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Leptin, hs-CRP and HOMA-IR in patients with type 2 diabetes: The role of different levels of vitamin D deficiency

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ABSTRACT

Background: To evaluate the effects of different levels of vitamin D deficiency on blood glucose, leptin, high sensitivity C-reactive protein (hs-CRP), and insulin resistance and their associations.

Methods: We quantified serum vitamin D level, hs-CRP, leptin, and fasting blood sugar (FBS) levels in 25 type-2 diabetic patients with deficient serum levels of 25-hydroxy vitamin D (≤15 ng/ml) and 25 type-2 diabetic patients with insufficient serum levels of 25-hydroxy vitamin D (16 to 30 ng/ml). The two groups were matched according to age, sex, and body mass index (BMI). Homeostasis model assessment of insulin resistance (HOMA-IR) was calculated by a formula based upon values of FBS and insulin concentrations.

Results: The mean value of vitamin D levels was 7.67±3.10 in the vitamin D deficient group and 23.20±9.97 in the vitamin D insufficient group. Patients with vitamin D deficiency had significantly higher FBS, postprandial glucose (PPG) and hemoglobin A1C (HbA1C) as compared to vitamin D insufficient group ($p<0.01$). We studied correlations of hs-CRP, HOMA-IR, and leptin in both vitamin D insufficient and deficient groups. There were significant positive correlations between leptin with hs-CRP ($r = 0.58$, $p<0.01$) and with HOMA-IR ($r = 0.49$, $p<0.05$) in vitamin D deficient group. These correlations remained significant after multiple adjustment for age, sex, BMI, systolic and diastolic blood pressure.

Conclusion*:* In conclusion, vitamin D deficient diabetic patients had elevated FBS, PPG and HbA1C compared with insufficient ones. The results also could possibly point the effect of vitamin D deficiency level on leptin associations with hs-CRP and insulin resistance.

Keywords: Vitamin D deficiency, type 2 diabetes, leptin, C reactive protein, homeostasis model assessment of insulin resistance

INTRODUCTION

Vitamin D deficiency is an increasing public health concern[1]. In type 2 diabetes, the prevalence of vitamin D deficiency is 20% higher than in non-diabetics[2]. Low vitamin D status can be caused by a number of factors, including insufficient cutaneous synthesis (due to limited sunlight exposure or aging), inadequate intake and absorption of vitamin D, or obesity[3]. High global prevalence and disease burden of vitamin D deficiency has led to the introduction of a roadmap for action in low and middle income countries[4]. Data from studies suggest that widespread vitamin D deficiency persists regardless of human development index of the country and fortification policies[5].Vitamin D is not only important in mineral homeostasis but it is also an anti-inflammatory hormone that can regulate immune responses, cell proliferation, and endothelial function[6,7]. Vitamin D deficiency is playing an important role in the genesis of coronary risk factors and cardiovascular disease, and seems to predispose to hypertension, diabetes and the metabolic syndrome [8,9]. Vitamin D is thought to have both direct (by the activation of the vitamin D receptor) and indirect (by the regulation of calcium homeostasis) effects on various mechanisms related to the pathophysiology of type 2 diabetes[10]. The effect of vitamin D on pancreatic cells and subsequent insulin release is mediated through vitamin D receptors [11]. Although the mechanisms are not fully understood, vitamin D deficiency impairs insulin secretion of pancreatic cells and increases insulin resistance, which are major factors in the pathogenesis of type 2 diabetes[12,13]. Animal and in vitro studies provide evidence that vitamin D indirectly impacts diabetes through insulin resistance[14]. The simplest and most commonly used surrogate estimate of insulin resistance is the homeostasis model assessment of insulin resistance (HOMA-IR). HOMA-IR is calculated by a formula based upon values of fasting blood sugar (FBS) and insulin concentrations[15].

Leptin is an adipocyte-secreted hormone that has been proposed to regulate energy homeostasis as well as metabolic, neuroendocrine, and immune functions. Leptin may also directly regulate glucose homeostasis independently of its effects on adiposity. Leptin affects glycemia at least in part via the central nervous system, but it may also directly regulate the physiology of pancreatic β-cells and peripheral insulin-sensitive tissues[16]. Vitamin D present a direct inhibitory effect on leptin secretion from human adipose tissue culture[17].

Active vitamin D also diminishes the expression of proinflammatory cytokines which can induce production of C-reactive protein (CRP) [18]. Clinical data underlie the importance of both CRP and leptin in estimating CVD risk especially in clinical states where chronically elevated CRP levels and leptin resistance coexist[19].

Here we aimed to study the effects of different levels of vitamin D hypovitaminosis on blood glucose, insulin resistance, leptin and high-sensitivity CRP (hs-CRP) and their associations.

MATERIALS AND METHODS

This is a descriptive cross-sectional study on 50 patients with type 2 diabetes referring to the diabetes clinic of Vali-asr Hospital, affiliated with Tehran University of medical science. The diagnosis of diabetes was made based on American Diabetes Association 2015[20]. Exclusion criteria were smoking, pregnancy, proteinuria, renal involvement (creatinine > 1.5 mg/dl or GFR < 70 cc/min), glomerulonephritis, congestive heart failure, insulin therapy, and hospital admission in recent months. None of the participants had any overt diabetic complication. Findings from studies in Iran have demonstrated that vitamin D deficiency is a highly prevalent problem among Iranians [21,22]. Even though, no optimal level of 25-hydroxy vitamin D [25(OH)D] has been agreed upon, Most agree that A circulating level of 25-OH-D

concentrations >30 ng/mL are considered to be sufficient and is required to maximize vitamin D's beneficial effects for health[23]. According to the baseline values, all of diabetic patients in our study were vitamin D deficient or insufficient and none of the patients had sufficient levels (>30 ng/ml) of serum 25(OH)D. A total of 25 type-2 diabetic patients with very low serum levels of 25-OH-D (≤15 ng/ml; deficient) and 25 type-2 diabetic patients with low serum levels of 25-OH-D (16 to 30 ng/ml; insufficient) were recruited[9]. We defined vitamin D deficiency and insufficiency according to the ranges proposed by the recently published K/DOQI guidelines. Thus, vitamin D stores are considered adequate when 25(OH)D levels are above 30 ng/ml. Levels between 16 and 30 ng/ml represent vitamin D insufficiency. Vitamin D deficiency is defined as 25(OH)D levels less or equal to 15 ng/ml[24,25].Two groups were matched according to age, sex, and body mass index (BMI). Diabetes mellitus was diagnosed according to the criteria of the American Diabetes Association[26]. Use of vitamin D supplements, drugs with known effect on serum concentrations of vitamin D, smoking, pregnancy, creatinine >1.5 mg/dl or GFR<70 cc/min, glomerulonephritis, congestive heart failure, use of antioxidant, statins, hormone replacement therapy and hospital admission in previous 6 months, history of rheumatologic, gastrointestinal, and liver dysfunctions were defined as exclusion criteria. Demographic and anthropometric data including age, sex, duration of diabetes, height, weight in light clothing, and blood pressure in sitting position were recorded.

Blood pressure was re-measured twice after 5 min and averaged. The BMI (kg/m2) was calculated according to the Quetelet formula. HOMA-IR was calculated according to; fasting insulin (U/mL) × FBS (mg/ld.)/405 [27]. The research was carried out according to the principles of the Declaration of Helsinki. The local ethics review committee of Tehran University of Medical Sciences approved the study protocol. All the patients received and signed written informed consent.

Blood samples

Blood samples were collected after 12 hours of fasting. They were centrifuged and kept at -70 ◦C until analysis. Serum creatinine, FBS, total cholesterol, triglycerides, high-density lipoprotein cholesterol (HDL-C), low density lipoprotein-cholesterol (LDL-C), and HbA1c were measured for all participants. Glucose measurements (intra-assay coefficient of variants [CV] 2.1%, intra-assay CV 2.6%) were carried out using the glucose oxidase method. Insulin was measured (intra-assay CV 4.7%, inter-assay CV 3.3%) by immune radiometric method (Immunotech, Finland). Cholesterol, HDL-C, LDL-C and triglycerides were determined using direct enzymatic methods (Parsazmun , Karaj, Iran). Serum leptin concentration was determined using an enzyme-linked immunosorbent assay (DRG Instruments GmbH, Germany), with an intra-assay coefficient of variation of 5.9-6.9% and an inter-assay coefficient of variation of 8.6-11.5%. Hs-CRP was assessed using a two-site, enzyme-linked immunosorbent assay (ELISA) (Diagnostic Biochem, London, Ontario, Canada). Sensitivity of the assay was 10 ng/L. Intra- and inter-assay coefficients of variation (CV) were 8% and 10% respectively. Serum 25(OH) Vitamin D was determined using enzyme-linked immunosorbent assay technique (DIAsource, Louvain-la-Neuve, KAP1971, Belgium) Intra- and inter-assay coefficients of variation (CV) were 7.8% and 7.4% respectively. Limit of detection was 2.81 ng/ml.

Statistical analysis

Data are analyzed using SPSS software (version16.0; SPSS, Chicago, Illinois, USA). Smirnov-Kolomogrov test was employed to test the normality of the variables in each group. The continuous variables are expressed as means ± standard deviation (SD). Quantitative variables

were compared between the two groups using t-test and Mann-Whitney test, while qualitative variables were compared with chi-square test. The magnitude of the relationship between continuous variables (leptin, hs-CRP and HOMA-IR) was measured using the Pearson correlation coefficient for linear relationships. Partial correlation coefficient after multiple adjustments for age, gender, BMI and systolic blood pressure (SBP) and diastolic blood pressure (DBP) were also calculated. P values < 0.05 were considered statistically significant.

RESULTS

Demographic and biochemical characteristics of the participants are illustrated in table1. There was no significant difference between groups with respect to age, sex, duration of diabetes, BMI, systolic and diastolic blood pressure, cholesterol, LDL-C, HDL-C, triglycerides and also medications (antidiabetic agents and hypertensive drugs).

Table 1: Baseline Characteristics of study population

	Vitamin D Deficient (≤15 ng/ml **)**	**Vitamin D Insufficient (**16 - 30 ng/ml)	**P-value**
Number	25	25	
Gender (male, female)	(13,12)	(7,18)	NS
Age(years)	56.56±11.79	55.60±14.20	NS
BMI (kg/m^2)	28.32±4.47	27.54±3.37	NS
Waist circumference (cm)	94.08±10.76	93.84±9.33	NS
Duration of diabetes (year)	9.42 ± 6.4	9.65 ± 5.31	NS
Blood pressure (mmHg)			
SBP	127.76±21.94	132.16±20.14	NS
DBP	79.92±11.63	76.12±12.80	NS
MAP	95.86±14.20	94.80±9.33	NS
Plasma glucose (mg/dl)			
FBS	199.61±54.74	146.78±46.4	**< 0.01**
PPG	284.1 ± 68.4	199.83 ± 40.74	**< 0.01**
HbA1C (%)	8.37±1.81	6.93±1.33	**< 0.01**
Insulin(µu/ml)	22.38±15.21	18.19±12.06	NS
HOMA-IR	9.16±5.83	7.16±5.47	NS
Hs-CRP(mg/ml)	4.23±0.43	3.09±0.24	NS
Creatinine(mg/dl)	0.96±0.22	0.99±0.27	NS
Urea(g/24h)	27.13±13.53	30.3±16.72	NS
Uric Acid(mg/dl)	5.27±1.07	5.23±1.25	NS
Plasma cholesterol (mg/dl)			
Total	187.38±29.72	190.78±52.72	NS
LDL	106.05±27.96	108.91±42.95	NS
HDL	43.75±9.71	44.13±10.86	NS
Plasma triglyceride (mg/dl)	194.67±86.82	155.95±70.51	NS
Vitamin D(ng/dl)	7.67±3.10	21.94±5.98	**< 0.001**
Leptin (ng/mL)	79.92±11.63	76.12±12.80	NS
Medication (%)			
OAD	80	76	NS
Insulin	15	16	
OAD + Insulin	8	4	
Antihypertensive drugs	32	34	NS

Abbreviations: BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; MAP, mean arterial pressure; FBS, fasting blood sugar; PPG, postprandial glucose; HbA1C, hemoglobin A1C; HOMA-IR, homeostasis model assessment- Insulin resistance; hs-CRP, high-sensitivity C-reactive protein; LDL, low density lipoprotein; TG, Triglyceride; HDL; high density lipoprotein; OAD: Oral antidiabetic drug.

The mean value of vitamin D levels was 7.67±3.10 in the vitamin D deficient group and 23.20±9.97 in the vitamin D insufficient group. FBS, PPG and HbA1C levels were significantly higher in vitamin D deficient patients ($P < 0.01$), whereas other covariates did not show any significant difference (Table 1). We studied correlations of leptin with hs-CRP and HOMA-IR in both vitamin D insufficient and deficient groups (Figure 1 and 2). There were only significant positive correlations between leptin with hs-CRP ($r = 0.58$, p value<0.01) and with HOMA-IR ($r = 0.49$, p value<0.05) in vitamin D deficient group; These correlations remained significant after multiple adjustment for age, sex, BMI, systolic blood pressure (SBP) and diastolic blood pressure (DBP) (Table 2).

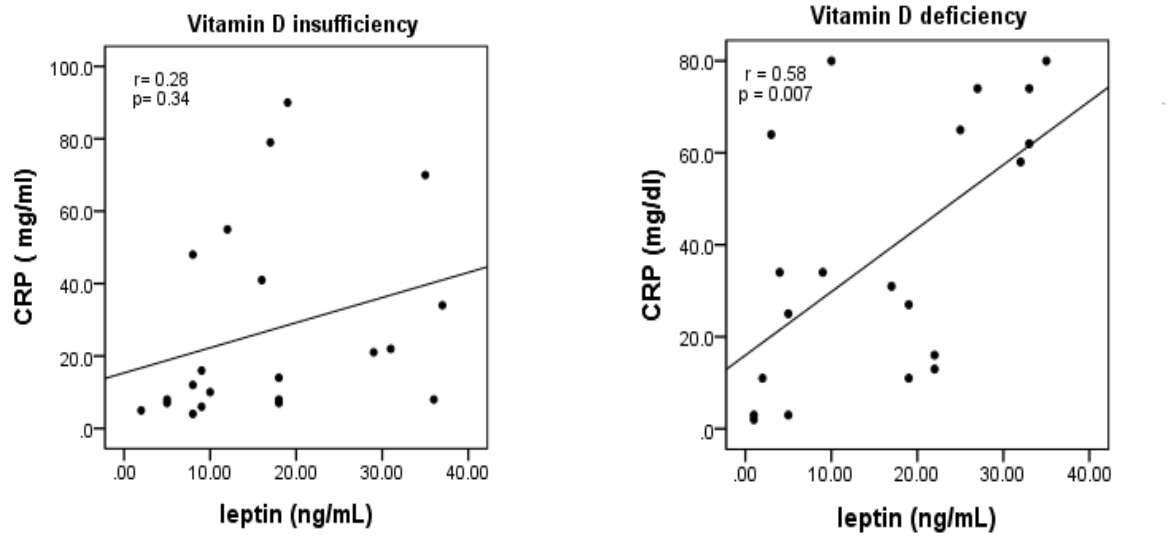

Fig 1: Scatter plot demonstrating the correlation between leptin and hs-CRP in vitamin D insufficient (16 to 30 ng/ml) and vitamin D deficient (≤15 ng/ml) type 2 diabetic patients.

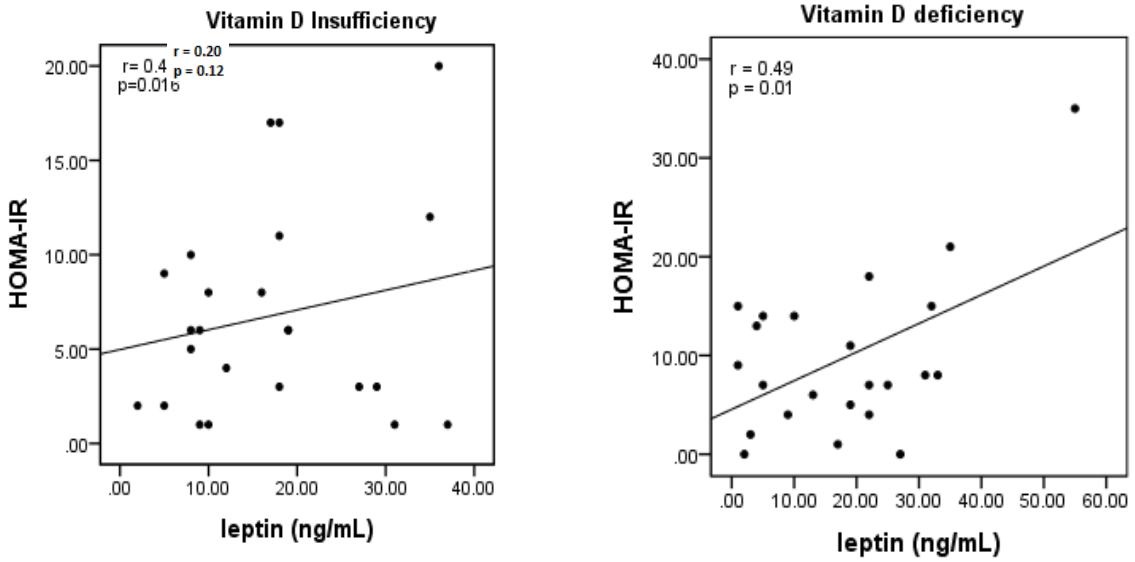

Fig 2: Scatter plot demonstrating the correlation between leptin and HOMA-IR in vitamin D insufficient (16 to 30 ng/ml) and vitamin D deficient (≤15 ng/ml) type 2 diabetic patients.

Table 2*:* Correlation coefficient (r) between leptin with hs-CRP and HOMA-IR in vitamin D deficient and insufficient type 2 diabetic patients before and after adjustment for age, sex, BMI, systolic blood pressure (SBP) and diastolic blood pressure (DBP).

Leptin correlation	**with**	Without adjustment	Adjusted for			
			Age and sex	BMI	SBP and DBP	Age, sex, BMI, SBP, DBP
Vitamin D Deficient (≤15 ng/ml)	**Hs-CRP**	0.58**	0.57*	0.57**	0.61**	0.59*
	HOMA	0.49*	0.58**	0.49*	0.52*	0.59**
Vitamin D Insufficient (16-30 ng/ml)	**Hs-CRP**	0.28	0.45*	0.09	0.38	0.40
	HOMA	0.20	0.20	0.19	0.30	0.32

*P<0.05 **P<0.01

Abbreviations: BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; hs-CRP, high-sensitivity C-reactive protein; HOMA-IR, homeostasis model assessment- Insulin resistance.

DISCUSSION

Vitamin D deficiency, as a vital problem in the new civilization, has attracted several investigations in the medicine. In a systemic review, the prevalence of vitamin D insufficiency in Iranian children and adolescents was 31% (CI 95% 30–31) [28].The present study demonstrated that serum FBS, PPG and HbA1C are higher in vitamin D deficiency in type 2 diabetic patients. Our results are generally consistent with previous reports [3,29]. It has been shown that deficiency in serum 25(OH)D levels decrease insulin secretion and increase peripheral insulin resistance and blood glucose[30]. With regard to glucose homeostasis, vitamin D affects pancreatic beta-cell proliferation and survival and its ability to respond to situations of increased insulin demand such as in type 2 diabetes.[3]. Vitamin D deficiency may cause disturbances in the vitamin D signaling pathway. In vivo study showed impaired insulin secretion in vitamin D-deficient rats and clearly established a molecular role of the Vitamin D receptor in the endocrine function of the pancreas[31].

We compared the correlations among leptin, CRP and HOMA-IR in vitamin D deficient and insufficient diabetic patients and we showed a significant positive association between leptin with CRP and HOMA-IR in vitamin D deficient group. These correlations remain significant after multiple adjustments for age, sex, BMI, systolic blood pressure and diastolic blood pressure. Similarly several studies have demonstrated a significant positive correlation between insulin resistance and leptin , independently of body weight or adiposity, in both normoglycemia and diabetes[32,33]. A population-based study by Zimmet et al. suggested that insulin resistance or concentration may contribute to the relatively wide variation in leptin levels or alternatively, leptin may play a role in the etiology of insulin resistance[34]. As stated earlier, vitamin D deficiency increases insulin resistance which has been found to induce leptin production [35]. Mechanism of both direct and centrally-mediated leptin action on the β-cell has been proposed to occur through sympathetic inhibition of β-cell (glucose stimulated) insulin secretion and improving peripheral insulin sensitivity [36-38] but the subjects with insulin resistance may be relatively resistant to these effects of leptin [32]. Effects of leptin on glucose homeostasis and the

ability of leptin to induce or improve insulin resistance suggests that a complex interplay exists between direct peripheral and centrally mediated effects of the hormone[39]. Interactions between leptin and insulin might have important implications in disruption of either insulin or leptin signaling associated with the metabolic syndrome[40,41]. Evidence from in vivo and in vitro studies supports the hypothesis that leptin and insulin signaling networks may overlap on several levels and play a crucial role in the regulation of glucose homeostasis. Leptin and insulin resistance occurring in the brain in pathological states such as diabetes are associated with insulin resistance in the periphery [16,41].

As mentioned above we observed a significant correlation between leptin and hs-CRP in the deficient group. This result was the same as previous studies [37,42,43]. In line with our study, Shamsuzzaman et al. demonstrated that increased leptin is associated with increased CRP independently of gender, measures of adiposity, and other variables[44]. Similarly Goya et al. showed that increased leptin was independently associated with significant increases in inflammatory markers such as CRP [45]. Leptin can be described as a cytokine-like hormone with pleiotropic actions which can stimulate CRP production by the liver[46]. This association is the results of adipo-hepato-regulatory loop that involves stimulation of CRP expression by leptin and the feedback inhibition of leptin functions by CRP[47]. In chronic inflammatory states, CRP directly inhibits the binding of leptin to its receptors and blocks its ability to signal and leptin itself is able to directly stimulate CRP synthesis from the liver and from the vasculature[19,47]. The interactions of leptin, HOMA-IR and hs-CRP in vitamin D deficient patients with type 2 diabetes were shown in Fig 3. No associations were found both between vitamin D and plasma glucose and between vitamin D and insulin resistance in our study. This result is consistent with Vilarrasa et al study[48].

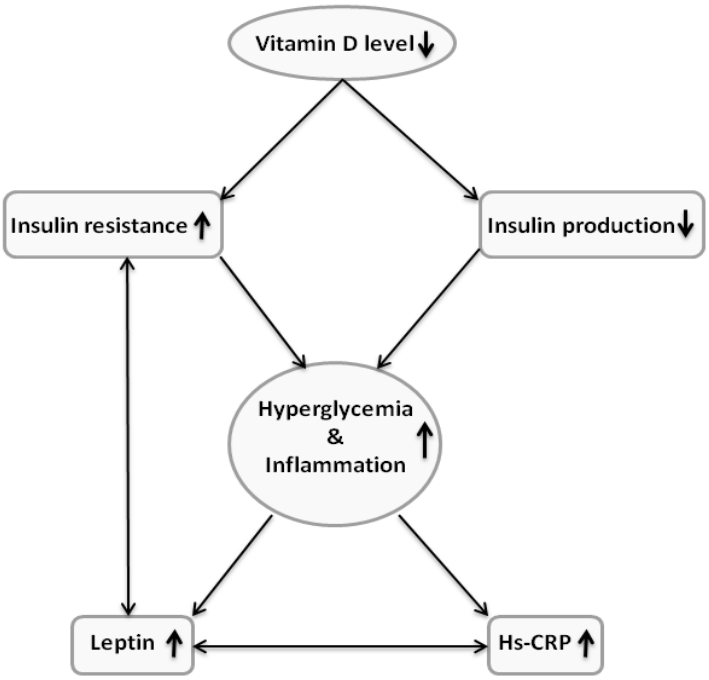

Fig 3: Theoretical framework for the interactions of leptin, HOMA-IR and hs-CRP in vitamin D deficient patients with type 2 diabetes. Vitamin D deficiency impairs insulin secretion and increases insulin resistance which results in aggravation of hyperglycemia (inflammatory state) and increasing the serum levels of hs-CRP and leptin. There are bidirectional positive associations between leptin and hs-CRP and also between leptin and Insulin resistance. Leptin and Insulin directly regulate each other.

CONCLUSION

Vitamin D deficient patients with diabetes had elevated FBS and PPG levels compared with insufficient ones. The findings presented herein have therapeutic implications. Diagnosis and treatment of vitamin D deficiency is easy and its correction may have a great therapeutic impact in diabetes. This study hypothesizes that, in patients with type 2 diabetes mellitus, normal levels of vitamin D in the blood may facilitate glucose control. The results also could possibly point the effect of vitamin D deficiency level on leptin associations with CRP and insulin resistance.

List of Abbreviations: HOMA-IR, Homeostatic Model Assessment for Insulin Resistance; hs-CRP, High-sensitivity C-reactive Protein; CRP, C-reactive protein; FBS, Fasting Blood Sugar; BMI, Body mass index; PPG, Postprandial Glucose; CVD, Cardiovascular disease; GFR, Glomerular Filtration Rate; HDL-C, High-density lipoprotein cholesterol; LDL-C, Low-density lipoprotein cholesterol; CV, coefficient of variation; SD, standard deviation; SBP, systolic blood pressure; DBP, diastolic blood pressure; HbA1C, hemoglobin A1c.

Competing interest: There are no conflicts of interest to declare.

Author's Contributions: M Nakhjavani designed the study. A Esteghamati, S Rabizadeh and SS Salehi participated in the data collection, statistical analysis and drafting of manuscript. H Mirmiranpour participated in the laboratory evaluation, clinical assessment and study design. S Karimpour participated to provide oversight when the manuscript was being drafted. Marjan Mouodi provided advice regarding development of the protocol for the study. All authors read and approved the final manuscript.

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