

Comparison of the Serum Immunoglobulin IgG level in Diabetic and Non-Diabetic patients of RCDSR having Periodontitis

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To evaluate and compare the serum IgG level in Diabetic and Non-Diabetic patients of RCDSR having Periodontitis” In this study, humoral immune responses were assessed in 60 patients. 15 patients each belonging to the four groups diabetic with periodontitis, diabetic without periodontitis, non-diabetic with periodontitis and non-diabetic without periodontitis (control) were analysed for the quantitative estimation of serum immunoglobulins G by Turbidometric Immunoassay. The serum of the Diabetic and non-diabetic patients was collected and Immunological assay was done by Turbidometric method by using Quantia IgG Turbidometric immunoassay for estimation of Immunoglobulin IgG in human serum, (Tulip diagnostics [P] Ltd., Goa, India). The data thus obtained were compared to the level of immunoglobulin found in clinically healthy gingiva. We estimated the sugar level by checking the Random Blood Sugar level by Glucose Test Kit based on end point and kinetic assay and also HbA1c percentage of the candidates, by using Nyco Card Reader. All the patients of Group A (Diabetic with Periodontitis) showed significant ($P < 0.01$) increase in serum IgG level as compared to controls (Group D- Non Diabetic, Non Periodontitis). Group B showed significance of $p = 0.006$. Group C showed significance of $p = 0.044$ and Group D showed significance of $p < 0.000$. With an increase in HbA1c percentage serum IgG showed significant ($P < 0.01$) increase. In the present study, the concentrations of the IgG in serum of diabetic and non-diabetic patients were found to be significantly high, when compared to the healthy subjects who had neither diabetes nor periodontitis. It was concluded that the IgG level in the serum of both diabetic and non-diabetic subjects with periodontitis were found to be significantly higher than that of healthy subjects. This study goes in accordance with the concept that the humoral immune response plays an important role in the pathogenesis of periodontal disease in diabetics. The significantly higher levels of immunoglobulin in the gingival tissues may be playing as a protective mechanism against the increased bacterial load in diabetic subjects. It may be postulated that increase in concentration of immunoglobulin in the diabetic group may be representing an enhanced response to diabetic state in periodontitis. The observations of the present study conclude the possible relationship associated with increased rate of tissue destruction in diabetic patients with periodontitis. The present study indicates that poor glycemic control may be associated with the increase in serum antibodies. Elevated antibody level explains why poorly controlled diabetes exacerbates periodontal disease.

Key words: Diabetes, periodontitis, IgG, HbA1c, Immunoassay

The link between diabetes and periodontitis has been well established and now it is well documented that persons having uncontrolled diabetes have an increased susceptibility to periodontitis. Patients with long past history of diabetes experience pathological

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changes in the tissues and organs proving that extent of diabetic complications is related to the degree of metabolic control. The major complications of diabetes, include retinopathy, nephropathy, neuropathy, and vascular degeneration, are the result of hyperglycaemia. In addition to this diabetes also causes some oral complications like xerostomia, tooth loss, gingivitis, changes in saliva's composition, taste alterations, burning mouth, tendency to buccal infections, delayed healing process, tooth decays, coated tongue, halitosis, periodontitis, odontogenic abscesses and soft tissue lesions of the tongue and oral mucosa¹. Both the types of diabetes are risk factors for periodontitis and are now a recognized complication of diabetes mellitus.

As per WHO, Diabetes Mellitus is a heterogeneous metabolic disorder, characterized by chronic hyperglycemia with disturbance of carbohydrate, fat, protein metabolism. Diabetes mellitus is a chronic disorder of carbohydrate metabolism due to inadequate endogenous production or utilization of insulin and is characterized by a tendency to hyperglycaemia. A disturbance in the metabolism of carbohydrates, proteins, and fat leading to Diabetes mellitus is a metabolic syndrome characterized by hyperglycemia^{2,3}. Insufficient insulin secretion and hepatic gluconeogenesis during Hyperglycemia is the main cause of diabetes⁴⁻⁷. Periodontal disease also induces elevation of chronic inflammatory state. It has been established that circulating immune complexes are significantly high in patients with diabetes as compared to control. Periodontitis is an inflammatory stage associated with bacterial infection and affects both the gum and the bone that supports the teeth and is caused mainly by anaerobic Gram negative microorganisms that are present in the bacterial plaque that adheres to the teeth. These bacteria produce a toxin that act locally like a stressing factor and starts the gum inflammation⁸. As the inflammation progresses, the gum gets detached from the teeth, and results in formation of periodontal pockets. In the untreated and neglected case there occurs worsening of the disease as these pockets spread and the plaque penetrates deeper, until it reaches the bone that can be destroyed with the loss of tooth support. The prevalence of periodontal disease among diabetics is well documented. Diabetes is

considered, by many authors as the sixth chronic complication. Carda et al. have performed a study with diabetic patients versus a control group and have found that 100% of diabetic patients presented periodontal disease versus 50% found in the control group⁹. Diabetic patients are more susceptible to develop periodontal disease because they present an impaired function of polymorphonuclear leucocytes, abnormalities in collagen metabolism and in the formation of final glycosylated products that adversely affect collagen stability and vascular integrity.

Diabetes is also known to alter the immune cell function. In diabetics the function of immune cells i.e., neutrophils, monocytes and macrophages usually metamorphose. During the same, neutrophilic adherence, chemotaxis and phagocytosis are altered suppressing the defence against bacteria in the periodontal pouch, which ultimately elevate the destruction of the periodontal membrane¹⁰. Proliferation of such pathogens ultimately facilitates the soft tissue deterioration. Large and deep pockets are usually developed in the periodontitis patients bacteria continuously grow, infect and inflame host tissue. Further, for periodontium, the Polymorphonuclear leukocytes (PMNs) always act as the primary defence cells. Unfortunately, in poorly controlled diabetes abnormalities in PMN functions are evidenced which makes the host more susceptible to infections¹¹. Up to far extent the implication process by which diabetes influences the periodontium is alike to the patho¹²⁻¹⁴. In other words, the bacterial biofilm alone is insufficient to explain disease initiation and progression. As per Offenbacher (1996) the periodontal tissues destruction is mainly due to the host's inflammatory response to the bacterial challenge. Besides other factors, diabetes mellitus are well characterized to modify the host response to the bacterial challenge and increases the risk for periodontal disease at any stage of life. Immuno-pathological studies have shown that, as human inflammatory periodontal disease progresses, the nature of the cellular infiltrates change¹⁵. Engebretson et al. reported that IL-1b levels in the GCF were twofold higher in diabetic patients with HbA1c levels >8% compared with those patients in whom HbA1c levels were 8%. Gingival fluid also contains antimicrobial substances including IgM, IgG, IgA, complement,

and leukocytes. These factors are primarily protective against microbial invasion, but, as seen above, the inflammation may become destructive, resulting in loss of periodontal attachment. The IgG, IgM, and IgA antibodies directed against a variety of oral microorganisms have been detected in plasma and crevicular fluid even in healthy individuals. These antibodies may influence the oral microbiota by interfering with adherence or by inhibiting bacterial metabolism. Furthermore, the IgG antibodies may enhance phagocytosis and killing of oral microorganisms through activation of complement or opsonisation. The immune response itself may contribute significantly to the periodontal destruction, sometimes even more than the pathogens. In periodontitis, the numbers of plasma cells frequently exceeds the number of infiltrating lymphocytes. The majority of plasma cells and IgG-bearing lymphocytes in periodontitis have cell-associated immunoglobulins IgG1, IgG3, or IgG4 subclasses¹⁵⁻¹⁷. An immunoglobulin test measures the level of certain immunoglobulins, or antibodies, in the blood. Antibodies are proteins made by the immune system to fight antigens, such as bacteria, viruses, and toxins. The body makes different immunoglobulins to combat different antigens. IgA, IgG, and IgM are frequently measured simultaneously. Evaluated together, they can give doctors important information about immune system functioning, especially relating to infection or autoimmune disease. Once an antibody is produced against a specific antigen, the next time that antigen enters the body; the immune system “remembers” its response and produces more of the same antibodies. In that way, checking for the presence of specific immunoglobulins in the blood can be helpful in diagnosing or ruling out infections or certain other illnesses.

Doctors also rely on the immunoglobulin test as one of the tools to help diagnose immunodeficiencies (when the immune system isn't working properly). A person can be born with an immunodeficiency or acquire it through infection, disease, malnutrition, burns, or as a side effect of medications. Immunoglobulin levels are also used as part of an evaluation for autoimmune conditions such as rheumatoid arthritis, lupus, and celiac disease.

MATERIAL AND METHODS

The study was carried out following the proper guidelines of the ethical committee of the Institute. Total 60 patients of age group 25 – 60 yrs including both genders were analyzed for their serum immunoglobulin level.

Source of data

For the study, 60 patients of both the genders were selected from the Out Patient Department of Periodontitis, Rungta College of Dental Sciences and Research, Kohka – Kurud Road, Bhilai, and Chhattisgarh.

1) The patients were screened and categorised into four groups according to their Blood Glucose level and Dental status using clinical parameters.

Group 1 Diabetic patients suffering from Periodontitis

Group 2 Diabetic patients not suffering from Periodontitis

Group 3 Non-Diabetic patients suffering from Periodontitis

Group 4 Non- Diabetic patients not suffering from Periodontitis (Control & Healthy Persons)

Clinical parameters

Following clinical parameters were recorded before commencement of the work:-

- 1) Presence of clinical inflammation
- 2) Clinical attachment loss (CAL) > 5mm – (Loe & Silness, 1963)
- 3) Probing depth > 5mm - (Silness & Loe, 1964)
- 4) Random Blood Sugar Level
- 5) HbA1c Level
- 6) Immunological analysis by Turbidometric method

a) IgG level

Collection of sample

Patient was explained previously about the procedure and written consent was taken. The blood is drawn from a vein, the skin surface where vein-puncture is to be done is cleaned with an antiseptic, and an elastic band (tourniquet) is placed around the upper arm to apply pressure and cause the veins to swell with blood. A needle is inserted into a vein (usually in the arm inside of the elbow or on the back of the hand) and blood is withdrawn and collected in a vial or syringe. After the procedure, the elastic band is removed. Once

the blood has been collected, the needle is removed and the area is covered with cotton or a bandage to stop the bleeding. Blood was withdrawn from the anterior cubital fossa using 24 gauge needles. About 5ml of blood was bled out of which 2 ml was added to anticoagulant for Random Blood Sugar and HbA1c tests and remaining was kept undisturbed for extracting serum, then centrifuged at 2000 rpm for 5 to 10 minutes to settle the erythrocytes and to finally extract and store the serum sample at 4°C till further processing.

Random blood sugar level

By Colorimetric method using serum sample using Span Diagnostic Glucose test Kit.

HbA1c Percentage

By Nycocard Reader

Immunological assay

Quantia IgG Turbidometric immunoassay for estimation of Immunoglobulin IgG in human serum, (Tulip diagnostics [P] Ltd., Goa, India) was used for Turbidimetric Immunoassay.

For IgG Estimation

Serum IgG was quantified by using above mentioned diagnostic kit. The standards used for the test was wavelength of 340 nm, reaction temperature at 37°C and cuvette of 1 cm path length. For estimation of serum IgG, the Quantia-IgG calibrator was reconstituted with exactly 1.0 ml of distilled water, wait for 5 minutes, mix the solution gently. Prepare 1.0 ml of 800 mg/dl IgG working standard from the reconstituted calibrator (888 µl) by adding saline (112 µl). Prepare dilutions of working standard for preparation of calibration curve. Take 500 µl of quantia IgG activation buffer and 5 µl of working standard in a clean cuvette. Mixed well and incubated for 5 minutes at 37 °C. Read Absorbance (A1) at 340 nm. Add 50 µl of Quantia- IgG reagent, mix gently, and wait for five

minutes. Read absorbance (A2). A calibration graph was plotted using absorbance of each dilution on the graph paper. Test Serum sample was diluted in 1:10 with normal saline. The diluted test serums were used in place of working standard and the absorbance was taken. [Fig. 1]

Calculations of immunological assay

Interpolate absorbance of diluted test serum on the calibration curve and obtain the concentration of IgG of the test serum. [Fig. 2]

Random blood sugar level

For in-vitro quantitative determination of Glucose in Human Serum / Plasma of the above serum samples of the 60 patients categorised into four groups, Glucose Test Kit was used based on end point and kinetic assay. Glucose oxidase (GOD) oxidises Glucose to Gluconic Acid and Hydrogen Peroxide. In presence of enzyme Peroxidase, released Hydrogen Peroxide is coupled with Phenol and 4- Aminoantipyrine (4-AAP) to form coloured Quinoneimine . Absorbance of coloured dye is measured at 505 nm and is directly proportional to Glucose concentration in the sample.

The 20ul of sample serum was mixed with 1500 ul of the Glucose Reagent and incubated at 37°C for 30 minutes. Then add 1500ul of distilled water and take absorbance at 490-550nm. Calculate the Serum Glucose level in mg/dl.

RESULTS

The patients in our study were in the age group of 30-50 years with the mean age being 47.60 years for Group A, 46.06 years for Group B, 43.13 years for Group C and 44.5 years for Group D with each group showing male predominance. Data were tabulated and statistically analysed using the Kruskal Wallis ANOVA ; $p < 0.000$; Sig test. Mann Whitney Comparison with Bonferroni Correction

Table 1. Statistical analysis of IgG levels between four Groups

IgG	N	Mean	SD	Median	95% CI	Min	Max
Group A	15	358.13	107.49	352.00	298.61 417.66	206	567
Group B	15	227.40	109.35	170.00	166.85 287.95	62	399
Group C	15	158.13	33.57	152.00	139.54 176.72	107	252
Group D	15	67.87	32.91	65.00	49.64 86.09	23	165
Total	60	202.88	132.39	165.00	168.68 237.08	23	567

Kruskal Wallis ANOVA ; $p < 0.000$; Sig

Table 2. Comparative Statistical analysis IgG levels between four Groups

	Group A	Group B	Group C	Group D
Group A		Sig	Sig	Sig
Group B	p = 0.006		Sig	Sig
Group C	p < 0.000	p = 0.044		Sig
Group D	p < 0.000	p < 0.000	p < 0.000	

Mann Whitney Comparison with Bonferroni Correction for $\alpha = 0.0083 (.05/6)$

Chart 1, 2 and 3: Comparative chart of serum IgG level between all 4 groups of patients

Chart Title

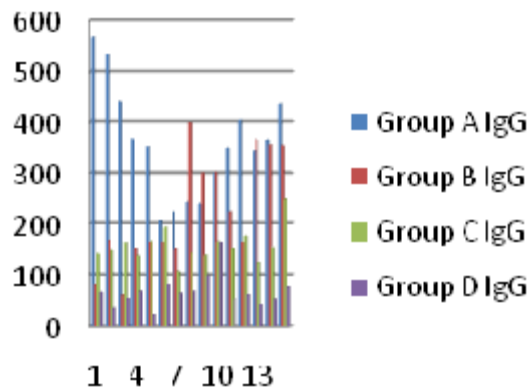


Chart 1.

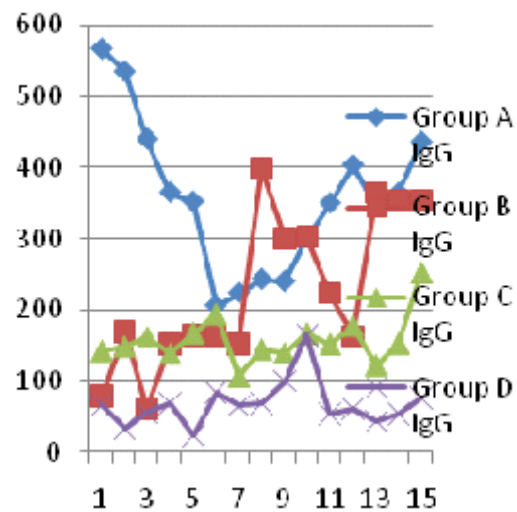


Chart 2.

IgG

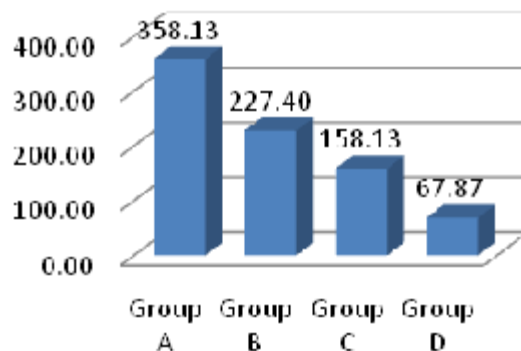


Chart 3.

for $\pm = 0.0083 (.05/6)$ was used to correlate the relationship between different parameters. Comparison of different parameters between controls and four groups was done by *t*-test. All the patients of Group A (Diabetic with Periodontitis) showed significant ($P < 0.01$) increase in serum IgG levels as compared to controls (Group D- Non Diabetic, Non Periodontitis). Group B showed significance of $p = 0.006$. Group C showed significance of $p = 0.044$ and Group D showed significance of $p < 0.000$. With an increase in HbA1c percentage serum IgG showed significant ($P < 0.01$) increase. [Table 1 and 2][Chart 1, 2 and 3]



Fig. 1. Immunological Assay

CONCLUSION

During initial investigation, we found higher levels of IgG in patients showing chronic hyperglycemia as compared to the control group. We assumed that possible immuno-inflammatory abnormalities were the underlying cause for the elevated immunoglobulins in our patients with elevated sugar level and its complications, may be documented.

There are many studies reporting association between periodontal disease and diabetes and is now well established fact that periodontal disease is more prevalent and severe in persons with diabetes than in non-diabetic patients^{18, 19}. It has also been focused that host immunological response of diabetic individuals are in compromised state. Many studies show that the polymorphonuclear leukocytes function in Type 2 diabetic patients with periodontal disease have shown incompetence in the chemotaxis and phagocytosis functions. It has been reported earlier that decreased chemotaxis, adherences, phagocytosis, and intracellular killing and are the consequences of bacterial infection. Diabetic patients with periodontitis have been shown to have depressed chemotaxis of peripheral blood leukocytes¹⁴⁻¹⁶.

High serum immunoglobulins and complements have been significantly reported in Type II diabetes patients suffering with

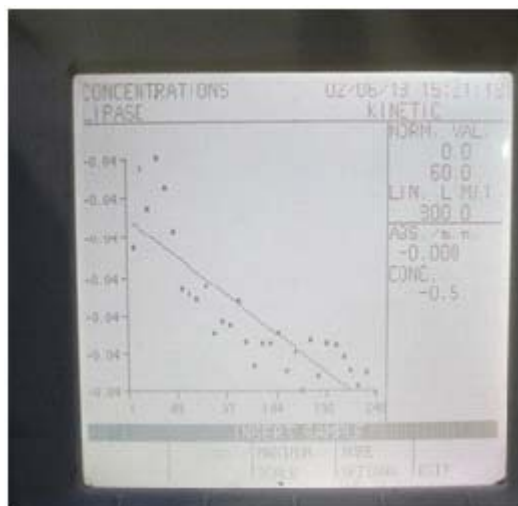


Fig. 2. Calibration graph

periodontitis. Fontana et al in their studies also observed similar findings. In the present study, the concentrations of the IgG in serum of diabetic and non-diabetic patients were found to be significantly high, when compared to the healthy subjects who had neither diabetes nor periodontitis. The results of the present study are in agreement with the results reported by Byers et al that the higher concentration of immunoglobulins IgA and IgG in inflamed human gingiva. Due to prolonged activity of bacterial antigens in the periodontitis patients there is rise in the level of Immunoglobulin, possibly due to stimulation of local production of Immunoglobulin²⁰. It may be postulated that increase in concentration of immunoglobulin in the diabetic group may be representing an enhanced response to diabetic state in periodontitis. The observations of the present study conclude the possible relationship associated with increased rate of tissue destruction in diabetic patients with periodontitis. The present study indicates that poor glycemic control may be associated with the increase in serum antibodies. Elevated antibody levels may explain why poorly controlled diabetes exacerbates periodontal disease.

Clinical significance

These findings demonstrate the importance of the immune system as well as good glycemic control, especially in patients diagnosed with periodontitis. The changes observed in

immune response may be the cause or the effect of periodontal disease in diabetic patients. The increased incidence of periodontitis in diabetic patients suggests that the alteration in immune response may contribute to the pathogenesis of periodontitis in patients with poorly controlled diabetes.

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