4.4 | 25 | 45.6 ± 3.5 |
| Periodontitis (P) | 42 | 49.9 ± 5.0 | 18 | 49.8 ± 5.8 |
| Diabetes (D) | 39 | 48.0 ± 5.1 | 21 | 51.5 ± 7.1 |
| Diabetes+Periodontitis ( D+P) | 42 | 50.2 ± 6.0 | 18 | 53.5 ± 5.3 |

n = number of subjects; SD= standard deviation

## Sample Collection and Storage

*Blood sampling:* A non fasting blood sample was collected from each subject into anticoagulant (heparin) containing sterile tubes for differential leukocyte count.

*Collection of serum sample:* 5-10 ml venous blood was collected into sterile tubes without anticoagulant and allowed to clot at room temperature for 2 hours. The serum sample was kept at 2-8$^{o}$C and tested within 7 days. The addition of 0.1% sodium azide was used for stabilization.

*Precipitation of Immune complexes by PEG (6000):* In this study the Circulating immune complexes (CIC) were investigated by precipitation of sera with Polyethylene glycol (PEG) [8] with minor modification. The techniques employed for immune complex detection depend on a few general principles in the region of antigen antibody equivalence of a precipitation curve, of course, the antigen antibody complex precipitate visibly. This precipitation or aggregate formation can be enhanced by cold or by the use of PEG. PEG is thought to enhance the activity of antigen antibody reactants [9]. PEG working solution was prepared by dissolving 4.2 gm of PEG (6000mw) in 100ml borate buffer working solution. Borate buffer solution contains boric acid (6.8 gm/dl), borex powder (9.4 gm/l) and sodium chloride (4.38 gm/l). The PH of the solution was adjusted to 8.4. 2ml of 4.2 % PEG was added to 0.2ml serum and the mixture was incubated at 4$^{o}$c for 2 hours and centrifuged at 2000g for 30 min. The pellet were then washed with 2% PEG and again centrifuged at 2000g for 30 min. The pellets were dissolved in 0.2 ml distilled water and diluted to 2.0 ml with 0.1 N NaOH. The concentration of CIC (immunoglobulins) is determined by radial immuno diffusion plates [10,11]. The statistical analysis was performed by using "Student t test" and one way ANOVA.

## Results and Discussion

Periodontitis patients exhibited higher number of leukocytes (WBC) when compared with control ($p<= 0.05$). This difference was attributable to higher neutrophil counts ($p<=0.01$). No significant difference was observed for monocyte, eosinophil and basophil numbers ($p>0.05$). The CIC (immunoglobulin, IgG, IgM,) concentration was elevated significantly in all four groups ($p<=0.001$). IgA in CIC was detected only in 8/60 subjects of control and 16/60 subjects of Periodontitis patients (Table II).

The concentration of WBC, neutrophils and monocytes in diabetic patients were higher compared to control ($p<0.001$). IgA was detected only in 17/60 diabetic patients. Diabetic patients with Periodontitis exhibited a greater number of leukocytes (WBC), neutrophils, lymphocytes, monocytes, eosinophils when compared with controls ($p<=0.001$). The concentration of IgG, IgM was much higher than control, Periodontitis and diabetic patients. Incidence of IgA in CIC of Diabetic + Periodontitis patients was 12/60.

Table II. Leukocyte count and Immunoglobulin concentration (CIC)

| Parameter | Group I (C) Mean ± SEM | Group II ( P) Mean ± SEM | Group III (D) Mean ± SEM | Group IV D+P Mean ± SEM | P value ANOVA |
|---|---|---|---|---|---|
| WBC,$10^9$/l | 6.93 ± 0.145 | 7.41 ± 0.14** | 8.09 ± 0.14**** | 8.327 ± 0.15**** | <=0.001 |
| Neutophils,$10^9$/l | 4.20 ± 0.073 | 4.6 ± 0.11*** | 4.72 ± 0.11**** | 5.213 ± 0.09**** | <=0.001 |
| Lympocytes,$10^9$/l | 1.97 ± 0.056 | 2.1 ± 0.04* | 2.64 ± 0.06**** | 2.552 ± 0.05**** | <=0.001 |
| Monocytes, $10^9$/l | 0.41 ± 0.015 | 0.42 ± 0.01* | 0.52 ± 0.08* | 0.49 ± 0.02**** | >0.05 |
| Eosinophils, $10^9$/l | 0.32 ± 0.0213 | 0.27 ± 0.02* | 0.28 ± 0.01**** | 0.28 ± 0.02**** | >0.05 |
| Basophils, $10^9$/l | 0.039± 0.0313 | 0.03 ± 0.002* | 0.04 ± 0.02* | 0.04 ± 0.002* | >0.05 |
| IgG, mg/dl | 7.79 ± 0.250 | 16.23 ± 0.5**** | 27.09 ±0.60**** | 29.28 ± 0.58**** | <=0.001 |
| IgM, mg/dl | 4.24 ± 0.165 | 8.04 ± 0.29**** | 18.8 ± 0.47**** | 22.86 ± 0.36 **** | <=0.001 |
| IgA, mg/dl | 0.206 ± 0.07 | 0.483 ± 0.11** | 0.492 ± 0.11** | 0.385 ± 0.10* | >0.05 |

*P value calculated by t test compared to control *>0.05, **<= 0.05, ***<=0.01, ****<=0.001, SEM standard error mean

Table III. Leukocyte count and Immunoglobulin concentration (CIC) in III & IV group

| Parameter | Group(III) Mean ± SEM | Group(IV) Mean ± SEM | Significance p-value |
|---|---|---|---|
| WBC,$10^9$/l | 8.09 ± 0.1359 | 8.327 ± 0.1463 | >0.05 |
| Neutophils,$10^9$/l | 4.72 ± 0.109 | 5.213 ± 0.085 | <=0.001 |
| Lympocytes,$10^9$/l | 2.64 ± 0.055 | 2.552 ± 0.046 | >0.05 |
| Monocytes, $10^9$/l | 0.52 ± 0.077 | 0.49 ± 0.015 | >0.05 |
| Eosinophils, $10^9$/l | 0.28 ± 0.014 | 0.28 ± 0.018 | >0.05 |
| Basophils, $10^9$/l | 0.042 ± 0.015 | 0.04 ± 0.002 | >0.05 |
| IgG, mg/dl | 27.096 ± 0.603 | 29.28 ± 0.584 | <=0.05 |
| IgM, mg/dl | 18.76 ± 0.466 | 22.86 ± 0.359 | <=0.01 |
| IgA,mg/dl | 0.492 ± 0.11 | 0.385 ± 0.10 | >0.05 |

P value calculated by using t test; neutrophils, IgG and Ig M significantly increased

## Conclusions

Most of the previous studies showed that systemic diseased patients are more susceptible to Periodontitis but there was still no sufficient evidence to claim a bidirectional causal association between Periodontitis and other systemic diseases. The present investigation supported a causal relationship between Periodontitis and diabetes. The circulating immune complexes (IgG, IgM) and neutrophils in the sera of diabetic patients with Periodontitis were found to be significantly higher compared to control, Periodontitis and diabetic patients. This increased level of neutrophils and immune complexes may be due to Periodontitis.

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