Impact of serum levels of vitamin D on lipid profiles, glycemic indices, and insulin resistance in obese type 2 diabetes patients: An observational study [version 1; peer review: awaiting peer review]

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Abstract

Background: Diabetes patients have a higher chance of developing dyslipidemia and increased release of free fatty acids, which participate in developing insulin-resistant fat cells. On the other hand, vitamin D insufficiency is linked to the evolution of type 2 diabetes mellitus (T2DM). This study examines the impact of vitamin D serum levels on lipid profiles and insulin resistance concerning glycemic indices in obese T2DM patients.

Methods: During the data collecting stage, 47 diabetes patients were chosen from the out-patient clinic. The control individuals were selected from the general population and were equivalent to the matching patients, with a total of 43 healthy participants. After an overnight fast, a venous blood sample was collected from each individual to test insulin and vitamin D3 levels using particular ELISA kits. In addition, by colorimetric test, serum was used to estimate total cholesterol, triglyceride, and high-density lipoprotein cholesterol. Aside from that, fasting serum glucose levels were measured (FSG).

Results: Fasting serum glucose (FSG), homeostatic model assessment-insulin resistance (HOMA-IR), total cholesterol, and triglycerides, all of these values were significantly elevated in people with diabetes as compared to controls (p-value <0.05) when the serum level of vitamin D was markedly low. In contrast, insulin and high-density lipoprotein values had decreased significantly in the diabetic population compared to controls (p-value <0.05) and were not correlated to vitamin D levels.

Conclusions: Diabetes patients had higher FSG, HOMA-IR, hemoglobin A1c (HbA1c), fasting insulin, triglycerides, total cholesterol to high-density lipoprotein cholesterol ratios (TC: HDL-C), triglyceride to high-density lipoprotein cholesterol ratios (TG: HDL-C), and low-density lipoprotein cholesterol to high-density lipoprotein cholesterol ratios (LDL-C: HDL-C) compared to controls.
cholesterol ratios (LDL-C: HDL). In obese diabetic individuals, vitamin D levels had a significant impact on total cholesterol, LDL-C, and the TC:HDL-C ratio.

**Keywords**
diabetes mellitus, triglyceride, cholesterol, insulin, vitamin D
Introduction
Diabetes mellitus (DM) is a metabolic disorder specified by persistent hyperglycemia with varying degrees of deterioration in the metabolism of carbohydrates, lipids, and proteins. Thus, diabetes patients have a higher chance of developing dyslipidemia and increased release of free fatty acids, which participate in developing insulin-resistant fat cells. Meanwhile, high concentrations of free fatty acids stimulate the creation of triglycerides; this boosts the release of apolipoprotein B (ApoB) and very low-density lipoprotein (VLDL), both have been related to an elevated risk of cardiovascular disease (CVD). Furthermore, hyperglycemia also has a negative effect on lipoproteins (especially low-density lipoprotein (LDL) and VLDL) by elevating their glycation and oxidation, thereby promoting the progression of aggressive atherosclerosis.

On the other hand, vitamin D insufficiency is linked to the evolution of T2DM. Therefore, mild vitamin D deficiency is linked to insulin resistance in several pathways, including low-grade inflammation caused by an unbalanced innate immune system, associated pro-inflammatory cytokines, and enhanced acute phase reactants.

This research aims to investigate the effects of vitamin D serum level on glycemic indices in relation to lipid profile in Iraqi obese type 2 diabetics compared to healthy controls with comparable vitamin D levels.

Methods
Ethical consideration
On 5 January 2022, the ethics committee of the College of Pharmacy at the University of Baghdad formally approved the research proposal (approval code: 542). In addition, all study participants gave written informed consent before participating.

Study design
An observational, case-control study that compared adults with type 2 diabetes mellitus with healthy controls.

Setting
From April to July 2022, a case-controlled study was carried out at two facilities in Baghdad, Iraq, including the Diabetes Centers at The Medical City Complex and AL-Kadhimiya Teaching Hospital.

Study groups
A total of 47 diabetic patients aged (35–64) years (nine male and 38 female) were selected from the out-patient clinic. Diabetic patients were diagnosed according to the American Diabetes Association ADA. The control subjects were comparable to the corresponding patients, with a total number of 43 healthy subjects (nine male and 34 female), aged (37–65) years, and were chosen from the general population as follows:

Group-1: Type 2DM patients have normal serum vitamin D levels ≥ 22.5 ng/ml, including 22 patients (four male, 18 female).

Group-2: Type 2DM patients have low serum vitamin D levels < 22.5 ng/ml, included 25 patients (five male, 20 female).

Group-3: Control subjects have normal serum vitamin D levels ≥ 22.5 ng/ml. It included 20 healthy subjects (four male, 16 female).

Group-4: Control subjects have low serum vitamin D levels <22.5 ng/ml, including 23 healthy subjects (five male, 18 female).

Eligibility criteria
Adults (>18 years), both sexes eligible, patients with an established diagnosis of type 2 DM according to the American Diabetes Association diagnostic criteria and not taking medication or on sulfonylureas only, subjects with BMI ≥ 30 (obese).

Exclusion criteria
Hepatic disease: liver disease accompanied by insulin resistance and hyperinsulinemia. Renal diseases: resulting in diminished 1,25(OH)2D (calcitriol) and elevated parathyroid hormone (PTH) levels occurring early in the course of renal function decline (patients with GFR <90). Malignancy, chronic autoimmune diseases, any history of the use of drugs such as insulin, metformin, antihyperlipidemic drugs, vitamin D, corticosteroids or hormone-containing drugs within the three months preceding the start of the study, and women who were pregnant.
Variables
The primary goal of this research was to assess the study groups' fasting serum glucose, HbA1c, insulin, HDL-C, triglyceride, total cholesterol, and LDL-C levels.

Bias
Prospective studies make it simpler to control selection bias since the target population or specimen specification may be precisely controlled to assure group homogeneity. One of the most effective ways to eliminate selection bias is randomization. It can potentially lessen the asymmetry of known and unknown characteristics among groups. As a result, it is effective in reducing random errors. In practice, this was accomplished using methods such as automated randomization services (GraphPad QuickCalcs (RRID:_SCR_000306)). However, the randomization sequence was unknown to the researchers. Therefore, discussing the results with our peers was also one method for eliminating reporting bias.

Sample size
The statistical tool G*Power (RRID:_SCR_013726) version 3.1.9.7 was utilized to determine the sample size. Using a 95% confidence level, the following were the calculated results: df = 88, non-centrality parameter = 3.66, critical t = 1.98. The sample size must be 90 individuals (f).

Study procedure
A venous blood specimen (6 ml) was obtained from each subject after overnight fasting; one milliliter of this specimen was put into an ethylene diamine tetra acetic acid (EDTA) tube to be used for the analysis of HbA1c. The remaining blood sample was placed into a gel tube (no anticoagulant) and left for 30 minutes at room temperature to allow it to clot, then centrifuged for 10–15 minutes at 4400 revolutions per minute (rpm) to get the serum. The resulting serum was then divided with a micropipette into three Eppendorf tubes to be kept frozen (-20 °C) until the assay for insulin and vitamin D₃ by using specific ELISA kits. The remainder of the serum was utilized for measuring total cholesterol (TC), triglyceride (TG), and high-density lipoprotein cholesterol (HDL-C) by colorimetric assay. Fasting serum glucose (FSG) was also measured. The homeostatic model assessment-insulin resistance (HOMA-IR) was obtained to estimate insulin resistance or sensitivity by using fasting serum glucose and insulin levels, using the formula: HOMA-IR = (Fasting Glucose (mg/ml) × Fasting Insulin (μU/ml))/405.

Materials and instruments
The purest materials were used for this study. Table 1 provides a summary of the chemical kits utilized in this investigation.

Statistical analysis
IBM SPSS version 23 (RRID:SCR_016479) was used in the statistical analysis process. First, the normality of data distribution was assessed because the dataset was smaller than the 2000 Shapiro-Wilk test used; the p-value was >0.05, which means the data is not normally distributed, and a non-parametric test must be used for analysis. Descriptive statistics were expressed as median (interquartile range (IQR)). Then, the non-normally distributed variables were managed by the analysis of variance (Kruskal-Wallis) test to compare the four studied groups and determine their degree of significance. Pearson's chi-squared test was carried out for categorical variables. A value less than 0.05 was accepted as a significant difference.

Results
The diabetics' groups had higher FSG, Insulin, HOMA-IR, and HbA1c levels compared to the control groups. However, for HOMA-IR, insulin, and HbA1c, there was no significant difference between the two diabetic groups or between the

| Chemicals            | Provider                          | Catalogue number | Reference |
|----------------------|-----------------------------------|------------------|-----------|
| Vitamin D            | MyBioSource (American)            | MBS264661        | NA        |
| Fasting serum glucose| LINEAR chemicals (Spain)          | NA               | 1129010   |
| Human Insulin Elisa Kit | MyBioSource (American)          | MBS704195        | NA        |
| Total cholesterol    | Human Diagnostic (Germany)        | NA               | 10028     |
| Triglycerides        | Human Diagnostic (Germany)        | NA               | 10724     |
| HDL-C                | Human Diagnostic (Germany)        | NA               | 10018     |
two control groups. The FSG patients with vitamin D level <22.5 ng/dl showed higher FSG level than patients with vitamin D level ≥22.5 ng/dl, as demonstrated in Table 2 and Figure 1.17

There were no marked differences in TC, and LDL-C levels between the two control groups and the DM group with vitamin D level ≥22.5 ng/dl, while the DM group with vitamin D level <22.5 ng/dl showed significantly higher TC and

Table 2. Glycemic measures among studied groups.

| Variables       | DM with vitamin D ≥22.5 ng/dl (N = 22) | DM with vitamin D <22.5 ng/dl (N = 25) | Control with vitamin D ≥22.5 ng/dl (N = 20) | Control with vitamin D <22.5 ng/dl (N = 23) |
|-----------------|----------------------------------------|----------------------------------------|-------------------------------------------|-------------------------------------------|
| FSG (mg/dL)     | 141(84)b                               | 182(67)a                               | 101(18)c                                  | 102(12.4)c                                |
| Insulin (U/ml)  | 7.889(6.79)a                           | 8.670(5.18)a                           | 5.0715(0.73)b                             | 4.287(0.85)b                              |
| HOMA-IR         | 2.728(2.93)a                           | 3.7505(2.33)b                         | 1.233(0.24)b                              | 1.223(0.33)b                              |
| HbA1c (%)       | 6.35(1.65)a                            | 7.7(2.95)a                             | 5.375(0.61)b                              | 5.210(0.7)                                |

N = number of subjects; DM = diabetes mellitus; FSG = fasting serum glucose; HOMA-IR = homeostatic model assessment-insulin resistance; HbA1c = glycated haemoglobin; superscripts (a,b,c) refer to significant differences among different groups such that (a) means the level is significantly higher than the level in (b) and the last is significantly higher than the level in (c).

Figure 1. HOMA-IR levels in studied groups.

Table 3. Lipid profile among the studied groups.

| Variables          | Diabetic patients with vitamin D levels >22.5 ng/dl (N = 22) | Diabetic patients with vitamin D levels <22.5 ng/dl (N = 25) | Control group with vitamin D levels ≥22.5 ng/dl (N = 20) | Control group with vitamin D levels <22.5 ng/dl (N = 23) |
|--------------------|---------------------------------------------------------------|---------------------------------------------------------------|----------------------------------------------------------|----------------------------------------------------------|
| TC mg/dL           | 196(70.5)b                                                  | 226(64)a                                                      | 172.5(40.5)b                                             | 170(41)b                                                 |
| TG mg/dL           | 119(114.5)a                                                 | 178(138.25)a                                                 | 103(53)b                                                 | 118(113.45)b                                             |
| HDL-C mg/dL        | 42.075(4.24)b                                               | 42.03(10.33)b                                               | 54.234(8.93)b                                           | 47(12.8)a                                                |
| LDL-C mg/dL        | 118.527(69.73)b                                            | 155.130(81.54)b                                             | 91.219(42.81)b                                          | 100(48.46)b                                              |
| TC: HDL-C Ratio    | 4.8245(1.91)b                                              | 5.4521(3.43)a                                              | 3.2905(0.95)c                                           | 3.8655(1.51)bc                                          |
| TG: HDL-C Ratio    | 3.8816(2.81)ab                                             | 4.1950(3.61)a                                              | 1.8686(1.79)c                                           | 2.37(2.61)bc                                            |
| LDL-C: HDL-C Ratio | 2.8270(1.83)b                                              | 3.6458(3.37)a                                              | 1.8569(0.92)c                                           | 2.1277(1.48)bc                                          |

TC = total cholesterol; TG = serum triglycerides; HDL = high density lipoprotein; LDL = low density lipoprotein; TC: HDL-C = total cholesterol to high-density lipoprotein cholesterol ratio; TG: HDL-C = triglyceride to high-density lipoprotein cholesterol ratios; LDL-C: HDL-C = low density lipoprotein cholesterol to high-density lipoprotein cholesterol ratios. Superscripts (a,b,c,d) refer to significant differences among groups, such that (a) means the level is significantly higher than the level in (b) and the last is significantly higher than the level in (c).
LDL-C levels than the other three groups. There was no marked difference in TG level between the two DM groups or between the two control groups. DM patients with vitamin D level ≥22.5 ng/dl showed no difference with DM patients with vitamin D level <22.5 ng/dl group or with the two control groups, while the DM group with vitamin D level <22.5 ng/dl showed a significantly higher TG level than the two control groups. No marked difference in HDL-C serum level between the two diabetic groups or between the two control groups was shown. However, both control groups had a higher significant HDL-C value than the patient groups. The diabetic group with vitamin D levels <22.5 ng/dl showed higher TC: HDL-C ratio and TG: HDL-C ratio levels than the diabetic group with vitamin D levels ≥ 22.5 ng/dl and the two control groups, while DM patients with vitamin D level ≥22.5 ng/dl showed significantly higher values than the control group with vitamin D level ≥22.5 ng/dl and showed an insignificant difference with the control group with vitamin D level <22.5 ng/dl. No marked difference in serum level of LDL-C: HDL-C ratio between the two diabetic groups nor between the two control groups was shown. The DM group with vitamin D level ≥22.5 ng/dl showed a non-significant difference in LDL-C: HDL-C ratio level with the control group with vitamin D level <22.5 ng/dl, while the DM group with vitamin D level <22.5 ng/dl showed a significantly higher LDL-C: HDL-C ratio level than the two control groups as shown in Table 3.17

**Discussion**

For the current study, there were no marked differences in confounder (age, gender, BMI, and smoking) among the four studied groups. The diabetics' groups had higher FSG, fasting insulin, HOMA-IR, and HbA1c levels compared to the control groups due to the effect of diabetes. The findings in this study showed that vitamin D levels had no impact on the level of fasting insulin, HOMA-IR, and HbA1c as demonstrated in Table 2, and this finding highly agreed with Al-Shoumer et al. and Luo et al. who suggested in their research that there was no link between glycemic control and vitamin D when confounders like age, gender, and BMI were controlled. This also agreed with research carried out by Olt S. et al. who suggested that vitamin D level wasn't correlated with glycemic control (For HbA1C correlation with vitamin D level, p = 0.33 (>0.05)). While Al-Timimi et al. reported that deficiency in vitamin D levels in patients with T2DM was significantly associated with glycemic control. The correlation between vitamin D and HbA1c is controversial; many previously published studies have shown a significantly weak negative correlation between HbA1c and vitamin D levels. Pittas et al., in a retrospective study, suggested that impaired B-cell activity and insulin resistance are among the pathophysiological factors associated with metabolic syndrome and type 2 diabetes (T2DM). Vitamin D levels may indirectly impact these factors by regulating calcium homeostasis or activating vitamin D receptors. In this study, patients with vitamin D levels <22.5 ng/dl showed higher FSG levels than patients with vitamin D level ≥22.5ng/dl, which agreed with a study done by Scragg et al. who found an inverse relationship between vitamin D and FSG levels in their research. While a Japanese study comparing DM type, two patients and healthy controls found that vitamin D insufficiency was present in 70% of cases without a difference in vitamin D levels between patients and controls. Two US studies suggest an increase in the mean vitamin D level in T2DM and the corresponding controls. The variation in outcome among studies indicates that vitamin D levels in patients with T2DM differ according to ethnicity or other unknown causes. In the present study, levels of vitamin D showed a pronounced impact on TC, LDL-C, and TC: HDL-C ratio levels, but it didn’t show an effect on TG, HDL-C: TG, HDL-C ratio, and LDL-C: HDL-C ratio levels. DM in this study showed an impact on all lipid profiles. This finding agreed with many studies that reported dyslipidemia as an independent predictor of a decrease in vitamin D level. TC was reported to correlate inversely with serum levels of vitamin D even after controlling for confounders.

This study's results disagree with many cross-sectional studies, where serum vitamin D levels correlate directly with HDL-C. Many investigations also revealed an oppositional relationship between TG and blood vitamin D levels. Although some research found a link between TG and blood vitamin D levels, other investigations found an opposite correlation. The current study findings are comparable to the research of John et al. on 170 Bangladeshi individuals born in the UK (101 female and 69 male) without a history of diabetes or other chronic illness. They found no link between vitamin D and HDL-C or TG levels. Due to enhancing intestinal calcium absorption, research considering lipid metabolism has found that vitamin D can decrease hepatic triglyceride production and boost uptake by peripheral tissues. Vitamin D boosts reverse cholesterol transport by increasing apo-lipoprotein A1, vitamin D also encourages the development of big HDL-C particles. Due to the interdependence of the metabolism of carbohydrates and lipids, blood lipid levels can be influenced by various variables in people with diabetes. As a result, every issue in lipid metabolism causes a disorder in glucose metabolism and vice versa. The primary defect in most people with type two DM is insulin resistance. Insulin resistance and hyperinsulinemia have a good predictive value for the development of T2DM in non-diabetic people. The TG: HDL ratio and other lipid profile ratios have been evaluated daily for many critical clinical uses. Prior studies have suggested a positive correlation between metabolic syndrome, negative cardiovascular risk factor profiles, and the prediction of diabetes or its consequences. This can happen because of the link between insulin resistance and TG: HDL-C ratio.
Limitations
Several selection criteria were used in the study to limit the confounding effect. Also, because the number of participants in our study was limited, we recommend increasing the number of patients in future research.

Conclusions
Elevated FSG, HOMA-IR, Hba1c, fasting insulin, triglycerides, TC: HDL-C ratio, TG: HDL-C ratio, and LDL-C: HDL-C ratio values were found in diabetes patients compared to healthy controls, with markedly diminished HDL-C levels in diabetes patients compared to healthy controls as a result of the diabetes effect. Vitamin D level significantly impacted total cholesterol, LDL-C, and TC: HDL-C ratio in obese diabetic patients.

Data availability
Underlying data
Zenodo: Underlying data for ‘Impact of serum levels of vitamin D on lipid profiles, glycemic indices, and insulin resistance in obese type 2 diabetes patients: An observational study’. ‘Laboratory findings’. https://doi.org/10.5281/zenodo.6989464.17

Data are available under the Creative Commons Attribution 4.0 International license (CC-BY 4.0).

Consent
Written informed consent for publication of the participants’ details was obtained from the participants.

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