Research Paper

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A Study of Prevalence and Vitamin D Impact in Diabetics

Medical Science

KEYWORDS : vitamin D3, Type-2 diabetes mellitus, HPLC.

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ABSTRACT

Background: Vitamin D deficiency is epidemic in India despite of plenty of sunshine. vitamin D has a major role on insulin secretion and preventing diabetic related complications. | AIM AND OBJECTIVES: To estimate the prevalence of vitamin D deficiency and understand the impact in type 2 diabetes mellitus by comparing this with healthy control group. | STUDY DESIGN-Cross sectional, case control observational study. | MATERIALS AND METHODS: This study was conducted in 50 patients of diabetic patients who are admitted to the medical wards of Gandhi hospital between May2012 and August 2013. All patients underwent a standard clinical examination and a blood drawn for Glucose, serum vitamin D3 assay. | STATESTICAL ANALYSIS: Z- Test is applied for comparisons of vitamin D and calcium levels in cases and controls. | Chi-square test is applied for comparisons of proportions. P value of < 0.05 is taken as significant. | RESULTS: The cutoff value of Serum 25-hydroxy vitamin D3 used in this study was< 20 ng/ml is taken as deficient. In our study 64%of the diabetic group are vitamin deficient (<20ng/ml) as compared to 36% of the comparison group (p<0.05). Mean vitamin-d3 levels in cases is 19.86ng/ml. mean vitamin-d3 level in controls is 28.35ng/ml, the difference between means of serum vitamin-d3 level of cases and controls is significant. (z=17.4, p<0.05). It shows vitamin D deficiency is statistically significant. | CONCLUSION: Our study shows that vitamin D deficiency is significantly more prevalent in Diabetics as compared to healthy Individuals in spite of plenty of sunshine in India. Diabetics with high HbA1C have higher prevalence of vitamin D deficiency.

INTRODUCTION:

Epidemiology of vitamin- D Deficiency and Diabetes Mellitus in India:

Vitamin D deficiency is epidemic in India despite of plenty of sunshine. Vitamin D deficiency has been reported in all age groups and adult males and females residing in rural and urban India.[1,2,3]

According to the ICMR – INDIAB study, there are 62.4 million people living with diabetes in India. Type 2 Diabetes (T2DM) is a progressive disease and hampers the quality of life of the patients due to micro and macro vascular complications.

Effect of vitamin D on insulin secretion:

Pancreatic islets have both VDR and vitamin D-dependent calcium-binding proteins (CaBP)[4,5] suggesting a role for vitamin D in insulin secretion. Its effect on the β cells is by increasing insulin response to glucose stimulation, but it does not affect basal insulin secretion [6].

The effects of vitamin D on insulin secretion may follow several pathways. Evidence exists that vitamin D influences β-cell insulin secretion through a rise in intracellular calcium concentration via non-selective voltage-dependent calcium channels [7]. As a consequence, a major mechanism of action of vitamin D on insulin secretion and synthesis is likely to involve the β-cell calcium dependent endopeptidases, which produce the cleavage that facilitates the conversion of proinsulin to insulin [8,9]. Moreover, calcium is not only necessary for insulin exocytosis but also for β-cell glycolysis, which plays a role in signaling circulating glucose concentration.[9]

Vitamin D and Peripheral Insulin Resistance:

In type 2 diabetes mellitus, abnormalities in many systemic inflammation markers have been found, such as tumor necrosis factor (TNF)-α and TNF-β, interleukin-6 (IL-6) and its receptor, C-reactive protein and plasminogen activator inhibitor. Some of these immune mediators, such as TNF-α and IL-6, may directly interfere with insulin signaling, resulting insulin resistance via a variety of pathways [10]. The existence of VDRs in activated T lymphocytes, macrophages and thymus tissue raised the idea that vitamin D might function as an immune modulator. [8,11,12,]

Vitamin D has been reported to down regulate the production of several cytokines: IL-2, IL-6 and IL-12, interferon-γ, TNF-α and TNF-β .Vitamin D deficiency is often associated with obesity and Type-2 DM[13,14] due to deposition of vitamin D in the fat stores where it becomes less bioavailable.[15]. Vitamin D-deficient obese subjects also have elevated PTH levels[16,17]. Increased PTH can decrease insulin sensitivity[18,19] .

Vitamin D role in Diabetes Mellitus Complications

In Diabetic retinopathy the possible inhibitory effect of vitamin D on retinal endothelial cell proliferation prevents a major cause of more severe retinopathy.[20] In diabetic nephropathy [21] Vitamin D negatively regulates the RAS by suppressing renin expression and thus plays a Reno protective role in DN. Vitamin D is a neurotropic substance and modulates neuromuscular function and neuronal growth and differentiation. Vitamin D insufficiency may potentiate diabetic nerve damage and may impair nociceptor function in diabetic neuropathy [22,23]

MATERIALS AND METHODS:

Patients attending the outpatient department or admitted to the general medicine wards of Gandhi Hospital in the period between May2012 and August 2013 were examined by history, physical examination and the relevant baseline investigations were done. Those patients with any chronic disorders like Cirrhosis of liver, CKD (GFR≤ 60) ,Morbid obesity-BMI≥30 ,taking vitamin D and calcium supplements, history of malabsorption, patients on AED, Type 1 diabetes mellitus are excluded. Those patients found eligible were enrolled into the study. Diabetes mellitus was diagnosed by American Diabetes Association, 2011 criteria [24]. The study sample size was 50. Comparison group comprised of 50 healthy volunteers. An informed consent is taken from both cases and controls.

BIOCHEMICAL ANALYSIS OF VITAMIN D

Venous blood sampling was performed. Blood samples were centrifuged for 20 minutes and aliquots of 200µl were frozen at --80°C for vitamin D estimation. 25-hydroxy vitamin D3 was measured in ng/ml by High Performance Liquid Chromatography (HPLC) method at National Institute of Nutrition, Hyderabad by UFLC SHIMADZU Machine (LC 20). The co-efficient of variation for this assay is 8%.

Procedure of chromatography: Serum 25-OH vitamin D3 is extracted with a suitable organic solvent after precipitation of the protein with absolute methanol and isopropanol (90:10v/v).An aliquot of organic phase (n-Hexane),which is evaporated under nitrogen and redissolved in mobile phase(methanol),is injected into a reverse phase HPLC column followed by an eluting solvent of suitable polarity. A reverse phase column has a non-polar stationary phase and polar mobile phase. 25(OH)D3 is detected at 265nm by a sensitive UV detector. An internal standard of [(H3)25(OH)D3] is used to account for processing loses and calculate recovery. The analysis was done by LC solution software.

SERUM 25(OH)D3 IN ng/ml = (AUC of sample/AUC of standard 1ng) x Dilution factor(4) x 2 x (100/recovery%) 27 25-hydroxyvitamin D3 values more than or equal to 30ng/ml was taken as normal. Values between 21 and 29ng/ml was taken as insufficient and values less than or equal to 20ng/ml was taken as sufficient. [25-31]

STATESTICAL ANALYSIS:

Z test is applies for comparisons of vitamin D and calcium levels in cases and controls.

Chi-square test is applied for comparisons of proportions. A P value of < 0.05 is taken as significant.

RESULTS AND DISCUSSION

The cases and controls are age and sex matched. Average age of cases is 52.8 and that of controls is 52.There are 29 males and 21 females in cases and controls (Table-1).

In our study (Diagram1) the Mean vitamin-D levels in cases-19.86ng/ml and that of controls mean is 28.35ng/ml. The difference between means of serum vitamin-D level of cases and controls is significant. (Z=17.4, P<0.05)

In our study 29 (18 deficient+11 insufficient) out of 50 controls had vitamin D deficiency, that accounts for 58% of healthy controls. In our study 37 (32 deficient+5 insufficient) out of 50 cases had vitamin D deficiency, that accounts for 74 %(64 % deficient+10% insufficient) in diabetics compared to 58% (36% deficient + 22% insufficient) of healthy controls.; which is significantly higher levels of vitamin D deficiency in diabetics compared to healthy controls($p < 0.05$) (Diagram 2 about here).

Our study showed 66.66% of female diabetic patients compared to 58.62% of males had vitamin D deficiency while 14.42% females and 6.89% males are vitamin D insufficient. The difference between was not significant (p>0.05).This is in concordance with that seen in healthy controls. 47.61% of female healthy controls compared to 27.58% of males are vitamin D deficient while 23.80% males and 20.68% females are vitamin D insufficient. This difference could be because of social factors prevailing in India.

Our study showed Mean vitamin-D in males (cases)-21.55ng/ml and Mean vitamin-D in females (cases)-18.18ng/ml. Though there is significant difference in serum vitamin D values between cases and controls, the difference is not significant between males and females. i.e is

Mean vitamin D in males (controls)-32.1ng/ml and Mean vitamin-D in females(controls)-24.6ng/ml.

Our study showed that cases recruited in winter(September to February) were more vitamin D deficient than those recruited in summer(march to august)(100% vs. 71%) and mean HbA1C in winters was 9.025 %as compared to 8.49% in summer months. The lesser vitamin D levels in winter months are because of less exposure to sun. The HbA1C rise in winters is because of more food intake and decreased activity [32] .Our study also showed higher vitamin D(Mean-24.79ng/ml) levels in patients with lower HbA1C levels(≤8) and vice-versa. A mean vitamin D of 15.32ng/ml was seen in patients with HbA1C >8.113.

42 out of 50 cases had their HbA1C measured.17 cases had HbA1C ≤ 8 and 25 cases had HbA1C > 8 & 12 out of 17 cases (70.58%) with HbA1C ≤ 8 had vitamin D insufficiency and deficiency.21 out of 25 cases (84%) with HbA1C > 8 had vitamin D deficiency. The difference is not statistically significant (p>0.05).

Our study showed an insignificant(p=0.71) correlation between the duration of diabetes, serum corrected calcium levels and deficiency of vitamin D; though vitamin D levels in diabetics of duration of > 5 years (Mean of 17.7ng/ml) is less than that of patients with duration ≤ 5years (Mean 21.66ng/ml). Mean serum calcium (corrected) in cases is 9.07mg%. Mean serum calcium (corrected) in controls is 9.04mg%. There is no significant difference in serum calcium between cases and controls.

Our study showed that, mean serum vitamin D levels in insulin treated group is 14.35ng/ml. The mean serum vitamin D levels in group treated with Oral anti diabetics is 18.35ng/ml. (diagram 3about here)There is no significant difference in serum vitamin D levels in insulin treated and those treated with oral hypoglycemic agents (OHA's) and also There is no significant difference (p=0.74) in serum vitamin D levels in controlled and uncontrolled diabetics.

According to our study 24 cases had macro vascular complications like CVA, CAD and PVD. Out of 24 cases 17(70.8%) had vitamin D deficiency and insufficiency. Though prevalence is more, the difference is not significant when compared to cases without macro vascular complication.

Our study showed a non-significant correlation (p=0.17) between vitamin D deficiency and micro vascular complications (retinopathy, nephropathy and neuropathy).17 cases had micro vascular complications.15 out of 17(88.23%) cases had vitamin D deficiency and insufficiency.23 out of 33 cases (69.7%) had vitamin D deficiency and insufficiency in cases without micro vascular complications. The difference is not statistically significant (p>0.05).

There is no significant difference in serum vitamin D levels between smokers and alcoholics than those with non-smokers and non-alcoholics (P=0.51&0.34 respectively). There is no significant difference in serum vitamin D levels between hypertensive's and

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non hypertensive's (p>0.05).

Mean vitamin D in cases residing in urban areas is 19ng/ml. Mean vitamin D in cases residing in rural areas is 23.2ng/ml. In urban areas 76.66% of patients are vitamin D insufficient &deficient. In rural areas 70% of patients are vitamin D insufficient & deficient. The difference is not statistically significant (p>0.05).

LIMITATIONS:

The main drawbacks of our study are:

1. Small sample size- As prevalence of vitamin D deficiency is high in general population, larger sample size would have given more statistically significant results.

2. Cases and controls are not strictly season matched; most of our cases and controls are taken during summer months and Confounding factors like cerebrovascular accident, hypertension were not excluded from cases.

3. It is an observational study and therefore no conclusion can be made as far as any cause and effect relationship is concerned between vitamin D deficiency and Diabetes mellitus type 2.

RECOMMENDATIONS:

1. In the present study, lower 25(OH)D3 levels were observed in a cohort of diabetes mellitus type 2 patients than in a control group and an inverse relationship was observed between glycosylated hemoglobin levels and 25(OH)D3 levels in the patient group, implying that 25(OH)D3 levels may affect glucose control in diabetes mellitus type 2. The prevalence of vitamin D deficiency in diabetics is significantly more than in healthy controls. Thus it is a fit case for supplementation trials to assess the need for vitamin D supplementation for glycemic control and prevention of complications of diabetes.

2. The association between the low serum 25(OH) D levels and elevated HBA1c in the study population may be inscribed into a wider context, portraying vitamin D insufficiency as a poor prognostic factor, which may play a vital role in impairing the glycemic control. Improving the vitamin D status will help in establishing a better glycemic control in people with DM type 2. It seems that the routine screening for vitamin D insufficiency may provide meaningful information and that it could be considered for diabetic care.

CONCLUSIONS

1. Prevalence of vitamin D deficiency is significantly higher in type 2 diabetic patients than in healthy control population (74%). Although Prevalence of vitamin D deficiency is also high in healthy population (58%).but statistically significant difference is found.

2. Vitamin D deficiency is more prevalent in urban areas than in rural areas. But has no significant differences between sexes, though females have more vitamin D deficiency than males, probably because of social factors.

3. Diabetics with high HbA1C have higher prevalence of vitamin D deficiency. But has no relation with duration of diabetes. Diabetics with macro vascular and micro vascular complications have more vitamin D deficiency than those without, though not statistically significant

4. There is no significant difference in Vitamin D levels either in treated and untreated groups or Patients treated with oral anti diabetics and insulin do not have a significant difference in vitamin D levels.

5. Diabetics with hypertension have no significant difference in vitamin D3 levels than those without hypertension. Serum calcium levels are not significantly different in diabetics and healthy controls.

Table:1 characteristics of patient profile

Characteristics	(vit-d3) ≤ 20 ng/ml	21–30ng/ml	> 30 ng/ml
Males(n=29)	18	2	9
Females(n=21)	14	3	4
Urban(n=30)	20	3	7
Rural(n=20)	12	2	6
Cases recruited between Sep.-Feb (n=4)	4	0	0
Cases recruited between Mar.-Aug (n=46)	28	5	13
Smokers(n=17)	10	1	6
Non-smokers(n=33)	22	4	7
Alcoholics(n=21)	12	2	7
Non alcoholics(n=29)	20	3	6
Denovo diabetics(n=17)	13	0	4
Diabetes of duration≤ 5yrs (n=20)	11	3	6
Diabetes of duration >5yrs (n=13)	8	2	3
With hypertension (n=29)	15	3	11
Without hypertension (n=21)	17	2	2
Diabetics treated with insulin (n=4)	3	0	1
Diabetics treated with OHA's(n=26)	17	4	5
HbA1C ≤ 8(n=17)	12	0	5
HbA1C > 8(n=25)	18	3	4
Cases with Macro-vascular complications(n=24)	14	3	7
Cases without Macro-vascular complications(n=26)	18	2	6
Cases with Micro-vascular complications(n=17)	11	3	3
Cases without Micro-vascular complications(n=33)	20	2	11

DIAGRAM: 1 comparison of 25 (OH) vitamin D3 between diabetics and non-diabetics

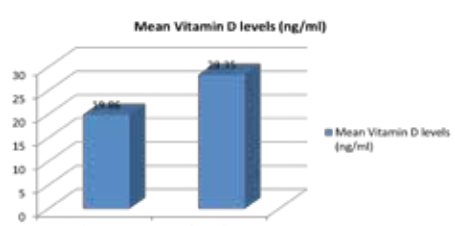


Diagram 2: showing vitamin D3 levels in cases and controls

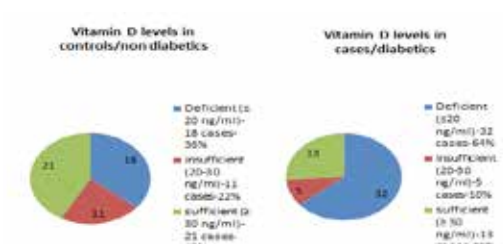
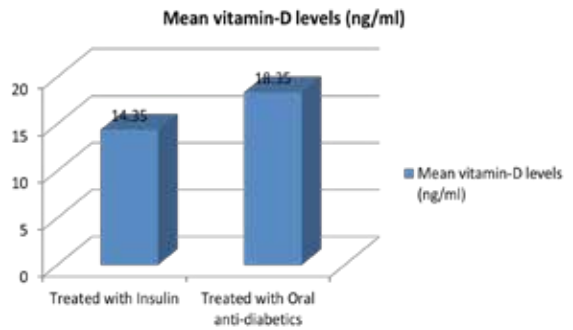


DIAGRAM 3: Relationship between treatment of diabetes and serum vitamin D levels.



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