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ORIGINAL RESEARCH

Comparison of the Serum Immunoglobulin IgM Level in Diabetic and Nondiabetic Patients with Chronic Periodontitis

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ABSTRACT

Aim: To evaluate and compare the serum IgM level in diabetic and nondiabetic patients with chronic periodontitis.

Materials and methods: A total of 60 patients were selected for the study and divided into four groups, diabetic with periodontitis, diabetic without periodontitis, nondiabetic with periodontitis and nondiabetic without periodontitis (control) were analyzed for the quantitative estimation of serum immunoglobulins M by turbidimetric immunoassay. The serum of the diabetic and nondiabetic patients was evaluated and turbidimetric method was used for immunological assay by using Quantia IgM turbidimetric immunoassay for estimation of immunoglobulin IgM in human serum. The data for the level of immunoglobulin thus obtained were compared with clinically healthy patient taken as control. Sugar level was estimated the by checking the random blood sugar level by glucose test kit based on end point and kinetic assay and compared with the HbA1c percentage of the patients, by using NycoCard Reader.

Result: The group A patients having diabetes with periodontitis showed nonsignificant increase in serum IgM level as compared to controls and other groups. Group B showed significance of p = 0.074. Group C showed significance of p = 0.982 and group D showed significance of p = 0.520. There was significant increase in HbA1c with an increase percentage serum IgM.

Conclusion: In the present study, significantly high concentrations of the IgM in serum of diabetic and nondiabetic patients were found as compared to the healthy subjects who had neither diabetes nor periodontitis.

Clinical significance: By this study, we can emphasize on the fact of the importance of the immune system and its correlation with glycemic control, especially in patients diagnosed with periodontitis, also suggests that the alteration in immune response in poorly controlled diabetic patients may contribute to the pathogenesis of periodontitis and is the cause of increased incidence of periodontitis in patients with diabetes.

Keywords: Diabetes, Chronic periodontitis, Immunoglobulin M, HbA1c, Immunoassay.

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INTRODUCTION

Several studies reveal that persons having uncontrolled diabetes have an increased susceptibility to periodontitis. Patients having long history of diabetes show demonstrable pathological changes in the organs and tissues showing that state of diabetic complications are related to the status of metabolic control.[1] Diabetes may damage many organs of the host such as eyes, kidneys, nerves and blood vessels and these complications of diabetes are retinopathy, nephropathy, neuropathy and vascular degeneration respectively. Other than 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 mucosa.[2,3]

In diabetes mellitus there is a deregulation of carbohydrate, fat, protein metabolism leading to heterogeneous metabolic disorder, characterized by chronic hyperglycemia, due to insufficient endogenous production or utilization of insulin and leading to tendency of hyperglycemia.[4] Insufficient insulin secretion and hepatic gluconeogenesis during hyperglycemia is the main cause of diabetes. In many studies it has been proved that periodontal disease also induces elevation of chronic inflammatory state. Significantly high circulating immune complexes has been established in patients with diabetes as compared to control.[5,6] Anaerobic Gram-negative microorganisms are mainly present in periodontitis, an inflammatory stage associated with bacterial

infection. Periodontitis affects both the gum and the bone that supports the teeth. The toxin produced by these bacteria starts the gum inflammation.[7] With the progression of the inflammation, the gum gets detached from the teeth, and results in formation of periodontal pockets.[8]

In a study with diabetic patients *vs* a control group Carda et al reported that 100% of diabetic patients presented periodontal disease *vs* 50% found in the control group.[9] It is also well documented that diabetic patients are more susceptible to develop periodontal disease due to impaired function of polymorphonuclear leukocytes, abnormalities in collagen metabolism and in the formation of final glycosylated products that adversely affect collagen stability and vascular integrity.

The immune cell function is also altered in diabetes. The function of immune cells, i.e. neutrophils, monocytes and macrophages usually metamorphose in diabetics. The neutrophilic adherence, chemotaxis and phagocytosis are altered in diabetics and suppress the defence against bacteria in the periodontal pouch, which ultimately elevate the destruction of the periodontal membrane.[10] Bacteria continuously grow, infect and inflame host tissue in large and deep pockets in the periodontitis patients. The polymorphonuclear leukocytes (PMNs) always act as the primary defence cells for periodontium and in poorly controlled diabetes abnormalities in PMN functions are evident making the host more susceptible to infections.[11,12] Immunoglobulin M is the basic antibody produced by the B cells and is dominantly secreted during primary immune response. It is found mostly in the serum of the host and is detected as elevated level during acute and chronic infection. Immunopathological studies have shown that, the nature of the cellular infiltrates change as inflammatory periodontal disease progresses.[13] Antimicrobial substances including IgM, IgG, IgA, complement and leukocytes are present in the gingival fluid. These primary factors are protective against microbial invasion; the inflammation may become destructive, resulting in loss of periodontal attachment. In plasma and crevicular fluid the IgG, IgM and IgA antibodies are directed against a variety of oral microorganisms and have been detected even in healthy individuals. The oral microbiota may influence these antibodies by interfering with adherence or by inhibiting bacterial metabolism. In addition the IgM antibodies may enhance phagocytosis and killing of oral microorganisms through activation of complement or opsonization. The immune response may contribute significantly to the periodontal destruction, sometimes even more than the pathogens. The numbers of plasma cells exceeds the number of infiltrating lymphocytes in periodontitis.[14,15]

MATERIALS AND METHODS

The study was carried out following the proper guidelines of the ethical committee of the institute. Total 60 patients of age groups 30 to 50 years including both genders were analyzed for their serum immunoglobulin M level.

Source of Data

For the study, 60 patients of both the genders were selected from the outpatient Department of Periodontitis. The patients were screened and categorized 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: Nondiabetic patients suffering from periodontitis.
- Group 4: Nondiabetic patients not suffering from periodontitis (control and healthy persons).

CLINICAL PARAMETERS

Following clinical parameters were recorded before commencement of the work:

1. Presence of clinical inflammation.
2. Clinical attachment loss (CAL) ≥ 5 mm (Loe & Silness, 1963).
3. Probing depth ≥ 5 mm (Silness & Loe, 1964).
4. Random blood sugar level.
5. HbA1c level.
6. Immunological analysis by turbidimetric method.
7. IgM level.

COLLECTION OF SAMPLE

Patient was explained previously about the procedure and written consent was taken. Blood was withdrawn from the anterior cubital fossa using 24 gauge needles. About 5 ml of blood was bleed out of which 2 ml was added to anticoagulant for HbA1c and other hematological tests and remaining was kept undisturbed for extracting serum, then centrifuged at 2,000 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 IgM turbidimetric immunoassay for estimation of IgM in human serum (Tulip diagnostics [P] Ltd., Goa, India) was used for turbidimetric immunoassay.

For IgM Estimation

For estimation of serum IgM, Quantia IgM calibrators were reconstituted with exactly 1.0 ml of distilled water, wait for 5 minutes, and mix the solution gently. Prepare 1.0 ml of 80 mg/dl IgM working standard from the reconstituted calibrator (800 µl) by adding saline (200 µl). Prepare dilutions of working standard for preparation of calibration curve. Take 500 µl of Quantia IgM activation buffer and 5 µl of working standard in a clean cuvette. Mix well and incubated for 5 minutes at 37°C. Read Absorbance (A1) at 340 nm. Add 50 µl of Quantia IgM reagent, mix gently and wait for 5 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.

CALCULATIONS OF IMMUNOLOGICAL ASSAY

Interpolate absorbance of diluted test serum on the calibration curve and obtain the concentration of IgG of the test serum.

RANDOM BLOOD SUGAR LEVEL

For *in vitro* quantitative determination of glucose in human serum/plasma of the above serum samples of the 60 patients categorized 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 colored quinoneimine. Absorbance of colored dye is measured at 505 nm and is directly proportional to glucose concentration in the sample.

The 20 µl of sample serum was mixed with 1,500 µl of the glucose reagent and incubated at 37°C for 30 minutes. Then add 1, 500 µl of distilled water and take absorbance at 490 to 550 nm. Calculate the serum glucose level in mg/dl.

RESULTS (TABLES 1A AND B, GRAPHS 1A AND B)

The patients in our study were in the age group of 30 to 50 years in each group and the mean age was 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 analyzed using the Kruskal-Wallis ANOVA; $p < 0.000$; significant test. Mann-Whitney comparison with Bonferroni correction for $\alpha = 0.0083$ (0.05/6) was used to correlate the relationship between different parameters. Comparison of different parameters between controls and four groups was done by t-test. The patients of group A having diabetes with periodontitis showed nonsignificant increase in serum IgM level as compared to controls and other groups. Group B showed significance of $p = 0.074$. Group C showed significance of $p = 0.982$ and group D showed significance of $p = 0.520$. There was significant increase in HbA1c with an increase percentage serum IgM. IgM response was found positive in and raised in group A patients may be due to the fact that severe chronic infections may develop in immunocompromised hosts with lymphocyte or gamma-globulin deficiencies which suggest that both cell-mediated and humoral immune responses are involved in resolution of infections and development of protection.

DISCUSSION

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

Tables 1A and B: Statistical analysis of IgM level among all the four groups

(A)

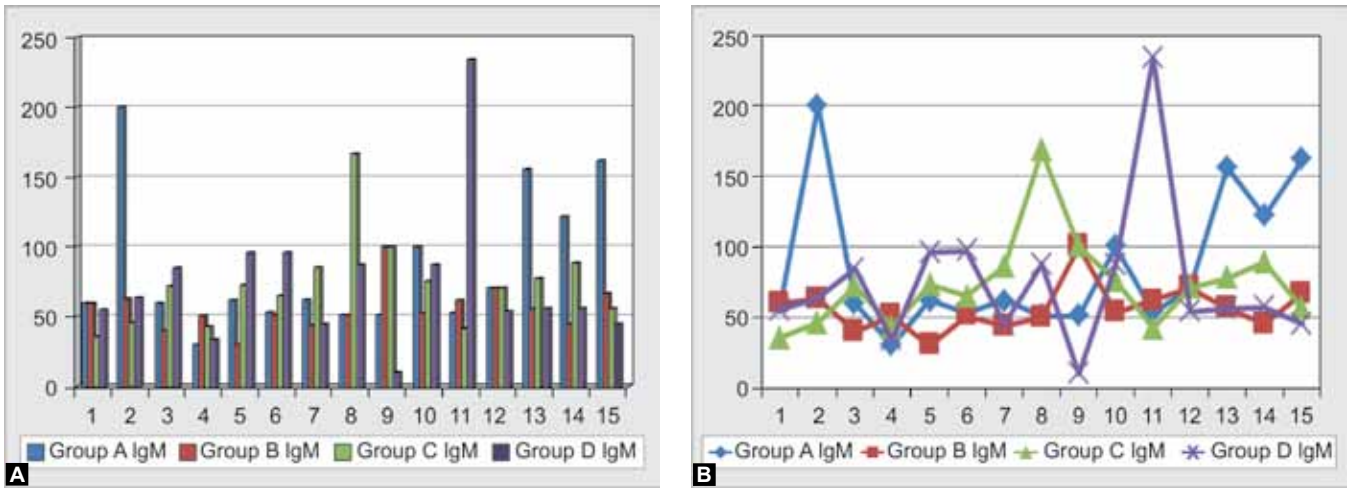
IgM	*N*	*Mean*	*SD*	*Median*	*95% CI*		*Minimum*	*Maximum*
Group A	15	86.20	50.47	62.00	58.25	114.15	30	200
Group B	15	56.20	16.20	53.00	47.23	65.17	30	100
Group C	15	73.40	32.21	72.00	55.56	91.24	36	168
Group D	15	73.87	50.69	57.00	45.79	101.94	10	234
Total	60	72.42	40.48	61.00	61.96	82.87	10	234

Kruskal-Wallis ANOVA; p = 0.247; Not significant

(B)

	Group A	*Group B*	*Group C*	*Group D*
Group A		S	S	S
Group B	p = 0.074		S	S
Group C	p = 0.983	p = 0.068		S
Group D	p = 0.520	p = 0.280	p = 0.740	

Mann-Whitney comparison with Bonferroni correction for α = 0.0083 (0.05/6); S: Significant



Graphs 1A and B: Comparative chart between serum IgG percentages in all four groups of test samples

nondiabetic patients.[16,17] It has also been established that host immunological response of diabetic individuals are in compromised state. Many studies show that in type 2 diabetic patients with periodontal disease, polymorphonuclear leukocytes functions have shown incompetence in the chemotaxis and phagocytosis functions. Decreased chemotaxis, phagocytosis, adherences and intracellular killing and are the consequences of bacterial infection. Diabetic patients with periodontitis have been shown to have depressed chemotaxis of peripheral blood leukocytes.[18-20] In type II diabetes patients suffering with periodontitis, high serum immunoglobulins and complements have been significantly reported. The studies of Fontana et al and in the present study, we observed similar findings, the concentrations of the IgM in serum of diabetic and nondiabetic patients were found to be significantly high, when compared to the healthy subjects who had neither diabetes nor periodontitis. There is rise in the level of immunoglobulin due to prolonged activity of bacterial antigens in the periodontitis patients. This may be due to stimulation of local production of immunoglobulin. It may be stated that increase in concentration of immunoglobulin in the diabetic group may be representing an enhanced response to diabetic state in periodontitis. The present study also goes along with the study conducted by Carda et al who reported that 100% of diabetic patients presented periodontal disease; in this study also significant correlation between diabetes and periodontitis is seen.

CONCLUSION

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. By observing the high antibody levels in the test serums it can be stated that 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. Immunoglobulin M being the natural antibody produced by the B cells and is dominantly secreted during primary immune response. It is found mostly in the serum of the host and is detected as elevated level during acute and chronic infection. It is helpful in activating the complement system and also contributes to opsonization. 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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