

# Co-relating HbA1c and serum IgA in diabetic and non-diabetic patients with and without periodontitis

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## ABSTRACT

**Objective:** Diabetics are more susceptible to the infections than the non-diabetics (healthy) one, suggesting that the immunologic capability may be unbalanced in diabetics. Periodontitis is associated with alterations in immune responses in both diabetic and non-diabetic subjects. Till date various attempts has been taken to monitor host immunological response of diabetic individuals. Therefore the need to study the local immune response in inflamed gingival showing Periodontitis is apparent. Since till date there are no well documented and significant baseline studies are available on the functional capacities of the lymphoid cells present in gingiva of the periodontitis patients, a study to check the level of immunoglobulin was investigated. **Methodology:** A study was carried out where HbA1c, Random Blood Sugar and Serum IgA level were assessed in 60 patients from the OPD of RCDSR. 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 Immunoglobulin A by Turbidometric Immunoassay and HbA1c was done by using Nyco card Reader. **Result:** The data thus obtained were compared with the level of immunoglobulin found in clinically healthy gingival of the control group. 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 groups are statistically highly differed from the value obtained against the normal/healthy controlled group 'D'. **Conclusion:** In our study it can be concluded that there was positive co-relation between the two parameters: elevated HbA1c and Immunoglobulin level with periodontitis. This co-relates to the possibility of an underlying immuno-inflammatory abnormality to be the cause for the elevated level of immunoglobulin in our patients with elevated sugar level.

**Key words:** Diabetes, Periodontitis, IgA, HbA1c, Immunoassay

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## INTRODUCTION

Diabetics are more susceptible to the infections than the non-diabetics (healthy) ones, suggesting the immunologic capability may be unbalanced in diabetics.<sup>1</sup> A close correlation exists between periodontal disease and diabetes, but the underlying mechanism is still not very well documented. Periodontitis is seen to be associated with functional alterations in immune responses in both diabetic and non-diabetic subjects. Many studies have proved that diabetics

in their life span show positive signs and symptoms of periodontitis. Recent studies have demonstrated a tight association between the level of glycemic control and periodontal disease. In diabetes, excess circulating glucose is seen to be involved in the glycosylation of haemoglobin and this process continues slowly throughout the lifespan of the erythrocyte.<sup>2</sup> 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.<sup>3</sup>

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Studies from different parts of the world revealed the fact that significant correlation was found between the HbA1c and the random plasma glucose levels.<sup>4</sup>

Being the second most common immunoglobulin in human serum (after IgG) and also the predominant immunoglobulin found in mucosal secretions, IgA is the most studied and investigated immunoglobulin. Many of the investigators have concluded that more IgA is actually produced than any other immunoglobulin because most of it is lost in secretions.<sup>5</sup> Akinlade and his team in a study concluded that the test patients when compared to control group showed elevated level of serum IgM and IgA in Type 2 diabetes.<sup>6</sup> It is evident that poor glycemic control may be associated with the increase in IgA and IgG serum antibodies,<sup>7</sup> while the case of IgM still remains unpredictable. Studies conducted in 70's 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.<sup>8</sup>

Many studies have been conducted to evaluate either serum or salivary immunoglobulin but it provides varying results.<sup>9</sup> Similar studies have evidenced increased serum Immunoglobulin-G, Immunoglobulin-A and Immunoglobulin-M in periodontitis patients,<sup>10,11</sup> while others reported no significant differences in serum immunoglobulin levels between periodontitis patients and healthy individuals.<sup>12,13</sup> The changes observed in immune response may be the cause or the effect of periodontal disease in diabetic patients. The increased incidence of periodontitis and gingival inflammation in patients showing hyperglycaemia suggests that the contribution of alteration in immune response leading to pathogenesis of periodontitis.

An immunoglobulin test measures the level of certain immunoglobulin, or antibodies, in the blood. The body makes different immunoglobulin 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.<sup>14,15</sup> 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 immunoglobulin 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 immuno-deficiencies (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.

## MATERIALS 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) > 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 Turbidometric method
- 7) IgA level

### Collection of sample

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

#### Immunological assay

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

##### 1) For IgA Estimation

Serum IgA 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 IgA, Quantia- IgA calibrator was reconstituted with exactly 1.0 ml of distilled water, wait for 5 minutes, mixed 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.

#### Calculations of immunological assay

Interpolate absorbance of diluted test serum on the calibration curve and obtain the concentration of IgA 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 categorised into four groups, Glucose Test Kit was used based on end point and kinetic assay. The 20 ul of sample serum was mixed with 1500 ul of the Glucose Reagent and incubated at 37°C for 30 minutes. Then add 1500 ul of distilled water and take absorbance at 490-550 nm. Absorbance of coloured dye was measured

at 505 nm and was directly proportional to Glucose concentration in the sample. Calculate the Serum Glucose level in mg/dl.

## RESULTS

In the present study the Immunoglobulin-A level in serum of Group A suffering from both the disease was found to be highly elevated in all the studied groups of patients and such group was statistically highly differed from the healthy control group. In our study it can be concluded that there was positive co-relation between the two parameters: elevated HbA1c and Immunoglobulin level with periodontitis. In our study we found the IgA level was highly elevated in Diabetic patients suffering from Periodontitis, 72.26 mg/dl Average, further the level was also seen to high in the group of Diabetic patient not suffering from Periodontitis, 74.6 mg/dl Average. However, the IgA was also found to be too high in non-diabetic patients also suffering from periodontitis, 57.3 mg/dl Average, whereas the control group showed 25.53 mg/dl as average IgA level in the serum of the control group. Conclusively, in any clinical problem the IgA level was found to elevated which were extreme in the group of patients, suffering from both of the disease, diabetes and periodontitis. The mean age group of the patients in our study were 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 for  $\alpha = 0.0083$  (.05/6) was used to correlate the relationship between different parameters. The data of serum IgA showed mean value of 72.26 and standard deviation of 17.6 in group A patients, mean value of 74.6 and standard deviation of 24.35 in group B patients, mean value of 57.33 and standard deviation of 23.13 in group C patients and mean value of 25.5 and standard deviation of 8.52 in group D patients. The ANOVA showed significant pattern in the results. The patients of Group A (Diabetic with Periodontitis) showed significant ( $P < 0.004$ ) increase in serum IgA level as compared to controls (Group D- Non Diabetic, Non Periodontitis). Group B showed non-significance of  $p = 0.209$ . Group C showed significance of  $p = 0.000$  (Figure-1). The HbA1c percentage in group A showed significant ( $P < 0.005$ ) as compared to group D, group B also showed significance ( $P < 0.001$ ), group C showed significance ( $P < 0.001$ ) as compared to control group (Figure-2). Comparison of different parameters between controls and four groups was done by *t*-test. With an increase in HbA1c percentage serum IgA showed significant ( $P < 0.01$ ) increase [Tables 1-4].

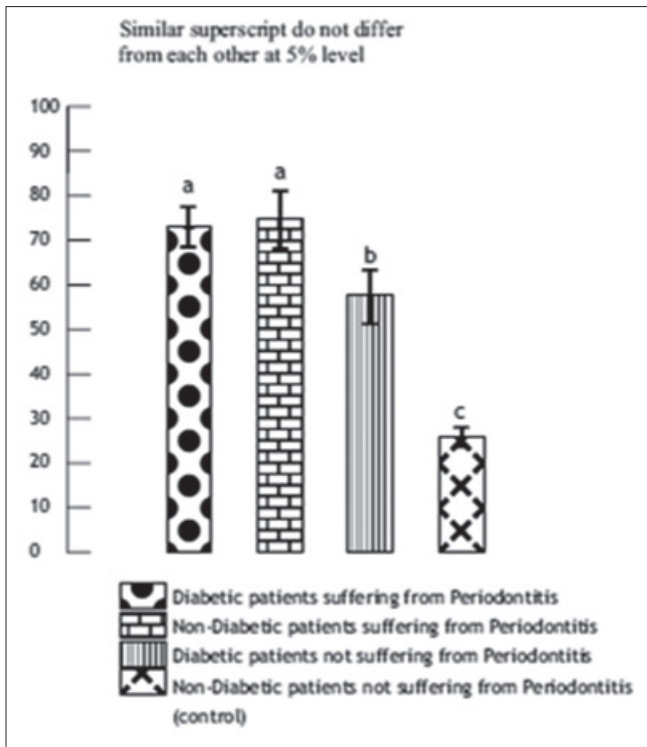


Figure 1: Graph showing HbA1C% of all four groups HbA1C

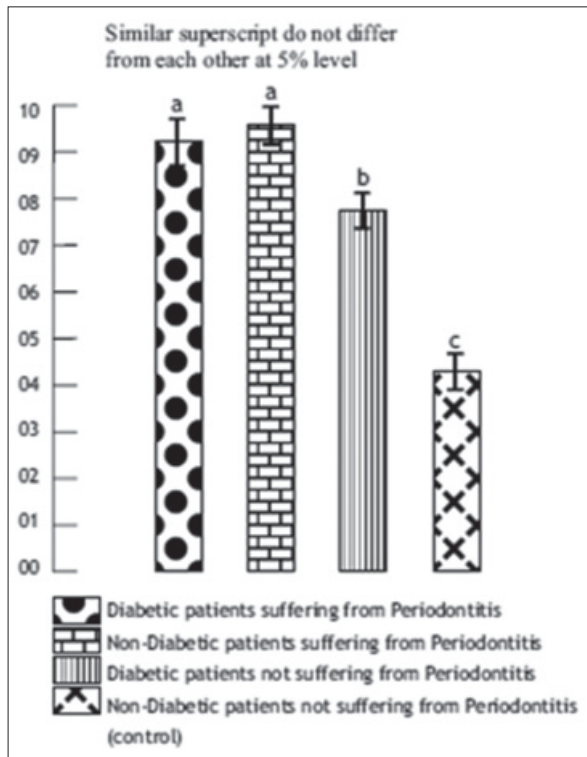


Figure 2: Graph showing IgA conc. of all four groups IgA

## DISCUSSION

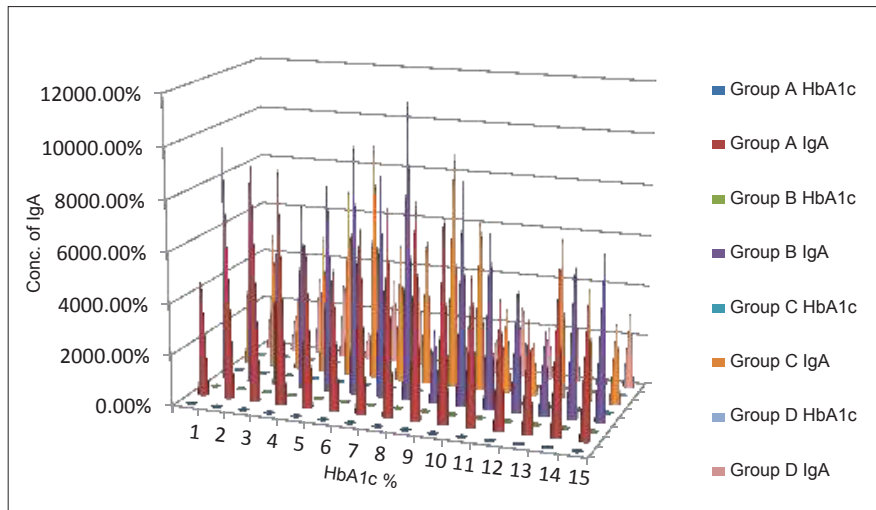
During investigation, we found higher levels of IgA in patients showing higher levels of HbA1c percentage

Tables 1-4: Comparative analysis of IgA and HbA1c among four studied group

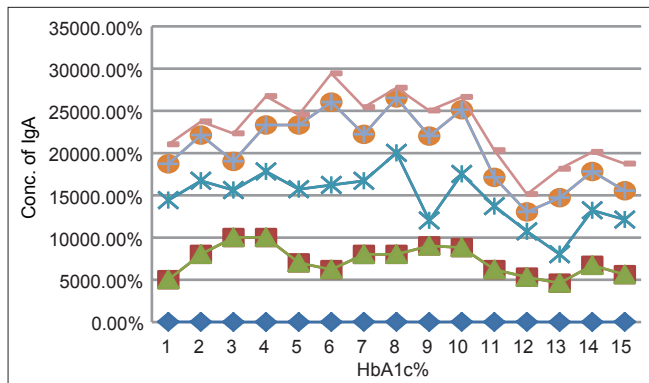
S.No.	Group A (Diab+Perio)		Group B (Diab+ Non-Perio)		Group C (non-Diab+ perio)		Group D (Non-Diab+ non-perio)	
	IgA	HbA1c	IgA	HbA1c	IgA	HbA1c	IgA	HbA1c
1	50	10.80%	94	9.80%	43	7%	23	4.30%
2	80	9.80%	87	9%	54	8%	16	5.60%
3	100	7%	56	8.60%	34	7.80%	33	3.10%
4	100	8.20%	78	8.80%	55	5.30%	34	5%
5	70	7.10%	87	12%	76	8%	12	3%
6	62	8.70%	100	11.60%	98	9%	34	5.20%
7	80	10.70%	87	6.20%	55	6.20%	32	3.50%
8	80	10.80%	120	8%	65	7.80%	12	3.30%
9	90	6.40%	30	11.20%	100	6.00%	30	6.90%
10	88	11.60%	87	10.70%	76	8%	15	5.50%
11	62	8.10%	75	11%	34	6.50%	32	3.35%
12	53	11%	54	9%	23	8.40%	21	3.80%
13	46	7.10%	34	9.80%	67	8%	34	3.20%
14	67	8%	65	8.50%	46	8.50%	23	4%
15	56	12.50%	65	9%	34	11.60%	32	4.40%

as compared to the control group. This co-relates to the possibility of an underlying immuno-inflammatory abnormality to be the cause for the elevated level of immunoglobulin in our patients with elevated sugar level. Our results evidenced that the level of glycosylated haemoglobin molecule (HbA1c) was comparatively very high in the both group of the patients; diabetics suffering from periodontitis and also diabetics not suffering from periodontitis. The result was 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 diabetic conditions, the disease periodontitis alone was 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 haemoglobin (Figure-3).<sup>13</sup> 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,<sup>16</sup> as the group of non-Diabetic patients suffering from Periodontitis was significantly differed from the control (normal health) group (Figure-4). 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.<sup>17,18</sup> 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,





**Figure 3:** Graph showing comparative values of HbA1c and IgA



**Figure 4:** Graph showing comparison between studied groups

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.<sup>19-21</sup> 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. In addition to a possible dysfunctional humoral response to infection, there is still the elevation of IgA levels in diabetic patients.<sup>22,23</sup> 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.<sup>24,25</sup>

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.<sup>26</sup>

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**Authors Contribution:**

**CD, KR, SP** – Contributed in designing the study, collecting samples and data and performing laboratory tests; **GV** – Contributing in analysing the periodontal patient and contributed in reviewing the manuscript; **AR and SV** – Contributed in analysing the data statistically and graphically.

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