

## TYPE 2 DIABETES MELLITUS PATIENTS WITH OR WITHOUT COMPLICATIONS CORRELATED WITH SERUM VITAMIN D LEVELS

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### ABSTRACT

**Background:** Evidence suggests that vitamin D plays a vital role in modifying the risk of diabetes. Vitamin D replenishment improves glycaemia in patients with type 2 DM with established hypovitaminosis D, thereby suggesting a role for vitamin D in the pathogenesis of DM. This study determines if there is any correlation between vit. D deficiency and T2DM. **Aim And Objective Of The Study:** To correlate serum Vitamin D levels in patients with T2DM with or without complications. **Materials And Methods:** This Cross-Sectional study was conducted at Dr. RPGMC Kangra over a period of 1 year. **Results:** Vitamin D level in patients without microvascular complications was  $25.63 \pm 13.52$  ng/ml. Out of the 60 patients with microvascular complications, 40 patients (66.7%) were deficient in vit D, 15 patients (25%) were insufficient in vit D. Only 5 patients (8.3%) had a sufficient level of Vit D. In patients without microvascular complications (total 40), 37.5% were deficient in Vit D, 30% were insufficient in Vit D and 32.5% had a sufficient level of vitamin D. **Conclusion :** Patients with type 2 diabetes mellitus had mean Vit D level of  $19.68 \pm 12.20$  ng/ml which is in the deficient range. The prevalence of vit D deficiency in patients with microvascular complications was more than in patients without microvascular complications (66.7% vs. 37.5%). Vitamin D levels in patients with poorly controlled diabetes ( $18.23 \pm 12.34$ ) were lower than patients with well-controlled diabetes ( $23.07 \pm 11.35$ ), but the difference was not significant. Vit D level decreased with increase in the duration of diabetes

**Keywords:** T2DM: Type 2 Diabetes Mellitus, microvascular complications, Vit D:Vitamin D

### INTRODUCTION

Type 2 Diabetes Mellitus (T2DM) is the most prevalent chronic metabolic disorder worldwide. Diabetes is the leading cause of death, disability and economic loss throughout the world. Large epidemiological studies suggest a link between T2DM and vitamin D. It has been proposed to be associated with worsening of T2DM in individuals (1). The human evidence comes primarily from many cross-

sectional and prospective observational studies, most of which showed an inverse association between vitamin D status and prevalence or incidence of type 2 DM. T2DM has consistently been shown to be prevalent in individuals with vitamin D deficiency. This in turns increases intracellular calcium in adipocytes stimulating lipogenesis with subsequent weight gain and impaired glucose intolerance (2).

Subclinical vitamin D deficiency is highly prevalent in both urban and rural settings and across all socioeconomic and geographic strata. Owing to its various implications on health, the epidemic of vitamin D deficiency in India is likely to significantly contribute to the enormous burden on the healthcare system of India.

Deficiency of vitamin D favors systemic inflammation and worsens glycemic control among people with diabetes enhancing their cardio-metabolic risk (3). Beneficial effects of administration of vitamin D in improving insulin sensitivity among people with diabetes are also reported. However, whether supplementation of vitamin D prevents the development of T2DM and its complications is not confirmed due to inconsistent results from clinical trials. In a post-hoc analysis of the RECORD study, a community-based effectiveness trial designed for bone outcomes, supplementation with 800 IU/day of vitamin D3 (given in a 2×2 factorial design with calcium carbonate) did not change the risk of self-reported type 2 diabetes; however, among study participants who were highly compliant with supplementation, there was a notable trend towards reduction in type 2 diabetes risk with vitamin D3 (OR 0.68; 95% CI 0.40–1.16). In that study, the decrease in FPG over 3-years was similar to the reduction in FPG achieved with metformin or lifestyle, in the Diabetes Prevention Program, which was associated with a 31–58% decrease in incident diabetes values.

Dr. RPGMC in H. P is the only major referral hospital in this part of the state catering to approximately 60% of the population of the state mainly of rural background. Most of the available literature on diabetes and its association with vitamin D is from studies based on urban population and studies in developed countries.

The scarcity of available data and the diversity of climate, culture and dietary habits prevalent in Himachal Pradesh prompted us to undertake this study in a sub-Himalayan region.

### **Aim And Objective Of The Study**

To evaluate serum Vitamin D levels in patients with type 2 diabetes mellitus with or without complications.

## **MATERIALS AND METHODS**

**Study Design-** It was a Cross-Sectional study. The study was conducted at Dr. Rajendra Prasad Government Medical College & Hospital Tanda.

The study was conducted over a period of 1 year from June 2016 to May 2017. Patients of T2DM >18 years, including freshly diagnosed patients of diabetes mellitus, who attended the diabetic OPD of the institution were enrolled for the study after taking their detailed history.

Diabetic Peripheral Neuropathy was diagnosed based on Revised Neuropathy Disability Score. Patients with NDS Score of  $\geq 6/10$  were taken as neuropathy

Diabetic Retinopathy was diagnosed by ophthalmologists and is characterized by microaneurysms, dot and blot hemorrhages, and exudates. Diabetic Nephropathy was diagnosed by urine albumin to creatinine ratio in a spot urine sample. Values  $> 30\text{ug/mg}$  creatinine was taken as nephropathy. It was further divided into microalbuminuria (30-300ug/mg creatinine) and macroalbuminuria ( $>300\text{ug/mg}$  creatinine).

Patients attending diabetic OPD of DR. RPGMCT and a who were either case of T2DM already on treatment or new patients were included in the study. Patients <18 years and with CKD stages 3-5 and patients on calcium, and vitamin D supplements were excluded

### **METHOD :**

Detail history of the patients attending diabetic OPD was taken regarding their age, occupation, duration of diabetes, treatment history, comorbid condition, and diabetes-related complications after obtaining informed consent. Proper clearance from the institutional ethics committee was taken. Patients were sent to ophthalmologists for fundus examination. After recording the weight, height, and BMI, all enrolled patients were subjected to following laboratory investigations: CBC, LFT, RFT, BGF, electrolytes. Vitamin D levels, HbA1c Level, Lipid profile, Urine analysis including microalbuminuria and urine for ACR, ECG

Vitamin D was analyzed using the ELISA technique based on the principle of competitive binding. The sensitivity of the test kit was 0.67ng/ml.

Two hundred fifty consecutive patients were enrolled initially after the history and physical examination. RFT was done of all the patients, and GFR was calculated. One hundred fifty patients were excluded because their GFR was less than 60. Finally, only 100 patients were included in the study. The patients finally selected underwent the remaining investigations and were evaluated for their diabetes-related microvascular and macrovascular complications.

### STATISTICS

Data collected were tabulated and analyzed using standard statistical methods. The mean and standard deviation was calculated. The p-value of less than 0.05 was taken statistically significant.

### OBSERVATIONS AND RESULTS

Between June 2016 and may 2017, 100 patients with T2DM were included in the study. Out of the total 100 patients, 56 were female (56%), and 44(44%) were male. The mean age of the patients included in the study was 53.08±7.98 years. The mean age of the males was 52.09±8.65 years, and the mean age of the females was 53.86±7.41 years. The mean BMI of the patient's were 23.52±2.08 which falls in the overweight category. 8.37±1.71 was the mean HbA1c of the patients. 52% of the patients were hypertensive. Thirty patients were known diabetic for 5 or fewer years. 26 patients had diabetes for 5 to 10 years, and only 2% of patients had diabetes for more than 20 years. Maximum patients (45%) were in the normal range followed by overweight(32%) and obese(22%), and only 1% of the studied population was underweight according to Indian classification for BMI.

**TABLE 1: Vitamin D classification with the prevalence**

Vitamin D classification	Number of patients	Percentage
Deficient ≤ 20 ng/ml	55	55
Insufficient 20-30 ng/ml	27	27
Sufficient 30-100 ng/ml	18	18
Excess >100 ng/ml	0	0

**TABLE 2: Microvascular complications with vitamin D**

	Microvascular complications				p value
	Present (60 patients)		Absent (40 patients)		
<b>Vit D mean level</b>	15.71±9.42		25.63±13.52		<0.05
<b>Vit D classification:</b>	no	%	No	%	
<b>Deficient</b>	40	66.7%	15	37.5%	0.126
<b>Insufficient</b>	15	25%	12	30%	0.143
<b>Sufficient</b>	5	8.3%	13	32.5%	0.793

Microvascular complications were present in 60 patients (60%), and 40 patients (40%) did not have any microvascular complications. (Table 2)

The mean age of the patients in the complication group was 56.32±7.43 years, and without complications, the mean age was 48.23±6.17 years, and this difference in age was significant (P=0.000)

BMI of the patients with microvascular complications was higher than the patients without microvascular complications (24.04 vs. 22.76 respectively). 41.7% of the patients (25 patients) with microvascular complications had normal BMI, 30% (18 patients) were overweight, and 28.3% (17 patients) were obese. Of the patients without microvascular complications 12.5% were obese, 50% had normal BMI, and 35% were overweight.

It was also seen that 77.3% of the obese patients had microvascular complications, 56.3% of the overweight patients had microvascular complications and 55.6% of patients with normal BMI had complications. The HbA1c level between the two groups(complication vs. without complication) was 8.603 and 8.025, and this was not significant (p≥0.05).

Total of 52 patients was hypertensive. Out of the 60 patients with microvascular complications, 41 patients (68.3%) were hypertensive. Among the 52 patients with hypertension, 41 patients had microvascular complications. Vitamin D level in patients with associated hypertension was 17.81±12.82 and in

patients without hypertension was  $21.71 \pm 11.27$ . ( $p \geq 0.05$ ).

48.3% (29 patients) of the patients with microvascular complications were more than 55 years of age, and 40% (24 patients) were between 50 to 55 years of age. Among patients without microvascular complications, 10% (4 patients) were aged more than 55 years, and 20% (8 patients) were between 50 to 55 years of age. None of the patients with microvascular complications were less than 40 years of age.

Only 3.3% of the patients with microvascular complications had diabetes for more than 20 years, and 23.3% of patients with complications had diabetes for 15 to 20 years. In contrast, the patients without microvascular complications none of them had diabetes for more than 15 years. 57.5% of the patients without microvascular complications had diabetes for

less than five years, and this difference was significant ( $p \leq 0.05$ ).

Prevalence of microvascular complications with duration of diabetes for 15 to 20 years (23.3%) was statistically significant ( $p \leq 0.05$ ) as none of the patients without microvascular complications had diabetes for more than 15 years.

With the increase in duration of diabetes, the frequency of microvascular complications increased. 100% of the patients with diabetes for more than 15 years had microvascular complications in one or the other form and only 23.3% of the patients with duration of diabetes for less than or equal to 5 years had microvascular complications, and 76.7% did not have complications. This was significant ( $p \leq 0$ ). (table 3)

**TABLE –3: Vitamin D level**

<b>VITAMIN D LEVEL</b>			
	No of patients	Mean value(ng/ml)	p value
<b>Sex: male</b>	44	$22.88 \pm 13.12$	0.019
<b>Female</b>	56	$17.16 \pm 10.90$	
<b>Microvascular complication:</b>			
<b>Present</b>			
<b>Absent</b>	60	$15.71 \pm 9.42$	0.000
	40	$25.63 \pm 13.52$	
<b>Diabetic peripheral neuropathy:</b>			
<b>Present</b>			
<b>Absent</b>	50	$16.56 \pm 9.55$	0.010
	50	$22.80 \pm 13.77$	
<b>Diabetic retinopathy:</b>			
<b>Present</b>	31	$11.14 \pm 6.92$	0.000
<b>Absent</b>	69	$23.52 \pm 12.14$	
<b>Mild NPDR</b>	15	$11.50 \pm 7.09$	
<b>Moderate NPDR</b>	15	$10.86 \pm 7.21$	
<b>Severe NPDR</b>	1	9.89	
<b>Diabetic nephropathy:</b>			
<b>Present</b>	36	$13.64 \pm 8.75$	0.000
<b>Absent</b>	64	$23.08 \pm 12.60$	
<b>Microalbuminuria</b>	26	$15.05 \pm 9.54$	
<b>Macroalbuminuria</b>	10	$9.98 \pm 4.95$	
<b>Hypertension:</b>			
<b>Present</b>	52	$17.81 \pm 12.82$	0.111
<b>Absent</b>	48	$21.71 \pm 11.27$	

	No of patients	Mean value(ng/ml)
<b>BMI :</b>		
<b>Normal</b>	45	22.34±13.83
<b>Overweight</b>	32	20.12±11.59
<b>Obese</b>	22	13.57±6.92
<b>Diabetes duration:</b>		
<b>≤5</b>	30	28.54±12.35
<b>&gt;5 - ≤10</b>	26	20.26±10.95
<b>&gt;10 - ≤15</b>	28	14.69±9.45
<b>&gt;15 - ≤20</b>	14	11.57±6.45
<b>&gt;20</b>	2	5.92±0.67

In males, the mean vitamin D level was 22.88±13.12ng/ml, and in females, the mean vitamin D level was 17.16ng/ml ±10.90. (Fig16). The difference in the vit.D level between the two sexes was statistically significant (p≤0.05)

Vit D level in patients with microvascular complications was 15.71±9.42 whereas it was 25.63±13.52 in patients without complications and this difference was significant (p≤0.05)

DPN was present in 50 patients(50%). Vitamin D level in patients with diabetic peripheral neuropathy(DPN) with or without other complications was 16.56±9.55, and in patients without neuropathy it was 22.80±13.77. This difference was significant(p≤0.05).

Diabetic retinopathy was present in 31 patients(31%). Fifteen patients had mild NPDR, 15 patients had moderate NPDR, and severe NPDR was present in only one patient. Vitamin D level in patients with mild NPDR was 11.50±7.09, in moderate NPDR was 10.86 ±7.21 and in severe NPDR the mean vitamin D level was 9.89.

Diabetic nephropathy was present in 36(36%)% of patients. Vitamin D level in patients with microalbuminuria was 15.05±9.54, and in patients with macroalbuminuria, the mean vitamin D level was 9.98±4.95ng/ml.

Vitamin D level decreased as the BMI increased. It was 22.34±13.83 in normal BMI, 20.12±11.59 in overweight and 13.57±6.92 in the obese category.

Among patients with microvascular complications 83.3% had DPN with or without complications, 51.7% had retinopathy with or without complications, and

nephropathy was present in 60% with or without complications.

17% had isolated neuropathy, 2 % had isolated nephropathy, and 3% had isolated had isolated had isolated retinopathy.10% had both neuropathy and nephropathy, 3% had both neuropathy and retinopathy and 5% had both nephropathy and retinopathy. Triopathy was present in 20% of patients (table 4)

**TABLE –4: Vitamin D levels in microvascular complications**

	No of patients	Vit D level(ng/ml)
<b>Neuropathy alone</b>	17	21.11±9.65
<b>Nephropathy alone</b>	2	13.80±10.38
<b>Retinopathy alone</b>	3	16.76±11.32
<b>Neuropathy + nephropathy</b>	10	21.10±9.22
<b>Neuropathy +retinopathy</b>	3	8.46±0.96
<b>Nephropathy + retinopathy</b>	5	7.38±1.81
<b>Triopathy</b>	20	11.64±7.16

VITAMIN D level in patients with triopathy was 11.64±7.16 and in patients with neuropathy and nephropathy combined was 21.10±9.22. in patients with neuropathy and retinopathy the mean vitamin D level was 8.46±0.96. In patients with nephropathy and retinopathy, the mean vitamin D level was 7.38±1.81.



vitamin D level in patients with isolated neuropathy was  $21.11 \pm 9.65$ , in isolated nephropathy was  $13.80 \pm 0.38$  and in isolated retinopathy was  $16.76 \pm 11.32$

Of the patients with neuropathy, 64% (32 patients) were deficient in Vit D, 26% (13 patients) were insufficient and sufficient level of Vit D was present in 10% (5 patients) (Table 5).

Among patients with nephropathy, Vit D was deficient in 75% (27 patients), insufficient in 19.4% (7 patients) and sufficient in 5.6% (2 patients) (Table 5).

Among the patients who were having retinopathy, Vit D was deficient in 83.9% patients (26 in no), insufficient in 16.1% patients (5 in no) and none of the patients with retinopathy had sufficient levels of Vit D (Table 5).

**TABLE –5: Prevalence of Vitamin D status with microvascular complications**

	Deficient ≤20		Insufficient 20-30		Sufficient >30	
	No	%	no	%	No	%
<b>Neuropathy:</b>						
<b>Present</b>	32	64	13	26	5	10
<b>Absent</b>	23	46	14	28	13	26
<b>Nephropathy:</b>						
<b>Present</b>	27	75	7	19.4	2	5.6
<b>Absent</b>	28	43.8	20	31.3	16	25
<b>Retinopathy:</b>						
<b>Present</b>	26	83.9	5	16.1	0	0
<b>Absent</b>	29	42	22	31.9	18	26.1
<b>Sex:</b>						
<b>Male</b>	19	43.2	14	31.8	11	25
<b>Female</b>	36	64.3	13	23.2	7	12.5

43.2% of males (19) as compared to 64.3% of females (36) were deficient in Vit D, 31.8% of males (14) and 23.2% of females (13) were insufficient in Vit D and sufficient level of Vit D was present in 25% males (11) and 12.5% of females (7)

Well controlled diabetes was present in 30 patients, and 70 patients were poorly controlled. VitD was lower in poorly controlled group ( $18.23 \pm 12.34$ ) as compared to the well-controlled group ( $23.07 \pm 11.35$ ), but it was not significant ( $P=0.069$ ).

Vit D levels were found to be inversely associated with HbA1c levels in the diabetic patients ( $p = 0.020$ ,  $r^2 = 0.054$ , linear regression).

## DISCUSSION

The mean level of vitamin d in this study was 19.68ng/ml. This level was similar to the study conducted by Bayani et al. where the mean concentration of Vit D in diabetic patients was  $18.7 \pm 10.2$  ng/dl (4). In a study by Bajaj et al. in India, the mean vitamin D level was  $19.046 \pm 6.614$  ng/ml in people with diabetes which was almost similar to our study (5). In our study, the mean vit d level in males was 22.88 ng/ml, and in females, it was 17.16ng/ml, and this difference in Vit D level was statistically significant ( $p < 0.05$ ). Mean vit. D Levels in men were significantly higher than women in T2DM subjects in two studies (6, 7).

.A meta-analysis with 3,612 diabetes cases (mean age 61.6 years) demonstrated an inverse association between circulating 25 (OH) D and incident type 2 diabetes (8).

Vitamin D levels were lower in patients of T2DM in our study with microvascular complications than those without complications. The mean vitamin D in our study was 15.71 ng/ml in people with diabetes with microvascular complications while it was 25.63 ng/ml in patients without microvascular complications. The difference in Vit D level between the two groups was significant ( $p \leq 0.05$ ).

The difference was significant in vit.D levels in patients with diabetic neuropathy with or without other microvascular complications than with patients without diabetic neuropathy ( $p < 0.05$ ) in our study. Mean vit.D levels in patients with diabetic neuropathy were found to be less in the study by Chaychi et al. (9). The mean level of Vit D in isolated neuropathy in the study by Soderstrom et al. was 22.2 ng/ml which is similar to our isolated neuropathy level (21.11 ng/ml) (10).

A meta-analysis by G.-B. Quet al. indicated that vitamin D deficiency is associated with the generation and development of DPN in Caucasians and Asians with T2DM (11). The study by Cui LJet al showed that vit. D deficiency is an independent risk factor for DPN (12).

Sánchez-Hernández et al showed that low vit. D status is associated with advanced diabetic nephropathy (13). Low vitamin D status is characteristically associated with advanced diabetic nephropathy (14). Vitamin D replacement also has beneficial effects on other diabetic nephropathy risk factors, such as hypertension and hyperlipidemia (15).

Levels of vit d in patients with mild NPDR was 11.50±7.09, in moderate NPDR it was 10.86±7.21 whereas in severe NPDR it was 9.89 and the prevalence of diabetic retinopathy was 31%. Mean vitamin D levels decreased with increased severity of diabetic retinopathy was shown by a study conducted by Aksoy et al (16). 25(OH)D level < 21 ng/mL was associated with increased risk of hypertension, diabetes, obesity, and high triglyceride levels—all associated with increased cardiovascular mortality (17).

Our study showed that vit. D level decreased with increase in duration of diabetes but the difference in the level was significant ( $p < 0.05$ ) only when it was compared in the group with duration of diabetes less than five years and more than five years. Vit. D level in patients with duration of diabetes for more than five years did not vary significantly. Only two studies regarding the association of vit D and the duration of diabetes was found, and they did not show any significant association between vit.D and duration of diabetes. It was also seen that with an increase in the duration of diabetes increased, the prevalence of microvascular complications increased.

Our study showed a deficiency of vit. D in people with diabetes which was correlating with duration of diabetes and other studies quoted. So it is prudent for the physician/internist to monitor vit. D level more so in patients with a long history of diabetes and patients having microvascular complications because they have got direct relation with vit. D levels.

## CONCLUSION

1. Patients with type 2 diabetes mellitus had mean vit. D level of  $19.68 \pm 12.20$  ng/ml which is in the deficient range. The prevalence of vit D deficiency in patients with microvascular complications was more than in patients without microvascular complications (66.7% vs. 37.5%). A significant difference in Vit D levels was present in patients with and without DPN ( $16.56 \pm 9.55$  vs.  $22.80 \pm 13.77$ ,  $p < 0.05$ ), with and without DN ( $13.64 \pm 8.75$  vs.  $23.08 \pm 12.60$ ,  $p < 0.05$ ), and in patients with and without DR ( $11.14 \pm 6.92$  vs.  $23.52 \pm 12.14$ ,  $p < 0.05$ ). Vitamin D levels in patients with poorly controlled diabetes ( $18.23 \pm 12.34$ ) were lower than patients with well-controlled diabetes ( $23.07 \pm 11.35$ ), but the difference was not significant. Vit. D level decreased with increase in the duration of diabetes. It was  $28.54 \pm 12.35$  in patients with duration of diabetes for five years,  $20.26 \pm 10.95$  for 6 to 10 years,  $11.57 \pm 6.45$  for 16 to 20 years and  $5.92 \pm 0.67$  ng/ml in patients with diabetes for more than 20 years.

Considering the high prevalence of vitamin D deficiency and the burden of diabetes and its complications, screening type 2 diabetes patients who are at risk of vitamin D deficiency should be considered.

## FINANCIAL DISCLOSURE

No cost was charged from the patient or his/her attendants for any investigations done under this study.

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