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RESEARCH ARTICLE

Study of vitamin D levels and its correlation with insulin resistance

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ABSTRACT

Background: Hypovitaminosis D has been reported as a risk factor for glucose intolerance. Although traditionally named as sunshine vitamin, in India, it is paradoxically deficient. Obesity is frequently associated with insulin resistance (IR) and prediabetes and other components of metabolic syndrome. Obesity is also commonly associated with hypovitaminosis D due to the capacity of adipose tissue to store 25-dihydroxy vitamin D making it biologically unavailable. **Aims and Objectives:** This study is designed to know the prevalence of hypovitaminosis D and study its relation with IR from Central India. **Materials and Methods:** A total of 594 patients were enrolled in the study. Of these, 560 met inclusion-exclusion criteria. Body mass index (BMI), Waist-hip ratio, and blood pressure were measured. Fasting sample was taken for the following investigations: Fasting blood sugar, fasting insulin, intact parathormone, lipid profile, and vitamin D were done. Homeostatic model assessment (HOMA-IR) was calculated. Based on BMI, they were divided into two groups: Cases (n = 168) with BMI >25 and controls (n = 392) BMI <25. **Results:** Overall vitamin D deficiency was seen in 413 (73.75%) with deficiency in cases 93.45% and 65.3% in control group. Severe vitamin D deficiency was seen in 14.88% cases and 11.99 controls. Mean serum vitamin D levels were 18.31 ± 11.53 in cases and 26.4 ± 12.28 0.002 (HS) in controls. Vitamin D sufficiency was seen in only 6.54% of cases and 34.69% of controls. Individuals with severe vitamin D deficiency have maximum IR. The parameters of IR were significantly seen to be associated with vitamin D levels. **Conclusion:** Vitamin D deficiency is rampant in Central India and shows strong independent association with IR.

KEY WORDS: Vitamin D3; Insulin Resistance; Hypovitaminosis D; 1,25-dihydroxy Vitamin D3

INTRODUCTION

Hypovitaminosis D has been reported as a risk factor for glucose intolerance.[1] Although traditionally named as sunshine vitamin, in India, it is paradoxically deficient.[2] Obesity is frequently associated with insulin resistance (IR) and prediabetes and other components of metabolic syndrome. Obesity is also commonly associated with hypovitaminosis D due to capacity of adipose tissue to store 25(OH) vitamin D making it biologically unavailable.[3] Insulin secretion is impaired by vitamin D deficiency and restored by 1,25-dihydroxycholecalciferol administration.[4]

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The incidence of type 2 diabetes mellitus (type 2 DM) is increasing at an alarming rate specially in India. Diabetes develops as a result of genetic and environmental factors. Defects in pancreatic β-cell function, insulin sensitivity, and systemic inflammation all contribute to the development of type 2 DM. IR is a major and modifiable risk factor for diabetes. Proper understanding of modifiable risk factors, which also

includes nutritional factors and their role in the development of IR and diabetes, is important. Obesity and other lifestyle factors such as exercise, alcohol consumption, smoking, and certain dietary habits have already been proved as contributing factors. Recently, a novel association between IR and vitamin D deficiency has been proposed. Vitamin D has *in vitro* and *in vivo* effects on pancreatic β-cells and insulin sensitivity.[5]

A meta-analysis was conducted recently to find out the association of vitamin D status and cardiometabolic disorders (cardiovascular disease, diabetes, and metabolic syndrome) which reviewed 28 independently published studies. The findings showed a significant 55% reduction in the risk of diabetes (9 studies), a 33% reduction in the risk of cardiovascular diseases (16 studies), and a 51% reduction in metabolic syndrome (8 studies) associated with a high serum 25-dihydroxy vitamin D (25(OH) D) concentration.[6]

Since vitamin D receptors are expressed in tissues like muscles and beta cells of pancreas, vitamin D level plays an important role in glucose metabolism and regulation.[7-10] A decreased amount of serum 25(OH)D, 1,25(OH)2 D and raised parathormone (PTH) can increase intracellular calcium in adipocytes which can stimulate lipogenesis predisposing a patient to further weight gain thus increasing the risk of diabetes.[11]

From India, data regarding association of vitamin D deficiency and IR are scanty, and there is no data from Central India. In a growing prevalence of diabetic pandemic, we found it important to study this association between vitamin D levels and IR. This study is designed to know the prevalence of hypovitaminosis D and study its relation with IR in Central India.

MATERIALS AND METHODS

Healthy family members of the patients attending various outpatient departments during the period August to October at NKPSIMS, Nagpur, Maharashtra, India, were randomly recruited for this cross-sectional study. The individuals who gave written consent and agreed to the study protocol were included in the study. They were asked to come in fasting state (10-12 h fast overnight). Inclusion criteria were as follows: Adults in the age group of 18-65 years (both ages inclusive) and those ready to give written consent. Diagnosed cases of DM, patients on treatment of obesity, ischemic heart disease, congestive cardiac failure, coronary artery bypass graft, liver and kidney dysfunction, other endocrine disorders, patients on beta blockers, angiotensin-converting enzyme inhibitors, angiotensin receptor blockers, other cardiac modulators, patients on treatment of osteoporosis, pregnant and lactating mothers, patients on calcium and vitamin D supplements in past one year, and any other concomitant medical conditions like osteosarcoma, etc., were excluded from the study. The study protocol was approved by the Institutional Ethics Committee of NKP Salve Institute of Medical Sciences and Research Center, Nagpur.

A proper history was taken and inclusion-exclusion criteria meticulously reviewed. The vital parameters were recorded. Blood pressure was recorded in sitting position on the right arm after giving rest of 15 min by mercury manometer. Anthropometric measurements included weight on Tanita scale, height on wall-mounted stadiometer, waist circumference and hip circumference were recorded. Body mass index (BMI) and waist-hip ratio were calculated. Blood sample (10 ml) was drawn and the following investigations were performed: Fasting blood glucose, fasting serum insulin levels, lipid profile, intact (PTH) iPTH, vitamin D levels, serum ionic calcium, serum phosphorus, and alkaline phosphatase. Vitamin D levels, i.e., 25(OH) D were measured by high-performance liquid chromatography (HPLC) method. iPTH was measured by chemiluminescence method using Siemens Centaur CPi kits. Structured questionnaire was given to them to assess ethnicity, physical activity, duration of sun exposure per day, smoking, use of sunscreen, and use of any drug supplements. Homeostatic model assessment (HOMA-IR) was calculated from fasting blood sugars (FBS) and insulin levels.

Statistical Analysis

Descriptive statistics are expressed as mean ± standard deviation. To study whether there is any significant difference in average levels of the various parameters under the study between cases (obese, insulin resistant) and controls (normal), we used Welch's *t*-test. Pearson's linear correlation was done between vitamin D levels and various parameters. Multivariate regression analysis was performed on pooled data to see independent associations of vitamin D with measures of IR.

RESULTS

A total of 594 patients were enrolled in the study after reviewing inclusion-exclusion criteria. Out of this, 34 were diagnosed to be diabetic hence excluded from the analysis. The final number of participants was 560. They were further divided into two groups: Cases (n = 168) with BMI >25 and controls (n = 392) with BMI <25. The prevalence of vitamin D deficiency in both cases and controls was found to be 413 (73.75%). The vitamin D deficiency in cases was seen to be 93.45% as against 65.3% in the control group. Based on vitamin D status, the individuals were divided into four groups, *viz.*: (1) Vitamin D sufficiency: (25(OH) D ≥30 ng/ml), (2) vitamin D insufficiency: (25(OH) D 20-30 ng/ml), (3) vitamin D deficiency: (25(OH) D 10-20 ng/ml), and (4) severe vitamin D deficiency: (25(OH) D ≤10 ng/ml). Table 1 shows that severe vitamin D deficiency was seen in 14.88% cases and 11.99% controls. Vitamin D sufficiency was seen in only 6.54% of cases and 34.69% of controls (Table 1).

Table 2 shows the anthropometric and biochemical parameters with comparison between two groups. Vitamin D levels (25(OH) D) in cases was seen to be 18.31 ±

11.53 ng/ml, and in controls, it was 26.4 ± 12.28 ng/ml with highly significant low levels in cases as compared with controls (P = 0.002 [HS]). Individuals with severe vitamin D deficiency having maximum IR (Table 3).

Table 3 shows that vitamin D levels show strong inverse relationship with all the parameters of IR except high-density lipoprotein levels with which it has strong positive correlation in both cases and controls.

In a pooled data, multivariate regression analysis was performed to see the independent relationship of vitamin D levels with insulin levels and HOMA-IR after adjusting for other confounders such as sex, age, BMI, and iPTH levels. The parameters of IR were significantly seen to be associated with vitamin D levels. Low vitamin D levels are an independent risk factor for IR (Table 4).

DISCUSSION

Our study reports the overall prevalence of vitamin D deficiency to be 73.75% which matches with the other studies from India. This is the first data reported from Central India about vitamin D status. Our study has found that the percentage of patients with severe vitamin D deficiency in insulin resistant cases (14.88%) is more than controls 11.99%. Vitamin D deficiency was found out to be 85 (50.59%) in cases as against 46 (19.38%) in controls which are highly significant.

The prevalence of vitamin D deficiency is reported to be between 70% and almost 90% in various studies from India.[12-15] The findings of our study match with these studies.

Notably, one of the clinical characteristics commonly associated with vitamin D deficiency is obesity. It has been proposed that vitamin D deficiency directly promotes IR.[16] The findings of our study support this theory. The data from various studies are discordant, while a study on 7904 individuals by Ford et al. showed negative correlation of vitamin D levels with metabolic syndrome.[17] Other studies did not find such association.[18,19] This difference from our study can be explained on the basis of very large sample size and difference in ethnicity of the sample. One more study by Sheena et al. had demonstrated independent associations of vitamin D with insulin sensitivity and beta-cell function in

Table 1: Vitamin D status

Category	Serum 25(OH) D in ng/ml			
	≤10 (%)	11-20 (%)	21-30 (%)	≥31 (%)
Cases (n=168)	25 (14.88)	85 (50.59)	47 (27.97)	11 (6.54)
Controls (n=392)	47 (11.99)	46 (19.38)	163 (41.58)	136 (34.69)

25(OH) D: 25-hydroxy vitamin D

Table 2: Anthropometric and biochemical parameters in obese subjects (cases n=168) and control (n=392)

Parameter	Cases n=168	Control n=392	P value
Age (in years)	38.68±10.86	37.38±11.21	0.925
BMI (kg/m^2)	29.14±7.64	23.69±2.7	0.0012 (HS)
Waist circumference	95.7±8.3	85.28±10.33	0.0000 (HS)
Hip circumference	98.8±10.38	91.44±12.18	0.006 (HS)
Waist-hip ratio	0.97±0.14	0.91±0.01	0.04 (S)
25(OH) D (ng/ml)	18.31±11.53	26.4±12.28	0.002 (HS)
iPTH (pg/ml)	55.38±16.41	48.51±10.33	0.04 (S)
Insulin (mIU/ml)	29.46±8.57	9.48±10.66	0.0001 (HS)
FBS (mg/dl)	90.37±9.28	85.3±10.48	0.004 (HS)
HOMA-IR	4.64±1.15	2.3±0.33	0.002 (HS)
Triglycerides (mg/dl)	146±13.86	114±18.42	0.003 (HS)
LDL (mg/dl)	113±16.48	102±14.63	0.02 (S)
HDL (mg/dl)	44±5.62	47.2±3.1	0.03 (S)

25(OH) D: 25-hydroxy vitamin D, BMI: Body mass index, FBS: Fasting blood sugar, HOMA-IR: Homeostatic model assessment-insulin resistance, LDL: Low density lipoprotein, HDL: High density lipoprotein, P<0.05 is taken as statistically significant, iPTH: Intact parathormone

Table 3: Correlation between vitamin D status, insulin levels and parameters of IR

Parameters	Magnitude of linear correlation (with P value)	
	Cases	Controls
Vitamin D versus FBS	−0.460 (P=0.02)	−0.374 (P=0.24)
Vitamin D versus serum insulin	−0.634 (P=0.01)	−0.547 (P=0.1)
Vitamin D versus HOMA	−0.876 (P=0.001)	−0.053 (P=0.804)
Vitamin D versus BMI	−0.7557 (P=0.000)	−0.304 (P=0.27)
Vitamin D versus WHR	−0.497 (P=0.019)	−0.233 (P=0.22)
Vitamin D versus systolic BP	−0.373 (P=0.34)	−0.021 (P=0.921)
Vitamin D versus diastolic BP	−0.573 (0.018)	−0.113 (P=0.607)
Vitamin D versus LDL	−0. 268 (0.199)	−0.276 (P=0.199)
Vitamin D versus HDL	0.765 (0.004)	0.863 (P=0.003)

HOMA-IR: Homeostatic model assessment-insulin resistance, FBS: Fasting blood sugar, BMI: Body mass index, LDL: Low density lipoprotein, HDL: High density lipoprotein, WHR: Waist-hip ratio, BP: Blood pressure

Table 4: Multivariate regression analysis

Per unit increase in baseline 25(OH) D	Coefficient of determination R	P value
Insulin	0.11	0.0002
HOMA-IR	0.14	0.0000

HOMA-IR: Homeostatic model assessment-insulin resistance, 25(OH) D: 25-hydroxy vitamin D

subjects without diabetes.[20] Another study in India by Dutta et al. has reported worsened state of vitamin D deficiency in insulin resistant individuals. Our study reports independent association of low levels of vitamin D with IR after adjusting for BMI and iPTH. This association clearly states role of vitamin D in regulating insulin sensitivity. These findings match the study from India by Dutta et al.

Longitudinal studies as done by Talaei et al. has shown FBS significantly reduced after vitamin D supplementation with significant reduction in HOMA-IR[21] as did Inzucchi et al. in diabetic patients.[22] Von Hurst showed that vitamin D supplementation significantly improved insulin sensitivity and IR.[23] though others have reported no improvement in IR after vitamin D supplementation.[24,25] Such prospective studies in future will further validate our finding of association of low vitamin D levels in IR.

Strengths of our study is a large sample size, estimation of vitamin D levels by HPLC, estimation of HOMA-IR as marker of IR instead of BMI and waist-hip ratio. Its cross-sectional nature is its limiting factor. The postulated mechanisms for the effects of vitamin D are the presence of vitamin D receptors on pancreatic cells, activation of 1 α hydroxylase by vitamin D, presence of vitamin D response element in insulin gene, presence of vitamin D receptors on skeletal muscle cells, and increase in transcription of insulin receptor genes by vitamin D.[7-10,21,26-28]

CONCLUSION

Vitamin D deficiency is rampant in Central India and shows strong independent association with IR.

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