Comparative evaluation of serum Immunoglobulin in the Diabetic and non-diabetic patients having Periodontitis.

Introduction:

In the Diabetic population some people appear to have elevated Immunoglobulin levels under the influence of some environmental factors and also due to the high burden of pathogens as people having high sugar level become prey for the pathogens very easily. In the present scenario it has been cleared that Periodontitis is considered as sixth complication of Diabetes and several studies have revealed regarding numerous alterations in oral flora as well as in the serum immunoglobulin levels as ascribed to diabetics. Bacteria that reside in the mouth shows the capability of infecting tissues surrounding the tooth leading to inflammation around the tooth and eventually resulting in to periodontal diseases (Marsh, 2000). The major etiology of the Periodontitis is the microorganisms that adhere to and grow on the tooth’s surfaces also involving aggressive immune response against these microorganisms (Baelum and Rodrigo, 2003). Several environmental and systemic factors along with the nature of agents (bacteria) systematize why people have varying susceptibility to periodontitis. Indeed, the combination of some risk factors such as diabetes, stress, smoking, neutrophil dysfunction, hyperlipidemia, hormonal, health care, socio-economic status, oral hygiene, diet and alcoholism confer susceptibility to periodontitis and which also varies from person to person depending on
one’s self ‘power’ (resistance) at different times and different place throughout a person’s life (Clarke and Hirsch, 1995).

People with diabetes are always at high risk of developing infections, including gum disease (Javed et al., 2005). Evidences show that periodontal disease may be associated with systemic diseases. The interrelationship between diabetes and periodontal disease is established through a number of pathways and is also bidirectional (Tan Wc et al., 2006; Grossi and Genco, 1998). Since diabetes impairs the immune defence mechanism so the patients are more susceptible to infection and thus the infections in diabetics are more severe when compared with non-diabetics (Mealey, 1999). Periodontitis causes an infectious burden in the host through bacteria and their products enter the systemic circulation and may result into health hazards such as atherosclerosis and cardiovascular diseases (Susanto et al., 2012). Periodontitis also play an important role in inducing impaired blood glucose control, there are evidences showing that periodontitis may induce insulin resistance (Taylor, 1999; Mealey, 2006; Li et al., 2000). Patients suffering from Periodontal disease are always having complicated blood sugar level and such high blood sugar levels always worsen the gum disease (Negrato et al., 2010; Taylor and Borgnakke, 2008; Carda et al., 2006).

All blood cells play an important role in the maintenance of a healthy periodontium but the circulating oral bacteria and lipopolysaccharides are able to stimulate hepatocytes to secrete Immunoglobulins and its isotype. Thus, recorded increased levels of immunoglobulins associated with periodontitis are perhaps due to inflammatory and infectious burden resulting from periodontitis (Pishdad and Faghiri, 1995). Further, the increase in antibody titer is the result of a host exposure to an antigen. Certain pathogens such as Actinobacillus actinomycetemcomitans can only be controlled by neutrophils when opsonised by IgG isotype. As stated earlier, the B cell produces Immunoglobulin isotype that can be divided into the different classes of Immunoglobulin like IgG, IgA, IgM, IgD and IgE. Amongst these Immunoglobulins, IgA is considered as anti-inflammatory isotype, IgM are produced in primary Immune response and IgG helps in opsonisation process (Ahmed and Gray, 1996; Walsh, 2003). To survive in the periodontal environment bacteria must neutralize and evade the host mechanisms involved in bacterial clearance and killing and to accomplish the same, periodontal bacteria are well equipped with many mechanisms. The immunoglobulins secreted by the host to facilitate phagocytosis of the periodontopathogens are degraded by the immunoglobulin - degrading proteases secreted by specific microorganisms. Patients having periodontitis often exhibit very high serum titers of IgG to some specific pathogens. Variability in the levels, types and strength of binding of the antibody were evident in different patient and the significance of the antibody were found to depend on its functional capabilities (Aukhil et al., 1988; Baker and Wilson, 1989).
Many systemic diseases and disorders have been implicated as risk indicators in periodontitis and for the same diabetes mellitus is an extremely important disease from a periodontal standpoint. The most striking feature in diabetes is the reduction in defense mechanisms and increased susceptibility to infections leading to destructive periodontal disease (Adler et al., 1973; Ainamo et al., 1990). An alteration in host responses and collagen metabolism clearly demonstrates the relation between diabetes and periodontitis. The prolonged tissue exposure to hyperglycemia which results in the production of advanced glycation end products (AGEs) plays an important part in the development of diabetic complications. This results in an increase in collagen cross-linking and the generation of free radicals. The thickening of the basement membrane occurs due to accumulation of modified collagen fibers in the tissues. This impairs oxygen diffusion, waste elimination, leukocyte migration and the diffusion of immune factors and may thereby contribute to the pathogenesis of periodontitis thereby enhancing the inflammatory response, leading to connective tissue damage, bone resorption and delayed wound repair. Immunoglobulin-G antibodies that are produced in response to the periodontopathogens are found in high titers in patients suffering with periodontitis, this confirms the subgingival colonization by the specific bacteria (Gunsolley et al., 1990; Nakagawa et al., 1994). Elevated serum IgG levels are demonstrated against P. gingivalis, A.actinomycetemcomitans and T. forsythensis in adult patients with periodontitis compared to subjects without disease (Kinane et al., 2000; Persson et al., 2000).

The aim of this study is to assess serum immunoglobulin levels (IgA, IgG and IgM) in non-insulin dependent diabetic (poor control and better control) and non-diabetic subjects with chronic periodontitis.

Materials and Methods:

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

Sources of Data:

For the study, 60 patients considering 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. The patients were further screened and categorized into four different groups, based on their respective Blood Glucose level and Dental status using prescribed clinical parameters.
Group A: Diabetic patients suffering from Periodontitis
Group B: Diabetic patients not suffering from Periodontitis
Group C: Non-Diabetic patients suffering from Periodontitis
Group D: 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) i.e., proper binding of gums with teeth, ≥ 5mm - (Loe and Silness, 1963)
3) Probing depth i.e., how much depth developed between tooth and gum ≥ 5mm - (Silness and Loe, 1964)
4) Random Blood Sugar Level
5) HbA1c (Glycosylated Haemoglobin) Level
6) Immunological analysis by Turbidometric method
   a) IgG level
   b) IgM level
   c) IgA level

Collection of Samples:
Patients were made aware about the study procedure and proper written consents were taken from them. Blood samples of the patients were taken from the anterior cubital fossa using 24 gauge needles. In each case, about 5ml was bled out of which 2ml was added to anticoagulant for Random Blood Sugar and HbA1c tests and remaining sample was kept undisturbed for extracting serum. The serum was extracted by centrifuging the blood sample at 2000 rpm for 5 to 10 minutes then allowed to settle the erythrocytes and finally stored the serum sample at 4°C till further processing.

Random Blood Sugar Level:
It was estimated by adopting the Colorimetric method (Dubois et al., 1951) using serum sample employing Glucose test Kit of Span Diagnostic Ltd (Surat, India).

Colorimetry is used in biochemical investigations involving the quantitative estimation of colours. The principle of colorimetry is to incorporate properties of the human colour vision system into the measurement and specification of visible light. This light is recorded
by a colour camera and stored in digital form. The digital image is processed by a computer and rendered on a colour monitor and the image is viewed by a human observer. If the quantity of a substance in a mixture is to be measured, the use of technique of colorimetry can be used, by allowing the substance to bind with colour forming chromogens. The difference in colour results in the difference in the absorption of light.

**Apparatus:**

The instrument used for colorimetry is colorimeter. This apparatus comprises of the following parts:

1. **Light source**
2. **Filter** (the device that selects the desired wavelength)
3. **Cuvette chamber** (the transmitted light passes through compartment wherein the solution containing the coloured solution are kept in cuvette, made of glass or disposable plastic)
4. **Detector** (this is a photosensitive element that converts light into electrical signals)
5. **Galvanometer** (measures electrical signal quantitatively)

According to Beer’s law when monochromatic light passes through the coloured solution, the amount of light transmitted decreases exponentially with increase in concentration of the coloured substance.

\[ I_t = I_o e^{-KC} \]

According to Lambert’s law the amount of light transmitted decreases exponentially with increase in thickness of the coloured solution.

\[ I_t = I_o e^{-kt} \]

Therefore, together Beer-Lambert’s law is:

\[ \frac{I_E}{I_o} = e^{-KCT} \]

where,

- \( I_E \) = intensity of emerging light
- \( I_o \) = intensity of incident light
- \( e \) = base of neutral logarithm
- \( K \) = a constant
- \( C \) = concentration
- \( T \) = thickness of the solution

**Normal Range of Random Blood Sugar** - 100-150 mg/dl
Steps for operating the photoelectric colorimeter:

1. Choose the glass filter recommended in the procedure and insert in the filter.
2. Fill two of the cuvette with blank solution to about three-fourth and place it in the cuvette slot.
3. Switch on the instrument and allow it to warm up for 4 - 5 minutes.
4. Adjust to zero optical density.
5. Take the test solution in another cuvette and read the optical density.
6. Take the standard solution in varying concentration and note down the optical density as S1, S2, S3, S4 and S5 and so on.
7. A graph is plotted taking concentration of standard solution versus the optical density.
8. From the graph the concentration of the test solution or the unknown solution can be calculated.

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 serum sample 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.

HbA1c Percentage:

By Nyco Card Reader: the NycoCard Reader is a small battery powered instrument, designed to measure all NycoCard products. It consists of two units: the instrument box, which is the operational and calculating unit, and the Reader pen, which detects the signal.

Patented measurement principle:

Each NycoCard test comprises a unique Test Device where the test is performed. The device has a central well for sample and reagent application, which is shaped to fit the Reader pen. When the pen is placed over the sample and the pen sleeve is pushed down, the measurement starts automatically and the test result appears on the display.

The NycoCard HbA1c test is a three minute Point of Care test for the measurement of glycated hemoglobin. NycoCard HbA1c provides an accurate and reliable method to monitor metabolic control in people with diabetes.
**Test specific information**

- Sample volume: 5 µL
- Assay time: 3 minutes
- Sample material: Capillary blood or anti-coagulated venous blood (EDTA, citrate or heparin)
- Measuring range: 4 - 15% HbA1c
- Reports DCCT aligned values
- NGSP-certified method
- Boronate affinity test principle
- No interference from Hb variants like HbC, HbE, HbF, HbJ and HbS
- No interference from carbamylated Hb
- Kit size: 24 tests

**Clinical use of NycoCard HbA1c**

- Improved diabetes management
- Better metabolic control
- Reduction in late complications
- Fewer hospital admissions
- Healthcare cost savings

Nyco Card Reader was used for in vitro measurement of glycated hemoglobin in human blood. Nyco Card HbA1c is a boronate affinity assay. The blood added to the reagent (Glycinamide buffer containing Zn ions, dye bound boronic acid and detergents), the erythrocytes immediately lyses. All hemoglobin precipitates. The boronic acid conjugate binds to the cis-diol configuration of glycated hemoglobin. An aliquot of the reaction is added to the test device (Plastic device containing a membrane filter), and all the precipitated hemoglobin, conjugate-bound and unbound, remains on the top of the filter. Any excess of coloured conjugate is removed with the washing solution (Morpholine buffered NaCl solution and detergents). The precipitate is evaluated by measuring the blue (glycated hemoglobin) and the red (total hemoglobin) colour intensity with the Nyco Card Reader, the ratio between them being proportional to the percentage of HbA1c in the sample.
**Immunological Assay:**

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

Quantia Ig is a diagnostic reagent for laboratory use. It contains ready to use Quantia-Ig activation buffer (R1), ready to use Quantia-Ig antihuman Ig antibody reagent (R2), Quantia - Ig calibrator containing lyophilized preparation of serum equivalent to the stated amount of Ig on a mg/dl basis, when hydrated appropriately. The Quantia - Ig calibrator is traceable to CRM 470. The test specimen is mixed with the activation buffer (R1) and then with antihuman IgG antibody reagent (R2) and allowed to react. Presence of Ig in the test specimen results in the formation of an insoluble complex producing a turbidity, which is measured at wavelength 340 nm. The extent of turbidity corresponds to the concentration of Ig in the test specimen.

**Principle:**

The method is an in-vitro diagnostic reagent based on Turbidimetric immunoassay for the detection of Immunoglobulin in human serum and is based on principle of agglutination reaction. The test specimen is mixed with the activation buffer and then with Anti - human Immunoglobulin reagent and allowed to react. Presence of Immunoglobulin in the test specimen results in formation of an insoluble complex producing a turbidity, which is measured at wavelength 340 nm. The extent of turbidity corresponds to the concentration of Immunoglobulin in the test specimen.

1) 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.

2) For IgM estimation: for estimation of serum IgM, a similar procedure was used except that instead Quantia- IgM calibrator was reconstituted with exactly 1.0 ml of distilled water, wait for 5 minutes, 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 25 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- IgM 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.

2) For IgA estimation: for estimation of serum IgA, a similar procedure was used except that instead Quantia- IgA calibrator was reconstituted with exactly 1.0 ml of distilled water, wait for 5 minutes, mix the solution gently. Prepare 1.0 ml of 144 mg/dl IgA working standard from the reconstituted calibrator (900 µl) by adding saline (100 µl). Prepare dilutions of working standard for preparation of calibration curve. Take 500 µl of quantia IgA activation buffer and 10 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- IgA 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.

Immuno - IgG/ IgM/ IgA estimation - units in mg/dl

Statistical Analysis:

To compare the overall differences among the studied parameters i.e., HbA1c, IgG, IgA and IgM in four different groups, Two-way ANOVA has been employed. Further Duncan’s multiple range test were employed to compare the level of differences in each parameter among four different groups.

Interpretation of the result:-

A calibration graph was plotted using absorbance of each dilution on the graph paper. Interpolate absorbance of diluted test serum on the calibration curve and obtain the concentration of IgG, IgA and IgM respectively. Calculate “A for each dilutions of the working standard (D1 to D5) by following formula:-

“A = A2 - A1

Plot a graph of “A Vs concentration of respective Immunoglobulins on a graph paper, taking dilutions on X-Axis and concentration in mg/dl on Y-Axis. This graph acted as calibration standard curve. Then interpolate the value of absorbance “A of test serum on the graph and calculate the level of Immunoglobulin in mg/dl. Multiply Immunoglobulin concentration ‘c’ with appropriate dilution factor (F) of the test.

Concentration of Ig in mg/dl (Test) = C x F

Normal Range in Healthy person:-

a) IgG- Person above 18 yeays- 767 - 1590 mg/dl
b) IgA- Person above 18 yeays- 90 - 450 mg/dl
c) IgM- Person above 18 yeays: 37 - 286 mg/dl

Absorbance of coloured dye is measured at 505 nm and is directly proportional to Glucose concentration in the sample. Calculate the Serum Glucose level in mg/dl.

Normal Range in Healthy person:
Random Blood Sugar value - 100 - 150 mg/dl.

For HbA1c level:
The result of concentration of glycosylated haemoglobin of test serum samples was interpreted in gm percentage.

3 - 6 % - Non- Diabetic
6 - 9 % - Diabetic (controlled)
More than 9 % - Diabetic (uncontrolled)

Results:
Results are summarized in Table and Figure 1-4.

1) Glycated haemoglobin HbA1c: Results of ANOVA revealed highly significant (<0.0001) ‘p’ value against overall tabulated parameters. The levels of HbA1c were noted in between 9 and 10 for both the groups having diabetic patients i.e., group ‘A’ (Diabetic patients suffering from Periodontitis) and group ‘B’ (Diabetic patients not suffering from Periodontitis) and both the the groups are not statistically differing from each other at 5% level. Further the group ‘C’ (Non-Diabetic patients suffering from Periodontitis) is significantly differed from group ‘A’ and group ‘D’ (healthy/normal or control). However group ‘D’ i.e., the control one highly differed from each other.

2) Immunoglobulin-G: Results of ANOVA revealed highly significant (<0.0001) ‘p’ value against overall tabulated parameters. The levels of IgG varied highly among each group of study. Highest value was recorded against group ‘A’ i.e., Diabetic patients suffering from Periodontitis and lowest in the control one. Thus, each group statistically differed highly from the others.

3) Immunoglobulin-A: Results of ANOVA revealed highly significant (<0.0001) ‘p’ value against overall tabulated parameters. The levels of IgA were found to be high in group ‘A’ (Diabetic patients suffering from Periodontitis) group ‘B’ (Diabetic patients not suffering from Periodontitis) and group ‘C’ (Non-diabetic patients suffering from Periodontitis) but the values are not differing statistically from each other. But, the values of all these
Figure: 1- Comparative HbA1c values among four studied groups.

Table: 1- One way ANOVA, showing overall HbA1c results

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<th>Mean Square</th>
<th>F</th>
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<td>HbA1c</td>
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<td>Between Groups</td>
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<td>Within Groups</td>
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<td>56</td>
<td>2.433</td>
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<td>Total</td>
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Figure: 2- Comparative IgG values among four studied groups.

Table: 2- One way ANOVA, showing overall HbA1c results

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<td>Between Groups</td>
<td>674035.383</td>
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<td>6429.943</td>
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Figure: 3 - Comparative IgA values among four studied groups.

Table: 3 - One way ANOVA, showing overall HbA1c results

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<td>Total</td>
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Figure: 4- Comparative IgM values among four studied groups.

Table: 4- One way ANOVA, showing overall HbA1c results

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groups are statistically highly differed from the value obtained against the normal/healthy controlled group ‘D’.

4) **Immunoglobulin-M:** ANOVA do not revealed any significant results (p<0.0.25) against overall tabulated parameters. Miscellaneous type of results in the levels of IgM has been obtained against each studied group. The highest level of IgM was occurred against group ‘A’ (Diabetic patients suffering from Periodontitis) and which statistically differed from the results obtained against group ‘B’ (Diabetic patients not suffering from Periodontitis). Further the values obtained against group ‘C’ (Non-diabetic patients suffering from Periodontitis) and group ‘D’ (Normal/healthy or Control) did not differed from each other nor from any other group.

**Discussion:**

Diabetics are more susceptible to the infections than the non-diabetics (healthy) ones, suggesting the immunologic capability may be unbalanced in diabetics (Toplak et al., 1996). It is now a well established fact that there exists a tight association between periodontal disease and diabetes, though the underlying mechanism has yet to be defined. Human periodontal disease is a chronic, inflammatory disease in which immune complexes are present in the serum. Sera from the patients with periodontal disease were found, however, to lack significant levels of circulating immune complexes. Till date various attempts has been taken to monitor host immunological response of diabetic individuals. Periodontitis is associated with alterations in immune responses in both diabetic and non-diabetic subjects. While diabetes is considered to be a risk factor for periodontal disease progression, few studies have demonstrated an association between the level of glycemic control and periodontal disease.

Excess circulating glucose in diabetes is a reactant molecule, involved in the glycosylation of various biomolecules like hemoglobin. This process continues slowly throughout the lifespan of the erythrocyte (Distiller and Zail, 1979). The higher the percentage of circulating HbA1c in the diabetes, poorer is the mean diabetic control. According to World Health Organization (WHO) HbA1c level of 7% or higher is taken diagnostic of diabetes (Peter et al., 1996). Our results evidenced that the level of glycosylated hemoglobin molecule (HbA1c) is comparatively very high in the group of both the patients; diabetics suffering from periodontitis and also diabetics not suffering from periodontitis. The result is highly comparable with the healthy control group i.e., subjects not at all suffering from both the referred diseases and also the group of non-diabetics patients suffering from periodontitis. It strengthened the general concept that irrespective to the periodontitis conditions, the disease diabetes alone is capable to increase HbA1c level. Our results support the earlier finding where chronic periodontitis
was found to be associated with a slight elevation in glycosylated hemoglobin (Rajan, et al., 2013). Further the results are also found to be consistent with earlier findings where chronic periodontitis was reported to be associated with elevated blood glucose in adults without diabetes and may increase one’s risk for type-2 diabetes (Wolff et al., 2009), as the group of non-Diabetic patients suffering from Periodontitis is significantly differed from the control (normal healthy) group.

A number of studies have indicated that cell-mediated immunity is depressed in the diabetic patient (MacCrush et al., 1974; Plouffe et al., 1978; Eliashiv et al., 1978). Periodontal disease lesions reportedly contain immunoglobulin-producing cells which manifest restrictive IgG subclass antibody responses. 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 of either IgG1, IgG3, or IgG4 subclasses (Mackler et al., 1978 a,b). Earlier studies revealed that, insulin do not reflect any effect in Immunoglobulin level in Diabetics as there were no significant differences in immunoglobulin levels between insulin-treated and non-insulin-treated diabetic groups. In our study we found the IgG level was highly elevated in Diabetic patients suffering from Periodontitis (368.13 Average), further the level was also seen to rise in the group of Diabetic patient not suffering from Periodontitis (227.40 Average). However, the IgG was also found to be too high in non-diabetic patients also suffering from periodontitis. Conclusively, in any clinical problem the IgG level was found to elevated which were extreme in the group of patients, suffering from both the diseases, diabetes and periodontitis.

Studies with evaluation of either serum or salivary quantification of immunoglobulins have provided varying results. Various studies evidenced increased serum Immunoglobulin-G, Immunoglobulin-A and Immunoglobulin-M in patients with periodontitis (Ołœanska-Seidlová et al., 1989; Anil et al., 1990; Wilton et al., 1992), regardless others reported no significant differences in serum Ig levels between periodontitis patients and healthy individuals (Ranney et al., 1981; Bokor and Bratic 1998; Srinivasan, 2012). Following the general rule the IgA (immunoglobulins-A) content in serum of any person suffering from any disease it was found to be highly elevated in all the studied groups of patients, either suffering from either Periodontitis, Diabetes or both the diseases and such groups statistically highly differed from the healthy control group. Nevertheless, we found an anomalous result when we compared (immunoglobulins-M) contents in all of our studied groups. Practically there were no difference in IgM level among all the studied group except the Group-B (Diabetic patients not suffering from Periodontitis) where we found very low level of IgM which significantly differed from the IgM level of Group-A (Diabetic patients suffering from Periodontitis). Anil et al., (1995) also reported little elevation of IgM in diabetics; the level was not significantly differed from the other studied group of
patients suffering from periodontitis. It is evident that poor glycemic control may be associated with the increase in IgA and IgG serum antibodies (Awartani, 2010), while the case of IgM is still remain unpredictable. Elevated antibody levels may explain why poorly controlled diabetes exacerbates periodontal disease. Many studies conclude that impaired IgM function may be caused by non-enzymatic glycation in diabetes mellitus with possible consequences for host resistance in the early phase of infection and may be because of this reason given by Hammes et al. (1990), the patients with high HbA1c percentage showed elevated levels of IgM in the present study. In our study it can be concluded that there was positive co-relation between the two parameters: elevated HbA1c and Immunoglobulin level with periodontitis.

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.

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 opsonisation. 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.

Elevation of immunoglobulin A may occur in monoclonal gammopathies such as multiple myeloma, primary systemic amyloidosis, monoclonal gammopathy of undetermined significance, and related disorders. In addition to a possible dysfunctional humoral response to infection, there is still the elevation of IgA levels in diabetic patients. It is possible that these patients have subclinical infections, or that the elevated IgA levels are secondary to a metabolic disturbance of diabetes. Immunoglobulins G, A, and M have been reported to be present in statistically significant higher levels in long-lasting (months of) poor control diabetic patients compared to healthy controls (Pishdad and Faghiri, 1995).