Evaluation and Comparison of Serum Vitamin D and Calcium Levels in Periodontally Healthy, Chronic Gingivitis and Chronic Periodontitis in Non Diabetics and Diabetes Mellitus Patients - A Cross Sectional Study

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LIST OF ABBREVIATIONS

SR. NO	SHORT FORM	FULL FORM
1	CP	Chronic periodontitis
2	T2DM	Type 2 diabetes mellitus
3	TNF-α	Tumor necrosis factor - α
4	IL	Interleukin
5	PI	Plaque index
6	GI	Gingival index
7	PPD	Periodontal pocket depth
8	CAL	Clinical attachment level
9	BOP	Bleeding on probing
10	HbA1c	Glycosylated hemoglobin
11	ELISA	Enzyme linked immunosorbent assay
12	CRP	C - reactive protein
13	NF-κβ	Nuclear factor kappa beta
14	SRP	Scaling root planing
15	RBS	Random blood sugar
16	GCF	Gingival crevicular fluid
17	NIDDM	Non insulin dependent diabetes mellitus
18	25 (OH)D	Vitamin D
19	1,25-(OH)2 D	Calcitriol
20	Ca	Calcium
21	NHANES III	Third National Health and Nutrition Examination Survey
22	IFG	Impaired fasting glucose
23	CPI	Community periodontal index
24	WHO	World health organization
25	PGE-2	Prostaglandin E2
26	IF-γ	Interferon - γ
27	S	Significant

28	NS	Non significant
29	HS	Highly significant
30	G	Gram
31	g/m^2	$Gram/meter^2$
32	mmol/L	Milli mol per litre
33	mg/l	Milli gram per litre
34	g/l	Gram per litre
35	Cm	Centimeter
36	mm Hg	Millimeter of mercury
37	mg/dl	Millimeter per decilitre
38	mg/ml	Milligram per millilitre
39	CVD	Cardiovascular disease
40	ng/ml	Nanogram per millilitre
41	µl	Microliter
42	rpm	Random blood sugar
43	DMFT	Decayed missing filled teeth
44	PDGF	Platelet derived growth factor
45	IFG	Impaired fasting glucose
46	P.gingivalis	Porphyromonas gingivalis

INTRODUCTION

Periodontal diseases are among the most prevalent dental diseases affecting people worldwide as well as the Indian community. Chronic periodontitis is a complex interaction between micro-organisms and the host tissues, associated with plaque and calculus accumulation. The bacteria's are the main cause of disease progression along with the host immuno-inflammatory response to chronic infection. It progresses with a slow to rapid rate .The increase in the rate of disease progression is caused by the impact of local, systemic or environmental factors that influence the normal host-bacterial interaction. Chronic periodontitis is also considered a potential risk factor for systemic diseases such as cardiovascular disease, type 2 diabetes mellitus (T2DM) etc.[1]

Diabetes mellitus is a metabolic disorder characterized by hyperglycemia due to defective secretion of insulin. Diabetes increases the glucose concentration in the

gingival crevicular fluid which further causes increase in plaque accumulation and change in its composition with an increased number of gram-negative anaerobes. As a result, there is impaired cellular functions, impaired host defense, vascular alterations, prolonged inflammation, impaired bone formation or repair ultimately resulting in tooth mobility and its loss. There is a two-way relationship between diabetes mellitus and periodontitis, [2] where local periodontal infection if present, can exacerbate and cause progression of diabetes. The consequences of which are alterations in glucose metabolism and regulation resulting in difficulties in maintaining optimal glycemic control. Also, chronic periodontal inflammation and pro-inflammatory cytokines such as interleukin-1 (IL-1) β, TNF-α and IL-6 lead to insulin resistance in T2DM. Conversely, diabetes influences the periodontium mainly due to the classic microvascular and macrovascular complications, changes in subgingival microbiota, and alterations in the host immuno-inflammatory response to potential periodontal pathogens.[3]

The discovery of receptors for 1α,25-dihydroxyvitamin D3 (1,25(OH)2D3), the activated form of vitamin D, in tissues with no direct role in calcium and bone metabolism (e.g. pancreatic beta cells and cells of the immune system) has broadened our view of the physiological role of this molecule. Associations between vitamin D status and cardio-metabolic diseases, for example, metabolic syndrome, obesity, diabetes, and hypertension had been reported previously. Vitamin D influences the expression of inflammation related cytokines and plays an important role in many chronic inflammatory diseases. There are evidence relating vitamin D deficiency with both periodontal disease and T2DM.[4]The prevalence of vitamin D deficiency in the general population varies by ethnic background, sunlight exposure, and the presence of

risk factors such as age, obesity, T2DM and other comorbidities. About one billion persons worldwide have been reported to have vitamin D deficiency or insufficiency.[5]

In addition to the central role of the optimal functioning of organ systems, including the cardiovascular, endocrine, and immune systems along with its well-known role in calcium/phosphorus homeostasis and bone physiology, it has been found that vitamin D can play a role in decreasing the risk of many chronic illnesses, including common cancers (e.g., breast, colon, prostate), autoimmune diseases, infectious diseases, hypertension, and cardiovascular diseases (CVDs). CVD risk factors are also affected by serum calcium and parathyroid hormone. The active metabolite of vitamin D, 1, 25 dihydroxy vitamin D inhibit cytokine production thus helping vitamin D exhibit potential anti-inflammatory effect. Thus, vitamin D deficiency results in bone loss and increased inflammation through its immuno-modulatory effects. Polymorphisms of the vitamin D receptor gene are found to be associated with periodontitis, alveolar bone loss, clinical attachment loss and/or tooth loss supporting the potential role of vitamin D in periodontal health. [6] The direct effect of vitamin D may be mediated by binding of its circulating active form, 1, 25-OHD to the β-cell vitamin D receptor. The indirect effects of vitamin D may be mediated via its important and well-recognized role in regulating extracellular calcium and calcium flux through the β –cell.[7] Thus vitamin D may improve insulin sensitivity and promote β -cell survival by directly modulating the generation and effects of cytokines.

There are evidence suggesting that calcium deficiency may also be one of the risk factors for periodontal disease.[8] As the chronically low intake of calcium along with vitamin D may lead to a negative calcium balance, thus causing a secondary increase in calcium removal from bone, including the alveolar bone. Such bone loss may contribute to the weakening of the tooth-attachment apparatus.

Also, calcium is essential for insulin-mediated intracellular processes in insulin-responsive tissues (skeletal muscle and adipose tissue) with a very narrow range of calcium needed for optimal insulin-mediated functions. Changes in calcium in primary insulin target tissues may contribute to peripheral insulin resistance leading to decreased glucose transporter-4 activity.[9] Insulin secretion is a calcium-dependent process and alterations in calcium flux can have adverse effects on β -cell secretory function. Inadequate calcium intake or vitamin D insufficiency may interfere with normal insulin release, especially in response to a glucose load by altering the balance between the extracellular and intracellular β -cell calcium pools. Thus, altered vitamin D and calcium homeostasis may also play a role in the development of T2DM. Also, the inverse association between vitamin D intake and prevalence of metabolic syndrome was dissipated after adjustment for calcium intake as observed in one of the study.[10]

Dietrich et al. (2005) [11] in a study concluded that lower levels of vitamin D are related to inflammation in the gums, a precursor to gingivitis. Vitamin D can be supplemented orally for 2 to 3 months to achieve the desired results for patients with gingivitis, although 2000 IU of vitamin D per day can produce the anti-inflammatory effect sooner. [12]

There is still need for more elaborate trials for better understanding the fluctuating vitamin D and calcium levels in inflammatory conditions like gingivitis, periodontitis and metabolic disease like diabetes mellitus which are co-related. Therefore the present study was undertaken to evaluate and compare vitamin D and calcium levels in serum of periodontally healthy, chronic gingivitis and chronic periodontitis patients with and without T2DM.

AIM AND OBJECTIVES

The present study aimed to evaluate and compare serum vitamin D and calcium levels in periodontally healthy, chronic gingivitis and chronic periodontitis in non-diabetics and diabetes mellitus (DM) patients. Along with the aim, the following were the objectives behind carrying out the study:

1. To evaluate the serum vitamin D and calcium levels in periodontally healthy patients.
2. To evaluate the serum vitamin D and calcium levels in chronic gingivitis in non diabetics and diabetes mellitus patients.
3. To compare the serum vitamin D and calcium levels in chronic gingivitis in non diabetics and diabetes mellitus patients.

4. To evaluate the serum vitamin D and calcium levels in chronic periodontitis in non diabetics and diabetes mellitus patients.
5. To compare the serum vitamin D and calcium levels in chronic periodontitis in non diabetics and diabetes mellitus patients.

REVIEW OF LITERATURE

Understanding the etiology and pathogenesis of periodontal diseases and their chronic, inflammatory and infectious nature necessitates recognizing the possibility that these infections may have effects somewhere else in the body. Microorganisms present in dental plaque and their metabolic products may enter the bloodstream, thereby causing many systemic diseases and sometimes resulting in degenerative conditions. Periodontal diseases and DM are closely associated and are highly prevalent chronic diseases with many similarities in pathobiology. Related antecedent conditions including vitamin D and calcium deficiencies may play an important role in this relationship. Deficient vitamin D and calcium alter the normal metabolic and endocrine function, resulting in increased hormones, cytokines, acute phase reactants and insulin resistance. Elevated circulating levels of several pro-inflammatory cytokines have been found in individuals with periodontitis.[13] Also, vitamin D and deficiency may place

subjects at risk for low mineral bone density/osteoporosis and osteopenia, as well as infectious and chronic inflammatory diseases.[14] It is important to highlight that type 2 diabetes mellitus occurs synergistically and/or concomitantly with other systemic diseases, jeopardizing the health status of affected individuals.

For the sake of better understanding, the review of literature has been divided into three parts

A. Studies on interrelationship between chronic gingivitis, chronic periodontitis and diabetes mellitus

B. Studies on interrelationship between vitamin D , calcium and diabetes mellitus

C. Studies on interrelationship between vitamin D, calcium and chronic gingivitis, chronic periodontitis.

A. Studies on interrelationship between chronic gingivitis, chronic periodontitis and diabetes mellitus

Shlossman M, Knowler WC, Pettitt DJ, Genco RJ. (1990)[15] presented a cross-sectional data of a population with a high prevalence of type 2 diabetes and with periodontal disease. In 2,878 subjects out of 3,219, data was collected from a bone score, probing examination or both and all the subjects were selected from the Gila River Indian Community, Ariz and standardized clinical examinations were performed in all of them. Each examination included evaluation of the oral mucous membranes by visual and digital examination, and recording of decayed, missing, and filled teeth and a panoramic radiograph (Siemens OP-IO). Six index teeth or their substitutes were used for the periodontal evaluation which included measurements of probing pocket depth and attachment levels. The panoramic films were used to determine interproximal crestal alveolar bone loss. A radiographic examination was performed by a trained

dental assistant for all eligible patients. The results showed that the prevalence of diabetes increased with age from 3% in the 5 to 24 year group to 60% in men and 71% in women aged > 45 years. The initial oral health survey and review of preoperative radiographs and dental records from patients who had lost teeth before showed that 72% of tooth loss were the result of periodontal disease. Also, preoperative radiographic examinations in 28 patients who lost their remaining teeth during the course of the study revealed the prevalence of the healthy category. The authors concluded that there are significant clinical implications that diabetes may be a risk factor for development of periodontal disease and that periodontitis is a complication of both type 1 and type 2 diabetes mellitus.

Katz PP, Wirthlin MR Jr, Szpunar SM, Selby JV, Sepe SJ, Showstack JA. (1991)[16] reviewed the epidemiological evidence of the relationship between diabetes and periodontal disease, possible physiological mechanisms for the association and effects of interventions on the occurrence and severity of periodontal disease among individuals with diabetes. Trends indicated that periodontal disease is more prevalent and more severe among individuals with diabetes which may be modified by factors such as oral hygiene, duration of diabetes, age and degree of metabolic control of diabetes. The authors suggested that association of diabetes and periodontal disease may be due to numerous physiological phenomena found in diabetes, such as impaired resistance, vascular changes, altered oral microflora and abnormal collagen metabolism. They also found that the subjects with diabetes who do not maintain good oral hygiene or good metabolic control of their diabetes or with other complications of diabetes, and teenagers and pregnant women appear to be particularly susceptible to periodontal disease.

Emrich LJ, Shlossman M, Genco RJ. (1991)[17] determined the relationship between diabetes mellitus and oral health status in Pima Indians from the Gila River Indian Community in Arizona. The probing attachment level, alveolar bone loss, age, sex, Calculus index, Plaque index, Gingival index, fluorosis, and DMFT as well as the diabetic status was assessed in 1,342 Pima Indians who were at least partially dentate. The prevalence and severity of destructive periodontal disease was determined by measuring probing attachment loss and radiographically apparent interproximal crestal alveolar bone loss, two independent but correlated indicators of periodontal destruction. Only diabetic status, age, and the presence of sub gingival calculus were significantly associated with both increased prevalence and greater severity of destructive periodontal disease in this population. Diabetic status was significantly and strongly related to both the prevalence and severity of disease after adjusting for the effects of demographic variables and several indices of oral health including the Plaque index. Authors found that, subjects with type 2 diabetes had an increased risk of destructive periodontitis with an odds ratio of 2.81 (95% confidence interval 1.91 to 4.13) when attachment loss is used to measure the disease. The odds ratio for diabetic subjects was 3.43 (95% confidence interval 2.28 to 5.16) where bone loss was used to measure periodontal destruction. The authors concluded that diabetes increases the risk of developing destructive periodontal disease about threefold. Furthermore, diabetes increases the risk of developing periodontal disease in a manner which cannot be explained on the basis of age, sex, and hygiene or other dental measures. Periodontitis should be considered a potential complication of diabetes in evaluation of patients.

Loe H. (1993)[18] reviewed from an ongoing longitudinal study of the periodontal condition in type II diabetes of the Pima Indians of the Gila River Community. The

Pima study population consisted of 2180 subjects 15 years old or older who had undergone one or more biennial clinical examinations, including a comprehensive oral and dental examination in the years between 1983 and 1988. For subjects seen more than once, the initial examination findings were used to determine the prevalence of periodontal disease. Incidence was computed for a subset of 746 subjects (295 men, 451 women) who were initially free of periodontal disease. Of this subset, 50 individuals had type II diabetes at the outset, whereas the remaining 696 did not have diabetes. Radiographic findings included panoramic films to measure interproximal crestal alveolar bone loss, which was scored as a percentage of bone loss from the cementoenamel junctions to the apex at the deepest point on the mesial or distal surface of each tooth present, excluding third molars. Periodontal disease was determined on the basis of the percentage of bone loss and on clinical findings of tooth loss which, in this population, is overwhelmingly attributable to periodontal disease. On the contrary, diabetes status was determined using the World Health Organization criteria for epidemiological studies, namely the 2-h, post load plasma glucose concentration >200 mg/dl. The authors found that approximately 8% of the non-diabetic subjects < 35 years of age had advanced periodontal disease as defined in the study. In contrast, the prevalence of this level of severity of disease in persons with type II diabetes in the same age group was 45-48%. Also, both loss of attachment and bone loss increase with age. However, diabetic subjects scored significantly higher for both of these indexes at all age levels, as compared with those with impaired glucose tolerance and those with normal glucose tolerance level. Periodontal disease affected >95% of individuals >55 years of age, regardless of their diabetes status. Measures of incidence were restricted to subjects 15-55 years of age. After controlling for age and sex, the rate of periodontal

disease in subjects with diabetes was 2.9 times, nearly threefold what it was in individuals without diabetes. The authors concluded that ,firstly the prevalence of advanced periodontal disease was substantially higher among type II diabetic persons than in non-diabetic persons of the Pima Indian community, secondly loss of periodontal attachment and alveolar bone started early in the diabetic population, thirdly the incidence rate of advanced periodontal disease was the same in men and women; higher age predicted a greater incidence rate, fourthly the rate of periodontal disease in subjects with type II diabetes was almost three rimes that in non-diabetic persons.

Tervonen T and Oliver RC. (1993)[19] compared the periodontal health specifically: tooth loss; the prevalence, extent and severity of periodontitis and the prevalence and extent of calculus of the general population to that of diabetic patients. A total of 114 patients, aged 20 to 64 years (mean age 40.6 years) volunteered to participate in the study. Out of total subjects, sixty-five were male and 49 female. The mean duration of their diabetes was 13.8 years. Of the 114 patients, 60 percent had type I diabetes. On the day of the examination, 39 patients were in good metabolic control of their diabetes with glycosylated hemoglobin's (HbAlc) of < 8 percent; 32 were in moderate control with HbAlc of 8 to 10 percent; and 22 had HbAlc >10 percent and were in poor control. While tooth loss and gingival attachment was similar for both groups, diabetics had a greater prevalence and extent of periodontal pockets. Diabetics with poor metabolic control and calculus also had more periodontitis. Tooth loss among this group of diabetics was similar to that of the employed adults. The rate of tooth loss was similar up to age 65. The differences between diabetics and employed adults were less when measurements from the mesial and buccal sites alone were considered, but

periodontal pockets were more prevalent and extensive among the diabetic group. The authors concluded that poor metabolic control, calculus and having diabetes for many years may increase the risk of periodontitis. Conversely, diabetics who control both their diabetes and oral health through self-care and regular professional care are at much lower risk for periodontitis and tooth loss.

Cutler CW, Machen RL, Jotwani R, Iacopino AM. (1999)[20] aimed to determine if diabetes cause a dysregulation of the gingival cellular and local cytokine response to adult periodontitis (AP) in humans. For this a total of 35 patients were selected who were categorized into the following groups based on the level of diabetic (type 2) control and presence or absence of adult periodontitis (AP): group 1, systemically and periodontally healthy (n = 6); group 2, systemically healthy with adult periodontitis (n = 7); group 3, well controlled diabetes and periodontally healthy (n = 6); group 4, well-controlled diabetes with adult periodontitis (n = 5); group 5, poorly controlled diabetes and periodontally healthy (n = 5); group 6, poorly controlled diabetes and adult periodontitis (n = 6). They all had to undergo a periodontal examination, including probing depths (PD), clinical attachment levels (CAL), gingival index (GI), plaque index (PI), and vertical bitewing radiographs. Peripheral blood, as well as gingival tissue (GT) and gingival crevicular fluid (GCF), was obtained from all subjects. Blood studies included levels of glycated hemoglobin (HbA1c), triglycerides (TG), cholesterol (CHL), low-density lipoproteins (LDL), and high-density lipoproteins (HDL). The levels of interleukin-1 beta (IL-1β) in GCF and GT, interleukin-6 (IL-6), and platelet-derived growth factor AB (PDGF-AB) in GT from patients in each experimental group were analyzed by enzyme-linked immunosorbent assay (ELISA).The results indicated that all clinical indices except PI were

significantly elevated in the poorly controlled and well-controlled diabetics, compared to systemically healthy patients. Pairwise linear regression analysis revealed significant (P□0.01) positive associations between periodontal inflammation (PD, CAL, PI, GI) and levels of GCF IL-1, GT IL-1, GT IL-6, but not GT PDGF; moreover, GT IL-6 levels were significantly associated (P□0.05) with GT IL-1. As TG levels increased in the non-AP patients (group 1 □ group 3 □ group 5), there was a trend, not significant, for increased GCF IL-1 □ levels and increased gingival inflammation. Interestingly, periodontitis resulted in increased PDGF-AB levels in the gingiva of systemically healthy and well-controlled diabetes patients, but this increase was obtunded in poorly controlled diabetes patients. The authors concluded a possible dysregulation of the normal cytokine/growth factor signaling axis in poorly controlled type 2 diabetics that may contribute to periodontal breakdown/diminished repair.

Taylor GW. (2001)[21] reviewed evidence for a bidirectional relationship between diabetes and periodontal diseases. Review of adverse effects of periodontal infection on glycemic control included reports of periodontal treatment studies and follow-up observational studies in which changes in glycemic control could be assessed. Observational studies reporting adverse effects of diabetes mellitus on periodontal disease provided consistent evidence of greater prevalence, severity, extent, or progression of at least one manifestation of periodontal diseases in the large majority of reports. Treatment studies provided direct evidence to support periodontal infection having an adverse, yet modifiable, effect on glycemic control. However, reviewers reported that not all investigations reported an improvement in glycemic control after periodontal treatment. Moreover, additional evidence to support the effect of severe periodontitis on increased risk for poorer glycemic control comes from 2 follow-up

observational studies. The evidence reviewed supports viewing the relationship between diabetes and periodontal diseases as bidirectional. The authors concluded that, further rigorous, systematic study is warranted to establish that treating periodontal infections can be influential in contributing to glycemic control management and possibly to the reduction of the burden of complications of diabetes mellitus.

Campus G, Salem A, Uzzau S, Baldoni E, Tonolo G. (2005)[22] evaluated the possible association between non–insulin dependent diabetes (T2DM) and clinical and microbiological periodontal disease among adult Sardinians. For this study, they selected a total of 212 individuals, 71 T2DM patients aged 61.0 ± 11.0 years and 141 non-diabetic controls in good general health aged 59.1 ± 9.2 years. Clinical periodontal examination for probing depth, attachment level, presence of calculus, bleeding on probing and assessment of plaque was done and subgingival plaque samples were obtained and P. gingivalis, P. intermedia and T. forsythensis were identified using multiplex polymerase chain reaction from all subjects. T2DM patients showed a significantly lower number of teeth with a significant increase in percent of pocket depths > 4 mm, periodontitis (P =0.046); bleeding on probing (P =0.02); and plaque index (P =0.01). Also, a significant association with diabetes was detected for plaque and bleeding on probing. Concerning bacteria prevalence, a positive association was detected for P. gingivalis and T. forsythensis. Presence of plaque was positively associated with case status and with prevalence of P. gingivalis and T. forsythensis. Thus the authors concluded that, patients with T2DM undoubtedly have a susceptibility for more severe periodontal disease.

Lalla E, Cheng B, Lal S, Tucker S, Greenberg E, Goland R et al. (2006) [23] in their study, evaluated the level of oral disease in children and adolescents with diabetes. Dental caries and periodontal disease were clinically assessed in 182 children and adolescents (6–18 years of age) with diabetes and 160 non-diabetic control subjects. The authors found that children with diabetes had significantly higher plaque and gingival inflammation levels compared with control subjects. Also, the number of teeth with evidence of attachment loss (the hallmark of periodontal disease) was significantly greater in children with diabetes. When controlling for age, sex, ethnicity, gingival bleeding, and frequency of dental visits, diabetes remained a highly significant correlate of periodontitis, especially in the 12 to 18-year-old subgroup. In the case group, BMI was significantly correlated with destruction of connective tissue attachment and bone. Thus authors concluded that periodontal destruction can start very early in life in diabetes and becomes more prominent as children become adolescents. Also, programs designed to promote periodontal disease prevention and treatment should be provided to young patients with diabetes.

Perrino MA. (2007)[24] reviewed the relationship between periodontitis and diabetes and reported that diabetics experience increased destruction of periodontal tissues as a result of an abnormal immune response, altered fibroblast function and levels of collagen, as well as the microvascular effects of advanced glycosylation end products (AGE). The accumulation of AGE in the periodontium is correlated with an increase in the level of inflammatory mediators, which are associated with tissue destruction. These inflammatory mediators may contribute to the severity of tissue destruction in diabetics with periodontal disease. The increased prevalence of periodontal disease in diabetics is an example of an oral/systemic relationship. They

stated that there is evidence that this relationship may be two-dimensional as well as diabetics with active periodontitis tend to have poor glycemic control when compared to patients without periodontitis. The reviewers also reported that an extensive amount of research has examined the relationship between periodontal disease and diabetes, and it is clear that they share many biological mechanisms. Thus, authors concluded that careful management of the diabetic patient may greatly reduce the potential for a decline in oral health as well as overall glycemic control and more research is needed to define the population that is most susceptible to the combined effects of periodontal disease and diabetes and which treatment is most beneficial for each of the diabetic patient.

Preshaw P. (2008) [25] demonstrated a clear link between periodontal disease and diabetes, and individuals with diabetes, particularly if poorly controlled, are at risk for advanced periodontitis. They stated that, diabetes is increasingly viewed as an inflammatory condition and dysregulated immune-inflammatory responses in diabetes may increase susceptibility to periodontal disease by disrupting local cytokine networks in the periodontium. Also, inflammatory cytokines such as interleukin-6 (IL-6) and tumour necrosis factor-α (TNF-α) are important in the pathogenesis of both diseases, and together with other pro-inflammatory cytokines and adipokines , may provide a mechanistic link between the two diseases. The authors also showed that altered neutrophil function and deposition of advanced glycation end-products (AGEs) are also likely to be important in the increased susceptibility to periodontal disease seen in people with diabetes. However, emerging data suggest that treating periodontal disease may have a beneficial effect on glycaemic control, and highlight the need to incorporate a full periodontal examination into management strategies for patients with diabetes.

Thus, in the author's opinion, it is clear that the dental team must become increasingly involved in the management of patients with diabetes, and it is recommended that periodontal screening of all patients diagnosed with diabetes is undertaken as a matter of routine.

Choi YH, McKeown RE, Mayer-Davis EJ, Liese AD, Song KB, Merchant AT. (2011)[26] investigated the relationship between chronic periodontitis, impaired fasting glucose (IFG) and diabetes in the U.S. population. For this, participants in the National Health and Nutrition Examination Survey III, aged 20 years, were grouped into quintiles of mean clinical attachment loss (CAL) and pocket depth, with the lowest category being the reference who received periodontal examinations and provided blood samples (n = 12,254). Plasma fasting glucose was categorized into three groups (normal < 100 mg/dL; IFG ≥100 but <126 mg/dL; and diabetic≥126 mg/dL). Socio-demographic factors and other potential risk factors were obtained by interview or examination. Participants in the top quintile category of CAL had higher prevalence odds of IFG and diabetes after adjustment for related confounders, compared with those in the bottom quintile. The highest quintile of pocket depth was positively associated with IFG and diabetes compared with the lowest quintile. Odds Ratios (ORs) for CAL increased from the lowest to the highest quintile for all outcomes. The ORs for pocket depth also tended to rise across quintiles. The authors concluded that chronic periodontitis measured by CAL and pocket depth was positively associated in a linear relation with IFG and diabetes in U.S. adults.

Padmalatha GV, Bavle RM, Satyakiran GV, Paremala K, Sudhakara M, Makarla S. (2016)[27] aimed to study the number of Porphyromonas gingivalis (P.gingivalis) in diabetes mellitus (DM) patients associated with periodontitis with and without tobacco-associated habits and to compare them with periodontitis patients having no other systemic pathologies. For this, they included in the study subgingival plaque samples from a total of seventy subjects. After this, DNA was isolated from the collected sample and was quantified using spectrophotometer for standardizing the polymerase chain reaction. The quantity of the isolated DNA was checked in a ultraviolet-visible spectrophotomer. The authors reported maximum score of P. gingivalis in periodontitis patients having DM, whereas the least score in periodontitis patients having DM with tobacco smoking habit compared to the other groups. Thus, P. gingivalis count is significantly reduced in periodontitis patients having DM with smoking habit. The authors concluded that P. gingivalis might not be a key causative organism responsible for the periodontal destruction in case of smokers despite the DM condition. Also, the decrease in counts may be attributed to change in the local environment like chemical and physical changes preventing the growth of P. gingivalis.

D'Aiuto F, Gable D, Syed Z, Allen Y, Wanyonyi KL, White S et al. (2017)[28] reviewed the nature of the association between poor oral health and diabetes. The reviews were undertaken by four groups each comprising consultant clinicians from medicine and dentistry, trainees, public health and academics. The methodology involved a streamlined rapid review process and synthesis of the findings. The results identified a number of systematic reviews of low to high quality suggesting that diabetes is associated with periodontal disease, tooth loss, and oral cancer in particular,

and that the management of oral diseases, most notably periodontal care, has a short-term beneficial influence on metabolic outcomes related to diabetes. Also, current evidence of mixed quality suggests a number of associations between oral diseases and diabetes mellitus (diabetes).

B. Studies on interrelationship between vitamin D, calcium and diabetes mellitus

Norman AW, Frankel JB, Heldt AM, Grodsky GM. (1980)[29] determined glucagon release in the isolated perfused rat pancreas by radioimmunoassay of the secreted proteins along with the effect of vitamin D deficiency on insulin. The author reported that during a 30-minute period of perfusion with glucose and arginine, pancreases from vitamin D deficient rats exhibited a 48 percent reduction in insulin secretion compared to that for pancreases from vitamin D-deficient rats that had been replenished with vitamin D. The authors concluded that, this result, along with the previously demonstrated presence in the pancreas of a vitamin D-dependent calcium-binding protein and cytosol receptor for the hormonal form of vitamin D, 1,25-dihydroxyvitamin D3, indicates an important role of vitamin D in the endocrine functioning of the pancreas.

Scragg R, Holdaway I, Singh V, Metcalf P, Baker J, Dryson E. (1995)[30] in a cross-sectional survey determined whether serum concentrations of 25 hydroxy vitamin D are altered in people with newly diagnosed diabetes mellitus and impaired glucose tolerance (IGT) in New Zealand Polynesian and Caucasian work force of 5677 staff aged 40-64 years. They found that, serum 25-hydroxyvitamin D concentration was significantly lower in newly detected cases with diabetes and IGT compared with controls individually matched by sex, age, and ethnicity. Also, among controls, serum

concentrations were significantly lower in Maori and Pacific Islanders compared with Europeans after adjusting for age, sex, and time of year. Thus the authors concluded that diabetes and IGT are associated with low serum concentrations of 25 hydroxy vitamin D and that low concentrations of this hormone in New Zealand Polynesians may partly explain their increased prevalence of diabetes/IGT compared with Europeans.

Yu JR, Lee SA, Lee JG, Seong GM, Ko SJ, Koh G et al. (2012)[31] aimed to evaluate the relationship between the serum 25-hydroxyvitamin D level and various parameters like height, fibrinogen, triglyceride, LDL-C, HbA1C in patients with T2DM and healthy controls. For this, the authors analyzed retrospectively data from 276 Korean patients with T2DM whose serum 25(OH) D level was measured. Non-diabetic healthy subjects who visited the hospital for health screening were selected as the control group (n=160). The authors found that, compared with control subjects, patients with T2DM had a lower serum 25(OH)D level out of which, eleven percent of T2DM patients were vitamin D insufficient (20-29 ng/ml) and 87% of the patients were vitamin D deficient (< 20 ng/ml). Also, the serum 25(OH) D level was significantly related to serum fibrinogen, triglyceride (TG), low-density lipoprotein cholesterol (LDL-C), ferritin, the urine albumin creatinine ratio and hemoglobin A1C (HbA1C). The authors concluded that the majority of Koreans with T2DM are vitamin D deficient and the serum 25(OH) D level in patients with T2DM is related to lipid and glucose parameters as among the various metabolic parameters, high levels of TG, LDL-C and HbA1C had a consistent association with vitamin D deficiency state in T2DM patients.

Oakley P, Chaney S, Persinger M, Chaney T. (2013)[32] explored the relationship of 25-hydroxylvitamin D blood levels in 106 randomly selected patients with diagnosed type 2 Diabetes Mellitus. The results showed 21% of the population were normal (32 ng/ml), 39% were found to have insufficient levels (20 - 30 ng/ml), and 35% were outright deficient (< 20 ng/ml). Clinically, 74% of the entire sample had significantly low vitamin D levels. Also, in total sample 94% had vitamin D levels at or below the optimal cut-off level. Body Mass Index was negatively correlated with vitamin D; that is, the greater the BMI of the patient, lesser their vitamin D level. The authors concluded that, both obesity and hypovitaminosis D are each mutually exclusive predictors for T2DM. Obesity and vitamin D deficiency may work synergistically to propel an individual into the diseased state of T2DM. As this study demonstrates that the majority of people with T2DM suffer from inadequate amounts of vitamin D, its testing should be routine for all people at risk for T2DM, prediabetics and those currently suffering with T2DM in order to elevate levels sufficiently to improve insulin sensitivity and improve long-term outcomes.

Afzal S, Bojesen SE, Nordestgaard BG. (2013)[33] tested the hypothesis that low plasma 25-hydroxyvitamin D levels is associated with increased risk of type 2 diabetes in the general population. For this, the authors measured 25(OH) D in 9841 participants from the general population, of whom 810 developed type 2 diabetes during 29 years of follow-up. Analyses were adjusted for sex, age, smoking status, body mass index, income, physical activity, HDL cholesterol. Lower 25(OH) D concentrations, by clinical categories or seasonally adjusted quartiles, were associated with higher cumulative incidence of type 2 diabetes. Also, multivariable adjusted hazard ratios of type 2 diabetes were 1.22 for 25(OH) D □5 vs ≥ 20 g/L and 1.35 for lowest vs highest

quartile. Also, the multivariable adjusted hazard ratio of type 2 diabetes for a 50% lower concentration of 25(OH) D was 1.12; the corresponding hazard ratio for those 58 years old was 1.26. The authors concluded that there is an association of low plasma 25(OH) D with increased risk of type 2 diabetes.

Elkassaby S, Harrison LC, Mazzitelli N, Wentworth JM, Colman PG, Spelman T, et al. (2014)[34] aimed to determine if high dose oral vitamin D3 (D) improves beta-cell function and glycaemia in type 2 diabetes subjects. For this study, 50 adults with type 2 diabetes diagnosed less than 12 months, with normal baseline serum 25-OH D (25D), were randomized to 6000 IU D (n=26) or placebo (n=24) daily for 6 months. Beta-cell function was measured by glucagon-stimulated serum C-peptide (DCP). Secondary outcome measures were fasting plasma glucose (FPG), post-prandial blood glucose (PPG), HbA1c and insulin resistance (HOMA-IR). The results showed that, in the diabetic group, median serum 25D (nmol/l) increased from 59 to 150 (3 months) and 128 (6 months) and median serum 1,25D (pmol/l) from 135 to 200 and 190. However, change in FPG (mmol/l) was significantly lower in D compared to placebo (P=0.007), as was change in PPG in D compared to placebo (P=0.005). The authors concluded that, oral D3 supplementation in type 2 diabetes was associated with transient improvement in glycaemia but without a measurable change in beta-cell function. This effect is unlikely to be biologically significant. Also, high dose D3 therefore appears to offer little or no therapeutic benefit in type 2 diabetes.

Al-Shoumer KA and Al-Essa TM. (2015)[35] in their review, suggested a possible link between abnormal vitamin D level and abnormal glucose homeostasis, two of the most common chronic medical conditions. Both conditions are associated with inflammation and the literature investigating the link between vitamin D and either

pre-diabetic states or diabetes was reviewed. In majority of observational studies, it has been shown that vitamin D is positively correlated with insulin sensitivity and its role is mediated both by direct mechanism through the availability of vitamin D receptors in several tissues and indirectly through the changes in calcium levels. Large number of, but not all, variable samples cross sectional human trials have demonstrated an inverse relation between vitamin D status and impaired glucose tolerance, insulin resistance or diabetes. The authors concluded that absence of both sizable prospective observational trials utilizing 25(OH) D as the main variable and the non-availability of randomized studies specifically designed to assess the effects of vitamin D on pre-diabetes and diabetes states, are the main obstacles to draw solid and conclusive relationships.

Lu Y, Zheng Y, Wang N, Chen Y, Li Q, Han B, et al. (2017) [36] aimed to reveal the extent to which vitamin D is present in the population in East China and to explore the relationship between serum 25(OH)D and type 2 diabetes mellitus. The cohort was selected based on a large investigation including 12,702 participants aged 21–92 years old. All the participants completed the questionnaire and went through a physical examination. Fasting blood samples were collected to test serum 25(OH) D and other metabolism-related indicators. The results sowed overall percentage of vitamin D deficiency was 80.55% (male 74.1%, female 85.0%). The authors concluded that men with lower serum 25(OH) D level had high value of insulin resistance and HbA1c. Also, the serum 25(OH) D level of those who were diagnosed with T2DM was higher than that in non-diabetics.

C. Studies on the interrelationship between vitamin D, calcium and chronic gingivitis, chronic periodontitis.

Nishida M, Grossi SG, Dunford RG, Ho AW, Trevisan M, Genco RJ. (2000)[37] evaluated the role of dietary calcium intake as a contributing risk factor for periodontal disease. For this, dietary calcium intake was determined from a 24-hour dietary recall, the presence of periodontal disease was measured by attachment loss. In addition, serum calcium was assessed using venous blood samples .After adjusting for covariants including age, gender, tobacco consumption, and gingival bleeding, logistic regression analysis was used to examine the association between periodontal disease and dietary calcium intake or serum calcium levels. The authors found the association of lower dietary calcium intake with periodontal disease for young males and females (20 to 39 years of age), and for older males (40 to 59 years of age). The relationship between low dietary calcium intake and increased levels of periodontal disease showed an estimated odds ratio (OR) of 1.84 for young males, 1.99 for young females, and 1.90 for the older group of males. These odds ratios were adjusted for gingival bleeding and tobacco consumption. The dose response was also seen in females, where there was 54% greater risk of periodontal disease for the lowest level of dietary calcium intake (2 to 499 mg) and 27% greater risk in females who took moderate levels of dietary calcium (500 to 799 mg) as compared to those who took 800 mg or more dietary calcium per day. A statistically significant association between low total serum calcium and periodontal disease was found in younger females aged 20 to 39 with OR = 6.11. Thus the authors concluded that low dietary intake of calcium results in more severe periodontal disease.

Dietrich T, Joshipura KJ, Dawson-Hughes B, Bischoff-Ferrari HA. (2004)[38] in the third National Health and Nutrition Examination Survey evaluated whether serum 25-hydroxyl vitamin D3 [25(OH)D3] concentrations are associated with periodontal disease. For this, the authors analyzed data on periodontal attachment loss (AL) and serum 25(OH) D3 concentrations from 11202 subjects aged 20 years. Mean AL was modeled in a multiple linear regression with quintile of serum 25(OH) D3 concentration as an independent variable. The model was stratified by age and sex and was adjusted for age within age groups, race or ethnicity, smoking, diabetes, poverty income ratio, body mass index, estrogen use and gingival bleeding. The authors found that 25(OH) D3 concentrations were significantly and inversely associated with AL in men and women aged ≥50 years. Compared with men in the highest 25(OH) D3 quintile, those in the lowest quintile had a mean AL that was 0.39 mm higher; in women, the difference in AL between the lowest and highest quintiles was 0.26 mm. The authors concluded that, low serum 25(OH) D3 concentrations may be associated with periodontal disease independent of bone mineral density.

Hildebolt CF. (2005)[39] commented on the relation of vitamin D and calcium with periodontitis that**,** most U.S. citizens have calcium intakes and serum levels of vitamin D far below recommended values. The reviewer stated that according to various authors, vitamin D and calcium deficiencies result in bone loss and increased inflammation, which are well recognized symptoms of periodontal disease. More recently there have been a number of studies in which investigators have suggested that calcium and vitamin D may benefit periodontal health, and it has been suggested that calcium deficiency may be a risk factor for periodontal disease. They also commented, as of 2002, there had been 70 randomized clinical trials on the effects of calcium and

vitamin D on the postcranial skeleton, and 68 of these studies (97%) reported positive effects for supplementation. Also, low levels of calcium intake and particularly vitamin D intake result in a low serum level of calcium that stimulates the parathyroid gland to produce PTH, which results in osteoclastogenesis. On another hand, periodontal disease results in the production of proinflammatory cytokines, which also result in osteoclastogenesis. It follows that alveolar bone in patients with periodontal disease and vitamin D and calcium deficient would be under a heavier osteoclastic load than are bones such as the femur and spine. They also found that, a number of infectious diseases have been linked with low levels of vitamin D, and it has been demonstrated that vitamin D can suppress cytokine production. Because of periodontal disease's unique periodontal-pathogen, hard-tissue environment, the effect of vitamin D and calcium on alveolar bone might be more pronounced than its effects in the spine and hip.

Dietrich T, Nunn M, Hughes M, Ferrari H. (2005)[11] determined the association between serum concentrations of 25-hydroxyvitamin D [25(OH)D] and gingival inflammation. For this, the authors analyzed data from 77503 gingival units (teeth) in 6700 never smokers aged 13 to 90 years. Multiple logistic regression models adjusted for subject and site-specific covariates included age, sex, race-ethnicity, income, body mass index, diabetes, use of oral contraceptives and hormone replacement therapy among women, intake of vitamin C, missing teeth , full crown coverage, the presence of calculus, the frequency of dental visits and dental examiner and survey phase. The authors found that compared with sites in subjects in the lowest 25(OH) D quintile, sites in subjects in the highest 25(OH)D quintile were 20% less likely to bleed on gingival probing. The association appeared to be linear over the entire 25(OH) D range. The authors concluded that, vitamin D may reduce susceptibility to

gingival inflammation through its anti-inflammatory effects and thus gingivitis may be a useful clinical model to evaluate the anti-inflammatory effects of vitamin D.

Miley DD, Garcia MN, Hildebolt CF, Shannon WD, Couture RA, Anderson Spearie CL, et al (2009)[40] reviewed whether the use of vitamin D and calcium supplements affects periodontal disease status. For this 51 subjects receiving periodontal maintenance therapy were recruited from two dental clinics out of which, 23 were on vitamin D (400 IU/day) and calcium (1,000 mg/day) supplementations and 28 were not on such supplementation. All subjects had at least two interproximal sites with 3 mm clinical attachment loss. Daily calcium and vitamin D intake (from food and supplements) were estimated by nutritional analysis. The following clinical parameters of periodontal disease were recorded for the mandibular posterior teeth: gingival index, probing depth, cemento-enamel junction–gingival margin distance (attachment loss), bleeding on probing and furcation involvement. To determine cemento-enamel junction – alveolar crest distances (alveolar crest height loss), posterior photo stimulable-phosphor bitewing radiographs were taken. The results suggested that compared to subjects who did not take vitamin D and calcium supplementation, supplement takers had shallower probing depths, fewer bleeding sites, lower gingival index values, fewer furcation involvements, less attachment loss, and less alveolar crest height loss. Also, the repeated measures analysis indicated that collectively these differences were borderline significant ($P = 0.08$). Thus the authors concluded that, in these subjects receiving periodontal maintenance therapy along with vitamin D and calcium supplementation had better periodontal health.

Hiremath V, Rao B, Naik V, Bhaskar K. (2013)[12] assessed the anti-inflammatory effect of vitamin D on gingivitis at various doses. For this, 96 subjects were recruited and divided into four groups and were given daily oral vitamin D supplementation in doses of 2000 IU for group A, 1000 IU for group B, 500 IU for group C and a placebo for group D over a 3-month period. The changes in gingival scores were measured after the 1st, 2nd and 3rd months. The authors found that, the gingivitis score changed in direct proportion to the dose of vitamin D supplementation. In group A, the mean gingival scores were 2.4 (baseline), 1.7 after the first month, 0.8 after the second month and 0.3 after the third month. The group B mean baseline gingival score of 2.3 decreased to 2.0 in the first month, 1.1 after the second month and 0.5 after the third month. In group C, the baseline gingival scores were 2.2 and 1.9 after one month, 1.4 after two months and 0.8 by the last visit. Comparing baseline gingivitis scores with the later-visit score using the Wilcoxon paired test, the significant anti-inflammatory effect was seen in group A after one month, in group B at two months and in group C at three months after oral vitamin D supplementation ($P < 0.0001$). The authors concluded that, there is a dose-dependent anti-inflammatory effect of vitamin D on gingivitis. Vitamin D is a safe and effective anti-inflammatory agent in doses ranging from 500 IU to 2000 IU.

Joseph R, Nagrale AV, Joseraj MG, Pradeep Kumar KM, Kaziyarakath JA, Chandini R. (2015)[41] aimed to find out the level of serum vitamin D in chronic periodontitis patients (CHP) with and without T2DM. In this case control study, 141 subjects were recruited, including 48 controls and 43 cases which consisted of chronic periodontitis patients with type 2 DM (CHPDM) and 50 CHP. Clinical parameters like pocket depth (PD), clinical attachment loss (CAL), modified gingival index (MGI),

plaque index (PI), and calculus index (CI) were recorded of all subjects. Laboratory investigations included serum 25-hydroxyvitamin D (25[OH] D) level estimation by electro-chemiluminescence immunoassay. Other laboratory investigations including fasting blood sugar (FBS) and serum calcium were also analyzed in all subjects. According to the results, the statistically significant difference was found between the mean serum 25(OH) D level of control (22.32 ± 5.76 ηg/ml), CHPDM (14.06 ± 4.57 ηg/ml) and CHP (16.94 ± 5.58 ηg/ml) groups respectively. The mean value of FBS was significantly high in CHPDM group as compared to CHP group. Periodontal parameters like MGI, PI, PD, and CI showed a significant difference between groups ($P < 0.05$) and a higher score was found in CHP group, while CAL and PI showed no statistically significant difference between CHP and CHPDM group ($P > 0.05$). The authors concluded that a low level of serum Vitamin D level is present in patients with CHP and CHPDM. As per their observations, the lower vitamin D level observed in case groups may be due to the diseases process rather than low vitamin D acting as a cause of the disease.

Harsha L, Vishnu V, Bedra A, Deepika V. (2015)[42] estimated serum calcium levels in patients with chronic periodontitis in Kanchipuram district. The study was conducted among 16 periodontitis patients and 15 control patients with healthy periodontium. 2ml serum sample of periodontitis patients was collected and the calcium levels were estimated using Arsenazo III method. In the current study, it has been elucidated that an increased level of serum calcium levels is observed in patients with periodontitis. Also, the mean serum calcium level observed in periodontitis patients is altered when compared to control. It has been observed that, there was a significant increase in serum calcium levels in patients with periodontitis. The authors concluded

that an increased level of serum calcium was observed in patients with periodontitis as compared to healthy subjects.

Rane MV, Suragimath G, Varma S, Zope S, Ashwinirani S. (2017)[43] evaluated salivary calcium levels in healthy subjects and patients with chronic gingivitis and chronic periodontitis. 150 subjects in the age range between 20 – 45 years were randomly selected and subjected to periodontal examination using the gingival index, plaque index, oral Hygiene Index and clinical attachment loss. Following the periodontal examination, subjects were divided into three groups of 50 patients each: Group A: healthy subjects, Group B: gingivitis patients, Group C: periodontitis patients. Saliva samples from the study subjects were collected and subjected to estimation of salivary calcium levels. The authors found that the levels of salivary calcium increased as the disease progressed from healthy to gingivitis and periodontitis. There was a statistically significant difference observed between healthy to gingivitis group and gingivitis to periodontitis group. Thus the authors concluded that, salivary calcium levels can be used as a biomarker to assess the periodontal disease progression. Also, according to authors, early diagnosis of periodontal disease by estimation of calcium levels in saliva can help in the prevention of gingivitis or periodontitis by various therapeutic measures.

MATERIALS AND METHODS

The present observational study was carried out to evaluate levels of vitamin D and calcium in the serum of periodontally healthy patients and to compare it with the vitamin D and calcium levels of chronic gingivitis and chronic periodontitis patients with and without T2DM. For this, a hundred patients visiting the Department of Periodontology of our institute were recruited. The study design was reviewed and approved by the Institutional Ethics Committee and is in accordance with the Helsinki declaration of 1975, as revised in 2013. Prior to the initiation of the study an informed consent was obtained from those who agreed to participate voluntarily.

Study Groups

A dental and medical history was recorded for the selected patients and an intraoral examination was conducted by a single examiner. Patients were then categorized into 5 groups of 20 patients each on the basis of periodontal parameters

including Probing pocket depth (PPD), Clinical attachment level (CAL), Plaque index (PI) [Silness and Loe 1964], Gingival index (GI) [Loe and Silness, 1963], radiographic evidence of bone loss, HbA1C levels and RBS levels.

1. **Group I (Systemically and Periodontally healthy)**

   GI = 0, PI = 0, PPD ≤ 3 mm,

   HbA1c levels ≤ 6.5%, RBS levels ≤ 200 mg/dl

2. **Group II (Chronic gingivitis patients without DM.)**

   GI ≥ 1, PI ≥ 1, PPD ≤ 3 mm,

   HbA1c levels ≤ 6.5 % and RBS levels ≤ 200 mg/dl

3. **Group III (Chronic gingivitis patients with DM.)**

   GI ≥ 1, PI ≥ 1, PPD ≤ 3 mm

   HbA1c levels ≥ 6.5 % and RBS levels ≥ 200 mg/dl.

4. **Group IV (Chronic periodontitis patients without DM)**

   GI ≥ 1, PI ≥ 1, PPD ≥ 3mm and CAL ≥ 5mm,

   Radiographic evidence of bone loss,

   HbA1c levels ≤ 6.5 % and RBS levels ≤ 200 mg/dl.

5. **Group V (Chronic periodontitis patients with DM)**

   GI ≥ 1, PI ≥ 1, PPD ≥ 3mm and CAL ≥ 5 mm,

   Radiographic evidence of bone loss,

   HbA1c levels ≥ 6.5 % and RBS levels ≥ 200 mg/dl.

In all the patients of the test and control groups clinical parameters (PPD,CAL,GI, PI) were evaluated along with RBS, HbA1c, calcium tests . Vitamin D was detected in serum.

Selection Criteria's

Inclusion Criteria

The inclusion criteria were patients with the presence of at least 20 natural teeth. Gingivitis was defined as the one with a probing pocket depth (PPD) ≤ 3 mm and that was positive for bleeding on probing. Chronic periodontitis (CP) was defined as the one with probing pocket depth (PPD≥ 3mm) and clinical attachment level (CAL≥ 5mm) and that was positive for bleeding on probing and radiographic evidence of bone loss when evaluated using the long cone technique. The glycemic status of patients was determined by using Random Blood Sugar (RBS) and diabetes mellitus if present, it was confirmed by glycated hemoglobin (HbA1c) test.

Exclusion Criteria

The patients which were excluded from the study were the one with:

1. The medical disorders such as cardiovascular or renal disease, malignancies, multiple sclerosis, vitamin D deficiency disorders including bone diseases.
2. Tobacco chewers and smokers.
3. Pregnant, post-menopausal and lactating females.
4. No history of antibiotic intake within 6 weeks.
5. No history of any periodontal therapy within 6 months.

Armamentarium (COLOUR PLATE IV)

Following armamentarium was used for the assessment of clinical parameters and for the collection of blood -

For examination of the patient

1. Mouth mirror
2. Straight probe
3. William's graduated (Hu-Friedy) periodontal probe.
4. Tweezer
5. Dental Explorer
5. Kidney tray
6. Disposable gloves
7. Disposable face mask

For drawing blood

1. Spirit
2. Sterilized cotton
3. 2 ml disposable syringe
4. 2 ml plastic vial
5. Tourniquet

Laboratory armamentarium for assessment of biochemical parameters (COLOUR PLATE IV and V)

Calibrated, volumetric transfer pipettes with 0-5 µl range, 5-50 µl range, 50- 200 µl range and 200-1000 µl range ,

Sterilized test tubes with test tube stand

Distilled water

Beakers, measuring cylinder

Sterile gloves

-80°c deep freezer (REMI Equipments Pvt. Ltd.)

Lab Centrifuge machine (REMI Equipments Pvt. Ltd.)

Vortex mixer (CM 101, REMI Equipments Pvt. Ltd.)

ELISA reader (LISA Microplate reader, REMI Equipments Pvt. Ltd.)

Additional materials and equipment that were required

The following material was required but not provided in the kit:

1. Distilled water
2. Pipettes for delivery of: 50 µl, 150 µl, 200µl and 1 ml (the use of accurate pipettes with disposable plastic tips is recommended)
3. Magnetic stirrer
4. Plate shaker (300 to 700 rpm)
5. Washer for microtiter plates
6. Microtiter plate reader capable of reading at 450 nm and 650 (bichromatic reading)

Assessment of Periodontal and Clinical Parameters

1. **Probing pocket depth (PPD)** It was measured using Hu Friedy William's graduated periodontal probe on four sites of all teeth. Patients were diagnosed as healthy (Group I) or gingivitis (Group II) or gingivitis with diabetes mellitus (Group III) as assessed by probing pocket depth (PPD) ≤ 3 mm. Patients were diagnosed with chronic periodontitis (Group IV) or chronic periodontitis with diabetes mellitus (Group V) as assessed by probing pocket depth (PPD) ≥ 3 mm.
2. **Clinical attachment level (CAL)** It was measured using Hu Friedy William's graduated periodontal probe on four sites of the concerned tooth from the

cement - enamel junction to the base of the pocket. Patients were diagnosed with chronic periodontitis (Group IV) or chronic periodontitis with diabetes mellitus (Group V) as assessed by Clinical attachment level (CAL) ≥ 5 mm.

3. **Plaque index (PI): (Silness and Loe, 1964)** PI was examined in the scoring units of teeth: distofacial, facial, mesiofacial and lingual surfaces. A mouth mirror and dental explorer were used to assess plaque index.

The criteria for scoring as follows:

SCORE	CRITERIA
0	No plaque in gingival area
1	A film of plaque adhering to the free gingival margin and adjacent area of the tooth. The plaque was recognized only by running a probe across the tooth surface
2	Moderate accumulation of soft deposits within the gingival pocket and on the gingival margin and/or adjacent tooth surface, which could be seen by the naked eye
3	Abundance of soft matter within the gingival pocket and/or on the gingival margin and adjacent tooth surface

A plaque index per person was obtained by adding all of the plaque scores and dividing by the number of surfaces examined.

$$\text{Plaque Index (PI)} = \frac{\text{Total PI scores per tooth}}{\text{No. of surfaces}}$$

The following suggested nominal scale was used for patient evaluation

Scores	Rating
0	Excellent
0.1-0.9	Good
1.0-1.9	Fair
2.0-3.0	Poor

4. Gingival index (GI): (Loe and Silness, 1963) This is a system for assessing the severity of gingivitis in four possible areas. The tissues surrounding each tooth were divided into four gingival scoring units: the distofacial papilla, the facial margin, mesiofacial papilla and the entire lingual gingival margin. A blunt periodontal probe was used (William's graduated) to assess the bleeding potential of the gingival margin according to the following criteria:

SCORE	CRITERIA
0	Normal gingiva
1	Mild inflammation, slight change in color, slight edema, no bleeding on palpation
2	Moderate inflammation, redness, edema and glazing, bleeding on palpation
3	Severe inflammation, marked redness and edema, ulcerations, tendency of spontaneous bleeding

The scores of all the surfaces were added and divided by a number of surfaces examined which provided the gingival index score per person.

$$\text{Gingival Index (GI)} = \frac{\text{Total GI scores per tooth}}{\text{No. of surfaces}}$$

The numerical score of the gingival index taken into consideration for varying degrees of clinical gingivitis was as follows

Gingival scores	Condition
0.1-1.0	Mild gingivitis
1.1-2.0	Moderate gingivitis
2.1-3.0	Severe gingivitis

Assessment of Biochemical Parameters

Blood and serum sample collection (COLOUR PLATE IV)

A total of 4ml of blood was collected from antecubital fossa by venipuncture using a 20 gauge needle. Blood was immediately transferred to the laboratory. 2 ml of blood sample was allowed to clot at room temperature and after 1 hour, serum was separated from blood by centrifuging at 300 rpm for 5 minutes. The extracted serum was immediately transferred to a plastic vial and stored at -80°c until assayed.

Evaluation of Vitamin D from serum

Samples were assayed for vitamin D levels using commercially available ELISA (Enzyme linked immune-sorbent assay) Krishgen Biosystem Vitamin D ELISA Kit **(COLOUR PLATE IV).**Samples were analyzed according to the instruction manual at the Department of Biochemistry. Briefly, serum samples were diluted with dilution buffer in the kit and the amount of vitamin D was determined. All samples were run in duplication.

Reagents

Reagents	96 Test Kits	Reconstitution
MT PLATE Microtiterplate with 96 Mab anti 25 OH Vit. D2 and D3 coated wells.	96 wells	Ready for use
CAL Calibrator 0 : biological matrix with gentamycin proclin	1 vials lyophilized	Add 2 ml distilled water
CAL Calibrators 1-5 in horse serum with gentamycin and proclin	5 vials lyophilized	Add 1 ml distilled water
CONTROL Controls N = 2 in human serum with proclin	2 vials lyophilized	Add 1 ml distilled water
INC BUF Incubation Buffer with casein and proclin	1 vial 20 ml	Ready for use
25OH Vit D CONJ 100x 25OH Vit D Concentrated Conjugate	1 vial 0.4 ml	Dilute 100 x with conjugate buffer
HRP CONJ 200x Concentrated HRP	1 vial 0.2 ml	Dilute 200 x with conjugate buffer
CONJ BUF Conjugate Buffer with casein and proclin	1 vial 30 ml	Ready for use
WASH SOLN 200x Wash solution (TRIS-HCL)	1 vial 10 ml	Dilute 200 x with distilled water
CHROMO TMB Chromogenic solution TMB (Tetramethyl benzydine)	1 vial 12 ml	Ready for use
STOP SOLN Stop solution HCL 1.5 N	1 vial 12 ml	Ready for use

Reagent Preparation

A. **Calibrator 0**: Reconstitute the calibrator 0 with 2 ml distilled water

B. **Calibrators 1 - 5**: Reconstitute the calibrators 1-5 with 1 ml distilled water

C. **Controls**: Reconstitute the controls with 1 ml distilled water.

D. **Working HRP conjugate solution**

The working HRP conjugate solution is to be prepared absolutely in the 15 minutes just after the first 2 hours incubation step is started (cf X.B.5). Adequate volume of working HRP conjugate solution was prepared by mixing concentrated conjugate, concentrated HRP and conjugate buffer according to the number of used strips, as indicated in the table below: for example for 6 strips (48 wells): 100 µl of concentrated conjugate and 50 µl of concentrated HRP to 10 ml of conjugate buffer. A vortex was used to homogenize. The working HRP conjugate was kept at room temperature and a brown glass vial was used for its preparation.

No. of strips	Volume of Concentrated Conjugate (µl)	Volume of Concentrated HRP (µl)	Volume of Conjugate Buffer (ml)
1	30	15	3
2	50	25	5
3	60	30	6
4	80	40	8
5	90	45	9
6	100	50	10
7	120	60	12
8	140	70	14
9	160	80	16
10	180	90	18
11	200	100	20
12	220	110	22

E. **Working wash solution**: An adequate volume of working wash solution was prepared by adding 199 volumes of distilled water to 1 volume of wash Solution (200x). A magnetic stirrer was used to homogenize. Unused working wash solution was discarded at the end of the day.

ASSAY PROCEDURE

1. Select the required number of strips for the run. The unused strips should be resealed in the bag with a desiccant and stored at 2-8°C.
2. Secure the strips into the holding frame.
3. Pipette 50 µl of each Calibrator, control and sample into the appropriate wells.
4. Pipette 150 µl of incubation buffer into all the wells.
5. Incubate for 2 hours at room temperature, on a plate shaker (300 to 700 rpm) Prepare the working HRP conjugate solution once the incubation is started (within 15 minutes)
6. Aspirate the liquid from each well.
7. Wash the plate 3 times by: dispensing 0.4 ml of Wash solution into each well aspirating the content of each well
8. Pipette 200 µl of the working HRP conjugate solution into each well, incubate the microtiterplate for 30 minutes at room temperature, on a plate shaker (300 to 700 rpm)
9. Aspirate the liquid from each well.
10. Wash the plate 3 times by: dispensing 0.4 ml of Wash solution into each well aspirating the content of each well.

11. Pipette 100 µl of the Chromogenic solution into each well within 15 minutes following the washing step.

12. Incubate the microtiterplate for 15 minutes at room temperature, on a plate shaker (300 to 700 rpm), avoid direct sunlight.

13. Pipette 100 µl of stop solution into each well.

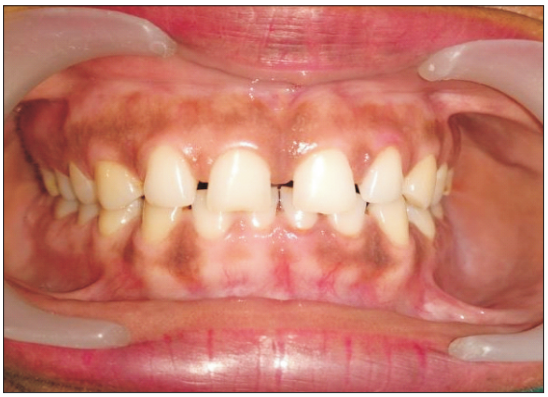
14. Read the absorbances at 450 nm (reference filter 630 nm or 650 nm) within 1 hour and calculate the results.

Summary of the Protocol

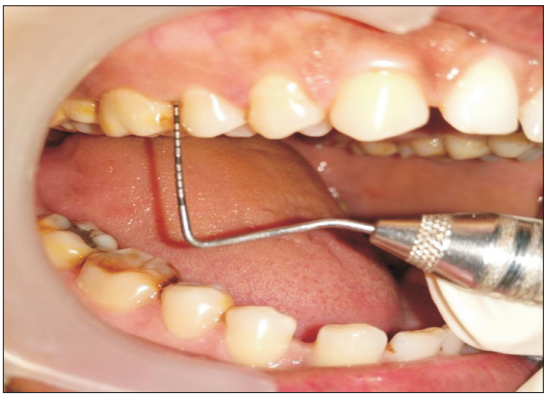
	CALIBRATORS (µl)	SAMPLE(S) CONTROLS (µl)
Calibrators (0-6)	50	-
Controls, Samples	-	50
Incubation Buffer	150	150
Incubate for 2 hours at room temperature with continuous shaking at 400 rpm. Aspirate the contents of each well. Wash 3 times with 400 µl of Wash solution and aspirate		
Working HRP Conjugate	200	200
Incubate for 30 minutes at room temperature with continuous shaking at 400 rpm. Aspirate the contents of each well. Wash 3 times with 400 µl of Wash solution and aspirate		
Chromogenic solution	100	100
Incubate for 15 mins at room temperature with continuous shaking		
Stop Solution	100	100
Read on a microtiterplate reader. Record the absorbance of each well at 450 nm (versus 630 or 650 nm).		

PLATE - I

Group I (Healthy)



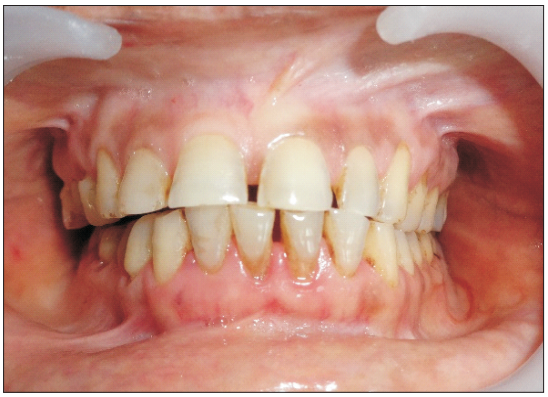
At baseline



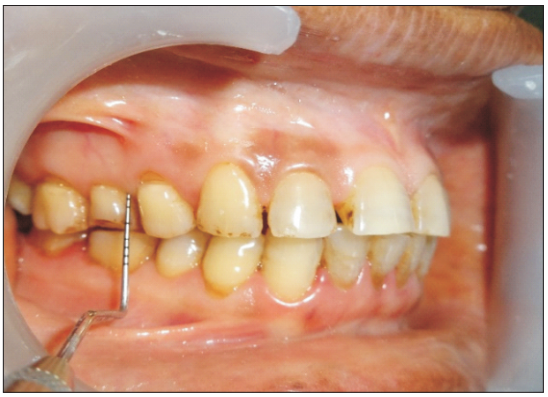
Probing depth at baseline

PLATE - II

Group II (Chronic Gingivitis)

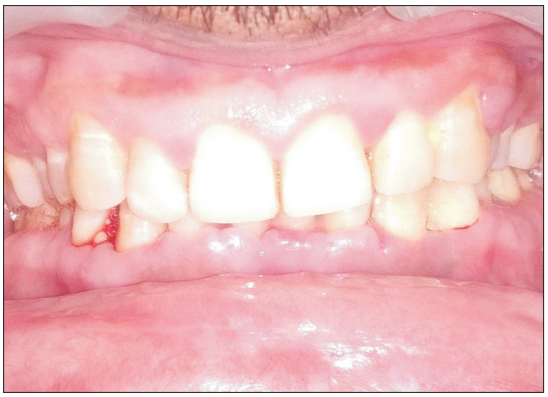


At baseline

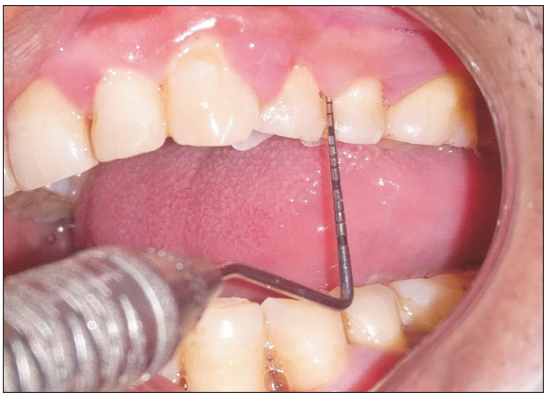


Probing depth at baseline

Group III (Chronic Gingivitis patients with DM)



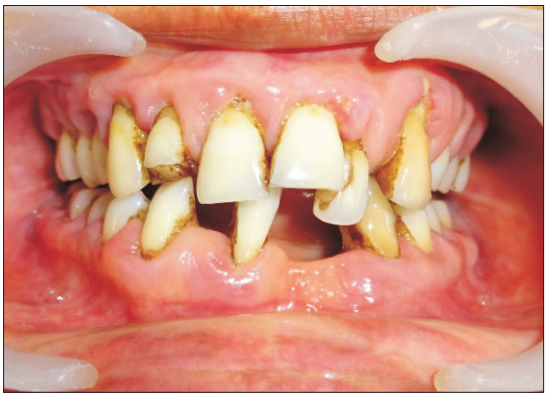
At baseline



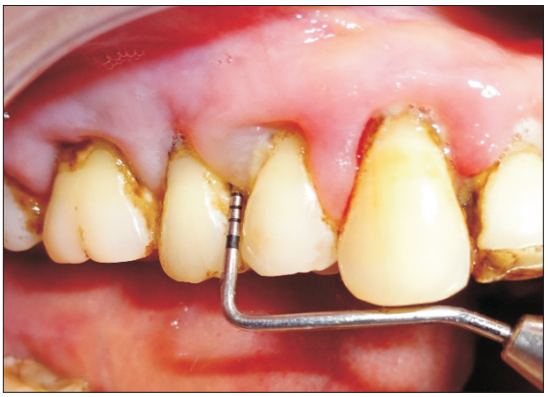
Probing depth at baseline

PLATE - III

Group IV (Chronic Periodontitis)

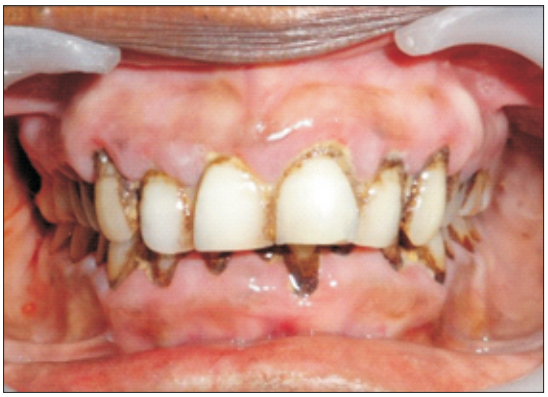


At baseline

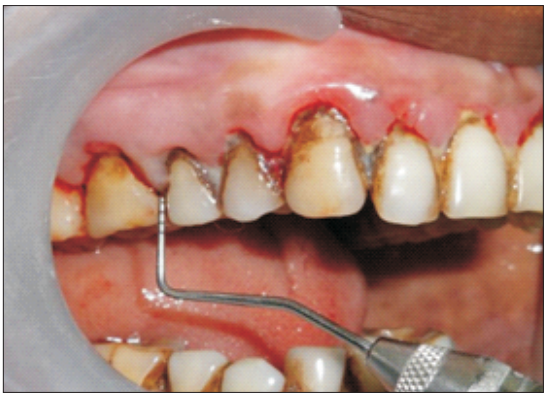


Probing depth at baseline

Group V (Chronic Periodontitis with DM)

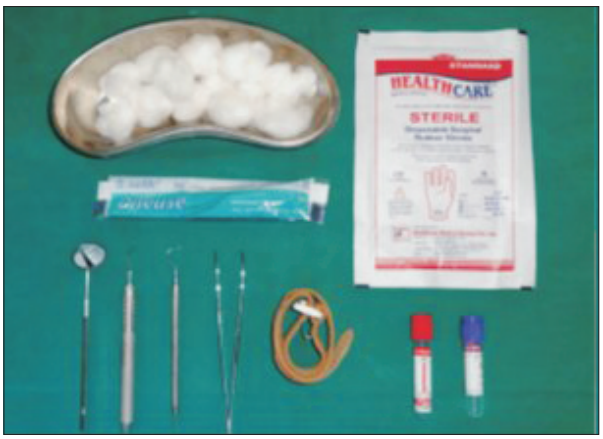


At baseline

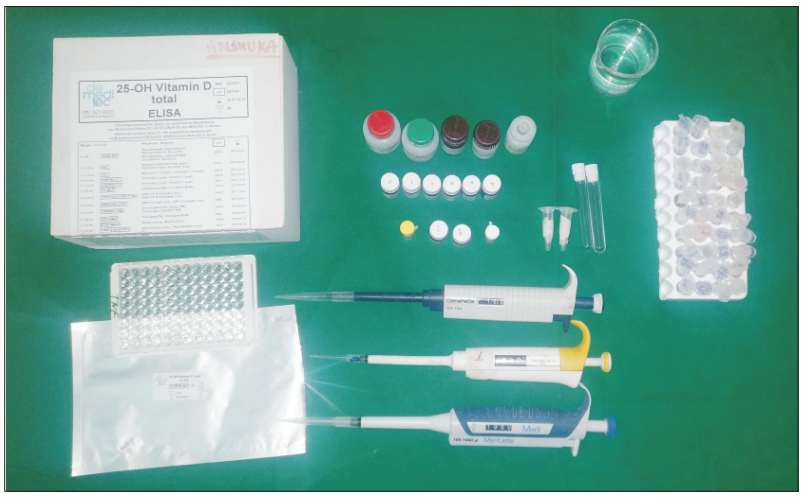


Probing depth at baseline

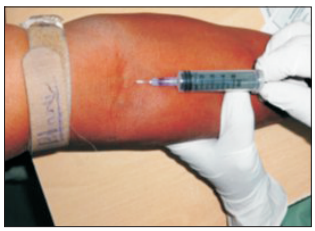
PLATE - IV



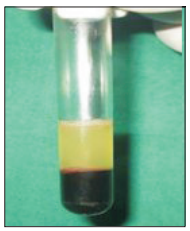
Armamentarium for clinical examination



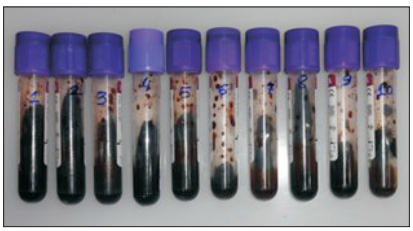
Krishgen Biosystems 25 – OH Vitamin D total ELISA kit



Collection of Blood



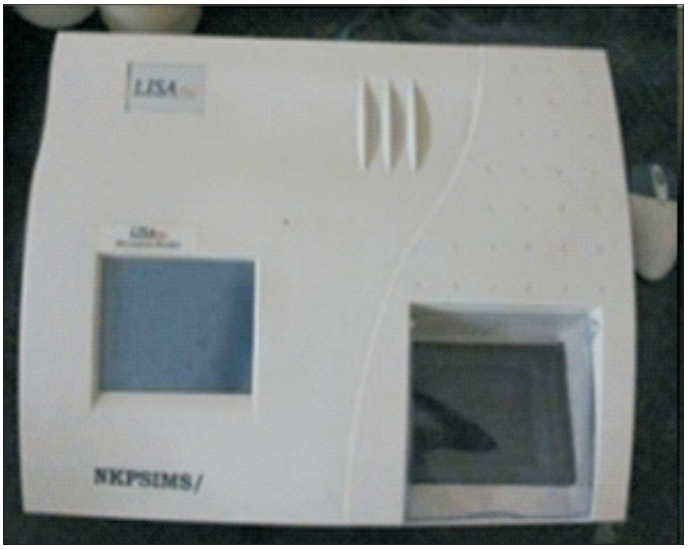
Collected serun sample



Blood samples for vitamin D analysis

PLATE - V

Centrifuge machine



Micro-plate reader machine

RESULTS

The present experimental study was aimed to evaluate the levels of vitamin D and calcium and its influence in varying conditions of patients. Vitamin D deficiency has been considered as a risk factor in the etiology of periodontal diseases. Vitamin D is also reported to decrease insulin resistance that further helps to improve the diabetic condition of an individual. In addition, calcium is found to be directly correlated with vitamin D levels, so we tried to assess that how the levels of vitamin D and calcium in the serum of periodontal disease alone and in the presence of T2DM subjects were affected. Incorporation of clinical, biochemical parameters enabled us to fulfill our objectives. For the present study, patients had been recruited that were examined clinically and biochemically for assessing periodontal health, diabetic and calcium status and then were categorized into five groups. Vitamin D levels were estimated

using ELISA in serum. After assessing the clinical and biochemical parameters in all the groups, inter-group comparisons were made.

STATISTICAL ANALYSIS

The software used in the analysis was SPSS 17.0 and $P < 0.05$ was considered as the level of significance.

Analysis of the data was carried out by using descriptive and inferential statistics both. The data regarding the periodontal parameters like Probing pocket depth (PPD), Clinical attachment level (CAL), Plaque Index (PI), Gingival index (GI), for patients in all the five study groups viz. Group I- Healthy controls, Group II- chronic gingivitis, Group III - chronic gingivitis with type 2 diabetes mellitus, Group IV – chronic periodontitis and Group V-chronic periodontitis with type 2 diabetes mellitus was obtained. Frequency distribution and descriptive statistics like mean were obtained for periodontal parameters. Also, descriptive statistics obtained were compared across the five groups.

All intergroup comparisons were done by using Student's unpaired t test. After intergroup comparisons, regression analysis was performed by keeping all the co- variates constant and then evaluating the association of vitamin D and calcium with gingivitis and periodontitis across the groups using multiple regression tests.

The severity of chronic periodontitis is mostly seen to be in age ≥ 35 years. To maintain the equality among all the groups and avoid debilitation of health related to older age patients, the patients selected were in the range of 30-50 years.

The mean of the demographic parameters, clinical parameters and biochemical parameters for all the five study groups is depicted in Table 1. The difference in clinical parameters (GI, PI, PPD, CAL) across study groups helped the authors to categorize patients into healthy (Group I), gingivitis (Group II and Group III and periodontitis groups (Group IV and Group V) respectively **(COLOUR PLATES I, II and III)**. Also, the mean HbA1c and RBS of Group III and Group V were greater as compared to other groups, on the basis of which patients were grouped into diabetics and non-diabetics according to the inclusion criteria. The mean vitamin D and calcium levels were found to be greater in Group I (66.87 ± 6.92 ng/ml and 10.99 ± 0.91mg/dL) and least in Group V (18.05 ± 3.79 ng/ml and 5.93 ± 0.84 mg/dL) respectively.

After finding the mean of individual parameters for all groups. Intergroup comparisons were made with respect to these parameters in order to find out the statistically significant differences.

Comparison of clinical parameters

Table 2a, 3a, 4a, 5a gives the Mean ± Standard Deviation of Periodontal parameters (GI,PI,PPD,CAL) of all the five study groups. The intergroup comparison calculated using Unpaired t test for independent samples between Group II and Group III, between Group IV and Group V, between Group II and Group IV and between Group III and Group V and also the comparison of each of these case groups with the control group i.e. Group I are depicted in 2b, 3b, 4b, 5b respectively.

The descriptive statistics for the periodontal parameters (GI, PI) of Groups I, Group II, Group III, Group IV and Group V are shown in Table 2a and 3a respectively. The mean GI,PI was highest i.e. 2.58 ± 0.35 and 2.62±0.31 mm in Group V and least

in Group I i.e. 0.81 ± 0.42 and 0.36 ± 0.30 respectively. The intergroup comparisons between periodontal parameters was calculated using Unpaired t test. Amongst which the intergroup comparisons between Group I and Group II (P value of 0.002), Group I and Group III (P value of 0.001) and Group II and Group III (P value of 0.001) for gingival index as shown in Table 2a and Graph 2a reveals a statistically significant difference.

The mean PI was highest i.e. 2.62 ± 0.31 in Group V, 2.57 ± 0.37 in Group IV,1.39 ± 0.34 mm in Group III followed by 1.38 ± 0.28 mm in Group II and 0.36 ± 0.3 mm in control Group I. The intergroup comparisons between Group I and Group II (P value of 0.001), Group I and Group III (P value of 0.001) for plaque index as shown in Table 2a reveals a statistically significant difference.

Similarly, the mean PPD was highest i.e. 6.38 ± 0.67 mm in Group V followed by 5.79 ± 1.22 mm in Group IV, 2.57 ± 0.8 mm in Group III, 2.28 ± 0.21 mm in Group II and 2.12 ± 0.65 mm in Group I . The intergroup comparison between Group IV and Group V as shown in Table 3a reveals a significant difference with P value of 0.034. Also statistically significant intergroup comparisons can be depicted between Group II and Group IV (P value = 0.002) and between Group III and Group V (P value = 0.046) in tables 4a, graph 4a and table 5a, graph 5a respectively.

The mean CAL was highest i.e. 6.70 ± 0.57 mm in Group V followed by 6.50 ± 1.35 mm in Group IV, 0.0 ± 0.00 mm in Group III, Group II and Group I. The intergroup comparison between Group IV and Group V as shown in Table 3a reveals a significant difference with P value = 0.041. Also statistically significant intergroup comparisons can be depicted between Group II and Group IV (P value = 0.001) and

between Group III and Group V (P value = 0.001) in Tables 4a, 5a and Graphs 4a, 5a respectively.

Descriptive statistics for biochemical parameters HbA1c, RBS, vitamin D and calcium levels in serum for all five study group patients as well as the intergroup comparison of all these parameters calculated using Unpaired t test is illustrated in Tables 2b, 3b, 4b and 5b respectively.

Comparison of HbA1c and RBS

HbA1c, levels were highest i.e. 10 ± 2.42 % in Group V, 9.35 ± 3.75 % in Group III followed by 6.60 ± 1.66 % in Group IV, 4.67 ± 0.37 % in Group II and 4.63 ± 0.36 % in Group I. The intergroup comparison was statistically significant between Group II and Group III (P value of 0.001) , in between Group IV and V (P value of 0.003), in between Group II and IV (P value of 0.001) and Group III and Group V (P value of 0.029) as depicted in Tables 2b,3b,4b and 5b respectively. Also when each of the case group was compared with the control Group I, statistically significant difference was found between Group I and Group IV (P value of 0.002) ,Group I and V (P value of 0.001), Group I and Group III (P value of 0.001) as depicted in Table 3b,graph 3b and Table 5b,graph 5b respectively.

RBS levels were highest i.e. 263.5 ± 72.34 mg/dL in Group V and 241.87 ± 98.54 mg/dL in Group III followed by 180.35 ± 22.27 mg/dL in Group II, 170.76 ± 41.59 mg/dL in Group IV and 97.65 ± 47.47 mg/dL in Group I. The intergroup comparison were found to be statistically significant in between Groups II and Group III (P value of 0.004) in Groups IV and Group V (P value of 0.003) and in Groups II and Group IV (P value of 0.003) as depicted in Table 2b,3b,4b respectively. When each

of these groups were compared with the control Group I, statistically significant difference was found in between Group I and Group III (P value of 0.001) , in between Group I and Group V(P value of 0.013) and in between Group I and Group II (P value of 0.091) as depicted in Tables 2b , 3b and 4b respectively.

Comparison of Vitamin D and calcium

The mean vitamin D levels were found to be least i.e 18.05 ± 3.79 ng/ml in Group V and 26.94 ± 1.84 ng/ml in Group IV followed by 37.22 ± 4.54 ng/ml in Group III, 49.05 ± 11.77 ng/ml in Group II and highest in Group I i.e 66.87 ± 6.92 ng/ml. The inter-group comparisons between Group II and Group III and in between Group IV and V was statistically significant with p-value of 0.001 as depicted in Tables 2b and 3b respectively. Also, the intergroup comparisons between Groups II and Group IV (P value of 0.001) and Group III and Group V (P value of 0.027) as shown in table 4b, graph 4c and table 5b, graph 5c are found to be statistically significant. When each of these groups were compared with the control Group I, statistically significant difference was found in between all groups (Group II, Group III, Group IV, Group V) with Group I i.e $P \leq 0.001$.

The mean calcium levels were found to be least i.e. 5.93 ± 0.84 mg/dL in Group V and 7.24 ± 0.71 mg/dL in Group IV followed by 8.38 ± 1.3 mg/dL in Group III, 10.41 ± 0.94 mg/dL in Group II and highest in Group I i.e 10.99 ± 0.91 mg/dL. The inter-group comparisons between Group II and Group III, in between Group IV and V with P value of 0.001 and Group III and Group V with P value of 0.014 as depicted in Tables 2b, 4b and 5b were found to be statistically significant. When each of these groups were compared with the control Group I, statistically significant difference was found with

respect to Group III (P value of 0.001) and Group V (P value of 0.036) as depicted in Table 2b,graph 2c and table 5b,graph 5c respectively.

When multiple regression analysis of vitamin D was done with chronic periodontitis parameters with T2DM, vitamin D was found to have a positive influence on CAL (Table 6, fig 1).Similarly, on keeping the other periodontal parameters constant in subjects with T2DM, calcium had positive influence on CAL (Table 7, fig 2).

DISCUSSION

Periodontal diseases are a group of bacterial infectious and inflammatory disease that result in the destruction of tooth supporting structures including gingiva, alveolar bone and the periodontal ligament eventually leading to tooth loss. Diabetes mellitus is one of the most commonly developing endocrine disorders and similarly among oral disorders chronic periodontitis is most frequently found one. The current literature indicates a positive association between the two disorders. Thus, the combination of hyperglycemic state and periodontal inflammation becomes a critical issue in regard to oral health.[44] Periodontal infection is not only confined to the oral tissues, but can also elicit a host immune response with systemic involvement. Mechanism being, periodontal inflammation can result into ulceration of sub-gingival epithelial cells which is clinically appreciated as pocket formation. These ulcerated areas are exposed to the microbial ingress and via these ulcerated cells or lysed cell lining micro-organisms get

direct access to the bloodstream. Microorganisms release toxins in the form of few enzymes and lipopolysaccharides into the blood circulation and it initiates host immune response and release of many pro-inflammatory mediators such as interleukin 1 (IL-1), Tumour necrosis factor α (TNF- α), prostaglandin E2, interferon γ etc.[45]

Vitamin D has also been found to have anti-inflammatory effects, as it inhibits antigen induced T-cell proliferation and cytokine production, specifically interleukin-2 and interferon – γ. Furthermore, vitamin D has marked effects on antigen presenting cells. In addition, few studies have shown beneficial effects of vitamin D and its analogs in animal models of autoimmunity and transplantation. Existing literature also reported the advantages of vitamin D on various outcomes other than bone health, such as muscle strength, colon cancer and inflammatory diseases.[46]

Vitamin D enters the circulation from the skin or diet .Within several hours, it accumulates in the liver where it undergoes hydroxylation to form calcitriol [1, 25 (OH)2]. It is hypothesized that vitamin D could affect periodontal disease in different ways, including Bone Mineral Density (BMD) mediated effects and BMD independent anti-inflammatory effects. Vitamin D has an important role in bone formation and preservation and successfully increased BMD and reduced non-vertebral fractures in several randomized clinical trials. [47,48, 49] Vitamin D has been found to inhibit cytokine production and cell proliferation in various tissues[50] and could thus affect the inflammatory resorption of alveolar bone.[51] **Dietrich et al. (2004)[38]** in his study suggested that higher serum vitamin D concentrations are associated with less attachment loss in elderly patients**.** Vitamin D deficiency also leads to the impaired secretion of insulin and induces glucose intolerance, while replenishment with vitamin

D rectifies the abnormalities. This impairment is primarily caused by the direct effect of vitamin D deficiency on the beta cell, but other effects of vitamin D deficiency, such as impaired food intake and hypocalcemia may also play a role.

One of the major functions of calcitriol is to maintain serum calcium and phosphorus concentrations within normal ranges by controlling absorption in the small intestine. The effects of calcium on periodontal disease are likely related to alveolar bone change, which eventually results in greater clinical attachment loss. The disturbed calcium/phosphorus balance could increase the production of parathyroid hormone, causing a loss of calcium from the skeleton and alveolar bone. There are many reports suggesting that oral bone mineral density correlates with skeletal bone density. **Wical and Brusse (1979)**[52] found that calcium supplementation reduced the rate of residual ridge resorption in the first year after tooth extraction. **Klemetti et al. (1994)**[53] studied the association between periodontal disease, diagnosed by radiographs and clinical probing depth and bone mineral status of the skeleton. Their study suggested that those who have high mineral value in the skeleton retain their teeth with deep periodontal pockets more easily than those with low mineral skeletal bone. However, a randomized controlled 180 day calcium supplement study without any periodontal treatment showed calcium supplementation did not affect periodontal disease.[54]

Alterations in calcium flux can also have adverse effects on insulin secretion, a calcium-dependent process. **Lundgren and Rosenquist (1992)**[55] in an animal study showed that calcium repletion alone normalized glucose tolerance and insulin secretion in vitamin D depleted rats. In people without diabetes, hypocalcemia is associated with impairment of insulin release. In diabetic patients, an oral calcium load augments

glucose-induced insulin secretion. Patients with resistance to vitamin D were found to have abnormal insulin secretion only if they were hypocalcaemic. Calcium is essential for insulin-mediated intracellular processes in insulin responsive tissues such as skeletal muscle and adipose tissue with a very narrow range of calcium needed for optimal insulin-mediated functions. Changes in calcium in primary insulin target tissues contribute to alterations in insulin action. Also, impairment of insulin receptor phosphorylation, a calcium-dependent process leads to impaired insulin signal transduction and decreased glucose transporter-4 activity. Changes in calcium modulated adipocyte metabolism, which may promote triglyceride accumulation via increased lipogenesis along with the inability to suppress insulin-mediated lipolysis lead to fat accumulation. Patients with diabetes mellitus thus exhibit impaired cellular calcium homeostasis including defects in skeletal muscle, adipocytes, and liver.

The clinical role of vitamin D and calcium as potential interventions for inflammatory diseases like gingivitis and periodontitis alone as well as with T2DM has not been defined yet, so the present study was planned with a hypothesis that vitamin D and calcium levels in serum of patients with gingivitis and chronic periodontitis with and without T2DM are reduced due to high inflammatory burden as compared to the healthy patients.

For this, patients were categorized into 5 groups of 20 patients each on the basis of periodontal parameters including Plaque index (PI), Gingival index (GI) ,Probing pocket depth (PPD), Clinical attachment level (CAL), radiographic evidence of bone loss, HbA1C levels and RBS levels.

1. Group I – Control group (Systemically and periodontally healthy)
2. Group II - Test group (Chronic gingivitis patients without DM)
3. Group III - Test group (Chronic gingivitis patients with DM)
4. Group IV - Test group (Chronic periodontitis patients without DM)
5. Group V - Test group (Chronic periodontitis patients with DM)

In all the patients of the test and control groups, clinical parameters were evaluated and RBS, HbA1c, calcium tests were done. Also, vitamin D levels were assessed in serum. The patients included in the study were 30-50 years of age. The mean age of patients in Group II and Group IV was 41.45 ± 8.02 years and 39.29 ± 10.11 years respectively. The mean age in Group III and Group V was 46.23 ± 10.41 years and 46.22 ± 10.64 years respectively **(Table 1)**. Periodontal disease and diabetes mellitus prevalence, or extent and severity of epidemiologic studies show more prevalence in older age group as compared to younger groups.[56]

Assessment of Clinical Parameters

Periodontal parameters evaluated were GI, PI, PPD, CAL in all the five groups. The mean PI and GI values in Group I (0.36 ± 0.30 and 0.81 ± 0.42), Group II (1.38 ± 0.28 and 1.18 ± 0.25), Group III (1.39 ± 0.34 and 1.50 ± 0.28), Group IV (2.57± 0.37 and 2.38 ± 0.53) and Group V (2.62 ± 0.31 and 2.58 ± 0.35) indicated that the patients demonstrated a gradual increase in these parameters and confirms with the inclusion criteria of the study for individual study groups. Also, the mean values of PI and GI in Group II (1.38 ± 0.28 and 1.18 ± 0.25) and Group IV (2.57 ± 0.25 and 2.38 ± 0.53) were less as compared to Group III (1.39 ± 0.34 and 1.50 ± 0.28) and Group V (2.62 ± 0.31 and 2.58 ± 0.35) respectively. These increased values of plaque and gingival

indices in patients with diabetes mellitus as compared to the ones with periodontal disease alone is in accordance with the findings of **Nayak S et al. (2017)**[57] who found that all the increased periodontal disease occurrence in diabetics may be due to xerostomia, which is very common among diabetics and elderly individuals. Xerostomia may lead to some oral problems, such as dental caries, bad breath, burning sensation in oral tissue, and increase in the plaque formation. This, in turn, can favor gingival inflammation in patients with compromised oral hygiene; diabetic individuals are more prone to severe inflammation due to weaker host defense mechanisms.

The higher mean values of PPD in Group IV (5.79± 1.22 mm) and Group V (6.38 ± 0.67 mm) as compared to Groups II and III indicated the inclusion criteria were strictly adhered to while the formation of study groups .

Also, the increased and statistically significant mean values of PPD and CAL in Group V **(Table 5a)** as compared to Group IV **(Table 4a)** , determines the increased periodontal destruction in periodontal disease patients with diabetes as compared to one without diabetes. This finding of our study is in accordance to **Choi et al**. **(2011)**[26] findings, who found that the highest quintile of pocket depth and clinical attachment loss were positively associated with IFG (Impaired Fasting Glucose) and diabetes. Also, **Bacic M et al. (1988)**[58] concluded in his study that, deep pockets (>4mm) and gingival inflammation were found to be more common in diabetic patients. **Taylor et al. (2001),**[21] in his study found that subjects with diabetes have increased prevalence, extent, severity, the progression of periodontal disease with more frequent and advanced attachment loss and bone loss.

Diabetes has been incriminated to be a risk factor for gingivitis and periodontal inflammation. Glycemic control may be one of the most important factors in the two way relationship between periodontitis and T2DM. Poor glycaemic control in diabetics have been proved to be a reason for increased inflammation and rapid destruction of periodontal tissue. Alveolar bone and attachment loss has also been shown to be increased many folds in periodontal disease associated with T2DM with uncontrolled hyperglycaemia.[59] The periodontal parameters of the present study co-relates with this as the periodontal pocket depth and clinical attachment level (CAL) was found to be more in chronic periodontitis patients with diabetes mellitus i.e 6.38 ± 0.67 mm and 6.70 ± 0.57 mm respectively, than in the patients without T2DM i.e 5.79 ± 1.22mm and 6.50 ± 1.35mm with a p value of 0.034 and 0.041 respectively **(Table 3a, Graph 3a).**

Also, evidence has accumulated proving the coexistence of severe form of gingivitis as well as periodontitis in patients with T2DM. Both of these conditions (gingivitis as well as periodontitis) may worsen the glycemic control in diabetic patients. The findings of the present study are in accordance with this, as the RBS and HbA1c levels in patients with gingivitis along with DM is found to be 241.87 ± 98.54 mg/dL and 9.35 ± 3.75 % respectively.

Similar statistically significant differences were revealed in the values of RBS and HbA1c in chronic periodontitis patients without T2DM (i.e 170.76 ± 41.59 mg/dL and 6.60 ± 1.66 %) and chronic periodontitis patients with T2DM (i.e 263.50 ± 72.34 mg/dL and 10.00 ± 2.42%) respectively. These findings indicate towards severe periodontal disease conditions which logically are influenced by the presence of T2DM **(Tables 2b, 3b)**.

RBS and HbA1C across Study Groups

To monitor diabetic control, RBS was evaluated among all five groups. The mean RBS values for Group V was 263.50± 72.34 mg/dL and for Group III was 241.87 ± 98.54 mg/dL which well differentiated diabetic patients from the non-diabetics (Group I,II and IV) .Similarly the mean RBS in Group III (241.87 ± 98.54 mg/dL) was more as compared to mean RBS of Group II (180.35 ± 22.27 mg/dL). However, HbA1c assay was chosen as a confirmatory test as it offers advantages over traditional blood glucose monitoring methods. The mean HbA1c values for Group III was 9.35 ± 3.75% and for Group V it was 10.00± 2.42% , from which it can be interpreted that in patients with T2DM, the progressive increase in HbA1c values are directly or indirectly linked with increased periodontal tissue breakdown **(Table 4b, Graph 4b)**.

In terms of mean HbA1c levels across these groups, our results are in accordance with **Apoorva S et al. (2013)**,[60] who studied the prevalence and severity of periodontal disease in T2DM patients and reported that as the glycated hemoglobin level increases, the severity of periodontal disease increases. These findings are similar to the ones of our study as the maximum destruction of periodontal tissues i.e CAL of 6.70 ± 0.57 mm was found in Group V i.e in patients with both diabetes mellitus and chronic periodontitis (Table 5a). Several interacting factors such as altered polymorphonuclear cell function and derangements of inflammatory protein response coverage at the periodontium result in a higher prevalence and severity of periodontitis. Also in the literature, it has been found that when appropriate periodontal therapy is provided, there is a considerable improvement in glycemic levels in patients with T2DM. This shows a two way relationship between periodontal disease and glycemic control. **Patil V et al. (2013)[61]** found that the risk factors like HbA1c, duration of

diabetes, personal habits and oral hygiene habits show a positive correlation with periodontal destruction.

Assessment of Vitamin D and Calcium across study groups

It seemed that people with vitamin D deficiency were more likely to experience T2DM in their old age. On the other hand, some studies have demonstrated just the opposite results. In an experimental study by **Bouillon R et al (2008)**,[62] vitamin D receptors (VDR) in knockout mice had a normal glucose homeostasis and bone mineralization and showed no increased susceptibility to diabetes. However, **Scragg R et al. (1995)**[30] found that vitamin D concentrations have an inverse correlation to blood glucose concentration.

Yu et al. (2012) [31] reported that in his study population, the majority of patients with T2DM were vitamin D deficient. A meta-analysis for observational studies showed a relatively consistent association between low vitamin D status and T2DM.[63] From these studies, it is evident that a relationship exists between insufficient serum vitamin D level and T2DM. Also, **Hidayat et al. (2010)**[46] suggested that vitamin D and its supplementation has improved glycemic control and insulin sensitivity in patients with type 2 DM. Thus, the low vitamin D levels in our study patients with T2DM as compared to the one without diabetes is in support to the findings of previous studies.

Dietrich et al. (2005)[11] evaluated the association between serum vitamin D and gingival inflammation and found a strong negative association. The author concluded that this inverse association may be due to the anti-inflammatory effect of vitamin D. In our study, the vitamin D levels in chronic gingivitis patients with diabetes mellitus were found to be less i.e 37.22 ± 4.54 ng/ml as compared to control 66.87 ± 6.92 ng/ml

and chronic gingivitis without T2DM 49.05 ± 11.77 ng/ml **(Table 2b, Graph2c).** Not only gingivitis, but the values of vitamin D in chronic periodontitis patients with T2DM i.e 18.05 ± 3.79 ng/ml was also less as compared to other groups suggesting the inverse correlation of vitamin D with chronic periodontitis **(Table 3b,5b,Graphs 3c,5c)**. These findings are in accordance with the study of **Joseph et al**. **(2015)**[41] who observed a low level of serum vitamin D level in patients with chronic periodontitis and chronic periodontitis with T2DM. Also, an inverse correlation between Vitamin D level and CAL was observed in our study i.e 18.05 ± 3.79 ng/ml and 6.70 ± 0.57 mm respectively **(Table 3b, Graph 3c)** which is in consistent with the study conducted by **Dietrich et al**. **(2004)**[38] who also reported an inverse association between serum vitamin D and periodontal attachment loss. So an inverse correlation of vitamin D levels was found with both diabetes mellitus and inflammatory conditions like gingivitis and chronic periodontitis.

Heaney RP et al. (2003)[64] found that, the vitamin D that is produced in the kidney in response to low serum calcium levels is transported to the small intestine and bone, where it interacts with vitamin D receptors to increase calcium absorption in the intestine and to release calcium from bone. It also travels to various tissues, where it binds to membrane receptors and opens calcium channels. This is in turn can result in obesity leading to diabetes. This study gives an evidence to the indirect influence of low calcium levels on diabetes by affecting the vitamin D levels in the body. This is in support to the findings of low calcium levels in diabetes mellitus i.e 8.38 ± 1.30 mg/dL and 5.93 ± 0.84 mg/dL in Group III and Group V as compared to 10.99 ± 0.91 mg/dL in Group I **(Table 5b, Graph 5c). Kanchana. N et al**. **(2014)**[65] observed a negative correlation between serum calcium levels and plasma blood glucose levels. Also the P

value was found to be less than 0.05 which confirmed that correlation was statistically significant. **Becerra-Tomas et al. (2014)**[66] concluded that a decrease in serum calcium concentrations is associated with an increased risk of T2DM in individuals at high cardiovascular risk.

In an examination of data on 12,000 adults who took part in the Third National Health and Nutrition Examination Survey, it was found that low dietary intake of calcium increased attachment loss in a dose-dependent fashion. Not only this, but for more than 40 years, investigators had suggested that low calcium intake may be associated with alveolar bone resorption and more recently there had been a number of studies in which investigators have suggested that calcium along with vitamin D may benefit periodontal health and it has been suggested that calcium deficiency may be a risk factor for periodontal disease. The findings of our study can be correlated to these studies as low level of calcium was found in our study patients i.e. 10.41±0.94 mg/dL and 7.24 ± 0.71 mg/dL in gingivitis and periodontitis patients respectively as compared to healthy patients i.e 10.99 ± 0.91 mg/dL**. (Table 4b, Graph 4c)**

According to **Pittas AG et al . (2006)**,[67] calcium intake had been associated with lower risk of incident diabetes previously and the combination of vitamin D and calcium may have been more beneficial than with either nutrient alone. Statistically significant results of calcium levels were found when compared between chronic periodontitis without T2DM 7.24 ± 0.71 mg/dL and chronic periodontitis with DM 5.93±0.84 mg/dL **(Table 3b, Graph 3c)** and 10.41±0.94 mg/dL in chronic gingivitis and 8.38± 1.30 mg/dL in chronic gingivitis with DM patients **(Table 2b, Graph 2c)**. From all these findings, it can be said that the calcium level found in chronic

periodontitis with the T2DM group is least as compared to other groups thus suggesting the inverse association of calcium with periodontitis and diabetes.

The results of our study depicts the decrease in vitamin D and calcium levels with increase in inflammation and glycated hemoglobin levels in serum, thus supporting the evidences with respect to the positive role that vitamin D and calcium plays in T2DM and inflammation by either improving the pancreatic β – cell function **(Bland R et al. 2004)**[7] by causing the direct effects by expression of 1-α- hydroxylase enzyme in pancreatic β cells and by transcriptional activation of the human insulin gene by vitamin D, also by improving insulin action **(Luquet S et al. 2005)**[68] by stimulating the expression of insulin receptor and enhances insulin responsiveness for glucose transport in vitro and thus helps in reducing inflammation by down regulating the nuclear factor– κβ which is an important regulator of genes encoding pro-inflammatory cytokines implicated in insulin resistance as suggested by **van Etten et al. (2005)** [69]

Bacterial plaque being the primary etiological factor for the periodontal diseases,[70] and by looking at the statistically significantly different values of vitamin D and calcium in periodontal disease patients alone as well as the one with T2DM, an inference can be made that nutrition also have impact on the inflammatory process. The study also supports the previous evidence, where **Miley D et al**. **(2009)**[40] in his study found that in patients receiving periodontal maintenance therapy, there was a trend for better periodontal health with vitamin D and calcium supplementation. The author also stated that more extensive longitudinal studies are required to determine the potential of this relationship.

Clinical Significance of the present study

Periodontal diseases are the widespread inflammatory diseases and it has been shown that the systemic disease like diabetes mellitus can have a worsening influence on the periodontal disease status. Under such circumstances, both the conditions need to be assessed in the initial stages of the disease, so that adequate measures are adopted to control the destruction of tissues. The results of our study depict that the levels of vitamin D and calcium lowers down in inflammatory as well as glycemic conditions, so we assume that if the supplements of vitamin D and calcium are given to the patients along with non-surgical periodontal therapy, it would not only help to improve the inflammatory conditions in an individual but also reduce the possibilities of complications associated with the altered glycemic status of the individual.

Limitations

Notwithstanding the significance of the present study, several limitations to be mentioned are:

1. The present study being a cross-sectional one, it is not possible to ascertain whether the periodontal disease is a cause or effect of hyperglycemic status in individual patients.

2. Our sample did not demonstrate any significant age and vitamin D level association. Age, however would be expected to negatively correlate with vitamin D status since older individuals are less efficient at producing vitamin D in sunlight as opposed to younger individuals as well as the fact that older individuals kidneys are less able to convert vitamin D into its active form.

3. Obesity is the risk factor for diabetes mellitus. Also, obesity and vitamin D deficiency may work synergistically to propel an individual into the diseased state of T2DM, pertaining to these two proven facts, Body Mass Index (BMI) was an important confounding variable in the present study, as it was not measured. Therefore, the role of obesity cannot be explained here.

4. Even though we took covariates into account, there could have been some other confounding variables such as smoking, sun exposure, dietary intake, regular exercise, all of which contribute to vitamin D synthesis, which were not considered in the present study.

Further studies are also recommended to elucidate the influence of factors such as gender, age and weight on the anti-inflammatory effect of vitamin D on gingivitis.

CONCLUSION

The present study was undertaken to evaluate whether vitamin D and calcium levels are associated with periodontal disease patients with and without type 2 diabetes mellitus. A total of 100 patients were recruited and categorized into 5 groups, with 20 patients in each. Group I being healthy, Group II being patients with chronic gingivitis, Group III being patients with chronic gingivitis with T2DM, Group IV being chronic periodontitis and Group V being chronic periodontitis with T2DM. All the patients were assessed clinically and biochemically for categorization into respective groups. Clinical parameters evaluated were PI, GI, PPD and CAL. Biochemical parameters included were RBS and HbA1C levels to assess the diabetic status of patients along with serum calcium levels. ELISA test was used to analyze vitamin D levels in serum of all the patients.

There was a gradual increase in the severity of clinical parameters from Group I to Group V and the significantly higher difference was observed among Group III, IV and V and they were found to be correlated with decreased levels of serum vitamin D and calcium. Along with this, when clinical and biochemical parameters were evaluated, an inverse correlation between RBS and HbA1C levels and serum vitamin D and calcium values was found.

From the analysis of the results, following observations can be drawn:

1. A negative and significant association was observed in vitamin D and calcium levels in chronic gingivitis and chronic periodontitis.
2. A negative and significant association was observed in vitamin D and calcium levels with RBS and HbA1c in gingivitis with DM and chronic periodontitis with DM.

Within the limitations of our study, it can be concluded that low vitamin D and calcium levels are associated with gingivitis and periodontitis. Not only inflammatory conditions, but it also affects the glycemic status of individuals. Therefore it can be stated that vitamin D and calcium levels are inversely correlated with random blood sugar and glycosylated hemoglobin along with probing pocket depth and clinical attachment level. Thus the patients diagnosed with both periodontal disease and type 2 diabetes mellitus can be vitamin D and calcium deficient. These findings suggest us that inadequate vitamin D and calcium levels might not be the cause but it might help to improve the inflammatory and glycemic status of an individual if supplemented along with the periodontal therapy. However, randomized interventional trials are needed before supplementation with vitamin D can be recommended for prevention of periodontitis with/without diabetes.

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Table 1. Mean of all the parameters for all groups

Parameters	N	Group I	Group II	Group III	Group IV	Group V
		Mean	Mean	Mean	Mean	Mean
Age (years)	22	44.65	41.45	46.23	39.29	46.22
GI	22	0.81	1.18	1.50	2.38	2.58
PI	22	0.36	1.38	1.39	2.57	2.62
PPD (mm)	22	2.12	2.28	2.57	5.79	6.38
CAL (mm)	22	0.0	0.0	0.0	6.50	6.70
HbAlc (%)	22	4.63	4.67	9.35	6.60	10.00
RBS (mg/dL)	22	97.65	180.35	241.87	170.76	263.5
Vitamin D (ng/ml)	22	66.87	49.05	37.22	26.94	18.05
Calcium (mg/dL)	22	10.99	10.41	8.38	7.24	5.93

Table 2a: Clinical parameters comparison of Group II and Group III with Group I

Parameter	N	Healthy (Group I) Mean ± Std. Deviation	Chronic gingivitis patients without DM. (Group II) Mean ± Std. Deviation	Chronic gingivitis patients with DM (Group III) Mean ± Std. Deviation	Comparison of Group I with Group II (p-value)	Comparison of Group I with Group III (p-value)	Comparison of Group II with Group III (p-value)
GI	22	0.81 ±0.42	1.18 ±0.25	1.50 ±0.28	0.002 (HS)	0.001(HS)	0.001(HS)
PI	22	0.36 ±0.30	1.38 ±0.28	1.39 ±0.34	0.001 (HS)	0.001(HS)	0.973

(S: significant ; HS: Highly significant)

Table 2b: Biochemical parameters comparison of Group II and Group III with Group I

Parameter	N	Healthy (Group I) Mean ± Std. Deviation	Chronic gingivitis patients without DM. (Group II) Mean ± Std. Deviation	Chronic gingivitis patients with DM (Group III) Mean ± Std. Deviation	Comparison of Group I with Group II (p-value)	Comparison of Group I with Group III (p-value)	Comparison of Group II with Group III (p-value)
HbAlc (%)	22	4.63± 0.36	4.67 ±0.37	9.35 ± 3.75	0.965	0.001 (HS)	0.001 (HS)
RBS (mg/dL)	22	97.65 ± 47.47	180.35 ± 22.27	241.87 ± 98.54	0.091	0.001 (HS)	0.004 (HS)
Vitamin D (ng/ml)	22	66.87 ± 6.92	49.05 ± 11.77	37.22 ± 4.54	0.019 (S)	0.001 (HS)	0.001 (HS)
Calcium (mg/dL)	22	10.99 ± 0.91	10.41 ± 0.94	8.38 ± 1.30	0.055	0.001 (HS)	0.001 (HS)

(S: significant ; HS : Highly significant)

Table 3a: Clinical parameters comparison of Group IV and Group V with Group I

Parameter	N	Healthy (Group I) Mean ± Std. Deviation	Chronic periodontitis patients without DM (Group IV) Mean ± Std. Deviation	Chronic periodontitis patients with DM (Group V) Mean ± Std. Deviation	Comparison of Group I with Group IV (p value)	Comparison of Group I with Group V (p value)	Comparison of Group IV with Group V (p value)
GI	22	0.81 ± 0.42	2.38 ± 0.53	2.58 ± 0.35	0.036 (S)	0.013 (S)	0.158
PI	22	0.36 ± 0.30	2.57± 0.37	2.62 ± 0.31	0.021 (S)	0.001(HS)	0.629
PPD(mm)	22	2.12 ± 0.59	5.79 ±1.22	6.38 ± 0.67	0.032 (S)	0.041 (S)	0.034 (S)
CAL(mm)	22	0.0	6.50 ±1.35	6.70 ± 0.57	0.001 (HS)	0.001(HS)	0.041 (S)

(S: significant ; HS : Highly significant)

Table 3b: Biochemical parameters comparison of Group IV and Group V with Group I

Parameter	N	Healthy (Group I) Mean ± Std. Deviation	Chronic periodontitis patients without DM (Group IV) Mean ± Std. Deviation	Chronic periodontitis patients with DM (Group V) Mean ± Std. Deviation	Comparison of Group I with Group IV (p-value)	Comparison of Group I with Group V (p-value)	Comparison of Group IV with Group V (p-value)
HbAlc (%)	22	4.63± 0.36	6.60 ± 1.66	10.00 ± 2.42	0.002 (HS)	0.001(HS)	0.003(HS)
RBS (mg/dL)	22	97.65 ± 47.47	169.76 ± 41.59	263.50 ± 72.34	0.484	0.013 (S)	0.003 (HS)
Vitamin D (ng/ml)	22	66.87 ± 6.92	26.94 ± 1.84	18.05 ± 3.79	0.001 (HS)	0.001 (HS)	0.001 (HS)
Calcium (mg/dL)	22	10.99 ± 0.91	7.24 ± 0.71	5.93 ± 0.84	0.088	0.036 (S)	0.001(HS)

(S: significant; HS : Highly significant)

Table 4a: Clinical parameters comparison of Group II and Group IV with Group I

Parameter	N	Healthy (Group I) Mean ± Std. Deviation	Chronic Gingivitis patients without DM (Group II) Mean ± Std. Deviation	Chronic periodontitis patients without DM (Group IV) Mean ± Std. Deviation	Comparison of Group I with Group II (p value)	Comparison of Group I with Group IV (p value)	Comparison of Group II with Group IV (p value)
PPD(mm)	22	2.12 ± 0.59	2.28 ± 0.21	5.79 ± 1.22	0.040 (S)	0.032 (S)	0.002 (HS)
CAL(mm)	22	0.0	0.0	6.50 ± 1.35	-	0.001 (HS)	0.001 (HS)

(S: significant; HS: Highly significant)

Table 4b: Biochemical parameters comparison of Group II and Group IV with Group I

Parameters	N	Healthy (Group I)	Chronic gingivitis patients without DM (Group II)	Chronic periodontitis patients without DM (Group IV)	Comparison of Group I with Group II (p value)	Comparison of Group I with Group IV (p value)	Comparison of Group II with Group IV (p value)
HbAlc (%)	22	4.63 ± 0.36	4.67 ± 0.37	6.60 ± 1.66	0.965	0.002 (HS)	0.001(HS)
RBS (mg/dL)	22	97.65 ± 47.47	180.35 ± 22.27	170.76 ± 41.59	0.091	0.484	0.003(HS)
Vitamin D (ng/ml)	22	66.87 ± 6.92	49.05 ± 11.77	26.94 ± 1.84	0.019 (S)	0.001 (HS)	0.001(HS)
Calcium (mg/dL)	22	10.99 ± 0.91	10.41 ± 0.94	7.24 ± 0.71	0.055	0.088	0.091

(S: significant ; HS : Highly significant)

Table 5a: Clinical parameters comparison of Group III and Group V with Group I

Parameter	N	Healthy (Group I) Mean ± Std. Deviation	Chronic Gingivitis patients with DM (Group III) Mean ± Std. Deviation	Chronic periodontitis patients with DM (Group V) Mean ± Std. Deviation	Comparison of Group I with Group III (p value)	Comparison of Group I with Group V (p value)	Comparison of Group III with Group V (p value)
PPD(mm)	22	2.12 ± 0.59	2.57 ± 0.80	6.38 ± 0.67	0.933	0.041 (S)	0.046 (S)
CAL(mm)	22	0.0	0.0	6.70± 0.57	-	0.001 (HS)	0.001 (HS)

(S: significant ; HS : Highly significant)

Table 5b: Biochemical parameters comparison of Group III and Group V with Group I

Parameter	N	Healthy (Group I) Mean ± Std. Deviation	Chronic gingivitis patients with DM (Group III) Mean ± Std. Deviation	Chronic periodontitis patients with DM (Group V)	Comparison of Group I with Group III (p value)	Comparison of Group I with Group V (p value)	Comparison of Group III with Group V (p value)
HbAlc (%)	22	4.63 ± 0.36	9.35 ± 3.75	10.00 ± 2.42	0.001 (HS)	0.001 (HS)	0.029 (S)
RBS (mg/dL)	22	97.65 ± 47.47	241.87 ± 98.54	263.50 ± 72.34	0.001 (HS)	0.013 (S)	0.127
Vitamin D (ng/ml)	22	66.87 ± 6.92	37.22 ± 4.54	18.05 ± 3.79	0.001 (HS)	0.001 (HS)	0.027 (S)
Calcium (mg/dL)	22	10.99 ± 0.91	8.38 ± 1.30	5.93 ± 0.84	0.001 (HS)	0.036 (S)	0.014 (S)

(S: significant ; HS : Highly significant)

Table 6: Regression analysis of vitamin D with chronic periodontics parameters with DM

Model		Unstandardized Coefficients		Standardized Coefficients	t value	p value
		B	Std. Error	Beta		
	PPD	-3.463	5.202	-0.541	-0.666	0.515
	CAL	3.622	5.596	0.526	0.647	0.526
R=0.160, R^2=0.025, Adjusted R^2= -0.089 F=0.222,p=0.803 > 0.05(not significant), Standardized Error of the estimate=3.62560						

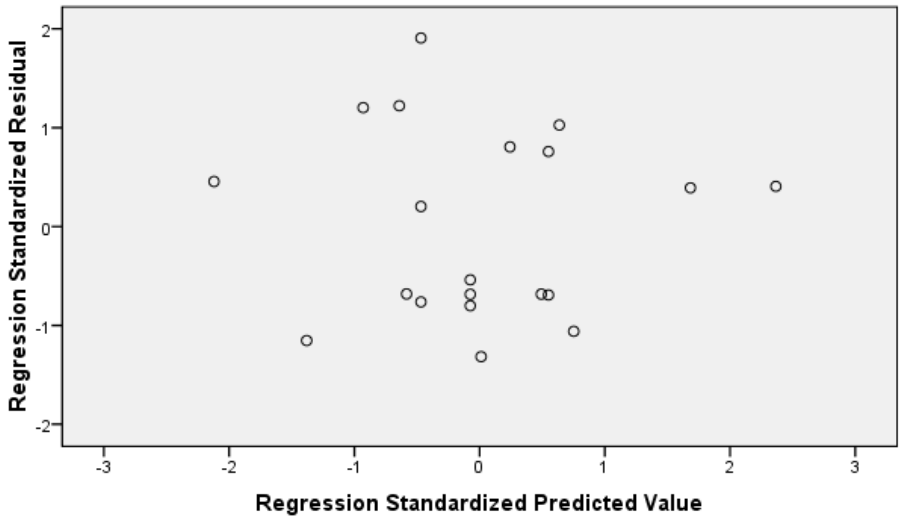


Figure 1: Regression analysis of vitamin D with chronic Periodontics parameters with DM

Table 7: Regression analysis of calcium with chronic Periodontics parameters with DM

Model		Unstandardized Coefficients		Standardized Coefficients	t value	p value
		B	Std. Error	Beta		
	PPD	-2.273	0.768	-1.978	-2.959	0.009 (HS)
	CAL	2.313	0.826	1.871	2.800	0.012 (S)
R=0.1583, R^2=0.340, Adjusted R^2= 0.263 F=4.383,p=0.029<0.05(Significant), Standardized Error of the estimate=0.53526						

(S: significant ;HS : Highly significant)

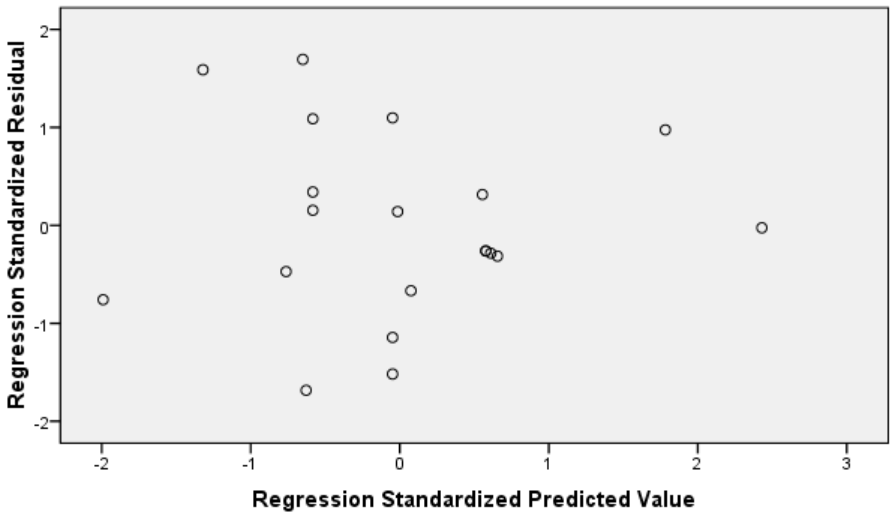
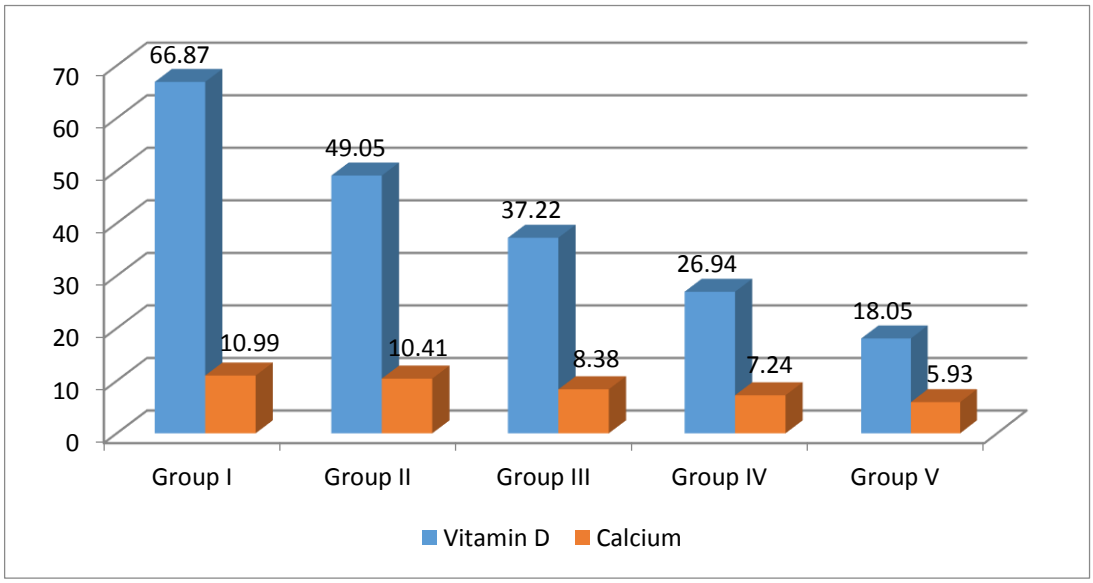
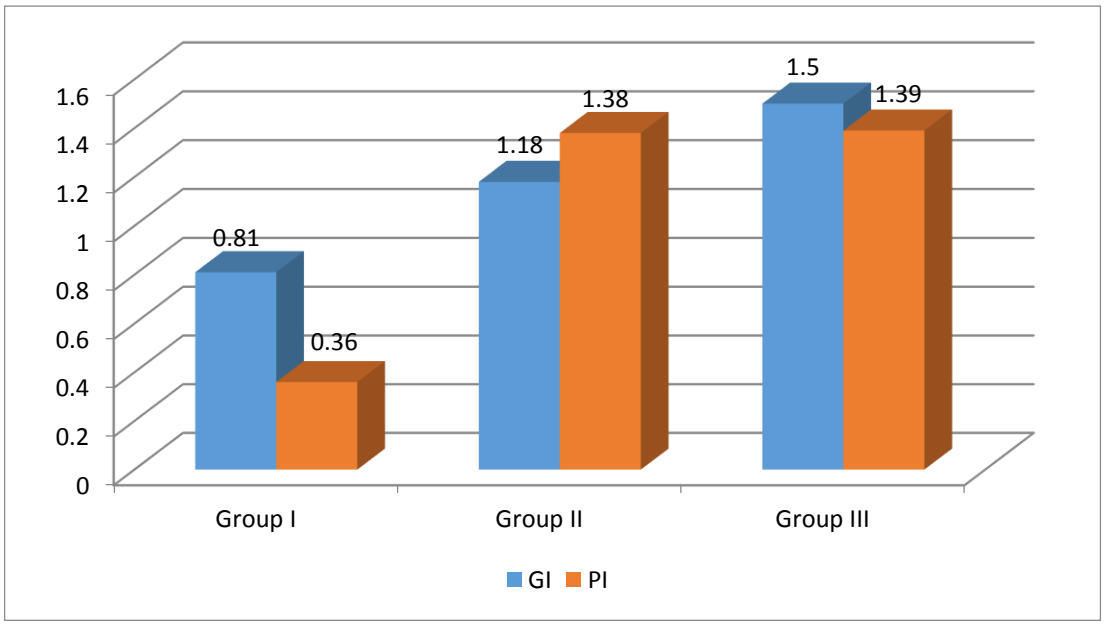


Figure 2: Regression analysis of calcium with chronic periodontics parameters with DM

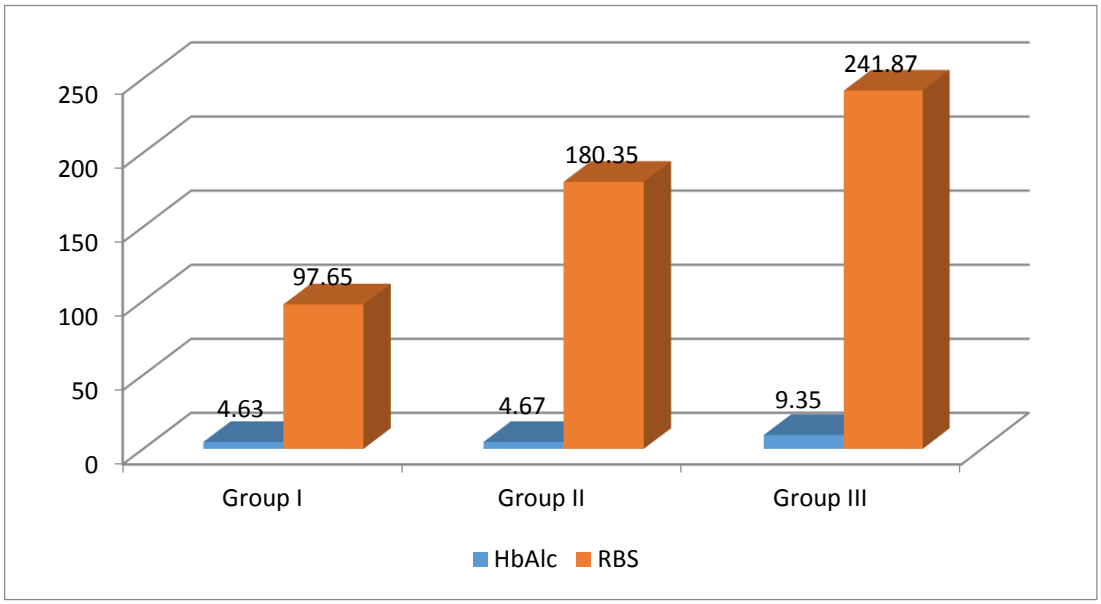
Graph 1: Mean of all the parameters for all groups



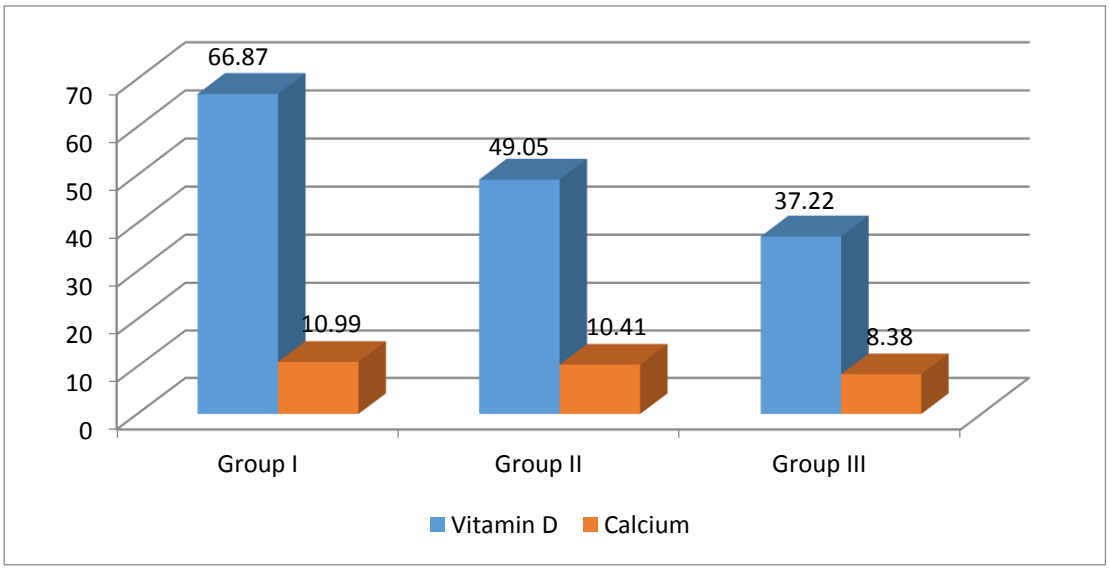
Graph 2a: Clinical parameters comparison of Group II and Group III with Group I



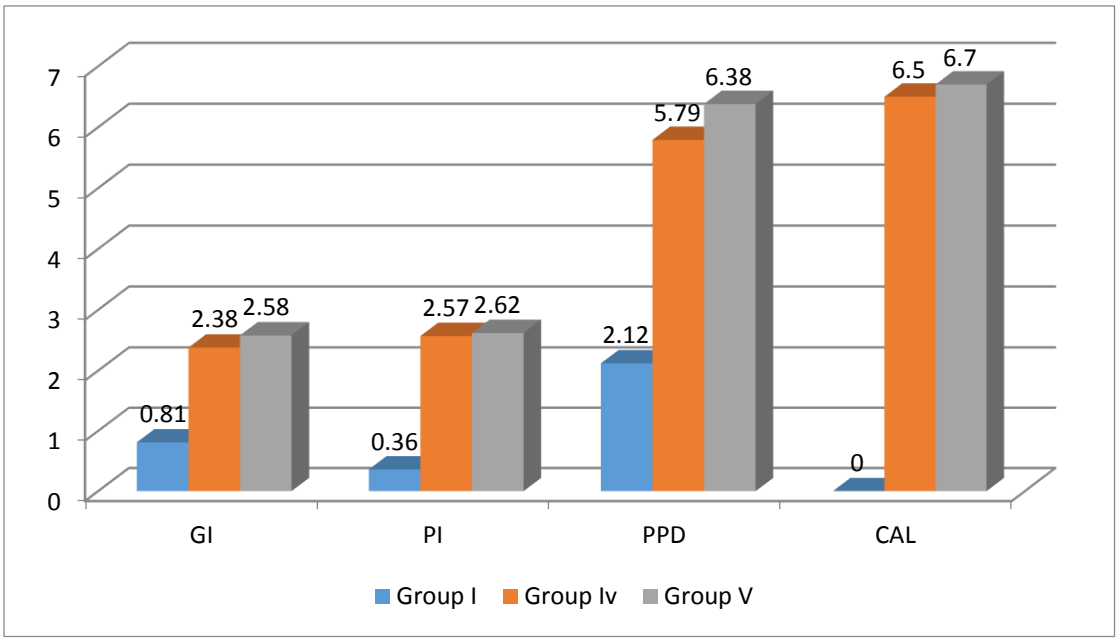
Graph 2b: RBS and HbA1c comparison of Group II and Group III with Group I



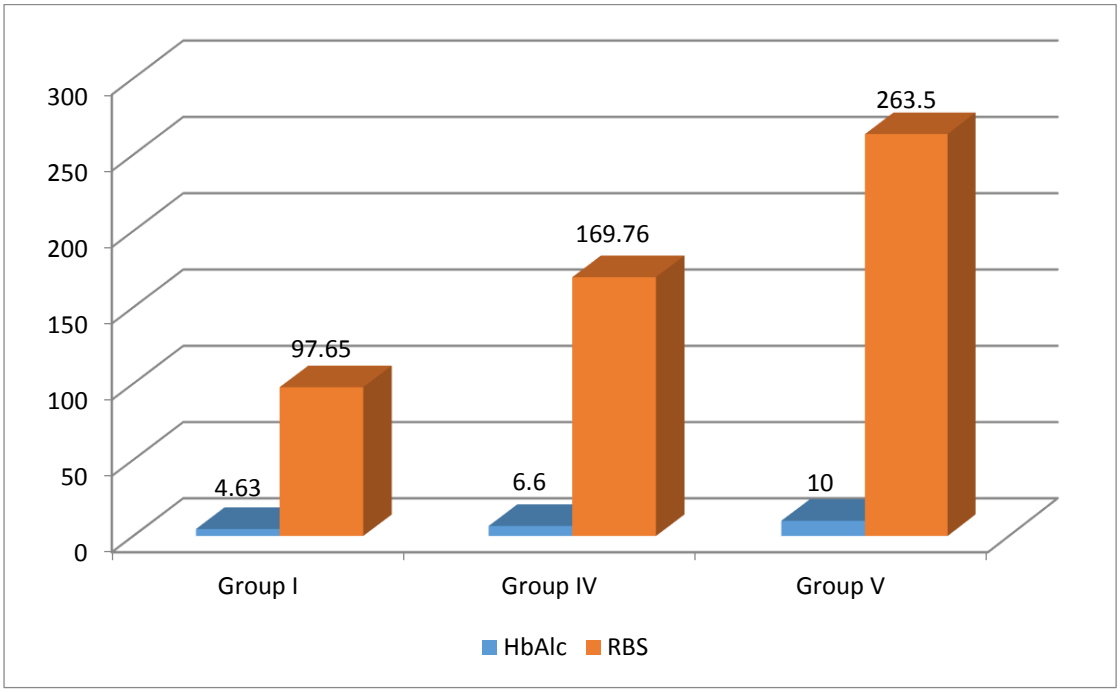
Graph 2c: Vitamin D and calcium comparison of Group II and Group III with Group I



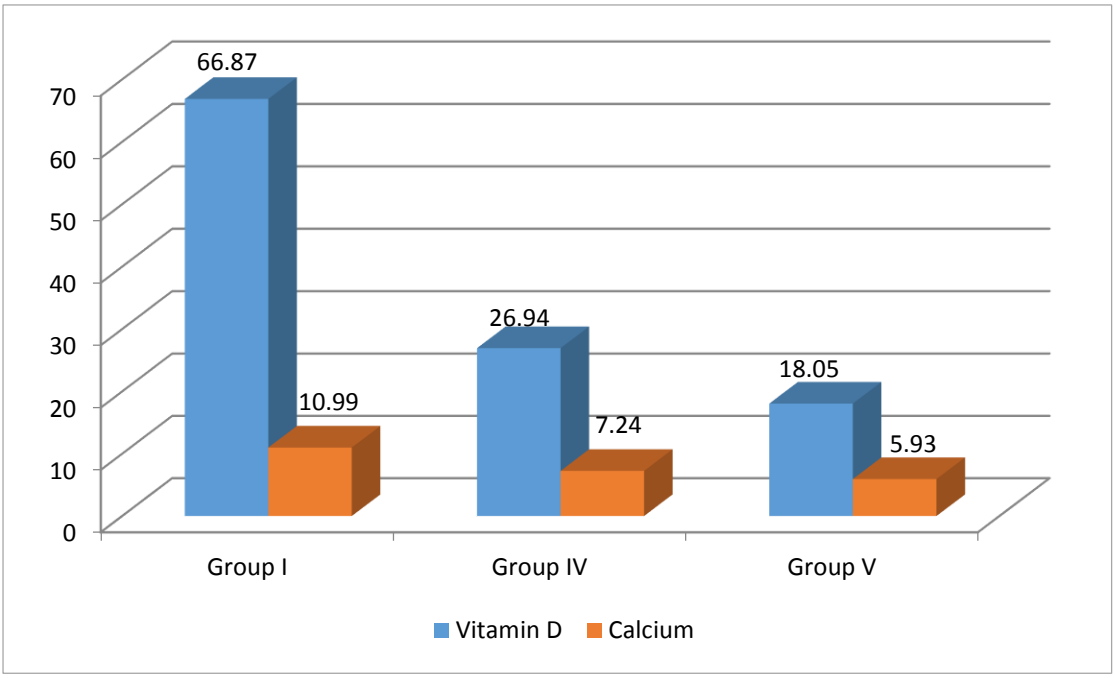
Graph 3a: Clinical parameters comparison of Group IV and Group V with Group I



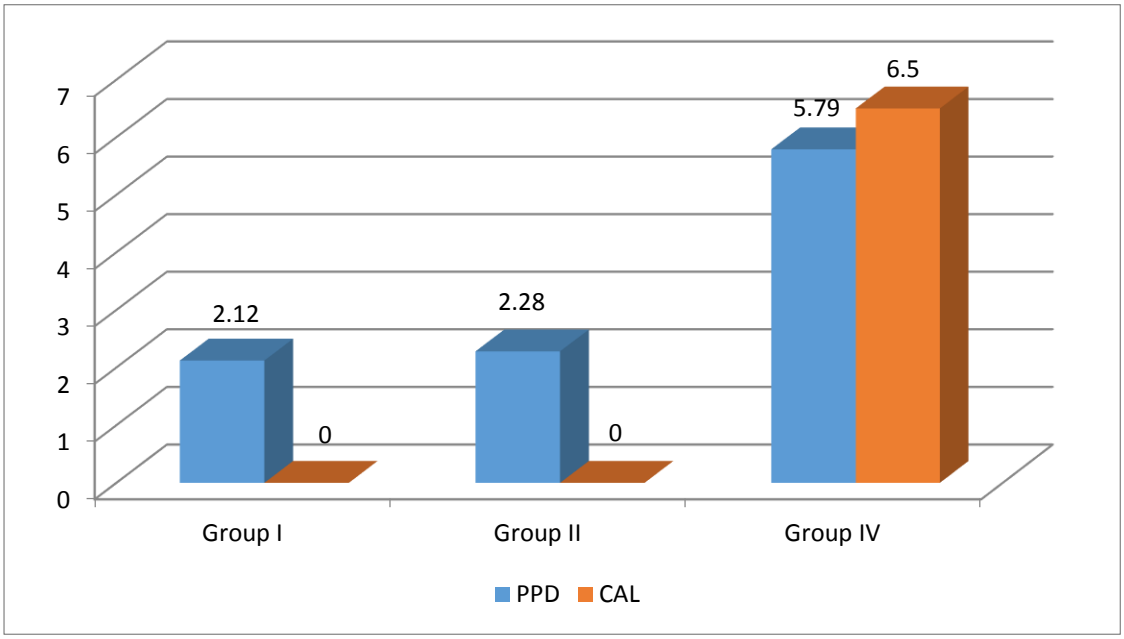
Graph 3b: RBS and HbA1c comparison of Group IV and Group V with group I



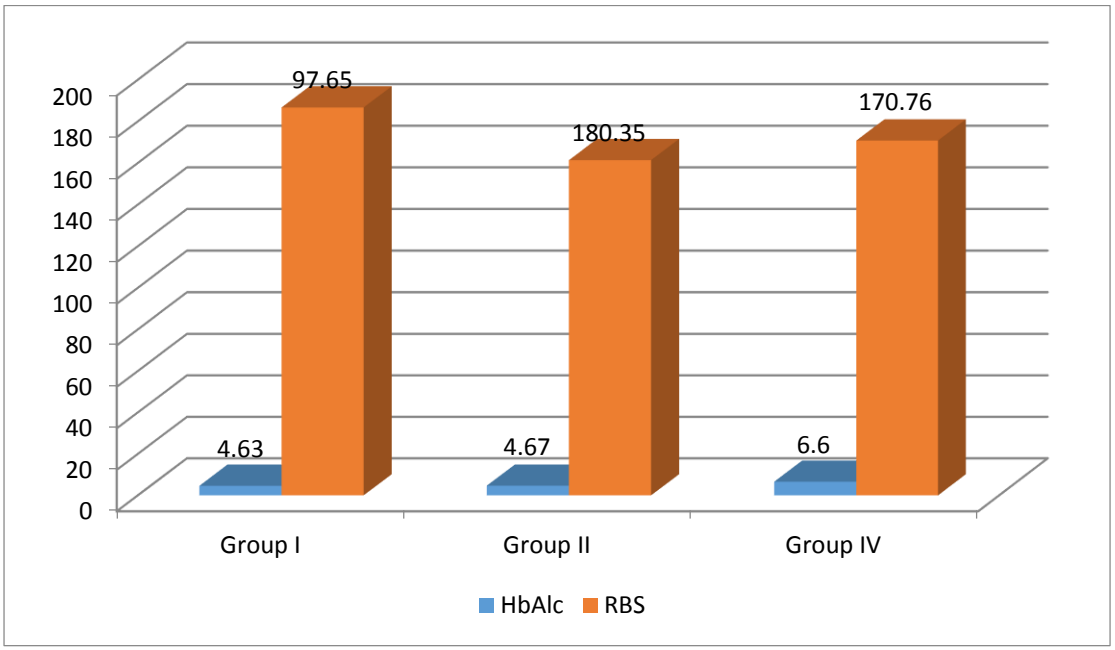
Graph 3c: Vitamin D and calcium comparison of Group IV and Group V with Group I



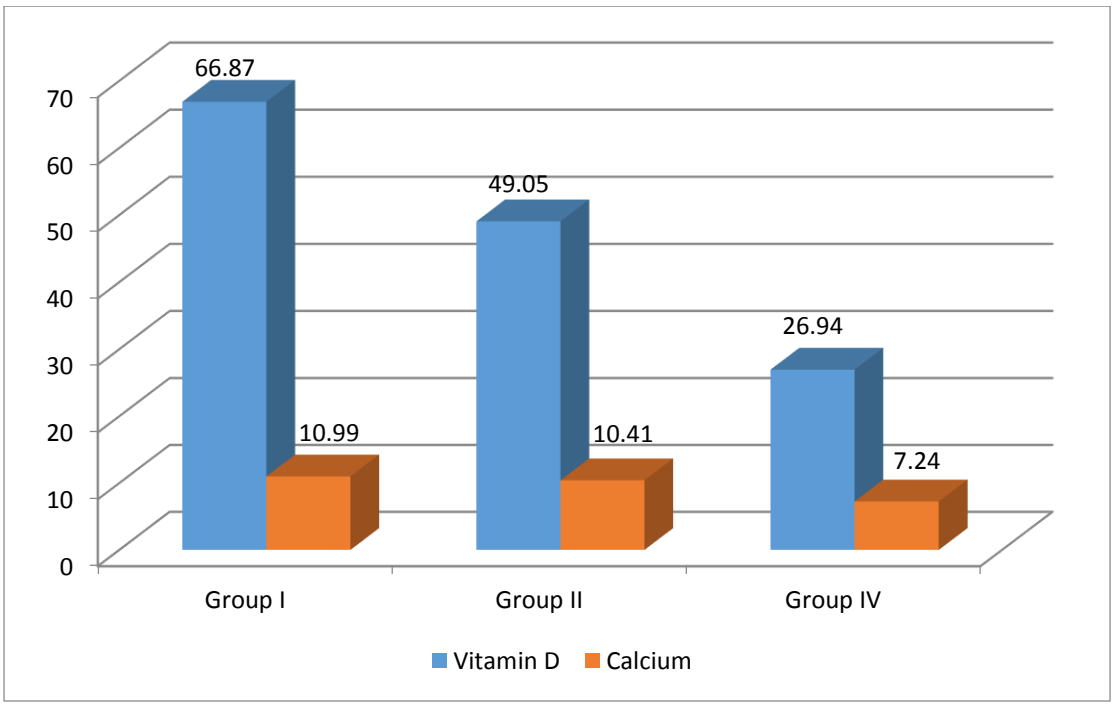
Graph 4a: Clinical parameters comparison of Group II and Group IV with Group I



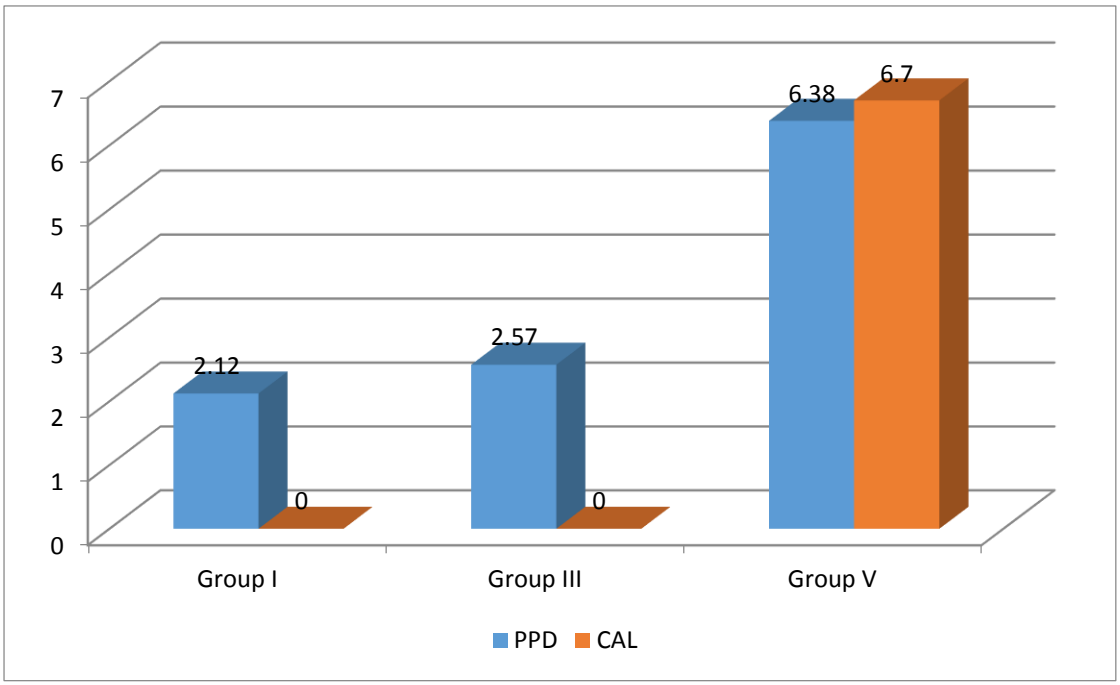
Graph 4b: RBS and HbA1c comparison of Group II and Group IV with Group I



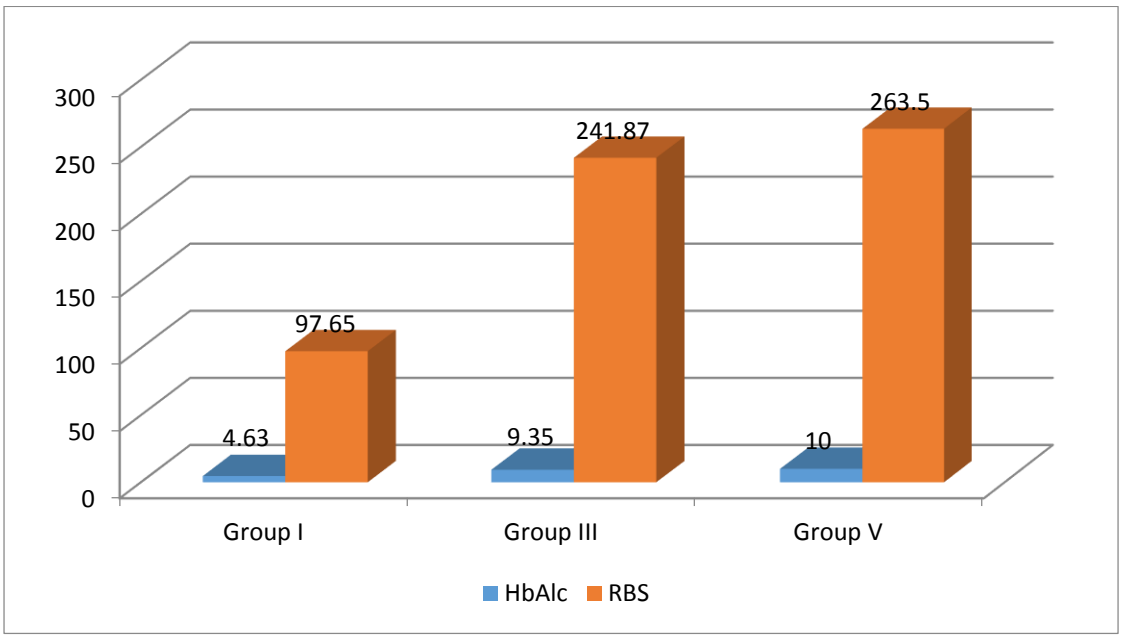
Graph 4c: Vitamin D and calcium comparison of Group II and Group IV with Group I



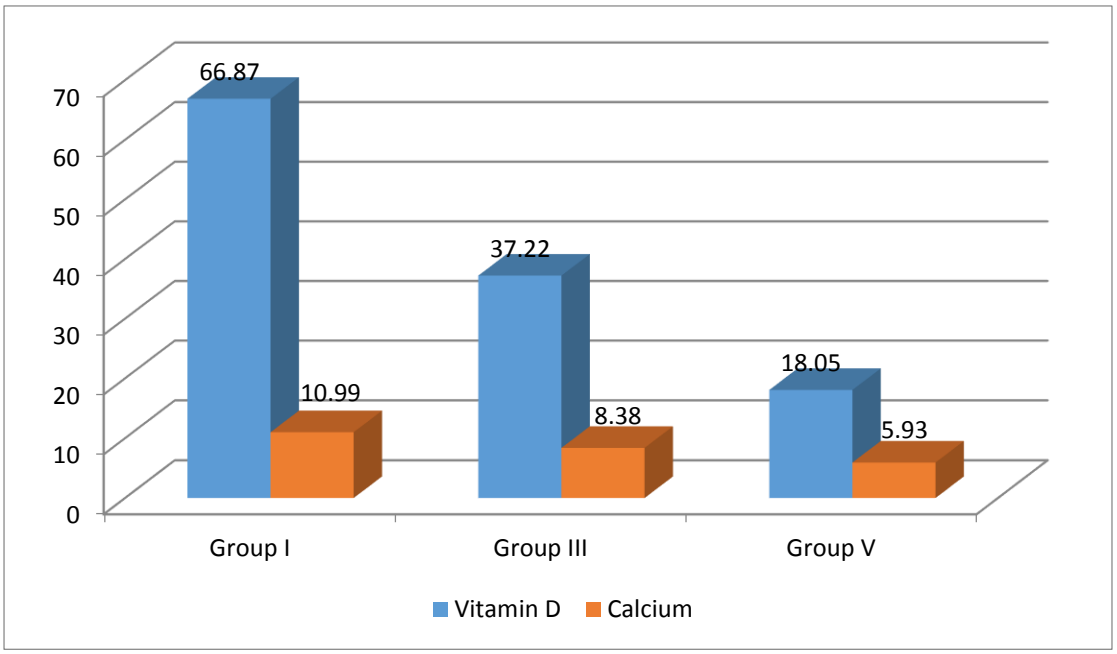
Graph 5a: Clinical parameters comparison of Group III and Group V with Group I



Graph 5b: RBS and HbA1c comparison of Group III and Group V with Group I



Graph 5c: Vitamin D and calcium comparison of Group III and Group V with Group I



MASTER CHART

GROUP I (HEALTHY)

Sr. No	Age (Years)	Gender	PPD (mm)	CAL (mm)	PI	GI	Vitamin D (ng/ml)	Calcium (mg/dl)	HbA1c (%)	RBS (mg/dl)
1	30	Male	2	0	0.1	2	69.11	12.2	4.20	71
2	29	Male	2.3	0	0.1	0.3	69.16	11.3	4.80	35
3	30	Female	1.2	0	0.2	1.8	60.57	9.1	5	69
4	66	Female	2.3	0	0.2	1.3	61.01	10.4	4.30	35
5	64	Female	2	0	0.4	0.2	60.28	11.1	4.50	179
6	50	Female	2.3	0	0.6	0.5	61.58	11.6	4	174
7	58	Male	2.2	0	1	0.4	60.94	11.7	5	103
8	46	Male	2.3	0	0.3	0.8	61.01	9.3	4.8	136
9	55	Female	2	0	0.2	0.5	70.94	10.7	5	106
10	50	Female	2.3	0	0.2	0.8	69.11	10.1	4.2	87
11	45	Male	2.4	0	1	0.7	69.16	11.9	4.8	176
12	40	Male	2.3	0	0.13	0.1	60.57	11	5	90
13	65	Male	2.3	0	0.2	0.9	61.01	10	4.8	60
14	26	Female	2	0	0.3	0.3	60.28	10.5	4.2	57
15	32	Female	2.3	0	0.1	0.3	69.16	11.5	4.8	41
16	35	Male	2.3	0	0.3	0.3	62.35	11.9	4.2	87
17	32	Male	2.3	0	0.4	0.6	70.69	11.7	5	78
18	50	Male	2.5	0	0.2	0.2	80.94	11.7	5	106
19	45	Male	2.3	0	0.2	1.3	71.01	10.1	4.2	87
20	46	Female	2.5	0	1	0.5	84.6	11.9	4.8	176
21	55	Male	2.3	0	0.3	0.2	69.11	12.2	4.8	136
22	55	Male	2.5	0	0.2	0.5	69.16	11.3	5	106

GROUP II (CHRONIC GINGIVITIS)

Sr. No	Age (Years)	Gender	PPD (mm)	CAL (mm)	PI	GI	Vitamin D (ng/ml)	Calcium (mg/dl)	HbA1c (%)	RBS (mg/dl)
1	44	Male	2.3	0	2	1.1	62.35	10.2	4.20	104
2	35	Male	2.3	0	1.3	1	61.64	11.4	4.80	106
3	42	Female	2.3	0	1.32	1	73.8	10.1	5	180
4	50	Female	2.3	0	1.2	1	64.52	11.6	4.30	176
5	38	Female	2.3	0	1.2	1.16	53.89	10.4	5.00	168
6	52	Female	2.3	0	1.28	1.18	63.992	11.9	4.00	187
7	46	Male	2.3	0	1.5	1.1	51.01	10.9	5.00	106
8	42	Male	2.3	0	1.22	1.19	47.29	10.1	4.80	135
9	52	Female	2.2	0	2.1	2	41.01	9.3	5.00	178
10	35	Female	2.1	0	1.23	1.1	53.87	9.5	4.20	187
11	30	Male	1.8	0	1.21	1.1	50.18	10.8	5.00	178
12	28	Male	2 .2	0	1.9	1.1	41.01	9.5	5.00	106
13	25	Male	3 .1	0	1.21	1.75	41.01	9.1	4.80	135
14	40	Female	2.3	0	1.29	1.17	50.94	9.3	4.20	187
15	42	Female	2.3	0	1.32	1	43.8	9.1	4.80	181
16	50	Male	2.3	0	1.2	1	44.52	11.6	4.20	176
17	38	Male	2.3	0	1.2	1.16	33.89	10.4	5	168
18	52	Male	2.3	0	1.28	1.18	33.992	11.9	5.00	87
19	46	Male	2.3	0	1.5	1.1	31.01	10.9	4.20	106
20	42	Female	2.3	0	1.22	1.19	37.29	10.1	4.80	135
21	35	Male	2.1	0	1.23	1.1	53.87	9.5	4.20	187
22	30	Male	1.8	0	1.21	1.1	50.18	10.8	5.00	178

GROUP III

(CHRONIC GINGIVITIS WITH DIABETES MELLITUS)

Sr. No	Age (Years)	Gender	PPD (mm)	CAL (mm)	PI	GI	Vitamin D (ng/ml)	Calcium (mg/dl)	HbA1c (%)	RBS (mg/dl)
1	65	Male	2.3	0	1.24	1.19	35.85	8	7.80	260
2	37	Male	3.5	0	2	1.8	37.29	8	8.70	287
3	40	Female	3.2	0	1.28	1.82	35.85	7.9	9.30	206
4	50	Female	3.1	0	2.2	1.45	34.8	8.3	7	215
5	55	Male	2.4	0	1.18	1.6	31.75	9.6	9.40	225
6	43	Male	3.3	0	1.1	1.6	33.61	9.1	9.40	261
7	48	Female	2.4	0	1.3	1.6	33.46	8.1	8.60	226
8	42	Female	2.2	0	1.3	1.58	35.85	8.8	12.70	262
9	57	Male	2.4	0	1.1	1.6	31.75	9.3	12.70	261
10	45	Female	3.2	0	1.2	1.91	34.8	6.3	9.40	235
11	32	Male	4.2	0	1.28	1.58	39.83	9.1	8.40	206
12	30	Male	1.2	0	1.2	1.2	39.4	9.1	9.70	269
13	43	Female	2.1	0	1.1	1.58	37.29	9.3	8.70	235
14	30	Male	1.2	0	1.2	1.8	35.45	9.1	9.20	269
15	55	Female	3.2	0	1.28	1.46	30.97	10.1	14.70	287
16	65	Female	2.8	0	1.28	1.58	36.94	8.3	9.40	206
17	47	Female	2.2	0	1.1	1	36.94	10.1	9.20	278
18	30	Male	1.2	0	1.2	1	31.75	6.1	9.20	229
19	48	Male	2.3	0	1.28	1.58	38.63	9.3	12.70	235
20	51	Male	3.5	0	1.7	1	38.98	6.1	9.40	287
21	56	Male	2.8	0	2	1.8	35.85	8	7.10	236
22	48	Male	2.1	0	2	1.29	37.29	8	10.30	362

GROUP IV

(CHRONIC PERIODONTITIS)

Sr. No	Age (Years)	Gender	PPD (mm)	CAL (mm)	PI	GI	Vitamin D (ng/ml)	Calcium (mg/dl)	HbA1c (%)	RBS (mg/dl)
1	48	Male	6.7	7.5	3	2.1	26.94	7.1	6.20	135
2	40	Male	5.3	6.5	3	2.1	26.94	7	6.3	106
3	30	Female	7.2	7.5	3	2.1	23.51	7.7	9.30	217
4	60	Female	6.7	6.9	2.8	2.58	24.4	7	9.90	215
5	51	Male	5.2	7.2	2.28	2.5	23.51	6.3	6.10	178
6	34	Male	6.2	6.5	2.1	2.91	26.41	6.5	5.20	106
7	43	Female	6.2	7.3	2.7	2.1	30.01	6.3	5.10	187
8	55	Female	5.3	6.8	2.28	2.58	24.6	6.1	5.30	178
9	28	Male	6.7	7.3	2.8	2.32	27.3	5.5	7.10	206
10	32	Female	5.5	7.5	2.1	2.58	25.9	6.3	5.70	187
11	33	Male	6.2	6.8	2.28	2.91	28.1	6.5	7.10	205
12	30	Male	1.2	1.2	3	2.1	27.6	5.1	5.90	169
13	52	Female	6.3	6.5	3	2.91	27.4	6.1	5.30	116
14	29	Male	5.2	7.3	2.3	2.8	28.18	6.5	7.10	207
15	30	Female	5.2	5.2	2.2	2.1	28.55	6.1	5.90	169
16	43	Female	6.5	6.7	2.5	2.91	29.98	6.5	10.30	207
17	30	Female	6.2	6.8	3	1.1	25.51	6.1	5.20	169
18	52	Male	6.3	6.5	2.26	2.58	27.1	6.5	5.90	106
19	30	Male	5.2	5.8	2.2	1.1	27.24	7	9.20	210
20	35	Male	6.5	6.8	2.2	2.91	28.1	6.1	5.30	187
21	40	Male	5.7	5.9	3	2.67	28.42	6.4	5.20	106
22	30	Male	7.2	7.5	3	2.1	23.51	7.7	9.30	217

GROUP V

(CHRONIC PERIODONTITIS WITH DIABETES MELLITUS)

Sr. No	Age (Years)	Gender	PPD (mm)	CAL (mm)	PI	GI	Vitamin D (ng/ml)	Calcium (mg/dl)	HbA1c (%)	RBS (mg/dl)
1	50	Male	6.1	6.5	3	2.18	20.1	5.3	10.70	420
2	40	Male	5.6	6.3	3	2.16	19.83	6.1	5.90	268
3	60	Female	5.5	6.1	2.8	3	19.4	6.4	5.90	269
4	55	Female	5.9	6.3	2.3	3	14.84	5.6	9.40	250
5	45	Male	5.9	5.9	2.2	2.2	17.52	4.1	10	391
6	33	Male	6.8	7.2	2.5	2.3	13.62	5.3	5.70	261
7	61	Female	6.2	6.3	2.4	2.1	12.1	5.6	9.60	276
8	50	Female	6.2	6.5	2.8	3	14.1	5.4	11.80	216
9	35	Male	7.1	7.3	2.5	2.8	14.02	5.2	5.20	291
10	58	Female	5.5	5.7	2.8	2.54	22.89	5.7	5.10	245
11	56	Male	7.1	7.3	2.7	2.24	17.52	5.1	5.90	335
12	40	Male	7.3	7.6	2.1	2.5	20.1	4.9	5.85	162
13	65	Female	7.1	7.3	2.9	2.8	23.7	5.6	9.60	276
14	42	Male	6.5	6.8	3	2.78	12.28	5.3	10.50	223
15	50	Female	6.4	6.8	3	2.58	21.12	6.3	14.20	259
16	48	Female	6.2	6.5	3	2.91	15.05	4.6	5.90	202
17	40	Female	6.2	6.5	2.6	2	24.52	5.8	14.20	410
18	52	Male	6.5	6.7	2.2	3	21.12	5.9	5.90	291
19	33	Male	6.7	6.9	2.3	2.58	14.25	5.1	14.20	287
20	35	Male	6.1	6.5	2.5	2.91	14.84	5.3	15.90	387
21	30	Male	6.2	6.5	2.2	2.1	20.46	6.1	14.20	269
22	55	Male	5.5	6.5	2.5	2.58	20.28	5.5	5.10	206

Evaluation and Comparison of Serum Vitamin D and Calcium Levels in Periodontally Healthy, Chronic Gingivitis and Chronic Periodontitis in Non Diabetics and Diabetes Mellitus Patients

A Cross Sectional Study

CASE HISTORY PROFORMA

NAME: **OPD NO:**

AGE/SEX: **DATE:**

ADDRESS: **PHONE NO**

OCCUPATION:

CHIEF COMPLAINT:

PAST DENTAL HISTORY:

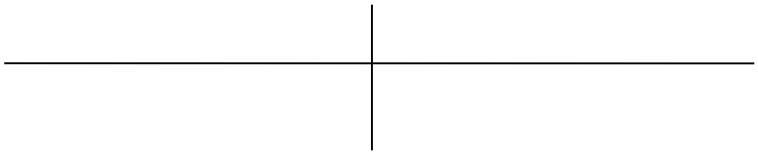
PAST MEDICAL HISTORY :

DRUG HISTORY :

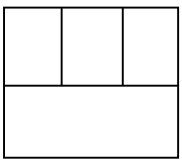
PERSONAL HISTORY :

ORAL HYGIENE HABIT:

TEETH PRESENT:



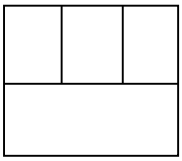
GINGIVAL INDEX *(*Loe & Sillness 1963)



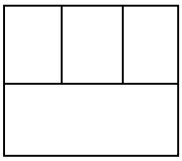
16

12

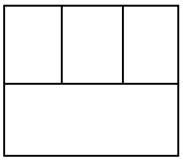
24



44



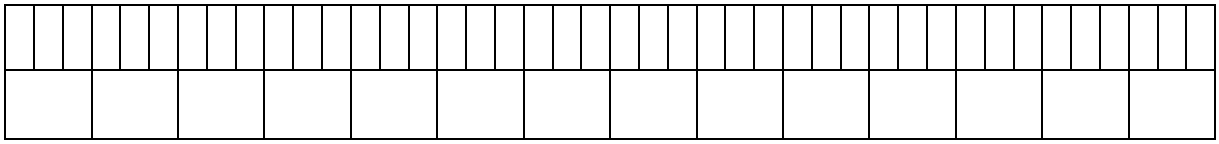
32



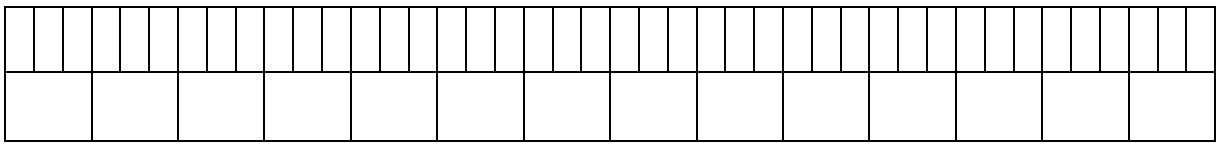
36

Score:

PLAQUE INDEX *(*Sillness and Loe 1964*)*



17 16 15 14 13 12 11 21 22 23 24 25 26 27

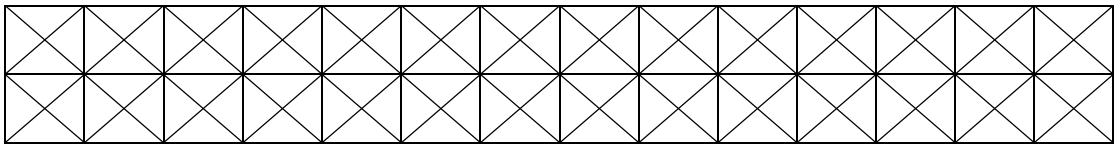


47 46 45 44 43 42 41 31 32 33 34 35 36 37

Score: $\frac{\text{Total scores of all teeth}}{\text{Total number of teeth examined}}$

PROBING POCKET DEPTH (mm):

17 16 15 14 13 12 11 21 22 23 24 25 26 27



47 46 45 44 43 42 41 31 32 33 34 35 36 37

CLINICAL ATTACHMENT LEVELS (mm):

17	16	15	14	13	12	11	21	22	23	24	25	26	27
47	46	45	44	43	42	41	31	32	33	34	35	36	37

BIOCHEMICAL ANALYSIS

1. HbA1c levels =
2. RBS levels =
3. Serum Vitamin D =
4. Serum Calcium =

Parameters	**Group I Healthy**	**Group II GINGIVITIS**	**Group III GINGIVITIS + T2DM**	**Group IV CP**	**Group V CP+T2DM**
GI					
PI					
PPD (mm)					
CAL (mm)					
Serum vitamin D (ng/dl)					
Serum CALCIUM					

CLINICAL DIAGNOSIS

Informed Consent Form

"Evaluation and Comparison of Serum Vitamin D and Calcium Levels in Periodontally Healthy, Chronic Gingivitis and Chronic Periodontitis in Non Diabetics and Diabetes Mellitus Patients - A Cross Sectional Study"

Mr./Master/Mrs./Miss._____

Resident of: _____

_____aged _____ years, exercising my free will/choice, without any pressure/lure of incentive in any form, hereby give my consent for the project to be conducted by _____.

I acknowledge the receipt of "patient's information sheet", and also the doctor has informed me about this research project suitably and sufficiently to my satisfaction. I agree to let my X-rays, photographs, blood investigations, other investigations to be taken as required. I agree to take part in this project and will not mix any other projects during the period of this trial. I shall report to the dental hospital or other place where called on given appointment dates and time. I shall inform the doctor on any adverse effects or unusual symptoms noticed by me. I shall co-operate with the doctors and paramedical staff, in all respects. I permit to publishing the results of my participation in this study. I shall not be given any reimbursement or compensation. I have been informed of my right to opt out of this research project at any time without giving any reason for doing so. I hereby record my consent for participation in the said trial.

_____	_____	_____	_____
Patient's name	Signature/thumbprint	Date	Time
_____	_____	_____	_____
Investigator's name	Signature	Date	Time