Journal of Diabetes and Metabolism
ISSN: 2155-6156
Bhosle et al., J Diabetes Metab 2018, 9:10
DOI: 10.4172/2155-6156.1000806
Research Article
Open Access

Evaluation of Effect of Vitamin D Supplementation on Glycemic Control in Patients of Type 2 Diabetes Mellitus

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Received date: September 19, 2018; **Accepted date:** October 09, 2018; **Published date:** October 15, 2018

Abstract

Background: In India the number of people with diabetes is increasing day-by-day. Due to a sole "Asian Indian Phenotype," Indians develop diabetes an era earlier and have an earlier onset of complications. Therefore, it is essential to evaluate more effective treatment strategies at an earlier stage of disease progression. WHO defines Diabetes Mellitus as a metabolic disorder characterized by hyperglycemia, glycosuria, hyperlipidemia, negative nitrogen balance and sometimesketonemia.

Aim: To assess and study the effect of Vitamin D supplementation on FBS, PPBS and HbA1clevel in diagnosed patients of Type 2 Diabetes Mellitus.

Methods: The present study was Prospective, open label, comparative, randomized, parallel group, two arm interventional study. Comparison of two active treatment groups over a period of six months. Total 120 patients of either sex in the age group of 30 to 60 years with Type 2 Diabetes Mellitus, with HbA1c level >7.0% and <8.5%. The effect of with/without Vitamin Dsupplementwith OHA observed on various parameters i.e. FBG, PPBS, HbA1c.

Results: In Group C the mean change in FBS from baseline to 6 months was 162.90 to 127.2 (-35.7); on the other hand, in Group Tfrom baseline to 6 months was 157.90 to 94.93 (-62.97). Whereas, PPBS from 213.40 to 176.17 (-37.23) in Group C; in GroupT from 209.70 to 149.03 (-60.67). On the other hand, in Group C, HbA1c 7.80 to 7.22 (-0.58) and in GroupT, 7.76 to 6.70 (-1.06). Group T statistically highly significant than GroupC in improving glycemic indices.

Conclusions: In Treatment Group Vitamin D supplement was responsible for improved levels of FBS, PPBS, HbA1c. In summation, it can be said thatwhen vitamin D levels were adequate control ofglycemic indices.The advantages of the study include: significant reductions, good efficacy, minimal rates of adverse reactions, no toxicity, good compliance.

Keywords: Diabetes; Vitamin D

Introduction

The World Health Organization defines Diabetes Mellitus as a metabolic disorder characterized by hyperglycemia, glycosuria, hyperlipidemia, negative nitrogen balance and sometimes ketonemia [1]. It is a group of heterogeneous disorders having varied etiologies. The pathophysiology involves absolute or relative insulin deficiency along with component of insulin resistance that results from defects in insulin action, insulin secretion or both [2]. The characteristic features include hyperglycemia with altered carbohydrates, proteins and lipid metabolism.

Progressing to epidemic proportions, there is alarming increase in prevalence of Diabetes Mellitus (DM) worldwide, with 246 million diabetics residing world over as of 2010 [3]. World Health Organization (WHO) projects an estimated 333 million diabetic patients all over the world by 2025, with India showing the steepest rise [3,4]. The International Diabetes Federation (IDF) recognizes India as Diabetes capital of the world, with around 40.9 million diabetics as of 2006. In India this projected rise of diabetic cases to 69.9 million by 2025 is attributed to genetic predisposition, ageing population, increased migration of susceptible patients, sedentary life style, dietary modifications and obesity [4]. Many trials have evidence of Asian Indians developing Diabetes Mellitus at least a decade earlier as compared to the western population [5,6]. This chronic and costly disease has been recently likened to the 'Black Death' of the 14th century [3,5].

The various types of Diabetes according to the eitopathogenesis are: [1,2,5].

- Type 1 characterized by complete or near total insulin deficiency.
- Type 2 involves various degrees of insulin resistance and impaired insulin secretion; Gestational Diabetes: raised blood sugar in $2^{nd}/3^{rd}$ trimester but resolves postpartum; Secondary DM: secondary to other hyperglycemic causes (drug and disease induced).

Type 2 diabetes mellitus (T2DM)

(T2DM) is a major problem confronting the health care system. It is characterized by progressive gradual loss of pancreatic β cell function,

insulin resistance and abnormal hepatic glucose production [7]. In its most severe form, ketoacidosis or non-ketotic hyperosmolar state may develop leading to stupor, coma and in absence of effective treatment even death [1,5].

Chronic glucotoxicity and lipotoxicity are considered as important predictors in the development of diabetic complications [6,8,9]. The microvascular complications may be shortlisted to include nephropathy, retinopathy, and neuropathy [4,5,7]. Few macrovascular complications can be enlisted as dyslipidemia, cardiovascular disease, cerebrovascular episode and peripheral vascular disease [9-11]. In most patient's inappropriate therapy results in inadequate glycemic control and devastating complications [12]. The increased morbidity and mortality rates are attributed to serious, disabling complications. This progressive, disabling disease affects the quality of life and reduces life expectancy by about 5-10 years [12-14].

American Diabetes Association (ADA) states a glycosylated hemoglobin (HbA1c) <7.0% as glycemic control target; IDF recommends a lower HbA1c range of 6.5% to 7% but American College of Clinical Endocrinologist recommend stringent target of 6.5% HbA1c [1,3,5,12]. Whether HbA1c level of <6.5% should be considered as an optional or primary diagnostic criterion remains controversial [15,16].

Vitamin

D is a fat-soluble secosteroid serving as hormone [6,12,14]. Richest dietary source is oily fish, available in fortified food as supplement [17,18]. It is synthesized by the body on exposure of skin to ultraviolet (UV) B radiation. By photolytic cleavage 7-dihydrocholesterol gets converted to pre-vitamin D3, this by spontaneous thermal isomerization is converted to vitamin D3 [12,14]. Types include: Ergocalciferol (Vitamin D2) made by UVB radiation of ergosterol and found in plants; Cholecalciferol (Vitamin D3) the preferred form for supplementation [19,20]. Calcitriol (physiologically active form) gets activated after hydroxylation in body [14]. It exerts its effects secondary to activation of Vitamin D Receptors (VDR) [8]. These are widely distributed in various body sites including osteoblast, pancreatic β cells, monocytes, cardiomyocytes, vascular endothelial cells, immune cells, and neurons [14,19,21].

Vitamin D3 exerts effect either by genomic or non-genomic action [22-26]. Direct action is via VDR (prototypical receptor) acting through nucleus and protein synthesis and mediates genomic transcription [25]. Activation by vitamin D3 ejects VDR from nuclear membrane to cytoplasmic membrane [19,21]. Non-genomic action is by translocation through the VDR present on plasma membrane, not requiring genetic transcription [23,24]. Both receptors mediate cell-type specific effects [25,26].

Vitamin D deficiency

Vitamin D deficiency (VDD) is globally recognized as a pandemic, affecting approximately 35-50% of world's population [22,27,28]. It is highly prevalent in India, modern day lifestyle being the culprit [27,30]. Research highlights >80 to 90% adults in sunny Middle East and South Asia get insufficient vitamin D [4,6,28]. Major causes are: inadequate sunlight exposure, dark skinned individuals, inadequate dietary intake of vitamin D, and inadequate vitamin D fortification of Indian dairy products [20,27,29]. This influence is also race and ethnic-specific [27,29,30].

Vitamin D Deficiency Syndrome (VDDS) is VDD in association with diseases like Osteoporosis, Diabetes, Obesity, Hypertension, Premature cardiovascular disease, Autoimmune disease, Depression, malignancies etc. [27-29].

Potential role of VDD in T2DM

T2DM pathology incorporates β cell dysfunction and IR. Low insulin levels are due to β cell dysfunction [12]. IR causes high glucose related to Zinc deficiency [19]. Zinc an integral part of insulin molecule is vitamin D dependent [30,31]. Low circulating vitamin D levels result in higher IR and β cell dysfunction relating VDD to DM [15,20]. Vitamin D supplementation improves β cell function; insulin sensitivity/secretion and glucose tolerance thus reducing risk of DM related morbidity and mortality [22,25,26].

Evidences suggesting role of vitamin D in glucose homeostasis and insulin secretion are: VDRs presence on β cells and skeletal muscle, VDR responsive element in human insulin gene promoter and expression of la hydroxylase enzyme [31].

Recent studies by researchers have related VDD with T2DM [32-37]. Studies reveal that 1% decrease in HbA1c is associated with 37% decrease in risk of micro vascular complications and 21% decrease in risk of diabetes related death [32]. An increase in serum 25(OH)D from 10 to 30ng/ml improves insulin sensitivity by 60% [6,12,14].

Study rationale

Sudden interest generated by varied role of vitamin D reflects in numerous recent studies investigating the relation between vitamin D inadequacy and incidence of T2DM. There is a lack of solid biological evidence in support of this mechanistic contribution of vitamin D supplement improving glycemic control and development of complications in diabetics [7].

In light of the conflicting results and methodological limitations of this evolving literature, the concern for definitive conclusive role of vitamin D in T2DM remains elusive. Moreover, majority of studies were carried out in Caucasian, American and Mexican populations, while Indian population despite being at higher risk is less investigated [14,32]. In concern with the widespread prevalence of both T2DM and VDD particularly in Indian scenario this potential relationship could hold enormous public health implications and hence demands further study to answer its unresolved questions.

Limited number of prospective studies investigating the relation between VDD and T2DM prompted the selection of this topic for my thesis [4,7,17,29,32,36-66]. The purpose of this study is to evaluate the potential association between vitamin D and Type 2 Diabetes Mellitus from both pathophysiologic and clinical perspective. Additionally, to assess effect of vitamin D supplementation on glucose homeostasis in terms of glycemic control in patients of T2DM not well controlled on a diet/ exercise regimen.

Material and Methods

Study design

- An open-label, randomized, single centre, comparative, prospective and parallel, two- arm interventional study.

- Total 120 patients suffering from T2DM were enrolled in study. They were randomly allocated into 2 groups of 60 each by using Random number table.
- Group 1 (control group) were patients not receiving any Vitamin D supplement during study period. They continued regularly with their OHA for diabetic control.
- Group 2 (treatment group) patients receiving OHA and supplemented with Vitamin D.
- The present study was carried out after obtaining approval of study protocol from Institutional Ethics Review Committee (IERC). It was conducted in compliance with protocol, informed consent regulations, IERC and as per ICH GCP Guidelines.

Study product

Vitamin D supplement: *Single sachet:* Dose equivalent to 1 gm containing 60,000 IU of Cholecalciferol. To be supplemented once a week for 2 months followed by once a month for 4 months. Additionally: 2000 IU of Cholecalciferol Capsule to be supplemented once daily.

Inclusion criteria

Willingly participated in the study and followed the study protocol, patients of either sex but within the age group of 30 to 60 years' patients of Type 2 Diabetes Mellitus with deranged blood glucose levels, patients receiving Oral Hypoglycemic Agents for diabetes control, patients with HbA1c level >7.0% and <8.5%, patients having serum 25(OH) D level <20 ng/ml, patients with BMI 18.5-30 Kg/m^2.

Exclusion criteria

- Patients not willing to give informed consent
- Patients with Type 1 Diabetes Mellitus (Insulin Dependent Diabetes Mellitus)
- Patients requiring Insulin for diabetic control
- Patient taking drugs that alter Vitamin D level.
- Patients with known allergy to study product.
- Patient within six month of post-operative period (major surgery).
- Pregnant or lactating women.
- Smoker, tobacco chewer, alcoholic
- Patients with
  a. Autoimmune disease
  b. Deranged hepatic enzymes
  c. Altered kidney functions
  d. Convulsive disorders
  e. Clinically significant cardiovascular disease (Coronary artery disease / ischemic heart disease/Arrhythmias), including history of CCF, Angina Pectoris or MI in last 1 year
  f. Known infection of Human Immunodeficiency Virus

Study visits

Clinic visits on day 0, at end of 3 months and at end of 6 months.

Baseline Investigations: FBS, PPBS, HbA1c, 25(OH)D, SGPT, S. Creatinine, ECG.

At end of 3 months' investigations performed were FBS, PPBS, HbA1c, and 25(OH)D

At 6 months evaluated for: FBS, PPBS, HbA1C, 25(OH)D, SGPT, S Creatinine, ECG.

Statistical Analysis

SPSS was applied for statistics using One-way ANOVA, Fischer's test and student's t test (Paired and unpaired) to measure the differences among the groups.

In the statistical analysis we have compared the study parameters in the groups (Group C and Group T) at all the levels by applying Analysis of variance (ANOVA), Fischer's test (F test) and Student t test.

ANOVA and F test was done to find out whether variation among the patients and variation during the time period were significant; results are presented in the form of ANOVA Tables. In order to compare the values obtained in control (Group C) and treated (Group T) groups for various parameters the data were subjected to student's t test. Paired t test was applied for comparisons within group while unpaired t test was applied for comparing values between the groups. Eventually calculation of correlation coefficient was done. The correlation studies indicated inter-relationship of all parameters with each other, as indicated by significant values of correlation coefficients (r). The p value denotes the level of significance in our study. Standard value is $p<0.05$ while the level of 0.0001 is considered as highly significant.

Observation and Results

Total number of 120 patients of Type 2 DM were enrolled and evaluated. They were randomly divided into two groups of 60 each. Group C as control received only OHA while Group T was on OHA and vitamin D supplementation being treatment group (Table 1 and Figure 1).

	Group C		Group T	
Age	**Male**	**Female**	**Male**	**Female**
Age 30-40 years	6	4	10	12
Age 40-50 years	17	17	18	12
Age 50-60 years	12	10	6	2
	32	28	34	26

Table 1: Shows age and sex wise distribution of the two groups under study Control Group (Group C) and Treatment Group (Group T). Both groups had 60 subjects each.

The table reflects that in both groups maximum number of patients belonged to age group of 40-50 years, 34 and 30 in Group C and Group T respectively. Group C had 22 patients aged 50-60 years and five aged 30 - 40 years as against Group T that had 22 patients aged 30-40 years and only eight patients in age group 50 to 60 years.

Of the total 120 diabetic patients selected 66 were male while 54 were female patients. Group C consisted of 32 male patients (53.33%) and 28 female patients (46.66%) while Group T had 34 male patients (56.66%) and 26 female patients (43.33%).

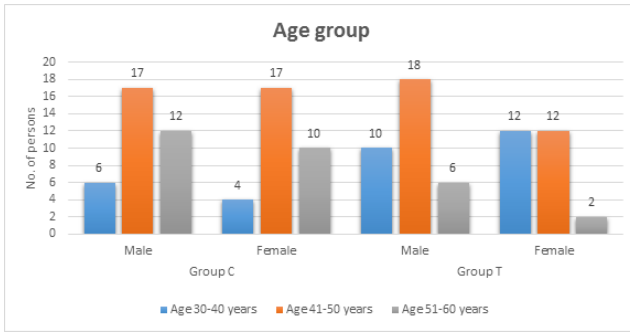

Figure 1: Group C consisted of 32 male patients (53.33%) and 28 female patients (46.66%) while Group T had 34 male patients (56.66%) and 26 female patients (43.33%).

Fasting blood sugar (FBS)

Table 2 shows the values of FBS as a study parameter at baseline, after 3 months and after 6 months of therapy in both study groups of 120 subjects each (Tables 2-4).

FBS (mg%)	Baseline Mean ± SD	After 3 Months Mean ± SD	After 6 Months Mean ± SD
Group C	162.90 ± 31.33	138.70 ± 34.05	127.2 ± 36.60
Group T	157.90 ± 37.27	124.07 ± 39.64	94.93 ± 15.35

Table 2: Values of FBS as a study parameter at baseline, after 3 months and after 6 months of therapy in both study groups of 120 subjects each.

ANOVA FBS Control group		
Source	F	Significance
Individual	9.658	* *
Months	33.786	* * *
(NS=Not Significant; *=Significant; ***=highly significant)		

Table 3: ANOVA FBS Control group.

ANOVA FBS Treatment group		
Source	F	Significance
Individual	5.631	**
Months	71.15	***
(NS=Not Significant; *=Significant; ***=highly significant)		

Table 4: ANOVA FBS Treatment group.

In Group C Mean FBS is reduced from 162.9 ± 31.33 to 138.7 ± 34.05 after 3 months and further to 127.2 ± 36.6 after 6 months of study reflecting total reduction of 21.6%. In Group T a sharper fall was seen in Mean FBS which reduced from 157.9 ± 37.27 to 124.1 ± 39.64 after 3 months and more pronounced in next 3 months to 94.9 ± 15.35 reflecting total reduction of 39.89%. This is graphically represented below (Figure 2).

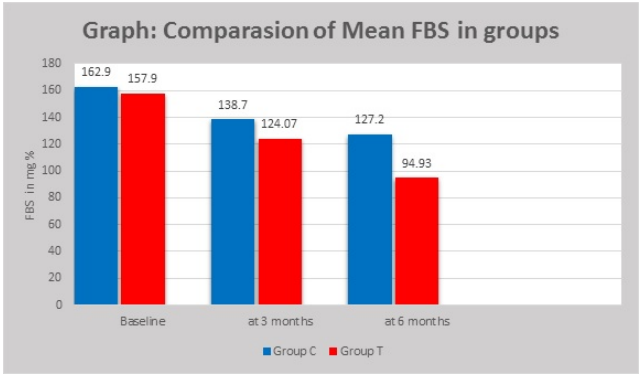

Figure 2: Group T a sharper fall was seen in Mean FBS which reduced from 157.9 ± 37.27 to 124.1 ± 39.64 after 3 months and more pronounced in next 3 months to 94.9 ± 15.35 reflecting total reduction of 39.89%.

Analysis of FBS in Control and Treatment group using ANOVA tables indicates a significant variation among individual patients and within the three time intervals. This was much more significant in Treatment Group T.

Comparison FBS	t-value	p-value	Significant /Not Significant
Group C at 6 Months	6.859	<0.0001	Significant
Group T at 6 Months	11.72	<0.0001	Significant
(if P>0.05 Not Significant; p<0.05 Significant; p<0.0001 highly significant)			

Table 5: Treatment Group T.

Postprandial blood sugar (PPBS)

The table shows the values of PPBS parameter at baseline after 3 months and after 6 months of therapy in both study groups of 60 subjects each (Tables 5 and 6).

PPBS	Baseline	After 3 Months	After 6 Months
(mg%)	Mean ± SD	Mean ± SD	Mean ± SD
Group C	213.40 ± 36.82	187.40 ± 31.72	176.17 ± 34.39
Group T	209.70 ± 41.30	175.93 ± 27.85	149.03 ± 16.86

Table 6: Values of PPBS parameter at baseline after 3 months and after 6 months of therapy in both study groups of 60 subjects each.

In Group C, Mean PPBS reduced from 213.4 ± 36.82 to 187.4 ± 31.72 after 3 months and further to 176.2 ± 34.39 after 6 months' study reflecting total reduction of 17.37% while in Group T Mean FBS reduction was comparatively more pronounced from 209.7 ± 41.30 to 175.9 ± 27.85 at 3 months and further pronounced to 149.0 ± 16.86 after 6 months reflecting total reduction of 28.7% (Tables 7 and 8, Figure 3).

ANOVA PPBS Control group		
Source	F	Significance
Individual	8.38	* *
Months	32.034	* * *
NS=Not Significant; *=Significant; ***=highly significant		

Table 7: ANOVA PPBS Control group.

ANOVA PPBS Treatment group		
Source	F	Significance
Individual	6.059	* *
Months	80.789	* * *
NS=Not Significant; *=Significant; ***=highly significant		

Table 8: ANOVA PPBS Treatment group.

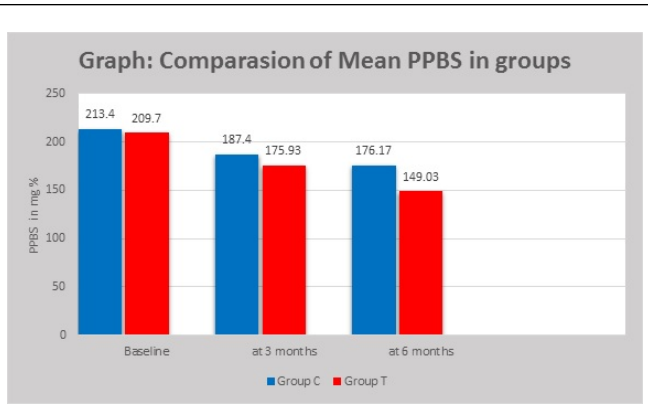

Figure 3: In Group C, Mean PPBS reduced, while in Group T Mean FBS reduction was comparatively more.

Analysis of PPBS in Control and Treatment group using ANOVA tables indicates a significant variation among individual patients and within the three time intervals. This was much more significant in Treatment Group T (Table 9).

Comparison PPBS	t-value	p-value	Significant /Not Significant
Group C at 6 Months	6.487	<0.0001	Significant
Group T at 6 Months	11.08	<0.0001	Significant
NS=Not Significant; *=Significant; ***=highly significant			

Table 9: Treatment Group T.

Glycosylated hemoglobin (HbA1c)

Table shows the values of HbA1c parameter at baseline after 3 months and after 6 months of therapy in both study groups of 60 subjects each (Table 10).

In control Group C there was minimal reduction in Mean HbA1c as it decreased from 7.8 ± 0.47 to 7.4 ± 0.51 in 3 months and then to 7.2 ± 0.59 at 6 months reflecting a total reduction of 7.43%. In contrast during the same period in Treatment Group T, the Mean HbA1c showed a decrease from 7.8 ± 0.51 to 7.2 ± 0.51 at 3 months and further to 6.7 ± 0.39 after 6 months reflecting a reduction of 13.66% (Tables 11 and 12, Figure 4).

HbA1c (%)	Baseline Mean ± SD	After 3 Months Mean ± SD	After 6 Months Mean ± SD
Group C	7.80 ± 0.47	7.42 ± 0.51	7.22 ± 0.59
Group T	7.76 ± 0.511	7.22 ± 0.513	6.70 ± 0.39

Table 10: Values at baseline, 3 months and 6 months in Group C and Group T.

ANOVA HbA1c Control group		
Source	F	Significance
Individual	10.6	* *
Months	39.95	* * *
NS=Not Significant; *=Significant; ***=highly significant		

Table 11: ANOVA HbA1c Control group.

ANOVA HbA1c Treatment group		
Source	F	Significance
Individual	10.124	* *
Months	151.24	* * *
NS=Not Significant; *=Significant; ***=highly significant		

Table 12: ANOVA HbA1c Treatment group.

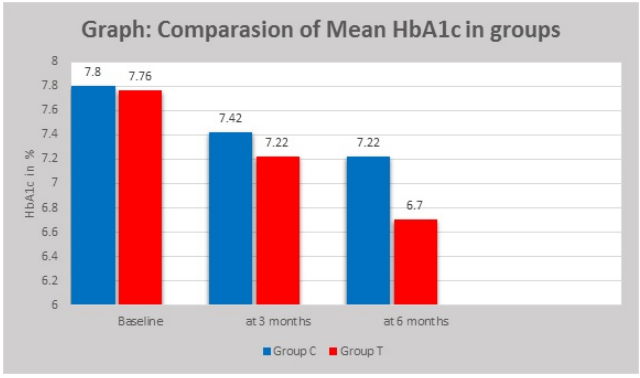

Figure 4: Comparasion of Mean HbA1c in groups.

Analysis of HbA1c in Control and Treatment group using ANOVA tables indicates a significant variation among individual patients and within the three time intervals. This was much more significant in Treatment group when time interval was seen (Tables 13 and 14).

Comparison HbA1c	t-value	p-value	Significant / Not Significant
Group C at 6 Months	7.403	<0.0001	Significant
Group T at 6 Months	17.18	<0.0001	Significant
if p>0.05 Not Significant; p<0.05 Significant; p<0.0001 highly significant			

Table 13: Treatment group when time interval was seen.

Vitamin D	Baseline Mean ± SD	After 3 Months Mean ± SD	After 6 Months Mean ± SD
Group C	13.40 ±3.26	13.03 ±3.11	13.07 ±2.26
Group T	13.17 ± 3.98	23.10 ±4.18	34.53 ± 4.08

Table 14: Values at baseline, 3 months and 6 months in Group C and Group T.

Tables show values of serum Vitamin D parameter at baseline, after 3 months and after 6 months of therapy in both study groups having 60 subjects each (Tables 15 and 16).

In Group C it was observed that at all the three levels of study the Mean Vitamin D levels remained almost constant from 13.4 ± 3.26 to 13.0 ± 03.11 after 3 months and to 13.0 ± 2.26 after 6 months. In Group T, Mean Vitamin D increased rapidly from 13.2 ± 3.98 at start of study to 23.1 ± 4.18 after 3 months of study and further rapidly increased to 34.5 ± 4.08 at the end of 6 months of study (Figure 5).

ANOVA Vitamin D Control group		
Source	F	Significance
Individual	10.487	* *
Months	0.605	NS
NS=Not Significant; *=Significant; ***=highly significant		

Table 15: Analysis of Vitamin D levels in both groups at completion of study.

ANOVA Vitamin D Treatment group		
Source	F	Significance
Individual	4.32	**
Months	433.62	***
NS=Not Significant; *=Significant; ***=highly significant		

Table 16: ANOVA Vitamin D Treatment group.

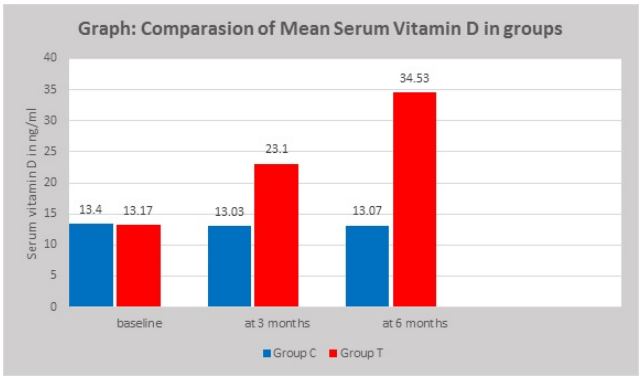

Figure 5: Comparasion of Mean Serum Vitamin D in groups.

Analysis of Vitamin D in Control and Treatment group using ANOVA tables indicates a significant variation among individual patients and within the three time intervals. This was very much significant in Treatment group. This variation in Vitamin D at various times was non-significant in control group (Tables17-19, Figure 6).

Comparison Vitamin D	t-value	p-value	Significant / Not Significant
Group C at 6 Months	1.109	<0.0001	Significant
Group T at 6 Months	26.19	<0.0001	Significant
if p>0.05 Not Significant; p<0.05 Significant; p<0.0001 highly significant			

Table 17: Variation in Vitamin D at various times was non-significant in control group.

	Baseline		At 3 months		At 6 months	
	Group C (Mean)	Group T (Mean)	Group C (Mean)	Group T (Mean)	Group C (Mean)	Group T (Mean)
FBS mg %	162.9	157.9	138.7	124.1	127.2	94.9
PPBS mg%	213.4	209.7	187.4	175.9	176.2	149
HbA1c %	7.8	7.76.	7.42	7.22	7.22	6.7
Vitamin D	13.4	13.17	13.03	23.1	13.07	34.53

Table 18: Comparison of study parameters at all levels in Group C and Group T.

The calculated values of t were compared with/the tabulated values and it was inferred that the differences in the values of FBS, PPBS and HbA1c obtained in control and treated groups were statistically non-significant up to 3 months. However, at the end of 6 months these values significantly decreased, the decrease was more pronounced in treatment group as compared to control group.

Test	Month	Group C	Group T	Difference	t-value	significance
FBS	0	162.93	157.9	5.03	0.566	NS
	3	138.73	124.07	14.67	1.537	NS
	6	127.17	94.93	32.23	4.494	**
PPBS	0	213.4	209.7	3.7	0.366	NS
	3	187.43	175.93	11.5	1.492	NS
	6	176.17	149.03	27.13	3.88	**
HbAIC	0	7.8	7.76	0.05	0.368	NS
	3	7.42	7.22	0.2	1.482	NS
	6	7.22	6.46	0.76	5.903	**
Vitamin D	0	13.4	13.17	0.23	0.248	NS
	3	13.03	23.1	10.07	10.58	***
	6	13.07	34.53	21.47	25.2	***
NS=Not Significant; *=Significant; ***=highly significant						

Table 19: Comparison & Analysis in Group C and Group T at study completion.

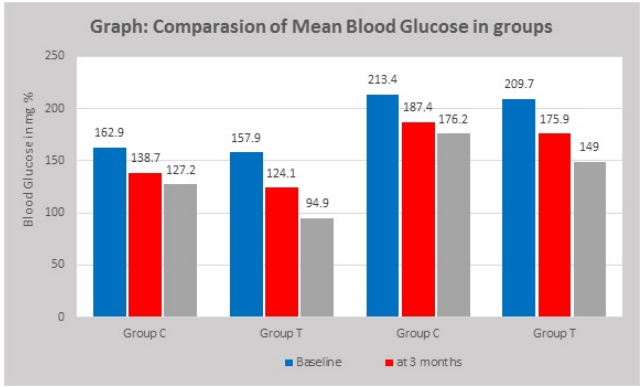

Figure 6: FBS and PPBS values in both groups at baseline, 3 months and 6 months.

It was also observed that the Vitamin D levels significantly increased in treated group from 3rd month itself and by end of 6 months was nearly 2.6 times the baseline levels. These levels remained almost constant in Control group all through the study.

The results obtained during present study were subjected to calculation of correlation coefficient (r) in order to understand the relationship among the various study parameters (FBS, PPBS, HbA1C and Vitamin D). The correlation matrix was then constructed for the two groups and presented in following Tables 20 and 21.

In control group the results clearly indicate that there was a significant well balanced positive relationship between FBS and PPBS; FBS and HbA1c; FBS and Vitamin D; PPBS and HbA1c; PPBS and Vitamin D and HbA1C and Vitamin D.

Control Group					
S.No		1	2	3	4
		FBS	PPBS	HbAIC	Vitamin D
1	FBS	XXX	0.956**	0.978**	0.254*
2	PPBS		XXX	0.980**	0.285**
3	HbA1c			Xxx	0.276**
4	Vitamin D				xxx

Table 20: Correlation coefficient (r) in order to understand the relationship among the various study parameters (FBS, PPBS, HbA1C and Vitamin D).

Treatment Group					
S.No		1	2	3	4
		FBS	PPBS	HbAIC	Vitamin D
1	FBS	xxx	0.995**	0.951**	** -0.535
2	PPBS		xxx	0.975**	**-0.572
3	HbA1c			Xxx	**-0.617
4	Vitamin D				xxx

Table 21: Treatment Group.

Almost similar results were obtained in treatment group for FBS, PPBS and HbA1c. However, Vitamin D levels had negative significant correlation with the remaining three parameters i.e. FBS, PPBS and HbA1c. This indicated that with the increase in Vitamin D content the values of these parameters decreased significantly thus reducing the blood sugar levels.

Discussion

The unprecedented increase in the prevalence of DM worldwide seen in the 21st century is described as reaching 'epidemic' proportions. According to recent WHO estimates India has presently 35 million diabetic patients, this is projected to increase to 100 million by 2035 (rise by 250%). Further support for rising prevalence comes from studies done in west and south India [4,54]. Earlier diseases was common in fourth decade of life but now is frequently seen in adolescents and younger children. This is mainly due to rise in new patients of T2DM, a consequence of lifestyle change, stress, obesity, lack of exercise, ageing population and increased migration of susceptible patients. It is characterized by inappropriate increase in blood glucose concentration due to inadequate insulin secretion and action in body. The onset is rarely recognized in the early phase of disease (IFG and IGT- Prediabetes). Nearly 50% cases present with one or more complication at diagnosis.

Non-enzymatic binding of the straight-chain glucose to proteins leading to glycosylation is a major cause of diabetic tissue damage. Chronic hyperglycemia is an important predictor of the development of diabetic complications (microvascular, macrovascular and acute metabolic complications) CUPS and CURES provided valuable data from India on diabetic complications [53,54]. Diabetes is known to

reduce life-expectancy by a decade. Devastating complications develop despite treatment, in most sufferers. Life-long burden imposed on individuals by diabetes is heavy. The costs to individuals and for health care are huge. Thus, any measure that could reduce the burden of diabetes is of importance socially, politically and economically.

Prevention and treatment of DM is a major public health challenge we face today. Although there is no known cure for diabetes, but complications can be prevented or delayed with healthy diet, regular exercise, oral medications and insulin when required for controlling blood glucose levels. Adequate treatment aiming at early control of hyperglycemia is important for reduction in these complications. This was well documented in the DCCT and in UKPDS [18,42,43]. Results from recent dies provide ample evidence for beneficial effects of early intervention to improve outcomes in diabetics [12,22,55].

Data accumulation over last 40 years' links lack of vitamin D to a wide range 0f disorders, including T2DM. The sun being the primary source of vitamin D, it is synthesized endogenously in skin to produce vitamin D3, with a small proportion (<20%) of vitamin D coming through diet from a limited range of foods (in form of vitamin D2 and vitamin D3) [67,68]. The main marker of vitamin D status is the metabolite 25(OH)D, which is synthesized in liver. Epidemiology of vitamin D status is inverse to that of diabetes; as blood levels of 25(OH)D decline with age, in obesity and also low in populations with increased skin pigmentation (African Americans and South Asians), while diabetes increases with age, obesity and is higher in these ethnicities [27,30]. Unfortunately huge part of our population is deficient in essential vitamin D. Asians and Black people are at higher risk of VDD, so are vegetarians and vegans. Surveys over several decades reveal this as a continuing global pandemic.

Health benefits of vitamin D are both preventative and reparative. Researchers in recent years have linked low vitamin D levels to diabetes and insulin resistance. There is evidence that vitamin D allows body to secrete more insulin and may also increase insulin sensitivity [35,38,41]. Overcoming insulin resistance, in particular, could be a way to head off T2DM before it sets in. Review of literature up to early 2000s provided evidence that vitamin D inadequacy has dose-effects on insulin resistance, insulin secretion and glucose homeostasis [57,59,61].

Dandona et al recognized this situation 25 years ago when he commented that '*whether vitamin D deficiency contributes to pathogenesis of diabetes or vice versa*' is an area ripe for investigation [69]. This comment unfortunately holds true even I today. Currently there is insufficient evidence of beneficial effect to recommend vitamin D supplementation as a means of improving glycemia or insulin resistance in patients with diabetes, normal fasting glucose or impaired glucose tolerance.

Recently figure of annual citations in PubMed database on vitamin D has witnessed a significant increase. This has almost doubled in last decade with a 20% increase in last three years. Publications show that vitamin D plays a crucial role in a plethora of physiological functions and associates vitamin D deficiency with many acute and chronic illnesses.

The principal driving forces for heightened interest can be traced to:

- The worsening, worldwide trend to nutritional vitamin D insufficiency & deficiency
- New role of non-hormonal, intracrine and paracrine actions of vitamin D metabolites
- Extraosseous / Non-skeletal benefits of vitamin D as in DM, HT, CAD, Obesity etc.
- VDD epidemic in India despite plenty of sunshine [20,52]. and also low dietary intake.

After reviewing many research papers, I opined that majority in the scientific community document an association of vitamin D deficiency in humans with T2DM. There is some research to the contrary. Hence this study was undertaken with an aim to evaluate effect of vitamin D supplementation on glycemic control in patients of T2DM. The glycemic control reflected in estimation of HbA1c and blood sugar levels.

We hypothesized that vitamin D deficiency may be related to glucose control in these patients. In our present study we enrolled patients of T2DM with vitamin D deficiency as per study protocol. The parameters of FBS, PPBS, HbA1c and serum 25(OH)D levels were evaluated at baseline, 3 months and 6 months of therapy.

Blood sugar levels

Results in Control Group (subjects received only OHA; were not supplemented with vitamin D) showed a decrease in Mean FBS levels from 162.9 mg% at start to 138.7 mg% in 3 months and to 127.2 mg% in 6 months. During same period PPBS levels fell from baseline level of 213.4 mg% to 187.4 mg% at 3 months end and to 176.2 mg% at end of study (6 months). The Mean reduction at study completion was 35.7 mg% in FBS and 37.2 mg% in PPBS in control group.

As against Treatment Group (subjects supplemented with vitamin D besides OHA) where Mean FBS decreased from 157.9 mg% at baseline to 124.1 mg% at 3 months and to 94.9 mg% at end of 6 months of therapy. PPBS values during same time reduced from an initial 209.7 mg% to 175.9 mg% at 3 months and to 149 mg% at 6 months. The Mean reduction calculated at study completion was 63 mg% in FBS and 60.7 mg% in PPBS in treatment group. On statistical analysis as $p<0.0001$. So we say the result is highly significant. The reduction was twice as much in Treatment Group who received vitamin D supplement during the study period.

This is comparable to studies conducted by Al-Daghri et al. [70]. 18 month prospective interventional study on T2DM Saudi patients with vitamin D supplement (2000 IU/day) oral as an adjuvant therapy. 25(OH)D levels remained below normal 18 months after treatment onset. Yet, this "suboptimal" supplementation significantly improved glycemic control with favorable change in blood sugar levels that were more pronounced in T2DM females.

Hurskainen et al. [71]. Mini Finland trial to study the associations between serum 25(OH)D status, glucose homeostasis and T2DM. A total of 850 men 906 women, aged 53-73 years, were analyzed. They concluded low level of serum 25(OH)D is associated with impaired glucose and insulin metabolism, Sugden et al. [72]. in a randomized, double-blind, placebo-controlled, parallel group trial in T2DM patients with low serum 25(OH)D levels showed oral vitamin D reduced FBS levels in patients with T2DM and vitamin D insufficiency.

The HbA1C levels in Control Group showed a decrease in Mean levels from 7.8% at start of study to 7.42% in 3 months and to 7.22% in 6 months. It reflects that the Mean reduction was 0.58 mg % in Control Group at study completion. During same period in Treatment Group Mean HbA1c levels reduced from baseline value of 7.76% to 7.22% at 3 months to 6.7% at 6 months of therapy. At study completion Mean reduction was 1.06% in treatment group. HbA1c is an indicator of

glycemic control hence the study shows that there was better glycemic control in Treatment Group as compared to Control Group at end of 6 months of therapy.

HbA1c is direct combination of glucose and adult hemoglobin. Amount of adult hemoglobin that becomes glycosylated to form HbA1c is directly related to average concentration of glucose in blood. Findings of DCCT show lowering HbA1c can delay/prevent development of serious complications and improves quality of life.

The results support various study trials by IK Athanassiou et al. [73]. It aimed to study 25(OH)D level relationship to glycemic control in 120 T2DM patients. 25(OH)D3 was measured by RIA and HbA1c by HPLC. They concluded lower vitamin D levels in T2DM than in control group (vitamin D level related to glycemic control). The therapeutic implications are supplementation may improve glycemic control.

Zoppini et al. [74] cross-sectionally examined association between HbA1c and serum 25(OH) D in 715 T2DM patients during 2011-2012. In T2DM patients they reported high HbA1c levels are associated with low serum 25(OH)D independent 0f duration of diabetes and diabetic treatment. Future studies are needed to clarify biological relation between glucose control and vitamin D metabolism in T2DM.

Vitamin D levels in Control Group remained almost constant throughout the study. The Mean values at baseline of 13.4 IU/dl were 13.03 IU/dl at end of 3 months and 13.07 at study completion. However, in Treatment Group baseline values of 13.17 increased to 23.10 at end of 3 months and further increased steeply to 34.53 IU/dl at end of 6 months of study. This means with adequate dose of vitamin D supplementation rise in serum vitamin D levels was nearly three times that resulted in achieving vitamin D sufficiency levels by study completion. The improvement is a result of vitamin D supplementation received by subjects in Treatment Group as an adjuvant therapy. This adequacy level of vitamin D was responsible for the improvement in glycemic control as reflected by reduction of blood sugar levels and HbA1c levels in Treatment Group.

The studies supporting this include Guo et al. [75] study to investigate relationship between 25(OH)D and pancreatic islet β cell function under different glucose tolerance statuses in 180 Chinese patients with T2DM concluded that 25(OH)D is closely related influential factors in functioning of β cells. Afsaneh [76] a before-after study on 100 patients with T2DM, who received 50,000IU vitamin D orally per week for eight weeks. The data showed significant improvements in serum FPG and insulin after vitamin D treatment suggesting vitamin D supplementation could reduce insulin resistance in T2DM [77]. An observational study, 171 T2DM patients were followed to evaluate vitamin D as a predictor of glycemic regulation. They inferred VDD predicts higher fasting and postprandial blood glucose and diabetes dysregulation. [78]. reported in 12 week RCT on T2DM subject's improvement in vitamin D levels and glycemic status in group receiving Vitamin D3 fortification. Other increasingly cited studies associating low vitamin D levels and glucose homeostasis in subjects at high risk for type 2 diabetes (one or more risk factors) for T2DM [32,43,47,79,80,81].

The observations can be summarized as significant improvement in blood sugar levels, Mean reduction in HbA1c and normalized Vitamin D levels in treatment group. The potential mechanism to explain protective effect of vitamin D against T2DM is based on vitamin D effect on glucose homeostasis (insulin resistance and β cell function). Glucose intolerance and type 2 DM is associated with defects in β cell function, insulin sensitivity and systemic inflammation. Adequate levels of 25(OH)D>30 ng/mL improves insulin sensitivity; eliminates burden on β cells and reverses abnormal glucose tolerance.

Potential mechanisms of vitamin D on Type 2 diabetes

- Vitamin D exerts its insulinotropic effect by the VDR, causing an increased calcium influx through β cell membrane (Kajikawa)
- Vitamin D affects insulin secretion by stimulating insulin biosynthesis in the Pancreatic beta cells (Bourlon)
- T2DM is associated with systemic inflammation (Duncan)
- Vitamin D may improve insulin sensitivity and promote beta cell survival by modulating the generation and effects of cytokines (Mathieu)

Conclusion

From the present study results it can be concluded that vitamin D deficiency as seen concomitantly in Type 2 DM patients.

In Treatment Group Vitamin D supplement was responsible for improved levels of FBS, PPBS, HbA1c and serum vitamin D levels among the individuals and within the time intervals. The near normal values of study parameters resulted in good control of blood sugar levels following 6 months of vitamin D supplementation. At the same time the serum Vitamin D values increased appropriately to reach adequacy levels from earlier insufficiency levels of at start of study. The improvement in all measures of glucose metabolism (fasting and 2-hour plasma glucose, HbA1c) with adequacy in serum vitamin D levels directly resulted in significant improvement in glycemic control in Treatment group that received the vitamin D supplementation.

In summation it can be said that when vitamin D levels were adequate the glycemic control was better with blood sugar levels in control.

The advantages of the study include: significant reductions, good efficacy, minimal rates of adverse reactions, no toxicity, good compliance.

The limitations may be enumerated as: pilot study with small sample size, double blind study couldn't be conducted, determining right dose of vitamin D supplement.

Another important question that still remains unanswered is: "Is low serum vitamin D level a factor that predisposes, or is it somehow a by-product of illness?" The Future scope encompasses

- Larger trials of longer duration are desirable
- Effect of higher dose: Additional Control?
- Specificity in different syndromes and long term effects
- due to Adverse effects of drugs only or independent risk factor

Despite evidence from the current study and prior mentioned published trials, doubts still remain about whether low vitamin status is an association or a cause of Type 2 DM. Further cohort studies are required, assessing baseline vitamin D status using serum 25(OH)D to be sure that the studies are not false-positive results. The exact mechanism for this beneficial effect yet remains to be explored. Interventional studies are needed to prove a causal relationship between vitamin D and glucose metabolism. Further studies on mode of action of Vitamin D in regulating diabetes may give interesting results. Additional studies are needed to evaluate the underlying mechanisms. Glucose clamp studies are also required because we are

still not sure of mechanism influenced by vitamin D: whether insulin resistance, secretion, or both.

But most importantly, given that nearly three decades have passed since the first studies linking vitamin D with insulin metabolism, well-designed clinical trials of the effect of vitamin D supplementation on glycaemia status and diabetes risk are urgently required to settle this question with the need to prevent past mistakes.

In particular, the vitamin D dose given in such trials needs to be high enough - above 2,000 IU per day to raise blood 25(OH)D levels above 80 nmol/l because diabetes risk is lowest at this level. If well-designed trials are carried out and confirm a protective effect from vitamin D, it could be used by the general population as a simple and cheap solution to help prevent the diabetes epidemic. The relationship between vitamin D status and glucose tolerance in Type 2DM needs further study.

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