Original Article

Association of 25 (OH) Vitamin D and Leptin in Individuals with Insulin Resistance

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Abstract

Introduction: Asian-Indian individuals with diabetes have been shown to have low vitamin D levels. Whether this hypovitamonisis D is associated with hyperleptinaemia is unclear. Also, whether this association is different in those with and without insulin resistance has not been ascertained. The present study aimed to investigate the association of 25-hydroxy vitamin D [25(OH) vitamin D] and leptin in individuals with and without insulin resistance. **Methods:** Ninety two individuals were recruited in two study groups (n = 46 each group). First group included individuals with insulin resistance (homeostasis model assessment of insulin resistance [HOMA-IR] ≥2.0). Second group included those without insulin resistance (HOMA-IR <2.0). Comparison of 25(OH) vitamin D, leptin, anthropometry, and biochemical parameters was done between two groups and correlations between 25(OH) vitamin D, leptin, and HOMA-IR were studied. **Results:** Individuals with insulin resistance were of simiar age (39.6 ± 5.3 years) and body mass index (24.4 ± 3.2 kg/m^2) as those without (39.5 ± 5.2 years and 23.6 ± 3.2 kg/m^2). Individuals with insulin resistance showed significantly lower 25(OH) vitamin D (17.8 ± 7.1 vs. 22.3 ± 11.6 ng/mL, P = .03) and significantly higher leptin levels (16.9 ± 15.8 vs. 9.6 ± 9.3 ng/mL, P = .09) compared to those without. Significant negative correlation was observed between 25(OH) vitamin D and leptin levels overall (r = -0.3, P = .008). HOMA-IR showed significantly negative correlation with 25(OH) vitamin D levels in individuals with insulin resistance (r = -0.33, P = .027). **Conclusion:** The present study found higher circulating leptin levels and lower 25(OH) vitamin D levels in individuals with insulin resistance. 25(OH) vitamin D levels were inversely associated with leptin levels particularly in women.

Keywords: 25(OH) vitamin D, diabetes, HOMA-I R, insulin resistance, leptin

Introduction

Studies have shown that vitamin D is associated with insulin resistance[1] and type 2 diabetes mellitus (T2DM)[2] and a few have also confirmed a pathological role in the development of insulin resistance and T2DM in obese individuals.[3] Vitamin D is also believed to regulate adipogenesis and energy metabolism.[3] It has been demonstrated to play an important role in regulation of leptin pathways and inhibition of adipogenesis.[4] Furthermore, invitro studies suggest that addition of 1,25 (OH) vitamin D3 to adipocyte culture inhibits leptin secretion.[5,6] A few clinical studies have also shown that leptin levels are inversely associated with vitamin D.[7-9]

Leptin resistance, marked by high circulating leptin levels, has been postulated as one of the mediators of insulin resistance and glycemic derangements in individuals with obesity.[10] Furthermore, studies have shown that pancreatic beta cells have leptin receptors[11] and that leptin inhibits insulin secretion from isolated rodent[12] and human islets[13] and from perfused rodent pancreas.[14] These studies suggest that leptin also influences insulin secretion through a direct action on beta cells. However, the inter-relationship of leptin and insulin sensitivity is confounded by the strong association between leptin and obesity and the effect of obesity on insulin

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sensitivity. Hence, there seems to be a complex inter-relation between leptin and insulin action as well.

Most of the studies which have attempted to elucidate the complex relationship of vitamin D with leptin have been conducted either in healthy individuals or individuals with T2DM who were either obese or nonobese.[7,9,15] There are very few studies on the association of vitamin D with leptin particularly in individuals with insulin resistance. The present study therefore aimed to investigate the association of 25 (OH) vitamin D and leptin in individuals with and without insulin resistance.

Materials and Methods

Institutional ethics committee–human research approved the study. Study procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation and with the Helsinki Declaration of 1975, as revised in 2000.

A total of 92 individuals in the age group of 30-50 years were recruited in two study groups (n = 46 each group). First group included individuals with insulin resistance (HOMA-IR ≥2.0). Second group included those without insulin resistance (HOMA-IR <2.0).

Inclusion criteria: Individuals with normal glucose tolerance (NGT), individuals with newly detected diabetes mellitus, and individuals with known diabetes. Exclusion criteria: Individuals with T1DM, individuals with T2DM for >5 years, those on insulin, individuals with chronic diseases, hyper/hypocalcemia, postmenopausal women, individuals with primary hyperparathyroidism, smokers, alcoholics (weekly intake of >130 g), individuals on antifungal, antiepileptic, or antitubercular drugs, and individuals on vitamin D/calcium supplements. Also, liver and kidney function tests, hemogram, calcium, and phosphorus were done to ascertain underlying diseases. Individuals with known diabetes were recruited from diabetes clinic of the institute. Individuals with newly detected diabetes mellitus and NGTs were recruited from the subjects attending Medicine OPD for minor ailments or attendants of patients admitted in medicine ward of the institute. Nearly equal number of males and females (22 and 24, respectively) was included in each group. NGT was confirmed on the basis of a 75g oral glucose tolerance test and American Diabetes Association criteria were followed for newly detected diabetes mellitus.

Fasting blood samples for biochemical estimations were collected in the morning after overnight fasting. Anthropometry, fasting and 2-h postprandial plasma glucose (post breakfast for individuals with known diabetes), A1c, fasting insulin, lipids, leptin, and 25(OH) vitamin D levels were measured in all study participants. Measure of insulin resistance (HOMA-IR) was calculated as follows: HOMA-IR = Fasting plasma glucose (mg/dl) × fasting insulin (μIU/ml)/405.

Serum insulin levels were measured by IRMA and 25 (OH) vitamin D levels by RIA (Beckman Coulter Inc, Czech Republic). Analytical sensitivity of insulin kit was 0.49 μIU/mL and coefficient of variation was <6% (inter-assay and intra-assay). Analytical sensitivity of vitamin D kit was 1.63 ng/mL and coefficient of variation was <7.5% (inter-assay and intra-assay). Serum leptin levels were measured by ELISA kit (BioVendor, Czech Republic). Analytical sensitivity of leptin kit was 0.2 ng/mL and coefficient of variation was <8% (inter-assay and intra-assay). Biochemical and haematological parameters were measured on auto-anylyzers (Beckman Coulter Inc, USA). Glycated hemoglobin (A1c) was measured by HPLC method (Bio-Rad D-10, USA).

Statistical analysis: Clinical and biochemical variables were compared between the two groups by unpaired *t*-test. Association between study variables was ascertained by Pearson's correlation test. Data were analyzed by SPSS Software (version 21.0) and differences were considered significantly different if *P* value was <.05.

Results

Details of clinical and biochemical parameters of study groups are given in Table 1. Study participants with insulin resistance showed significantly lower 25(OH) vitamin D and significantly higher leptin levels compared to those without insulin resistance [Table 2]. Female study participants showed significantly lower 25 (OH) vitamin D and significantly higher leptin levels compared to male participants overall [Figure 1]. However, 25(OH) vitamin D and leptin levels were comparable in men with and without insulin resistance and in women with and without insulin resistance.

Table 1: Details of clinical and biochemical parameters in two study groups

Variable	Individuals with insulin resistance (HOMA-IR ≥2.0)	Individuals without insulin resistance (HOMA-IR <2.0)	*P*
Age (years)	39.6±5.3	39.5±5.2	0.9
Male: Female (*n*)	1:1.1 (22/24)	1:1.1 (22/24)	-
BMI (Kg/m^2)	24.4±3.2	23.6±3.2	0.3
Waist (cm) - Male	92±8	87±11	0.014
Waist (cm) - Female	85±10	81±11	0.07
SBP (mmHg)	125±8	121±10	0.017
DBP (mmHg)	75±5	76±6	0.5
FPG (mg/dL)	161±42	86±9	< 0.001
2-h PPPG (mg/dL)	245±64	113±15	< 0.001
A1c (%)	8.9±1.6	5.2±0.4	< 0.001
Serum Insulin (μIU/mL)	10.3±6.8	6.2±3.9	< 0.001
HOMA-IR	4.3±2.1	1.3±0.7	< 0.001
CHL (mg/dL)	164±25	157±32	0.2
TG (mg/dL)	138±73	113±49	0.06
HDLc (mg/dL)	40±8	42±12	0.8
LDLc (mg/dL)	96±5	93±10	0.07

Significant negative correlation was observed between serum 25(OH) vitamin D and leptin levels. A significant negative correlation was also observed between serum 25 (OH) vitamin D and leptin levels in female study participants but not in male participants. HOMA-IR showed significantly negative correlation with 25(OH) vitamin D levels only in those with insulin resistance [Table 3]. Significant positive correlation of HOMA-IR was also found with leptin levels overall (r = 0.26, *P* = .013, n = 92).

Discussion

The present study found higher leptin levels and lower 25 (OH) vitamin D levels in individuals with insulin resistance compared to those without. There was a significant negative association of 25 (OH) vitamin D with leptin. Although HOMA-IR correlated positively with leptin levels in all study participants, a significant but negative association with 25 (OH) vitamin D was observed only in individuals with insulin resistance.

Our study included individuals of both genders with varying weight categories and glucose tolerance. Categories of weight ranged from nonobese to overweight to obese and categories of glucose tolerance included normal glucose tolerance, newly detected diabetes mellitus, and knwon T2DM. In contrast, most of the earlier studies that have attempted to investigate the association of vitamin D with leptin[7,9,15] included either healthy individuals or individuals with T2DM who were either obese or nonobese and their results may not be universally applicable.

The finding of significant negative association between serum 25 (OH) vitamin D and leptin levels of our study confirms the findings of few recent studies which have also reported a significant association between vitamin D and leptin.[9,15] Although the underlying cause for the negative correlation cannot be ascertained from our study, there can be several explanations. First, this may be due to a direct inhibitory effect of vitamin D on leptin secretion from adipose tissue;[5] second, vitamin D deficiency has been shown to contribute to leptin resistance[16,17] leading to hyperleptinemia and insulin resistance. It is also possible that obesity-induced hyperleptinemia may lead to lower 25-hydroxy vitamin D levels due to a decrease in conversion of vitamin D to 25-hydroxy vitamin D.[7] Effects of vitamin D supplementation on leptin levels have not been consistent as leptin levels improved in some studies[8,18] but not in others.[19,20] It has been suggested[21] that vitamin D–mediated inhibition of leptin secretion from adipocytes may be relevant only during early stages of obesity while other mechanisms may be more important with progressive and persistent obesity. Most of our study participants were either nonobese or mildy obese. Hence, it would appear that at this stage it is more likely that vitamin D deficiency and the concommitent increase in leptin secretion causes hyperleptinemia. It is possible that as the degree of obesity increases, the effect of obesity-related hyperleptinemia may become more important resulting in lowering of vitamin D. However, the exact mechanism by which vitamin D interacts with leptin secretion and its role in in-vivo regulation of leptin levels is not fully understood and needs to be ascertained. Whether low vitamin D levels lead to hyperleptinemia or hyperleptinemia leads to lowering of vitamin D levels cannot be answered with certainty from the

Table 2: Comparison of 25(OH) vitamin D and leptin levels between two study groups

Variables	Individuals with insulin resistance (HOMA-IR ≥2.0)	Individuals without insulin resistance (HOMA-IR <2.0)	*P*
25 (OH) vitamin D (ng/mL)	17.8±7.1	22.3±11.6	0.03
Leptin (ng/mL)	16.9±15.8	9.6±9.3	0.009

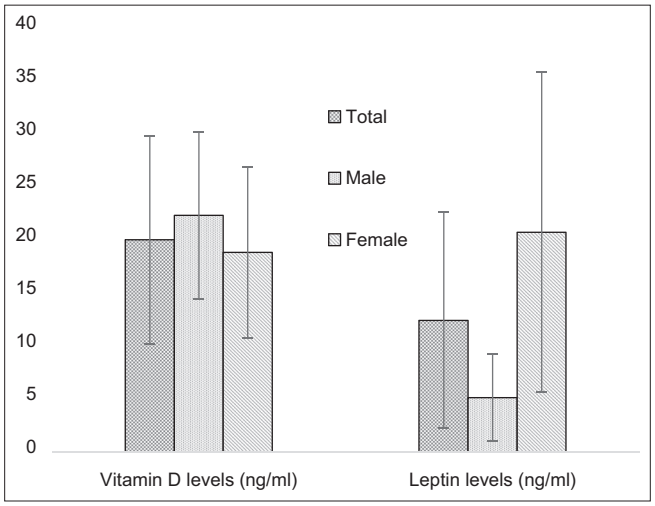

Figure 1: Comparison of vitamin D and leptin levels between male and female study participants

Table 3: Correlations between HOMA-IR, 25(OH) vitamin D, and leptin levels

	25(OH) vitamin D*		Leptin		HOMA-IR (>2.0)	
	Correlation coefficient (*r*)	Statistical significance (*P*)	Correlation coefficient (*r*)	Statistical significance (*P*)	Correlation coefficient (*r*)	Statistical significance (*P*)
25(OH) vitamin D	-	-	-0.3	0.008	-0.33	0.027
Leptin	-0.3	0.008	-	-	0.13	0.4
HOMA-IR (>2.0)	-0.33	0.027	0.13	0.4	-	-

*on subanalysis by gender, significant correlation of 25(OH) vitamin D with leptin was observed only in females (*r*=-0.3, *P*=0.038) but not in males (*r*=-0.047, *P*=0.7).

present study and further studies are required to clarify this complex association.

We also found higher circulating leptin levels in individuals with insulin resistance and a significant association of leptin with HOMA-IR. Similar association has also been reported earlier[22,23] and may be explained by obesity-associated leptin resistance at hypothalamus that results in insulin resistance at different peripheral tissues like liver and skeletal muscles.[24,25]

Our study shows lower 25-hydroxy vitamin D levels in individuals with insulin resistance and a significant inverse association of serum 25 (OH) vitamin D levels with HOMA-IR only in those with insulin resistance. This finding suggests that the effect of 25 (OH) vitamin D levels, if any, on insulin sensitivity are significant only at higher levels of insulin resistance. It could also mean that insulin resistance secondarily causes hypovitaminosis D. However, we cannot comment on this issue with certainity as this was a cross-sectional study. Also, literature on this issue is divided. Epidemiological studies have largely demonstrated that lower vitamin D levels are associated with an increased risk of insulin resistance.[26] Therefore, further studies are warranted to address this specific issue.

This study has few limitations. First, this was a cross-sectional study, where a single measurement of serum vitamin D and leptin levels was done, so a causative association cannot be ascertained. Second, iPTH was not measured which could have provided information on the functional significance of vitamin D deficiency, and third, inclusion of individuals who were lean or overtly obese (body mass index >30 kg/m^2) could have further clarified the association of leptin with vitamin D across the entire spectrum of weight categories.

In conclusion, the present study found a significant negative association of serum 25 (OH) vitamin D with leptin levels overall. Circulating leptin levels were higher and 25 (OH) vitamin D levels were lower in individuals with insulin resistance.

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Conflicts of interest
There are no conflicts of interest.

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