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Comparison of serum IGG levels in chronic periodontitis patients with and without diabetes mellitus

[1]Dr. Pallavi Samatha. Y, Professor, Department of Periodontics and Implantology, Drs. Sudha & Nageswara Rao Siddhartha Institute of Dental Sciences, Chinaoutpally- 521286, India.

[2]Dr. Sounica. K, Post Graduate Student, Department of Periodontics and Implantology, Drs. Sudha & Nageswara Rao Siddhartha Institute of Dental Sciences, Chinaoutpally- 521286, India.

[3]Dr. Divya Sri. G, Post Graduate Student, Department of Periodontics and Implantology, Drs. Sudha & Nageswara Rao Siddhartha Institute of Dental Sciences, Chinaoutpally- 521286, India.

[4]Dr. Mounika. D, Post Graduate Student, Department of Periodontics and Implantology, Drs. Sudha & Nageswara Rao Siddhartha Institute of Dental Sciences, Chinaoutpally- 521286, India.

Corresponding Author: Dr. Pallavi Samatha. Y, Professor, Department of Periodontics and Implantology, Drs. Sudha & Nageswara Rao Siddhartha Institute of Dental Sciences, Chinaoutpally- 521286,India.

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Abstract

Background & objectives: Periodontitis is a mixed infection where the pathogens act directly or indirectly in destruction of tooth supporting structures. Diabetic patients have compromised ability to respond to infectious challenges that predisposes them to bacterial infections such as periodontal disease. In periodontitis the major aspect of adaptive host response is the production of antibodies. The study aims to compare the serum IgG levels in chronic periodontitis patients with & without diabetes mellitus.

Method: This study was conducted on 60 patients. The periodontal status was assessed by recording clinical parameters (Plaque index, gingival index, probing pocket depth, clinical attachment level). The patients were divided into 3 groups of 20 each namely, Group I: Healthy group, Group II: Chronic periodontitis patients without diabetes, Group III: Chronic periodontitis patients with diabetes mellitus. Serum levels of IgG was estimated by immunoturbidimetry method. The statistical analysis was done using ANOVA and Tukeys test.

Interpretation and conclusion: The mean serum levels of IgG was significantly high in group III compared to other two groups thus suggesting that there was increased serum IgG levels in chronic periodontitis with diabetes mellitus. IgG can be a potential biochemical marker for periodontal destruction.

Keywords: Chronic periodontitis, diabetes mellitus, immunoglobulin G

Introduction

Chronic periodontitis is a progressive disease affecting gingiva, periodontal ligament and alveolar bone resulting in gingival inflammation subsequently affecting the supporting structures of tooth resulting in resorption of alveolar bone[1]. Microbial challenge is an important etiological factor in initiating an inflammatory response in susceptible hosts. The host responds with immediate inflammatory and immune response to the invading bacteria by production of chemokines and cytokines that cause destruction of connective tissue and bone. Genetic, environmental and various other acquired factors influence in mediating the progression of periodontal disease[2].

Diabetes mellitus is a group of metabolic diseases characterized by chronic hyperglycemia resulting from defects in insulin secretion, insulin action or both. Type 1 or insulin dependent diabetes results from failure of pancreas to produce enough insulin and Type 2 or non-insulin diabetes dependent results where cells fail to respond to insulin adequately. Longitudinal studies have demonstrated a two way relationship between diabetes and periodontitis[3]. There is severe periodontal tissue destruction in diabetic patients. This may be due to the abnormalities in polymorphonuclear neutrophils function such as altered activation, adherence and defects in neutrophil chemotaxis. Diabetic patients are more susceptible to infection and periodontal disease is a common manifestation in diabetics.

The primary function of immune system is to eliminate infectious agents and minimize the damage caused by them. Antibodies belong to the third fastest migrating group of serum globulins which are the gamma globulins. Five classes of immunoglobulins have been recognized- IgG, IgA, IgM, IgD & IgE based on physicochemical & antigenic differences. They inhibit bacterial adherence, colonization, enhance phagocytosis, detoxify bacterial toxins & play a major role in defense against bacterial infections. Among the five classes of immunoglobulins, IgG is the predominant immunoglobulin in human serum & IgA is predominant in seromucous secretions. IgG is considered as anti-inflammatory isotype & helps in opsonization process. To survive in periodontal environment, bacteria must neutralize & evade the host defense mechanisms involved in bacterial clearance & killing. The immunoglobulins secreted by the host to facilitate phagocyosis of periodontal pathogens are degraded by immunoglobulin-degrading proteases secreted by specific micro organism. Certain pathogens such as Actinobacillus actinomycetamcomitans can only be controlled by neutrophils when opsonised by IgG isotype. Periodontitis patients often exhibit very high serum titers of IgG to some specific pathogens. Increase in antibody titer is the result of host exposure to an antigen[5].

Diabetic patients are always at high risk of developing infections because of impairment in host defense mechanism. Inflammatory signaling pathway also becomes activated by metabolic stress leading to insulin resistance. Increased glucose metabolism leads to increase in mitochondrial production of reactive oxygen species enhancing activation of inflammatory pathways. The increased level of immunoglobulins is due to inflammatory stress[6].

There are few studies regarding the estimation of serum levels of IgG immunoglobulins in chronic periodontitis with or without type 2 diabetes. The aim of this study is to compare serum immunoglobulin IgG levels in chronic periodontitis patients with & without type 2 diabetes.

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Materials & Methods

Diabetic patients who are attending the department of Periodontics & Implantology at Drs. Sudha & Nageswar Rao Siddhartha Institute of Dental Sciences were included in the study. A total of 60 subjects between age 40-60 years were included in the study & divided into 3 groups consisting of 20 healthy subjects, 20 subjects with chronic periodontitis, 20 chronic periodontitis subjects with diabetes in each group. Subjects having probing pocket depth of >4mm with clinical attachment loss of >2mm in each quadrant with 20 permanent teeth were included in the study. Patients maintaining good oral hygiene who had not received periodontal treatment & antibiotic therapy within the preceding six months were included in the study. Patients who were suffering from systemic diseases other than diabetes or conditions like pregnancy that could aggravate periodontal manifestations were excluded from study.

Clinical examination

Periodontal examination was carried out using mouth mirror & explorer. Simplified oral hygiene index (OHI-S) was used to assess oral hygiene. Periodontal status was recorded by using William's periodontal probe. All the subjects were examined clinically for presence of plaque, gingival bleeding, clinical attachment level, probing pocket depth.

Estimation of serum IgG immunoglobulin

Five milliliter of venous blood was collected from each patient by venipuncture in anticubital fossa. The blood samples were collected in vacuum tubes. Samples were centrifuged at 2500 rpm for 10 minutes to separate serum. The serum samples were transferred to plastic vials & stored at -20℃ for estimation of immunoglobulin G. Immunoglobulin G levels was quantitatively estimated by IgG immunoglobulin kit (Euro Diagnostic Systems Pvt. Ltd, Spinreact. S.A). Immunoglobulin values were assessed in semi automatic analyzer (Erba Chem-7 technologies, India).

Data analysis

The demographic analysis for distribution of age and sex was performed respectively using ANOVA and Chi square test. The comparison of serum IgG levels in all three groups were analysed using ANOVA and the multiple comparison between the groups was done using Tukey's test.

Results

The data collected was analyzed to determine the levels of serum IgG in chronic periodontitis patients with and without diabetes.

A total of 60 subjects were included in this study and divided into three groups: healthy (20), chronic generalised periodontitis patients (20) and periodontitis patients with diabetes (20). The demographic data (percentage of distribution in each group, mean and standard deviation for three groups are shown in table 1).

Table 1, Graph 1 shows distribution of males & females in three study groups. The percentage of distribution of males & females in healthy group (group 1) is 40% and 60%. The percentage of distribution of males & females in chronic periodontitis group (group 2) is 50%. The percentage of distribution of males & females in periodontitis with diabetes group (group 3) is 45% & 55%.

Table 2, Graph 2 shows comparison of three groups with mean age by one way ANOVA. The mean age in group 1 (healthy) was 39.20 with standard deviation of 3.41, in group 2 (chronic generalized periodontitis) the mean age was 45.35 with standard deviation of 5.94 and in group 3 (periodontitis with diabetes) the mean age was 45.25 with standard deviation of 4.70. Comparison of the three study groups in relation to mean age compared by one way ANOVA showed that there was a statistical significant difference among the groups with a p value of 0.0001.

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Pairwise comparison with mean age by Tukeys multiple posthoc procedure showed that there was statistical significant difference between group 1(healthy) and group 2 (periodontitis), group 1 (healthy) and group 3 (periodontitis with diabetes). There was no statistical significant difference between group 2 (periodontitis) and group 3 (periodontitis & diabetes) with p value of 0.99 (Table 2).

Table 3, Graph 3 shows the Mean and SD of serum Ig G levels in three study groups. The mean serum IgG level in group 1 (healthy) was 1207.75 with standard deviation of 262.92 in group 2 (periodontitis) the mean serum IgG level was 1657.60 with standard deviation of 90.20 and in group 3 (periodontitis with diabetes) the mean serum IgG level was 1892.30 with standard deviation of 109.34. There was a significant increased levels of IgG in group 3 (periodontitis with diabetes)

Table 4 shows the comparison of the three study groups for IgG levels by one way ANOVA. There was statistically significant difference among the groups with p value 0.0001. Table 5 shows Pair wise comparison of three groups for IgG levels by Tukey's multiple posthoc procedures. There was statistically significant difference between group 1 (healthy) and group 2 (periodontitis) with p value of 0.0001 and group 1(healthy) and group 3 (periodontitis and diabetes) with p value of 0.0001 and group 2 (periodontitis) and group 3 (periodontitis with diabetes) with p value 0.0003.

Correlation among different groups showed that there was a positive association between IgG levels and periodontitis patients with diabetes. The results showed that there was significantly higher serum IgG levels in periodontitis patients with diabetes.

Discussion

Gingivitis and periodontitis are chronic inflammatory diseases affecting the supporting structures of the tooth induced by bacterial biofilm on the teeth. An imbalance between a localized infection and an exaggerated inflammatory response of host plays a pivotal role in determining the tissue damage. Other factors, such as drugs, tobacco smoking, immunodeficiency, and various systemic diseases, are known to influence their pathogenesis. Periodontitis is associated with alteration in immune response in both diabetic & non diabetic subjects[7].

Prevalence of periodontitis is more in diabetic subjects compared to non diabetic subjects. This is due to reduced polymorphonuclear neutrophil (PMN) chemotaxis, defect in phagocytosis, and depressed humoral response. Hyperglcemic state in diabetic patients leads to the formation of advanced glycation end products (AGEs) contributing to tissue changes within the periodontium[8]. A recent meta-analysis conducted to evaluate the association between diabetes and periodontal disease concluded that subjects with diabetes had significantly greater severity of periodontal disease (Khader Y S et al)[9].

Immunoglobulin G (IgG) molecules are family of glycoproteins which defend the body against invading pathogens. The constant (Fc) domain of antibody is very potent in initiating pro-inflammatory pathways by activation of innate immune effector cells via cellular receptors specific for antibody constant region (FcR) & activation of complement pathway. Injury/infection causes influx of inflammatory cells to the site leading to inflammation which is characterized by drop in the P^H & calcium levels in its microenvironment. Ficolins are innate pattern recognition receptors & are structurally similar to mannose binding lectin (MBL). It initiates lectin pathway of complement system & plays an important role in innate immunity by acting as phagocytic receptor for pathogens. During inflammation, ficolins bind to pathogen & recruit IgG to form immune complexes. IgG interacts with

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pathogen associated ficolins through its Fc region, collaborates with MBL, clear it by receptor mediated phagocytosis & thereby control inflammation by reducing pro-inflammatory IL-6,8, TNF & increasing anti-inflammatory IL-10[20].

A recent longitudinal study conducted by Salvi et al[15] observed more severe gingival inflammation in subjects with type 2 diabetes than in control subjects without diabetes, despite similar quantitative & qualitative bacterial plaque characteristics suggesting an increased inflammatory gingival response in patients with diabetes. Ervasti et al [16] observed more gingival bleeding sites in patients with poorly controlled diabetes compared to subjects with controlled diabetes with despite similar plaque scores in them. In our study we found that the levels of IgG were highly elevated in periodontitis patients with diabetes. The probable cause of increased IgG levels may be due to their increased production to neutralize bacterial toxins. Major antibody response in periodontitis is IgG presumably in response to the outer membrane proteins of gram negative periodontal pathogens & bacterial polysaccharides. Higher serum levels of IgG especially reactive with A. actinomycetamcomitans is considered as protective to periodontal breakdown[10].

Studies with evaluation of either serum or salivary quantification of immunoglobulins have provided varying results. Olsanska Seidlova et al (1989)[11] conducted a study to examine a range of systemic immunological parameters (IgA, IgG, IgM, IgD) in patients with chronic periodontitis and found that the levels of IgG, IgM, IgD increased significantly in periodontitis patients.

Anil et al (1990)[12] conducted a study to assess the humoral response by estimation of serum serum IgA, IgG, IgM, IgD, IgE in chronic periodontitis patients by single radial immunodiffusion technique. They observed that all immunoglobulins except IgD were found to be elevated significantly in chronic periodontitis patients.

Fatin Atarwani (2010)[13] conducted a study to assess serum immunoglobulin levels (IgA, IgG, IgM) in chronic periodontitis patients with & without type 2 diabetes. Serum immunoglobulin levels were estimated by immunoturbidimetry method. They found that IgG & IgA levels were found to be significantly higher in diabetic patients with chronic periodontitis. This elevation in serum IgG may be due to increased antibody production to neutralize bacterial toxins.

In our study, elevated serum IgG levels were observed in periodontitis patients with diabetes (group 3) compared to periodontitis patients (group 2) & healthy patients (group 1) & supports that there is increased serum immunoglobulin levels in periodontitis patients with diabetes. The higher levels of immunoglobulins could be protective mechanism against increased bacterial challenge in diabetic subjects.

Anil et al (2006)[18] found that IgG & IgA levels were increased in the gingival tissues of diabetic subjects with periodontitis compared to healthy subjects. They concluded that these findings correlated to the concept that humoral immune response plays an important role in pathogenesis of periodontitis in diabetic subjects. This elevation is significant & perhaps the higher levels of immunoglobulins in gingival tissues could be a protective mechanism against increased bacterial load in diabetic subjects

In contrast, the studies conducted by Ranney et al (1981)[14] and Bokor & Bratic et al (1998)[17], found no significant differences in serum IgG levels between periodontitis patients & healthy individuals. Lalla et al [19] found that the rate of periodontal destruction is related to inappropriate glycemic control in diabetic patients indicating that

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accurate glycemic control is an important factor to prevent periodontal complications.

The findings of our study demonstrate the role of the immune system in maintaining good periodontal health especially in diabetic patients. The changes observed in immune response may be the cause or the effect of periodontal disease in diabetic patients.

Conclusion

This is a cross sectional designed study with a small sample size with variation in age, gender & limited in its ability to conclude the relation between IgG levels & periodontal disease. Further longitudinal studies with larger sample size are required to accurately establish the relation between them. Based on the results obtained in our study, it can be concluded that there was statistically significant increase in serum IgG levels in periodontitis patients with diabetes compared to periodontitis patients & healthy controls.

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Legends Table and Figure

Table 1: Distribution of male and females in three study groups (Healthy, CGP, Periodontitis with DM)

Groups	Male	%	Female	%	Total
Healthy	8	40.00	12	60.00	20
Chronic generalized periodontitis	10	50.00	10	50.00	20
Periodontitis patients with diabetes mellitus	9	45.00	11	55.00	20
Chi-square=0.4042 P = 0.8171					

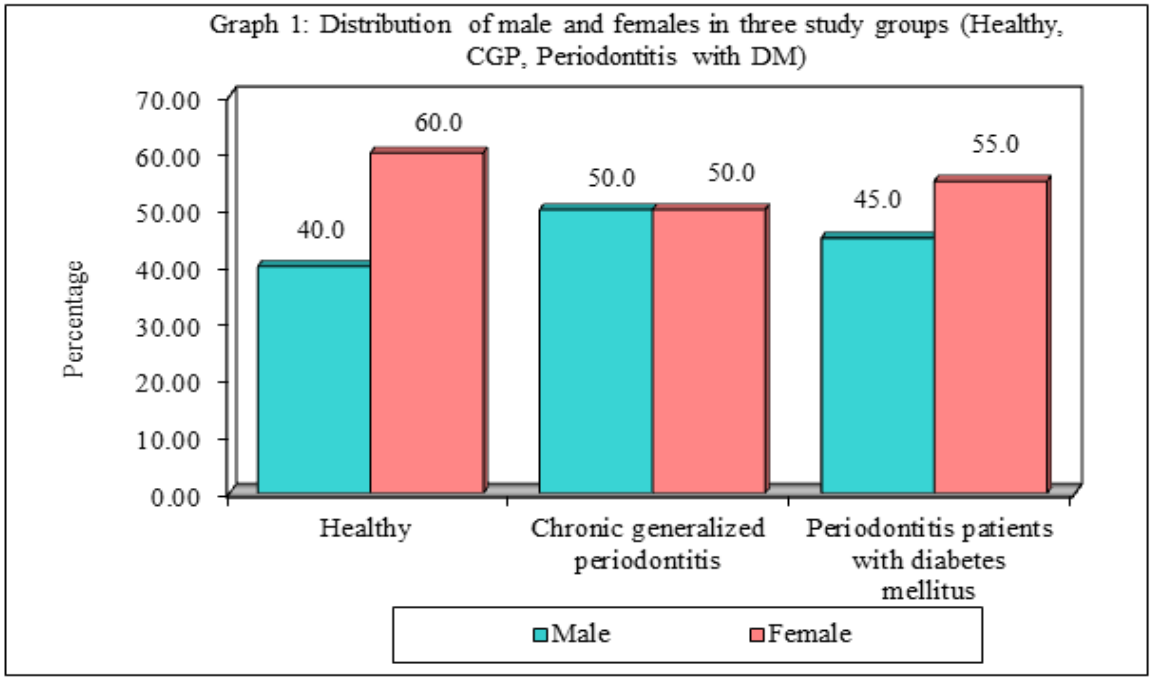

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Table 2: Comparison of three study groups (Healthy, CGP, Periodontitis with DM) with mean age by one way ANOVA

Groups	Mean	SD
Healthy	39.20	3.41
Chronic generalized periodontitis	45.35	5.94
Periodontitis patients with diabetes mellitus	45.25	4.70
F-value	10.7836	
P-value	0.0001*	
Pair wise comparisons by Tukeys multiple posthoc procedures		
Healthy vs Chronic generalized periodontitis	p=0.0005*	
Healthy vs Periodontitis patients with diabetes mellitus	p=0.0007*	
Chronic generalized periodontitis vs Periodontitis patients with diabetes mellitus	p=0.9977	

*p<0.05

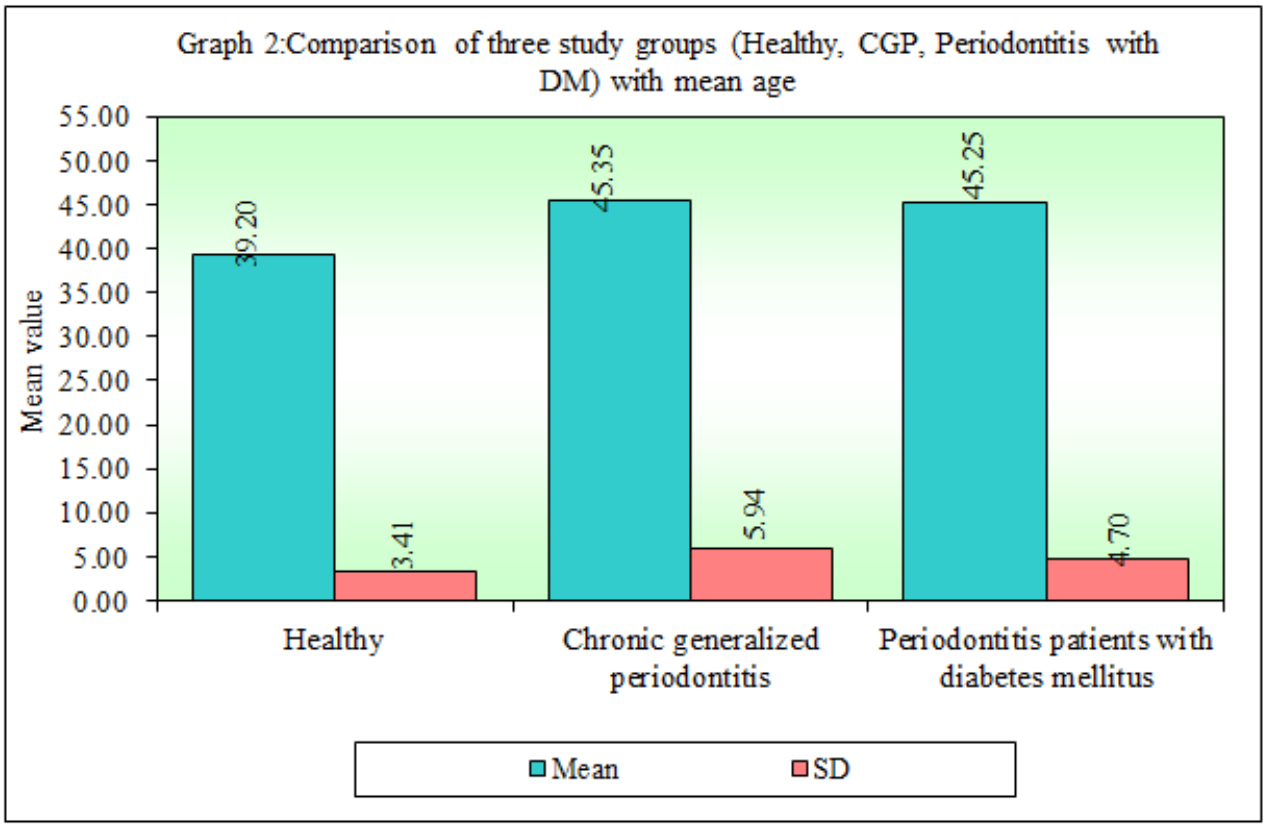

Table 3: Mean and SD of serum Ig G levels in three study groups (Healthy, CGP, Periodontitis with DM)

Groups	Mean	SD	SE
Healthy	1207.75	262.92	58.79
Chronic generalized periodontitis	1657.60	90.20	20.17
Periodontitis patients with diabetes mellitus	1892.30	109.34	24.45

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Table 4: Comparison of three groups (Healthy, CGP, Periodontitis with DM) with mean serum Ig G levels by one way ANOVA

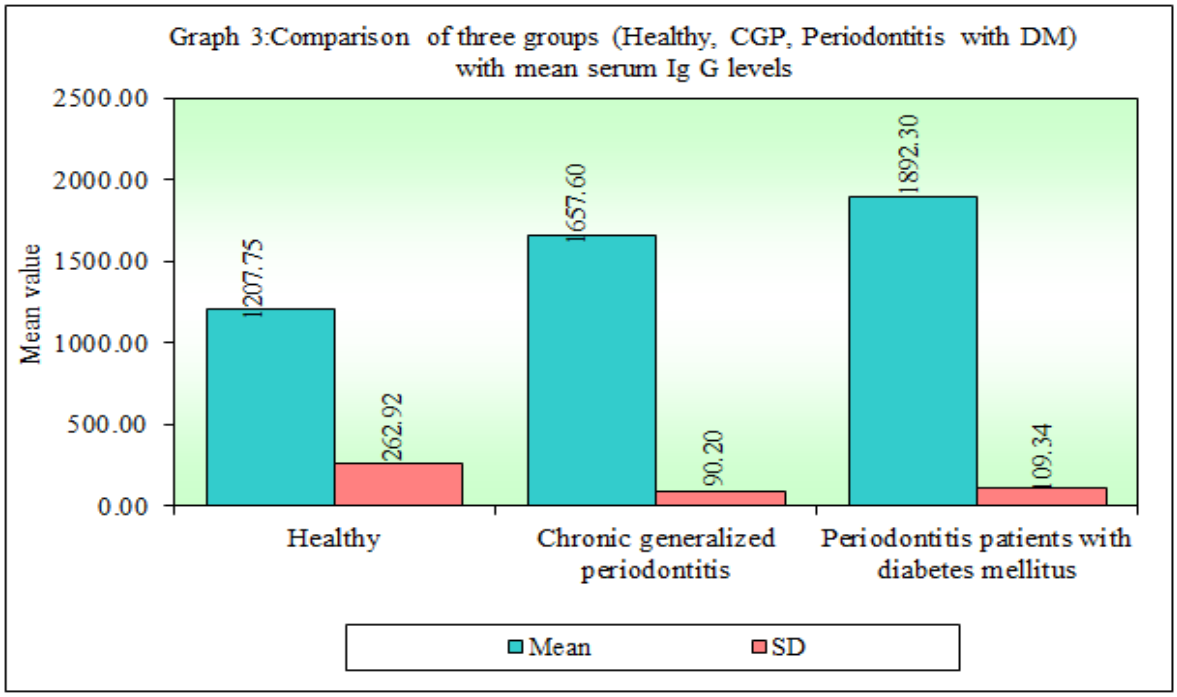
Sources of variation	Degrees of freedom	Sum of squares	Mean sum of squares	F-value	p-value
Between groups	2	4840385.43	2420192.72	81.3801	0.0001*
Within groups	57	1695144.75	29739.38		
Total	59	6535530.18			

*p<0.05

Table 5: Pair wise comparison of three groups Healthy, CGP, Periodontitis with DM) with mean serum Ig G levels by Tukeys multiple posthoc procedures

Groups	Healthy	Chronic generalized periodontitis	Periodontitis patients with diabetes mellitus
Mean	1207.75	1657.60	1892.30
SD	262.92	90.20	109.34
Healthy	-		
Chronic generalized periodontitis	p=0.0001*	-	
Periodontitis patients with diabetes mellitus	p=0.0001*	p=0.0003*	-

*p<0.05



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