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"Periodontitis And Diabetes Interrelationship: Assessment Of C-Peptide And Glutamic Acid Decarboxylase Antibody Titer (Gad) – A Case Control Study"

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Abstract:
Diabetes mellitus is a disease of metabolic dysregulation, primarily of carbohydrate metabolism, characterized by hyperglycemia (elevated blood glucose) that results from defects in insulin secretion, impaired insulin action, or both.
It is generally accepted that there is an association between diabetes and periodontitis.Various studies were done to find the mechanism behind this reciprocal relationship.Chronic periodontal infection leads to an increase in serum tumor necrosis factor α, interleukin-1, and interleukin-6 and C-reactive proteins. The increase in these cytokines may increase insulin resistance by interfering with glucose and lipid metabolism and antagonizing insulin action. The increased insulin resistance will ultimately cause an increase in the risk for type 2 diabetes. it seems that in individuals with type 2 diabetes and periodontitis, an elevated chronic systemic inflammatory state induced by periodontal disease may contribute to insulin resistance through a "feed-forward" mechanism, worsening glycemic control.
C-peptide was initially thought to be just a by-product of insulin production and biologically inert; however, research findings indicate that it does have important biological activity, especially in relation to diabetes mellitus.
*Type 1diabetes is an autoimmune disease and 98% of new-onset type 1 diabetics test positive for one or more autoantibodies. Glutamic acid decarboxylase (GAD) antibodies are very useful as predictive markers for type 1 diabetes. GAD antibody is detected in about 70% to 80% of newly diagnosed type 1 diabetes patients and in 70% of slowly progressive insulin-dependent diabetes mellitus patients, who initially develop type 2 diabetes and then progress to type1 diabetes.**TakayakuKono et al. 2001**concluded that GAD is ubiquitously expressed by human gingival and periodontal ligament fibroblasts. Also, there is evidence that periodontal inflammation may result in higher levels of GAD and influence GAD antibody titre. Thus, severe periodontitis may lead to an extent GAD autoimmunity induced Type 1 diabetes mellitus.*
*Thus, in the present study we evaluated the levels of C-peptide, GAD-antibody, and HbA1c in Experimental **(CH-SH)** and Control **(SH)** group.*

Aim of study
Aim of the study was assessment the interrelationship between periodontitis and diabetes by evaluating the levels of c-peptide, gad autoantibody and hba1c in serum of systemically healthy patients with (cp-sh) and without chronic severe periodontitis (sh).
Objective of study
The objectives of this study were to compare the levels of C-peptide in systemically healthy patients withand without chronic severe periodontitis, compare the levels of GAD autoantibody in systemically healthy patients with and without chronic severe periodontitis, compare the levels of HbA1c in systemically healthy patients with and without chronic severe periodontitis and to evaluate whether chronic periodontitis can act as a risk factor for the development of type I diabetes mellitus.
Result and Summary:
A total of 30 systemically healthy patients were included in the study, of which 15 patients were of chronic generalized periodontitis patients (experimental group) (CP-SH) and 15 were periodontally healthy patients (control group) (SH). The periodontal parameters and laboratory tests were recorded and results were calculated.
With the present study it was found thayC-peptide levels was found to be less in experimental group than control group, however the differences were not statistically significant,suggesting that chronic periodontitis might be associated with disturbed β-cell activity, GAD-antibody levels, in experimental was found to be more than control group. However, no statistically significant difference was found between both the groups. In experimental group, out of 15 patients 2 patients expressed very high GAD anti-body titers, suggesting that chronic periodontitis may influence the level of this antibody which is widely used as a predictive marker for slowly progressive insulin dependent diabetes mellitus and HbA1c levels were found to be more in experimental group than control group. However, the difference was not found to be statistically significant.

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I. Introduction:

Diabetes mellitus is a disease of metabolic dysregulation, primarily of carbohydrate metabolism, characterized by hyperglycemia (elevated blood glucose) that results from defects in insulin secretion, impaired insulin action, or both[(1)].

According to the Indian Council of Medical Research-Indian Diabetes study (ICMR-INDIAB), a national diabetes survey, India currently has 62.4 million people with diabetes. This is set to increase to over 100 million by 2030. The majority of people with diabetes (>90%) in India are diagnosed with Type 2 diabetes (T2DM)[(2)]. Diabetes (Type I and Type II) are associated with microvascular (i.e. retinal, renal, possibly neuropathic), macrovascular (i.e. coronary, peripheral vascular) and neuropathic (i.e. autonomic, peripheral) complications [(1)].

Type 1 diabetes mellitus usually results from cellular-mediated immune β-cell destruction, frequently leading to total loss of insulin secretion [(3)]. It is mainly reported in children and young adolescents; however it also develops in adults, earlier known as LADA (latent autoimmune diabetes in adults). LADA describes a subgroup of patients who develop phenotypic type 2 diabetes but with markers of autoimmunity. American Diabetes Association (ADA) currently does not sub classify LADA as a separate diagnostic entity but considers it as type 1 diabetes occurring in adults [(4)]. Some studies have demonstrated 15–30% of all cases of type 1 diabetes are diagnosed after 30 years of age. In this older group of type 1 patients the β-cell destruction occurs more slowly than in children, with a less abrupt onset of symptoms. However, based on age criteria only, many cases of type 1 diabetes mellitus are misdiagnosed as type 2[(3)].

Type 2 diabetes mellitus, previously defined as Non insulin- dependent diabetes, results from insulin resistance and to a certain extent altered insulin production. It often occurs in obese people over the age of 35. Hyperglycemia results from lack of endogenous insulin, which is relative in type 2 diabetes mellitus. Relative insulin deficiency usually occurs because of resistance to the action of insulin in muscle, fat and the liver, and an inadequate response by the pancreatic β cells.Type 2 patients have altered insulin production as well. In many patients, especially early in the disease, insulin production is increased, resulting in hyperinsulinemia. As the condition progresses, insulin production often decreases and patients have a relative insulin deficiency in association with peripheral insulin resistance. However, autoimmune destruction of β cells does not occur, and patients retain the capacity for some insulin production[(3)].

This metabolic syndrome(Diabetes) is now viewed as an inflammatory condition, and its development is preceded by low-grade systemic inflammation. As such, elevated plasma concentrations of pro-inflammatory mediators / markers such as C-reactive proteins, cytokines (interleukin-1β, interleukin-6, tumor necrosis factor α) and prostanoids (prostaglandin E2) are seen in patients suffering from diabetes mellitus[(4)].

Periodontal diseases are considered as world-wide prevalent diseases and affect 90% of Indian population. Advanced periodontal disease with pocket formation and bone loss leading to tooth loss affects 40-45% of population [(5)].

Periodontitis is a chronic inflammatory disease of tissues surrounding the teeth caused by specific anaerobic pathogens. The destructive process of periodontitis is thought to begin with the accumulation of biofilms which contain significant bacterial masses on the tooth surface at or below the gingival margin. It is characterized by signs of inflammation such as, bleeding upon probing, red and/bluish-red colour gingiva, enlarged gingival contours due to edema/fibrosis, increased gingival exudates, increased probing depths, alveolar bone loss and increased tooth mobility[(5)].

It is generally accepted that there is an association between diabetes and periodontitis. Majority of clinical and epidemiological evidence demonstrates that individuals with diabetes (Type 1 and Type 2) tend to have a higher prevalence and more severe/rapidly progressing forms of periodontitis than non-diabetics. Additionally, patients with poor control of diabetes experience more periodontitis than well-controlled diabetics. It has also been shown that effective treatment of periodontitis may actually improve some diabetic complications, especially hyperglycemia. Severe periodontitis is associated with poor glycemic control and exacerbated hyperglycemia. Additionally, periodontitis confounded diabetic control as increasing disease severity necessitated larger dosages of hyperglycemic medications. Effective treatment of periodontal infection and reduction of periodontal inflammation were found to be associated with lower levels of glycated hemoglobin. Accumulating evidence suggests that effective periodontal therapy does improve diabetic metabolic control, suggesting a reciprocal relationship may actually exist [(6)]. Contrary to this, in some studies no such improvement was seen in diabetic metabolic control [(7)].

Various studies were done to find the mechanism behind this reciprocal relationship.Chronic periodontal infection leads to an increase in serum tumor necrosis factor α, interleukin-1, and interleukin-6 and C-reactive proteins. The increase in these cytokines may increase insulin resistance by interfering with glucose and lipid metabolism and antagonizing insulin action. The increased insulin resistance will ultimately cause an increase in the risk for type 2 diabetes. Given these mechanisms promoting insulin resistance, it seems that in individuals with type 2 diabetes and periodontitis, an elevated chronic systemic inflammatory state induced by periodontal disease may contribute to insulin resistance through a "feed-forward" mechanism, worsening glycemic control. This might explain why periodontitis increases the risk of poor glycemic control among patients with type 2 diabetes [(8)].

It has been proved that Proinflammatory cytokines and peroxynitrite, the stable marker of the labile reactive nitrogen species nitric oxide, are detectable in the pancreatic islets where T1DM (Type 1 Diabetes Mellitus) is developing. One of these cytokines, interleukin 1 (IL-1), a prototypic and evolutionarily strongly conserved mediator of innate immunity with multiple biological actions has long been known to cause β-cell dysfunction and death. Pancreatic islet cells express the highest density of IL-1 receptors among all body tissues. In contrast to other cells, β cells are more susceptible to IL-1-triggered apoptosis, partly because they have higher IL-1-receptor density than other cell types. β cells are particularly vulnerable to the effects of IL-1 exposure, since the biochemical mechanisms of action underlying the toxic effects of IL-1 target organelles in β cells[(9)]. The immunopathogenesis of type 1 diabetes mellitus is also associated with T-lymphocyte autoimmunity. However, there is growing evidence that B lymphocytes play a role in many T-lymphocyte–mediated diseases[(10)]. Cumulative effects of inflammatory cytokines & B cells leads to destruction of pancreatic β- cells and reduced insulin production in type 1 Diabetes mellitus which can be assessed by C – peptide level.

C-peptide was initially thought to be just a by-product of insulin production and biologically inert; however, research findings indicate that it does have important biological activity, especially in relation to diabetes mellitus.C-peptide is produced by a series of enzymatic cleavages of the precursor molecules preproinsulin and proinsulin. Enzymatic cleavage of proinsulin by proconvertases and carboxy-peptidases produces insulin and C- peptide, which are released in equimolar amounts from β-cells into the portal circulation. Since C-peptide and insulin are released in equimolar amounts from the β-cells of the pancreas, the measurement of C-peptide has been used as a marker of β-cell function and an index of insulin secretion.C-peptide measurements have also been used to classify diabetes mellitus. Type 1 diabetes mellitus is characterized by β-cell destruction, which leads to very low or undetectable levels of C-peptide. In contrast, type 2 diabetes mellitus is associated with insulin resistance and typically initially has normal or elevated levels of C-peptide, which can decrease over the course of the disease. Monitoring residual β-cell function through the measurement of C-peptide is a strategy endorsed by the American Diabetes Association (ADA)[(11)].

Type 1diabetes is an autoimmune disease and 98% of new-onset type 1 diabetics test positive for one or more autoantibodies. These autoantibodies such as islet cell antibody (ICA), insulin autoantibody (IAA), and glutamic acid decarboxylase (GAD) antibody are very useful as predictive markers for type 1 diabetes. GAD antibody is detected in about 70% to 80% of newly diagnosed type 1 diabetes patients and in 70% of slowly

progressive insulin-dependent diabetes mellitus patients, who initially develop type 2 diabetes and then progress to type1 diabetes [(12)].

GAD is an intracellular enzyme required for the synthesis of the major inhibitory neurotransmitter, gamma-aminobutyric acid (GABA)[(13)]. Two isoforms of GAD have been reported, GAD 65 and GAD 67. GAD 65 is primarily expressed in pancreatic β cells, while GAD 67 is expressed in brain tissues. Because patients with type 1 diabetes often develop autoantibody against GAD 65 (GAD antibody), it is widely used as a diagnostic tool in predicting early destruction of the pancreas. Furthermore, some patients who originally developed type 2 diabetes that later progressed into type 1diabetes are also known to develop GAD antibody when pancreatic β cells are destroyed. Thus, the GAD antibody titer is believed to be an important predictive marker for the early phase of pancreatic destruction due to autoimmune reaction [(12)].

TakayakuKono et al. 2001[(12)] concluded that GAD is ubiquitously expressed by human gingival and periodontal ligament fibroblasts. Also, there is evidence that periodontal inflammation may result in higher levels of GAD and influence GAD antibody titre. Thus, severe periodontitis may lead to an extent GAD autoimmunity induced Type 1 diabetes mellitus.

Glycohemoglobin is formed continuously in erythrocytes as a product of the non-enzymatic reaction between the hemoglobin protein, which carries oxygen molecules, and glucose. Binding of glucose to hemoglobin is highly stable; thus, hemoglobin remains glycated for the life span of the erythrocyte, approximately 123 ± 23 days [(14)]. Determination of glycohemoglobin levels provides an estimate of the average blood glucose level over time, with higher average blood glucose levels reflected in higher HbA1c values[(15)].Although many studies reported more severe periodontal disease in subjects with diabetes than those without diabetes, few examined the association between periodontitis and glycemia or the level of glycosylated hemoglobin in adults without diabetes[(16)].

Since both age and the prevalence of chronic periodontitis and diabetes type 2 occurs at almost similar time period i.e 35 – 65 yrs, we can hypothesize that inflammatory chronic periodontitis may lead to β cell dysfunction and thus cause decreased production of insulin, which is characterized by low C-peptide levels and increased GAD antibody titre. This can be an alternative mechanism other than insulin resistance by which periodontal disease acts as a risk factor for developing type 1 diabetes mellitus in adults or exacerbate the progression of type 2-diabetes to type 1-diabetes.

Thus, in the present study we evaluated the levels of C-peptide, GAD-antibody, and HbA1c in Experimental **(CH-SH)** and Control **(SH)** group.

II. Material And Method:

This is a case control study and the subjects included for this study were selected from patients visiting outpatient **Department of Periodontology, Rishiraj College of Dental Sciences and Research Centre, Bhopal**. This study was carried out over a period of 1 year. Ethical Committee clearance was obtained from the Ethical Committee of Rishiraj College of Dental Sciences and Research centre, Bhopal.

Criteria For Patient Selection

Inclusion Criteria

A total of 30 patients belonging to age group 35-65 years were selected, among which 15 patients were categorized into systemically healthy chronic generalized severe periodontitis group**(CP-SH)(Experimental group) (according to AAP criteria 1999)[(17)]** and 15 patients into a group of systemically healthy individuals without periodontitis**(SH)(control group).**

1. The selected subjects in experimental group (CP-SH), had the presence of more than 16 teeth, ≥ 5 mm clinical attachment loss (CAL) in more than 30% sites and radiographic evidence of bone loss.
2. The selected subjects in control group (SH) had the presence of more than 20 teeth, Probing depth ≤ 3 mm, with no clinical attachment loss and no radiographic evidence of alveolar bone loss.

Exclusion Criteria

1. Patients with history of diabetes mellitus, hypertension or any other systemic diseases.
2. Patients with family history of Diabetes
3. Pregnant& lactating females.
4. Patients having habit of tobacco chewing, smoking and intake of alcohol.
5. Patients were excluded if they had taken antibiotics one month prior to the inclusion for the study.
6. Patients who have received periodontal treatment during the past 6 months.

All the subjects in the study were matched for age, gender and other parameters. Written informed consent was obtained from the subjects before starting the study.

Through an interview process, an extensive history was recorded.

Clinical Measurements

Clinical pocket depth and clinical attachment loss was recorded using a pressure sensitive probe with UNC markings. All measurements were performed by a single calibrated examiner and a calibration exercise was performed to ensure acceptable intra-examiner reproducibility. Calibration exercise was performed by recording pocket depth and clinical attachment loss at two different occasions, 1 hr apart. Obtained data was subjected to Kappa statistics, with values 0.81- 0.99which comes under perfect agreement.

The following parameters were recorded:

1. Gingival bleeding index was recorded by using**Ainamo and Bay gingival bleeding index.**
2. Probing pocket depth and clinical attachment loss at mesio-buccal, mid-buccal, disto- buccal and mesio-palatal, mid-palatal, disto-palatal aspects of all teeth.

Pressure Sensitive Probe

This probe has a probe tip with a diameter of 0.5 mm. A controlled probing pressure of 20 gm is usually applied. It has a visual guide and a sliding scale where two indicator lines meet at a specified pressure. Probes are color-coded, having demarcation between 5-10-15 mm markings**.**

Gingival Bleeding Index (Ainamo and Bay 1975)[(18)]

The presence or absence of gingival bleeding is determined by gentle probing of the gingival crevice with a periodontal probe. The appearance of bleeding within 10 seconds indicates a positive score that is expressed as a percentage of the total number of gingival margins examined. Each tooth was scored in 4 areas: Facial, Palatal/Lingual, Mesial, and Distal.

Bleeding score = Total score/ Number of surfaces examined

Periodontal Parameters

Probing pocket depth

Probing pocket depths were measured from the crest of gingival margin to the base of the pocket.

Each tooth was scored in 6 areas: mesio-buccal, mid-buccal, disto-buccal, mesio-palatal, mid-palatal, and disto-palatal surfaces.

Clinical attachment loss

Clinical attachment loss was measured from the cemento-enamel junction to the base of the pocket.

Each tooth was scored in 6 areas: mesio-buccal, mid-buccal, disto-buccal, mesio-palatal, mid-palatal, and disto-palatal surfaces.

Radiographic findings

Orthopantamographs (OPGs) of systemically healthy subjects with chronic generalized periodontitis were taken, to show the radiographic evidence of bone loss.

Laboratory parameters

GAD antibody titer, HbA1c and C-peptide levels analysis

Analysis of the sample will be done by **using Glycosylated Proteum technology in Diabetomics laboratory (CLIA), 20,000 N.W. Walker road, Beaverton, Oregon 97006 (USA).**

1 ml of intra venous blood from antecubital vein was collected. Full drop of blood was placed at the centre of a circle on the card. Out of 8 circles minimum of 3 full circles are needed for complete testing. After air drying the card at room temperature for 3 hours, the card was placed in the foil pouch and sent for testing(Phtograph 1)**.**

III. Result:

A total of 30 systemically healthy patients were included in the study, of which 15 patients were of chronic generalized periodontitis patients (experimental group) (CP-SH) and 15 were periodontally healthy patients (control group) (SH). The periodontal parameters and laboratory tests were recorded and results were calculated.

The statistical analysis was done using SPSS software version 19.0 IBM USA, using the following tests:

MEAN: mean of the data was calculated as follows:

(X1 + X2 + X3 + ... +XN) / N

X1, X2, X3, XN are the values of the observations being averaged and N equals the number of observations.

STANDARD DEVIATION: Standard deviation of the data was calculated as follows:

$$s = \sqrt{\frac{\sum(x - \bar{x})^2}{n - 1}}$$

UNPAIRED t-TEST: Unpaired t-test wasused for comparison of changes in quantitative variables i.e. age, number of teeth, bleeding index, CAL, PPD, C-peptide, GAD autoantibody, HbA1c of one group to another. It was calculated as follows:

$$t = \frac{\bar{x}_1 - \bar{x}_2}{\sqrt{s^2\left(\frac{1}{n_1} + \frac{1}{n_2}\right)}}$$

$$s^2 = \frac{\sum_{j=1}^{n_1}(x_j - \bar{x}_1)^2 + \sum_{i=1}^{n_2}(x_i - \bar{x}_2)^2}{n_1 + n_2 - 2}$$

Where x bar 1 and x bar 2 are the sample mean, s^2 is the pooled sample variance, n_1 and n_2 are the sample sizes and t is a student t quantile with $n_1 + n_2 - 2$ degrees of freedom.

Demographic Characteristics

Table 1, Graph 1 and Table 2, Graph 2

The subjects were in age range of 35-65 years with mean age of (control group) being 42.0 ± 8.4 years and experimental group being 43.80 ± 7.01 years. The control group consisted of 6 males and 9 females and experimental group also consisted of 6 males and 9 females.

Table 1: Demographic Distribution Of Study Subjects According To Gender

GENDER	HEALTHY (SH)	DISEASE (CP-SH)	TOTAL
MALE	6	6	12
FEMALE	9	9	18
TOTAL	15	15	30

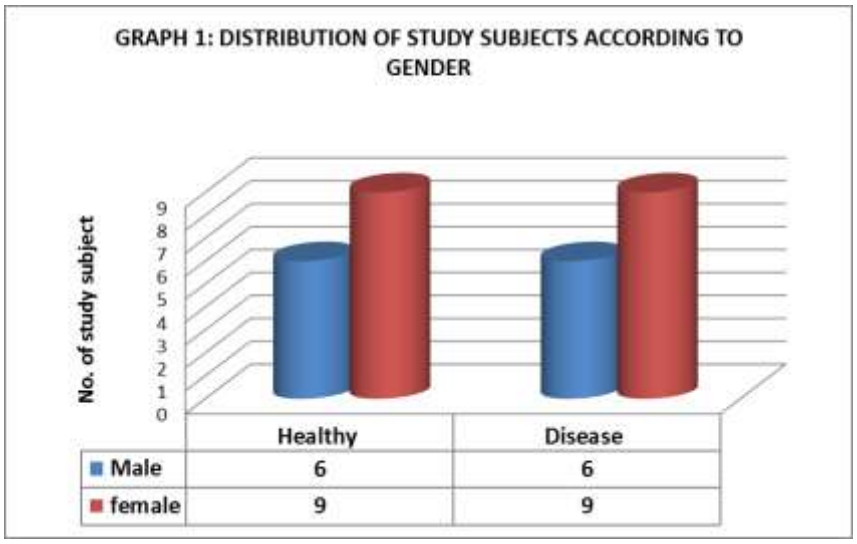
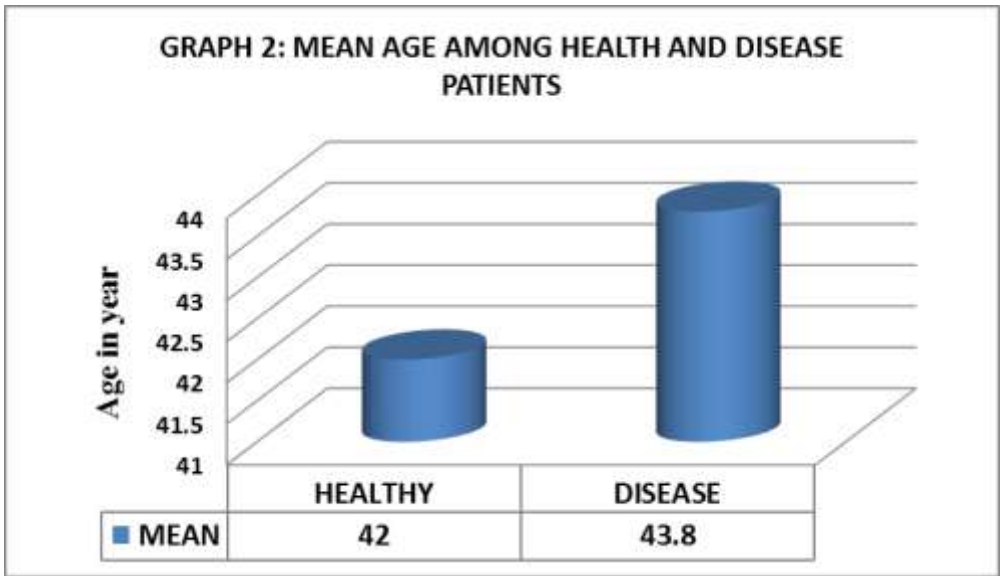


TABLE 2: MEAN AGE AMONG HEALTHY (SH) AND DISEASE (CP-SH) PATIENTS

SUBJECTS	NUMBER	MEAN	S.D	MEAN DIFFERENCE	Unpaired student 't' test value	P VALUE
HEALTHY (SH)	15	42.0	8.4	1.8	0.633	0.532
DISEASE (CP-SH)	15	43.80	7.01			



Clinical And Laboratory Parameters

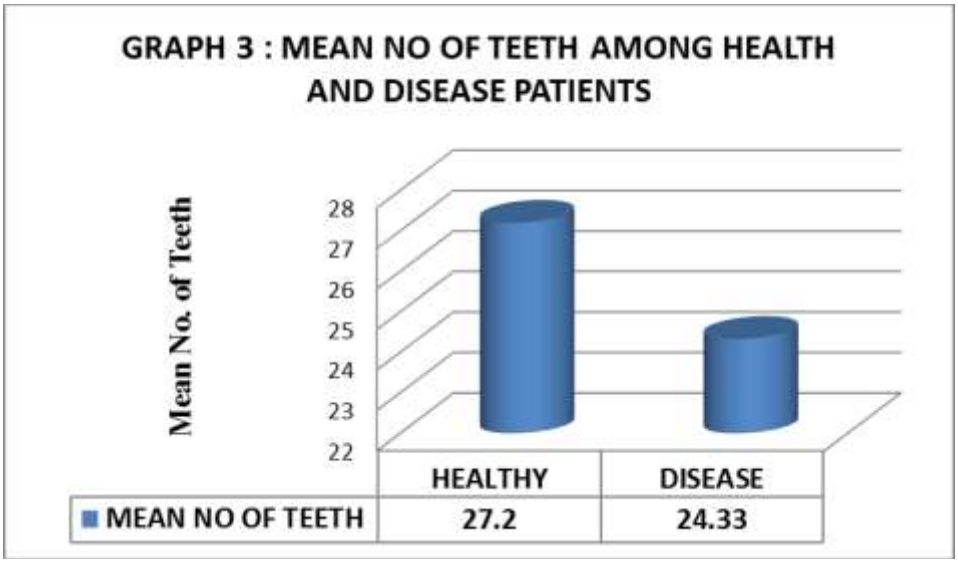
Periodontal Parameters

Number of teeth (Table 3, Graph 3)

In experimental group, the number of teeth was found to be 24.33 ± 2.66 versus 27.20 ± 1.14 in control group, the difference being highly significant (P = 0.001)

Table 3: Mean Number Of Teeth Present Among Healthy (Sh) And Disease (Cp-Sh) Patients

SUBJECT	NUMBER	MEAN	S.D	MEAN DIFFERENCE	Unpaired student 't' test value	P VALUE
HEALTHY (SH)	15	27.20	1.14	2.86	3.829	0.001(HS)
DISEASE (CP-SH)	15	24.33	2.66			

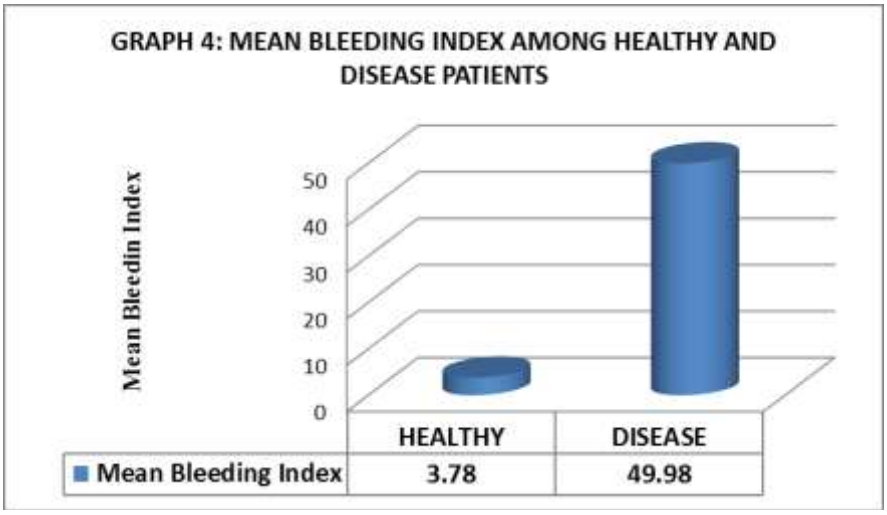


Bleeding index (Table 4, Graph 4)

In experimental group, the bleeding index was found to be 49.98 ± 11.89% versus 3.78 ± 1.96 % in control group, the difference being highly significant ($p < 0.005$).

Table 4: Mean Bleeding Index Among Healthy (Sh) And Disease (Cp-Sh) Patients

SUBJECT	NUMBER	MEAN	S.D	MEAN DIFFERENCE	Unpaired student 't' test value	P VALUE
HEALTHY (SH)	15	3.78	1.96	46.19	14.839	0.001
DISEASE (CP-SH)	15	49.98	11.89			

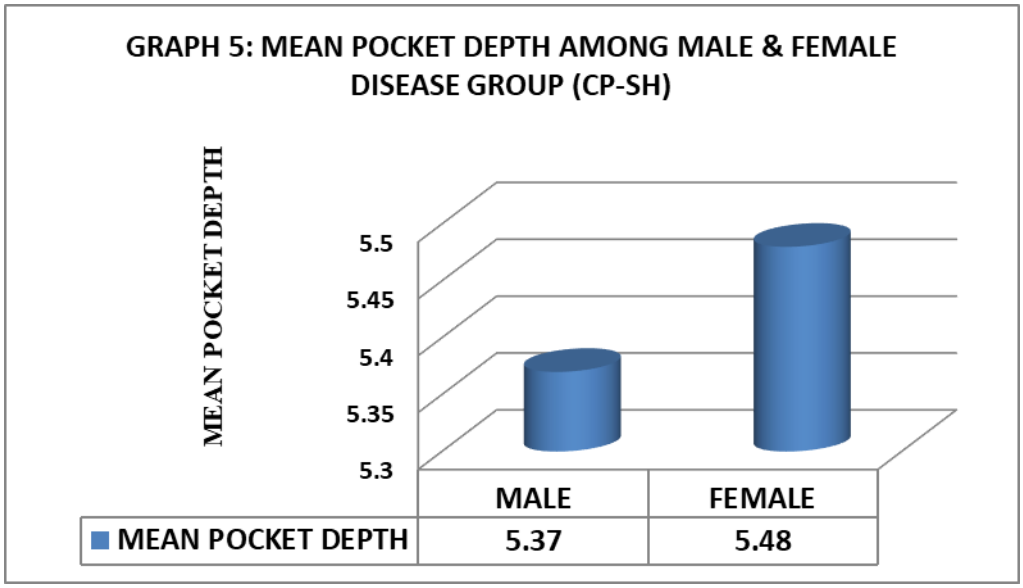


Probing pocket depth (PPD) (Table 5, Graph 5)

In experimental group, the mean PPD was found to be 5.43 ± 0.828 mm. mean PPD in female patients was found to be 5.48 ± 1.05 mm versus 5.37 ± 0.36 mm in male patients.

Table 5: Mean Pocket Depth Among Males& Females Diseased(Cp-Sh) Group

GENDER	NUMBER	MEAN	S.D.	MEAN DIFFERENCE	Unpaired student 't' test value	P VALUE
MALE	6	5.37	0.36	0.113	0.251	0.806
FEMALE	9	5.48	1.05			

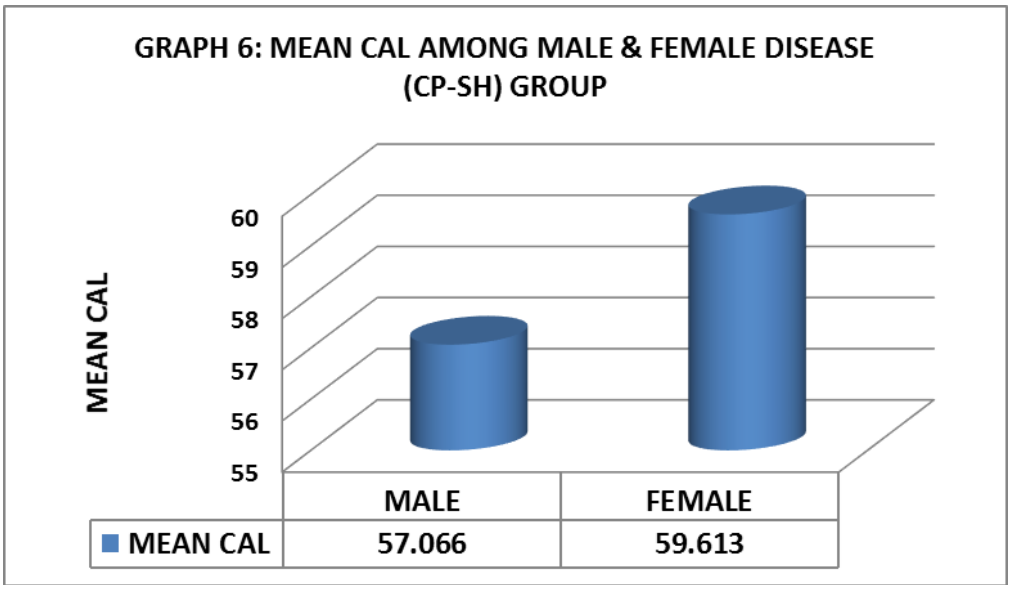


Clinical attachment loss (CAL) (Table 6, Graph 6)

In experimental group, the mean CAL was found to be 58.59 ± 10.65 mm. Mean CAL, in female patients was found to be 59.613 ± 7.99 mm versus 57.066 ± 14.43 mm in male patients.

Table 6: Mean Cal Among Males& Females Diseased (Cp-Sh) Group

GENDER	NUMBER	MEAN	S.D.	MEAN DIFFERENCE	Unpaired student 't' test value	P VALUE
MALE	6	57.066	14.53	2.54	0.440	0.667
FEMALE	9	59.613	7.99			



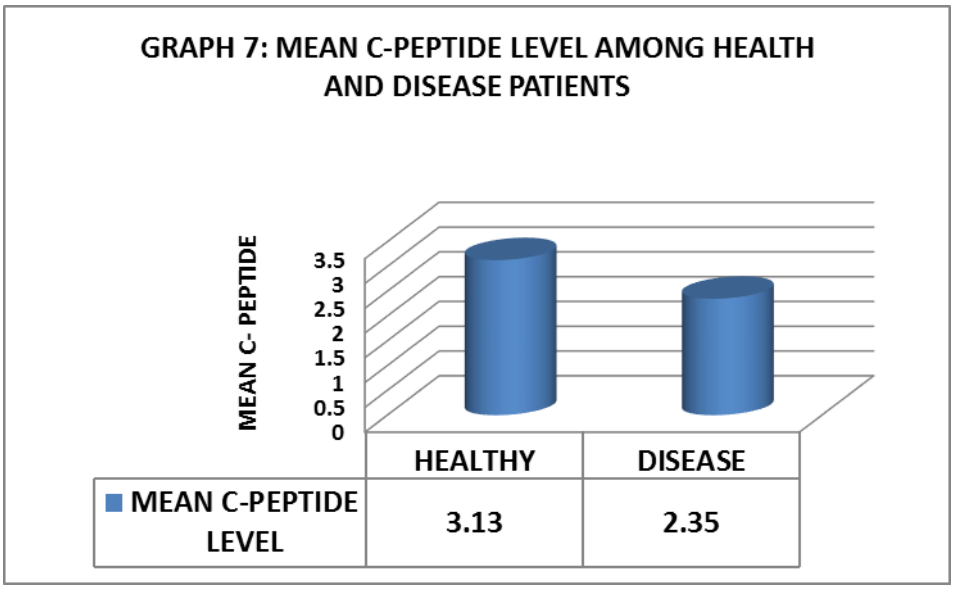
Laboratory parameters

C- peptide (Table 7, Graph 7)

C-peptide levels were found to be 2.35 ± 0.98 ng/ml in experimental control versus 3.13 ± 1.79 mg/ml, in control group, with difference being statistically non-significant (P = 0.152).

Table 7: Mean C Peptide Level Among Healthy (Sh) And Diseased(Cp-Sh) Group

SUBJECT	NUMBER	MEAN	S.D.	MEAN DIFFERENCE	Unpaired student 't' test value	P VALUE
HEALTHY (SH)	15	3.13	1.79	0.78	1.473	0.152
DISEASED (CP-SH)	15	2.35	0.98			

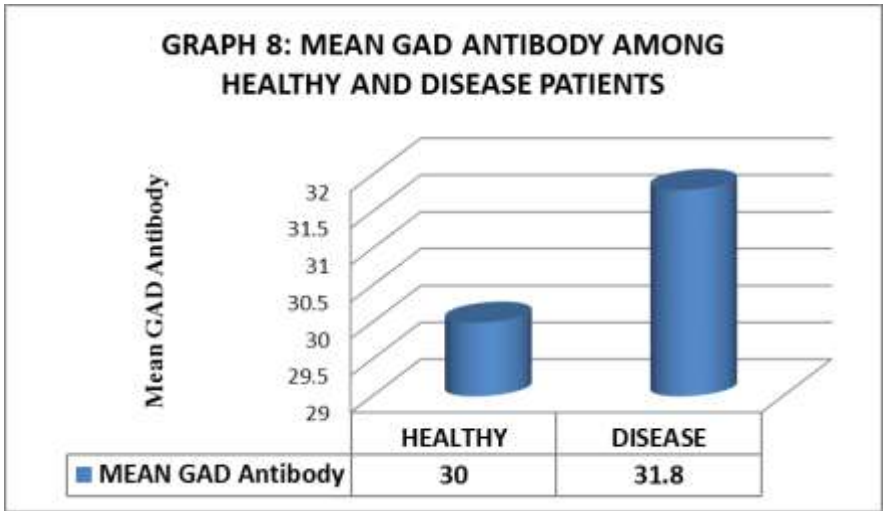


GAD – antibody (Table 8, Graph 8)

In experimental group GAD antibody were found to be 31.8 versus 30 ± 4.84 IU/ml, with difference being statistically non-significant. (P = 0.161)

Table 8: Mean Gad Antibody Among Healthy (Sh) And Diseased(Cp-Sh) Group

SUBJECT	NUMBER	MEAN	S.D.	MEAN DIFFERENCE	Unpaired student 't' test value	P VALUE
HEALTHY (SH)	15	30	4.84	1.80	1.439	0.161(NS)
DISEASED (CP-SH)	15	31.8	0.00			

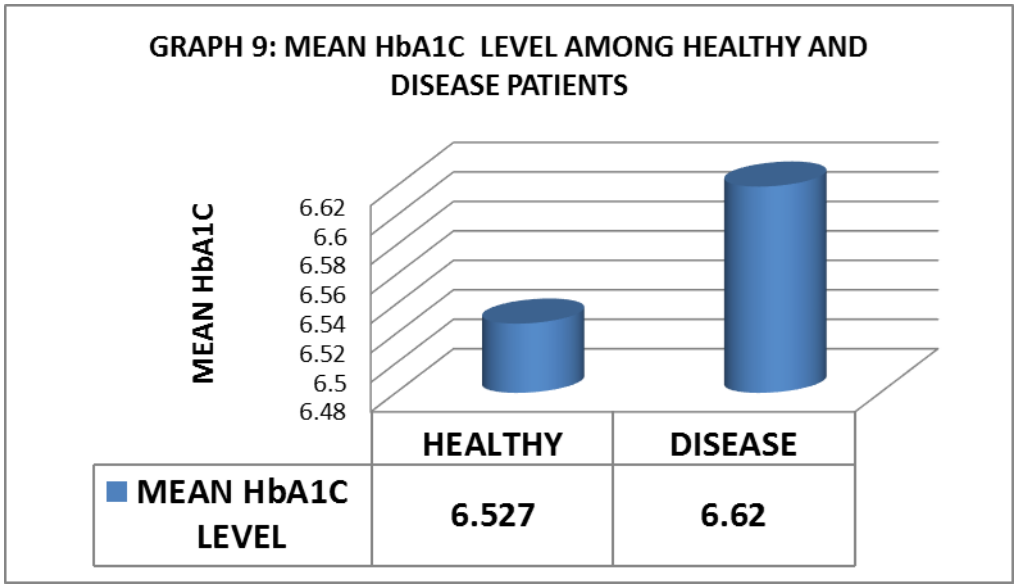


HbA1c (Glycated haemoglobin) (Table 9, Graph 9)

In experimental group HbA1c levels were found to be 6.620 ± 0.50 % versus 6.527 ± 0.44 %, in control group, with difference being statistically non-significant (P = 0.59).

Table 9: Mean Hba1c Level Among Healthy (Sh) And Diseased(Cp-Sh) Group

SUBJECT	NUMBER	MEAN	S.D.	MEAN DIFFERENCE	Unpaired student 't' test value	P VALUE
HEALTHY (SH)	15	6.527	0.443	0.0933	0.541	0.593
DISEASED (CP-SH)	15	6.620	0.50			



IV. Discussion:

Diabetes mellitus comprises a clinically and genetically heterogeneous group of metabolic disorders manifested by abnormally high levels of glucose in the blood [(19)].

The diabetes epidemic is more pronounced in India, as the World Health Organisation (WHO) reports show that 32 million people had diabetes in the year 2000[(20)].The International Diabetes Federation estimates the total number of diabetic subjects to be around 40.9 million in India and this is further set to rise to 69.9 million by the year 2025[(21)]. "India is thus the diabetes capital of the world"[(22)].

The two main types of diabetes are classified primarily on the basis of their underlying pathophysiology – Type 1 (T1DM) and Type II Diabetes mellitus (T2DM)[(23)].Type 1 diabetes, which results from autoimmune destruction of insulin-producing β-cells in the pancreas, leading to total loss of insulin secretion. Insulin is used by the body to facilitate the transfer of glucose from the bloodstream into the target tissues, such as muscle, where glucose is used for energy. Because a person with type 1 diabetes no longer produces endogenous insulin, glucose is unable to enter target cells and remains in the bloodstream, resulting in sustained hyperglycemia[(24)].

Type 2-diabetes, which results from insulin resistance rather than from total absence of insulin production. Autoimmune destruction of β-cells does not occur in type 2 diabetes, and patients retain the capacity to secrete some insulin, although production often diminishes over time. Patients with type 2 diabetes can remain undiagnosed for years because hyperglycemia appears gradually and often without symptoms. Insulin resistance results in a decreased capacity to transfer glucose into target cells; thus, hyperglycemiadevelops[(24)].

Periodontal disease is an entity of localized infections that involve tooth supporting tissues, the structures that make up the periodontium (i.e., gingiva, periodontal ligament, root cementum, and alveolar bone)[(1)].Gingival and Periodontal diseases affect 90% of Indian population. Advanced periodontal disease with pocket formation and bone loss, leading to tooth loss affects 40-45% of the population [(25)].World health organization (WHO) reported that 10-15% of world populations suffer from severe periodontitis[(26)].

Diabetes mellitus is, as previously stated, a systemic disease associated with serious complications that can affect the quality of life and life expectancy of the patient.Periodontitis is considered as the sixth complication of diabetes and the association between periodontitis and diabetes mellitus is well established [(27)].

Periodontal disease is more prevalent among diabetic patients than among non-diabetic patients. More poorly controlled diabetes is, the more severe the periodontal disease [(28)]. **Nelson et al. (1990)**[(29)] in a longitudinal Pima Indian Population studies showed increase in the prevalence of attachment and bone loss in diabetic patients with poor metabolic control.

Several studies have also shown that control of periodontal infection through mechanical therapy combined with systemic antibiotics improved glycemic control, leading to reduced requirement of insulin in Insulin Dependent Diabetes Mellitus (IDDM) patients [(28, 30)].

Thus, a bidirectional relationship exists between diabetes mellitus and periodontitis[(31)].Diabetes mellitus not only influence the pathophysiology of periodontal disease in a one way fashion, but periodontal disease in turn influence the diabetic status in reciprocal fashion[(32)].Inflammation is a common link between diabetes and periodontal disease.

It has been suggested that subclinical inflammation is linked to insulin resistance [(33)]. Due to the dynamic nature of the inflamed periodontium in chronic periodontitis, the tissue may serve as an endocrine-like source of inflammatory mediators [(34)]. Chronic periodontal infection leads to an increase in serum tumor necrosis factor α, interleukin-1, interleukin-6 and C-reactive proteins[35]. The increase in these cytokines may increase insulin resistance by interfering with glucose and lipid metabolism and antagonizing insulin action. The increased insulin resistance will ultimately cause an increase in the risk for type 2 diabetes [(34)]. Tumor necrosis factor α has been considered as the causative factor in insulin resistance and Type 2 Diabetes mellitus in animal models and human studies [(36)].

Infection induced insulin resistance syndromes, if long standing or chronic, are considered to be precursors toactive diabetes due to destruction of pancreatic β-cells that results from sustained elevations of IL-1β and tumor necrosis factor α [(36)]. Some studies have also suggested that a pro-inflammatory imbalance created by excess of TNF α/ IL-1β is one of the most critical determinants of β-cell loss in diabetic patients[(37)].Elevated level of IL-1β are also thought to play a role in development of Type 1 diabetes mellitus[(6)]. It has been demonstrated that IL-1β facilitates protein kinase C activation leading to pancreatic β- cell destruction through apoptotic mechanisms, as the pancreatic islet cells express the highest density of IL-1 receptors among all body tissues[(9)]. Additionally, IL-1β has been shown to be cytotoxic to β-cells in culture and animal models through depletion of cellular energy and production of nitric oxide [(38)].

All these inflammatory markers (TNF-α, IL-1β, and IL-6) are also elevated in chronic periodontitis[(3)]. Thus, here we hypothesized that this can be an alternative mechanism other than insulin resistance by which periodontal disease leads to type 1-adult onset diabetes or exacerbate the progression of type 2 diabetes to type 1 diabetes mellitus.

Hence, in our present study we analysed the β-cell function, by evaluating C-peptide levels in Group I (CP-SH) (Experimental group) and Group II (SH) (Control group) through Proteum technology (Diabetomics labs, USA). C-peptide levels was found to be less in experimental group than control group, however the differenceswere not statistically significant **(0.98 vs. 1.79, P value: 0.152)(Table 7, Graph 7).**

Merchant et al. (2011) [39] conducted a study to evaluate C-peptide levels in young diabetic patients (Type 1 and 2 diabetes) with and without periodontitis.They found that among youth with type 2 diabetes, those with periodontal damage had lower fasting c-peptide (2.3 vs. 3.4 ng/mL, p-value=0.01) levels and concluded that Periodontal damage is associated with impaired beta cell function and metabolic syndrome components. They concluded by saying that these findings need to be confirmed in larger, prospective studies. Their findings are similar to findings of our study.

Recently Hasaan G. Mohamed et al. (2015) [40] conducted a study, to compare the levels of 10 glucoregulatory biomarkers including C-peptide in GCF, of152 subjects non diabetics, with (G1) and without chronic periodontitis (G2). They concluded that,the level of C-peptide was lower among subjects with periodontitis (G1) than in those without periodontitis (G2) and the results were statistically significant. Moreover, the amount of C-peptide was negatively correlated with the number of diseased sites with PD ≥ 4 mm, suggesting **that chronic periodontitis might be associated with disturbed β-cells activity**. The statistical difference observed in this study may be due to large sample size compared to our study. Hence further studies with large sample size are needed.

C-peptide and insulin are released into the circulation in comparable amounts, but C-peptide is a more reliable indicator of β-cell activity, as it has a longer half-life.Monitoring residual β-cell function through the measurement of C-peptide is a strategy endorsed by the **American Diabetes Association (ADA)**, and it recommends the use of C-peptide measurements as the outcome measure in clinical trials investigating methods to preserve β-cell function [11].

Thus, in our present study, C-peptide value was evaluated.

In our present study we evaluated the GAD-antibody levels, in both experimental (CP-SH) and control group (SH). However, no statistically significant difference was found between both the groups but in experimental group out of 15 patients 2 patients expressed very high GAD anti-body titers**(Table 8, Graph 8))**.In a similar study,**Takayuki Kono et al (2001)[12]** evaluated if certain cells in periodontal tissue could express GAD antibody in periodontitis patients as compared to systemically healthy without periodontitis. They found that 2 out of 62 periodontitis patients expresses GAD antibody in comparison to healthy patients, however it was not statistically significant**(31.8 vs 30 ± 4.84, P = 0.161)**. Thus, they concluded that periodontal inflammation may result in higher level of GAD, and influence GAD antibody titer.

Nishimura F et al. (2000)[41] in a review on Negative effects of chronic inflammatory periodontal disease on diabetes mellitus concluded that gingival and periodontal ligament fibroblasts were found to express glutamate decarboxylase, and some otherwise healthy periodontitis patients develop anti-glutamate decarboxylase antibody. Chronic periodontitis may influence the level of this antibody which is widely used as a predictive marker for slowly progressive insulin dependent diabetes mellitus.

Autoantibodies such as islet cell antibody (ICA), insulin autoantibody (IAA), and GAD anti body are predictive markers for type 1 diabetes. Since the GAD antibody is detected in 70-80% of slowly progressive insulin dependent diabetes patients who develop type 2 diabetes and then progress to type 1 diabetes, and since it tends to be observed for longer period of time after the onset of type 1 diabetes, this antibody is most commonly used for diagnosing type 1 diabetes.

In chronic periodontitis, as a result of continuous chronic inflammation and destruction of periodontal connective tissue, the intracellular components of periodontal tissues may receive exposure to the local immune system during which time the autoantibodies against such antigens could be developed [12].

Several studies have shown that periodontal treatment of diabetic patients decreased HbA1c levels. However it remains unclear whether periodontal status affects HbA1c in non-diabetics [42].

Thus, in our study we evaluated HbA1c levels in experimental (CP-SH) (with periodontitis) and control (SH) (without periodontitis) groups. HbA1c levels was found to be more in experimental group than control group. However, the difference was not found to be statistically significant **(6.62 vs 6.52, p-value: 0.593) (Table 9, Graph 9).** This result was expected as none of the patients included in our study werediabetics.

Ryan E. Wolff et al. (2009)[43]conducted a pilot study to evaluate HbA1c levels in periodontitis cases (59 subjects) and healthy cases (53 subjects) without diabetes. Unadjusted mean HbA1c levels did not differ significantly between cases and controls (5.66% –0.56% versus 5.51% – 0.44%; P = 0.12). However, after adjusting age, gender, BMI, and current smoking, mean HbA1c was significantly higher in periodontitis cases (between-group difference, 0.21%; P = 0.046) than in control group but the difference was very less. Thus, they concluded that Periodontitis is associated with a slight elevation in glycosylated hemoglobin. The clinical significance of this difference remains to be determined. This preliminary finding is consistent with earlier reports and from our study that periodontitis is associatedwith elevated blood glucose in adults without diabetes

and may increase one's risk for diabetes.They suggest that, there may be a threshold, above which the periodontitis affects the HbA1c values in the general population, and this finding needs to be confirmed in larger studies.

Padma Rajan et al. (2013)[(44)]conducted a study to determine if glycosylated haemoglobin (HbA1c) is elevated in systemically healthy chronic periodontitis (70 patients) in comparisonto systemically healthy patients without periodontitis patients (70 patients) and also to compare the HbA1c levels that were obtained with lab and chairside test kit. They found that, The unadjusted mean HbA1c levels with kit in cases were 5.51 ± 0.53, while in controls they were 5.44 ± 0.27, but the difference was statistically not significant (P = 0.38). The unadjusted mean HbA1c levels with lab in cases were 5.50 ± 0.74, while in controls they were 5.48 ± 0.29. However, the difference was statistically not significant (P = 0.841).

Ruchika et al. (2015) [(42)] conducted a studyto determine if glycosylated hemoglobin is elevated in patients with periodontitis who are non-diabetic adults.A total of 36 patients were selected and were divided into test and control groups. They found that, both the groups showed similar HbA1c levels, but there was a marginal increase in levels in the test group (cases), which was not statistically significant (cases- 6.06%, controls-5.8%; P=0.101). They thus concluded that these data suggest a possible link between periodontitis and glycemic control in non-diabetic individuals. Periodontal disease may be a potential contributor to development ofdiabetes.

Our study shows a positive correlation between the periodontitis and HbA1c values. Further studies with a larger sample size may give statistically significant results.

Glycohemoglobin is formed continuously in erythrocytes as a product of the non-enzymatic reaction between the hemoglobin protein, which carries oxygen molecules, and glucose. Binding of glucose to hemoglobin is highly stable; thus, hemoglobin remains glycated for the life span of the erythrocyte, approximately 123 ± 23 days.Measurement of HbA1c is of major clinical valueand accurately reflects the mean blood glucose concentration over the preceding 1–3 months.

V. Summary And Conclusion:

The aim of this study was to assess the interrelationship between periodontitis and diabetes by evaluating the levels of C-peptide, GAD autoantibody and HbA1c in serum of systemically healthy patients with and without chronic severe periodontitis, without any risk factors.

From the observations of this study following conclusions were drawn:

1. C-peptide levels was found to be less in experimental group than control group, however the differences were not statistically significant,suggesting that chronic periodontitis might be associated with disturbed β-cell activity.
2. GAD-antibody levels, in experimental was found to be more than control group. However, no statistically significant difference was found between both the groups. In experimental group, out of 15 patients 2 patients expressed very high GAD anti-body titers, suggesting that chronic periodontitis may influence the level of this antibody which is widely used as a predictive marker for slowly progressive insulin dependent diabetes mellitus.
3. HbA1c levels were found to be more in experimental group than control group. However, the difference was not found to be statistically significant.

With the above findings, we conclude thatchronic periodontitis can act as a risk factor for slowly progressive Type 1 diabetes mellitus or progression of Type 2 diabetes to Type 1 diabetes mellitus. In some of the studies, where after periodontal therapy no improvements were found in glycemic control of diabetic patients, probably Type 2 diabetes mellitus may have progressed to Type 1 diabetes mellitus.

Further randomized clinical trials (RCT's), and prospective studies with larger sample size, are required to confirm the above drawn conclusions.

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