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# Serum Levels of 25-Hydroxyvitamin D, Vitamin D Binding Protein, and Vitamin D Receptor in Type 2 Diabetes: A Case-Control Study from Libya

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# مستويات 25-هيدروكسي فيتامين د، وبروتين الارتباط بفيتامين د، ومستقبل فيتامين د في مصل الدم لدى المصابين بداء السكري من النوع الثاني: دراسة حالات وشواهد من ليبيا

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**Abstract:**
Type 2 diabetes mellitus (T2DM) is a complex condition marked by disturbance in the way the body handles carbohydrate, lipid, and protein metabolism. 25 Hydroxyvitamin D (25(OH)D) is known to play an essential role in blood sugar regulation by influencing both insulin secretion and the body's response to insulin. This study aimed to evaluate the relationship between 25(OH)D, vitamin D-binding proteins (VDBP), and the Vitamin D Receptor (VDR) in Libyan Patients with T2DM. The study was carried out in Benghazi City between June 2022 and March 2023. A total of 44 patients with T2DM and 44 matched healthy controls, who had no history of T2DM. Clinical examinations and laboratory evaluations were performed on all subjects. Our study demonstrates a significant decrease ($p<0.001$) in the levels of 25(OH)D, VDR, Ca, P, and HDL-C in patients with T2DM compared to the control group. In contrast, no significant difference in the serum VDBP was observed between the two groups ($P=0.07$). In addition, patients with T2DM had remarkably high levels of (TC), (TG), and (LDL-c) compared to controls ($p<0.001$). Correlation analysis revealed a strong negative association between 25(OH)D and HbA1c levels ($r = -0.737$, $p<0.05$), alongside a strong positive correlation between 25(OH) D and VDR ($r = 0.979$, $p<0.05$). Vitamin D deficiency is a commonality in T2DM patients and is closely associated with poor glycemic control, however, due to the study's cross-sectional design, causality cannot be inferred. These results emphasize the possible role of 25(OH)D in the pathogenetic mechanism, progression, and management of T2DM.

**Keywords:** Type 2 diabetes mellitus, Vitamin D, Vitamin D binding protein, Vitamin D receptor.

**الملخص:**
يُعتبر داء السكري من النوع الثاني (T2DM) حالة مرضية معقدة تتميز باضطراب في آلية تعامل الجسم مع أيض الكربوهيدرات والدهون والبروتينات. ويعرف فيتامين د بصورته 25-هيدروكسي فيتامين د (25(OH)D) بدوره الأساسي في تنظيم مستوى سكر الدم من خلال تأثيره على إفراز الإنسولين واستجابة الجسم له. هدفت هذه الدراسة إلى تقييم العلاقة بين مستوى 25(OH)D، وبروتين ارتباط فيتامين د (VDBP) ومستقبل فيتامين د (VDR) لدى المرضى الليبيين المصابين بداء السكري من النوع الثاني. أُجريت هذه الدراسة في مدينة بنغازي خلال الفترة من يونيو2022 إلى مارس

2023. شملت الدراسة 44 مريضًا مصابًا بداء السكري من النوع الثاني و44 شخصًا سليمًا كمجموعة ضابطة متطابقة، دون تاريخ مرضي للإصابة بالسكري من النوع الثاني. أُجريت الفحوصات السريرية والتحاليل المخبرية لجميع المشاركين. أظهرت نتائج الدراسة فروقا ذات دلالة معنوية (p<0.001) في مستويات 25(OH)D ومستقبل فيتامين د (VDR) والكالسيوم (Ca) والفوسفور(P) والكوليسترول عالي الكثافة (HDL-C) لدى مرضى السكري من النوع الثاني مقارنة بالمجموعة الضابطة. في المقابل، لم يُلاحظ أي ذات دلالة معنوية في مستويات بروتين ارتباط فيتامين د في المصل (VDBP) بين المجموعتين (P=0.07). بالإضافة إلى ذلك، سُجل ارتفاع ملحوظ في مستويات الكوليسترول الكلي (TC) والدهون الثلاثية (TG) والكوليسترول منخفض الكثافة (LDL-C) لدى مرضى السكري مقارنة بالأصحاء (p<0.001 ). كما أظهر تحليل الارتباط وجود علاقة عكسية قوية بين مستويات 25(OH)D والهيموغلوبين السكري (HbA1c) (r = -0.737, p<0.05) إلى جانب علاقة طردية قوية بين 25(OH)D ومستقبل فيتامين د (VDR) (r = 0.979, p<0.05) .ان نقص فيتامين د يُعد شائعًا بين مرضى داء السكري من النوع الثاني ويرتبط ارتباطًا وثيقًا بسوء التحكم في مستوى السكر في الدم، إلا أنه وبسبب التصميم المقطعي للدراسة، لا يمكن استنتاج علاقة سببية مباشرة. وتؤكد هذه النتائج الدور المحتمل لـ 25(OH)D في الية حدوث المرض وتطوره، وإدارة داء السكري من النوع الثاني.

**الكلمات المفتاحية:** داء السكري من النوع الثاني، فيتامين د، البروتين الرابط لفيتامين د، مستقبل فيتامين د.

**Introduction:**

Diabetes mellitus is an incurable disease that rapidly propagates around the world and is linked to significant morbidity, increased mortality, rising costs for healthcare, and has emerged as a pressing global health challenge. According to projections, the prevalence of affected people worldwide is expected to rise significantly from approximately Growing from 171 million cases worldwide in 2000 to close to 366 million expected by 2030 [1]. T2DM poses a significant threat to public health worldwide. 38 million people globally had diabetes in 2016, and 2.8 million of them lived with a disability [2]. Evidence from observational studies suggests that vitamin D deficiency has been associated with the development and progression of type 2 diabetes as well as subsequent microvascular catastrophes [3], [4]. T2DM represents a major public health concern in Libya, recent estimation indicating that approximately 10–16% of adults are affected, making the country among those with the highest prevalence rates in North Africa. Dietary habits are considered the most likely contributing factor [5]. Diabetes represents one of the most pressing global health emergencies of this century, ranking among the top ten leading causes of mortality worldwide, alongside cardiovascular disease (CVD), respiratory disease, and cancer [6]. Individuals with T2DM face a high risk of developing both microvascular complications, such as retinopathy, nephropathy, and neuropathy, and microvascular complications, including cardiovascular comorbidities. These risks are largely driven by chronic hyperglycemia and metabolic disturbance associated with insulin resistance [7].

Vitamin D (calciferol) represents a group of fat-soluble ketosteroids that play a key role in human health. It is naturally present in a few foods, including fish liver oils, fatty fish, egg yolks, mushrooms, and animal liver. In vertebrates, 7-dehydrocholesterol is photosynthesized in the skin by ultraviolet (UV) B-light. Although there are other forms of vitamin D, the two physiologically significant forms are vitamin D2 (ergocalciferol), derived mainly from plant sources, and vitamin D3 (cholecalciferol), obtained primarily from animal sources and skin synthesis [8]. a prohormone that promotes bone health protection and makes calcium and phosphorus equilibrium easier [9]. In both children and adults, vitamin D deficiency is still very common. Inadequate vitamin D status in pregnancy and childhood may inhibit growth, cause skeletal abnormalities, and potentially lead to a higher risk of hip fractures in adulthood or old age. In adult's vitamin D deficiency can aggravate osteopenia and osteoporosis, lead to muscle weakness and osteomalacia, and elevate the risk of fractures. (VDBP), also called the group-specific component protein (Gc), is a multifunctional serum glycoprotein. It serves as the primary carrier of 25(OH)D metabolites in the bloodstream and is essential for both intracellular uptake and transportation [10]. Therefore, changes in VDBP may affect vitamin D levels and activity, which may change insulin secretion, β-cell dysfunction, and glucose metabolism [11]; [12]. VDBP protects the cell from actin-mediated harm by interacting with other serum proteins [13].

VDBP cooperates with other circulating proteins to protect cells from actin-induced damage [13]. 25(OH)D performs its cellular effects by attaching to the intracellular hormone receptor known as the Vitamin D receptor (VDR) [14], belonging to the steroid hormone receptor superfamily [15].

VDR is the key protein to mediate the biological function of vitamin D, and growing evidence suggests that it may play an important role in the development of T2DM [ 16]. VDR can directly influence insulin secretion, and altered transcription of the VDR gene in pancreatic β-cells has been shown to modify β-cells insulin output [17]. Furthermore,

Low vitamin D levels combined with reduced VDR activity are associated with an increased risk of developing T2DM [18]. Although VDBP has several biological functions independent of vitamin D, its

primary role is to regulate the circulating free and total concentrations of vitamin D metabolites. Among these metabolites, 25-hydroxyvitamin D (25(OH)D) is the most widely studied because it is considered the best indicator of overall vitamin D status [19]. Since the availability of vitamin D depends on DBP (VDBP), higher DBP levels can reduce the amount of free vitamin D, leading to decreased activation of the VDR [19][20].

In Libya, Benghazi, to date, no studies have explored the relationship between 25(OH) D, VDBP, and VDR and T2DM. Therefore, this study is the first to assess serum 25(OH) D level, VDBP, and VDR in Libyan patients with T2DM. Supplementing with vitamin D can help patients avoid T2DM, together with the serious complications that may impact bone and cardiovascular disease.

**Aim of the study:**

This study aims to investigate the association between 25(OH) D, VDBP, and VDR in type 2 diabetic patients and healthy controls.

**Methods:**

This study utilized a case–control approach conducted at the National Center for the Treatment and Diagnosis of Diabetes. The study population consisted of 44 patients previously diagnosed with T2DM (20 males and 24 females) with a mean age of 44.52 ± 12.01 years. And age- and sex-matched 44 apparently healthy volunteers (25 males and 19 females) with a mean age of 43.97 ± 11.59 years.

**Exclusion Criteria:**

Individuals who had type 1 diabetes were not included. Participants were excluded if they had thyroid or parathyroid problems, liver or kidney dysfunction, or any other significant endocrine abnormalities. Additionally, patients using corticosteroids, antiepileptics, antidepressants, vitamin D, or calcium supplements, drugs known to disrupt vitamin D metabolism or bone health—were not included. Furthermore, the study excluded participants with acute or chronic inflammatory illnesses, cancer, recent fractures, and pregnant or lactating women.

**Sample collection and processing:**

After an overnight fast of 12 hours, 8 mL of venous blood was drawn from each participant. Of this volume, 1 mL was placed in a sodium fluoride (NaF) tube for the assessment of fasting blood glucose using an enzymatic colorimetric method on the COBAS INTEGRA analyzer (Roche Diagnostics, Germany). An additional 1 mL was transferred into an EDTA tube for the determination of glycated hemoglobin (HbA1c) as a proportion of total hemoglobin, which was measured using the same system.

The remaining 6 mL was allowed to clot in a plain tube and centrifuged at 3000 rpm for 10 minutes, and the separated serum was stored at −80 °C until analysis. Serum calcium ($Ca^{2+}$), phosphorus (P), triglycerides (TG), total cholesterol (TC), LDL-c, and HDL-c were determined using an automated chemistry analyzer (COBAS INTEGRA, Roche Diagnostics, Germany).

Serum concentrations of 25(OH)D, VDR, and VDBP were determined using enzyme-linked immunosorbent assay (ELISA) with commercially available kits, following the manufacturer's instructions. (CUSABIO, USA; 25(OH)D: Cat.) No. CSB-E08003h, VDR: Cat. No. CSB-EL021944HU, VDBP: Cat. No. CSB-EL010409HU) with horseradish peroxidase (HRP)-based colorimetric detection.

In the study, we measured serum VDR concentration using a quantitative sandwich ELISA. A96-well microplate coated with a VDR-specific capture antibody was used. Samples were added to the wells, followed by a horseradish peroxidase (HRP)-conjugated detection antibody to form an antibody-antigen-enzyme complex. The color developed was measured spectrophotometrically, and VDR concentrations were calculated according to the standard curve. Instructions for Manufacture were followed, and we ran quality control per plate. The Manufacturer reports no cross-reactivity with serum proteins.

**Sample size and power:**

No formal prospective sample size calculation was performed before recruitment. Based on the observed mean difference in 25(OH)D between T2DM and control groups (mean difference = 26.63 ng/mL, pooled SD = 7.81), a post-hoc power analysis showed that the sample size 44 participants per group provided approximately 100% power to detect a difference at $\alpha = 0.05$. smaller effects, such as those observed for VDBP, may require a large sample size.

**Statistical Analysis:**

In this study, data were entered and analyzed using Statistical Package for the Social Sciences (or SPSS software version 23; SPSS INC., Chicago, USA). The results are expressed as Mean ± Standard Deviation (SD) to indicate variability and range. Comparison of continuous variables between two groups was performed using the unpaired t-test for parametric data. Diagnostic performance was assessed by contrasting the "Receiver-Operating Characteristic" (ROC) curve and calculating the area under the curves. From these curves, sensitivities, specificities, and the optimal cut-off values were

determined as the points maximizing the sum of sensitivity and specificity were determined. Sensitivity was further used to evaluate diagnostic validity. A p-value < 0.05 was considered statistically significant.

**Table (1)**: Demographic profile of the studied groups

| Variable | | Group IT2DM group | Group II Control group | p-value |
|---|---|---|---|---|
| Number | | 44 | 44 | - |
| Gender | Males No. (%) | 20(45%) | 25(56%) | - |
| | Females No. (%) | 24(55%) | 19(44%) | - |
| Age (Mean ± SD) (years) | | 44.52±12.01 | 43.97±11.59 | >0.05 |
| BMI (Mean ± SD) ($kg/m^2$) | | 32.5±5.9 | 26.9±4.7 | >0.05 |
| Duration of DM (years), mean±SD | | 11.54±10.03 | - | |

**Table 1** presents the main characteristics of the patients and controls, including the number of participants, male-to-female ratio, age, and BMI (expressed as Mean± SD). No statistically significant differences between patient groups and control groups in terms of age and gender (P>0.05).
As shown in Table 1, the mean BMI was significantly higher in the T2DM group compared to the control group. As BMI differed between groups (mean 32.5 vs 26.9 $kg/m^2$), and obesity is known to influence vitamin D metabolism, the BMI represents a potential confounder. To address this, we adjusted for BMI in multivariate models; however, the residual confounding may remain.

The mean serum total cholesterol level was higher in T2DM patients (165±35.90 mg/dl) compared with the control group (145±25.62 mg/dl). The difference is statistically significant (t = -3.10, P = 0.03), as presented in Table 2 and illustrated in Figure 1.

The mean serum HDL-C level was significantly lower in T2DM patients (36.75±9.43 mg/dl) compared with the control group (42.68±11.78 mg/dl), with a statistically significant difference (t = -2.60, P = 0.011) as shown in Table 2 and illustrated in Figure 2.

The mean serum LDL cholesterol level was significantly higher in T2DM patients (106.12 ± 33.38 mg/dl) compared with the control group (70.13 ±21.24 mg/dl), with the difference being statistically significant (t = 6.03, P = < 0.01), as shown in Table 2 and illustrated in Figure 3.

As shown in Table 2, the mean serum TG level was significantly higher in T2DM patients (152.36 ± 51.12 mg/dl) compared with the control group (62.09 ±21.27 mg/dl), with the difference being statistically significant (t = 10.81, P < 0.01), as illustrated in Figure 4.

**Table 2:** Lipid profiles in diabetic and healthy control subjects.

| Parameter | Group | N | Mean± SD | t-value | p-value |
|---|---|---|---|---|---|
| Cholesterol (mg/dl) | T2DM | 44 | 165±35.90 | 3,10 | 0.003 |
| | Control | 44 | 145±25.62 | | |
| HDL-c (mg/dl) | T2DM | 44 | 3.75±9.43 | -2,60 | 0.011 |
| | Control | 44 | 42,68±11.78 | | |
| LDL (mg/dl) | T2DM | 44 | 106.12±33.38 | 6,03 | <0.01 |
| | Control | 44 | 70.13±21.24 | | |
| Triglycerides (mg/dl) | T2DM | 44 | 152.36±51.12 | 10,81 | <0.01 |
| | Control | 44 | 62.09±21.27 | | |

**N**: number of the subjects; **SD**: standard deviation; **TG**: Triglyceride; **HDL-C**: high-density lipoprotein cholesterol; **LDL-C**: low-density lipoprotein cholesterol & **t**: Student's t-test. *P-value significant at P ≤ 0.05.

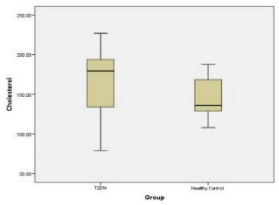

**Figure (1):** Distribution of the mean Cholesterol among controls and cases.

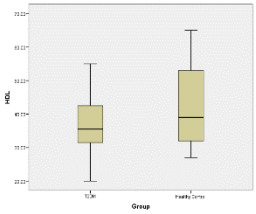

**Figure 2:** Distribution of the mean HDL-c among controls and cases.

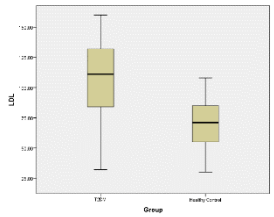

**Figure (3):** Distribution of the mean LDL-c among controls and cases.

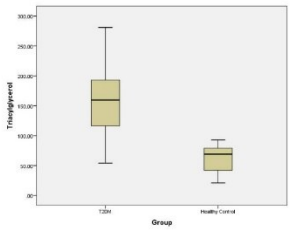

**Figure (4):** Distribution of the mean TG among controls and cases.

The FBG results were higher in T2DM cases (144.50±30.20 mg/dl) in contrast to controls (84.20±8.06 mg/dl), as shown in Table 3 and illustrated in Figure 5; the difference is also statistically significant (t = 12.77, P < 0.01).

The mean HbA1c percentage was significantly higher in T2DM patients (7.81±1.46) compared with the control group, which had a normal mean value (4.91±0.42) (t = 12.60, P< 0.01), as shown in Table 3 and illustrated in Figure 6.

**Table 3:** Fasting blood glucose (FBG) and glycosylated hemoglobin (HbA1c) in diabetic and healthy control subjects.

| Parameter | Group | N | Mean± SD | t-value | p-value |
|---|---|---|---|---|---|
| FBG (mg/dl) | T2DM | 44 | 144,50±30,20 | 12,77 | <0.01 |
| | Control | 44 | 84.20±8.06 | | |
| HbA1c (%) | T2DM | 44 | 7.81±1.46 | 12,60 | <0.01 |
| | Control | 44 | 4.91±0.42 | | |

P-value is the difference between the T2DM group and the control group, P < 0.05 (significant), P > 0.05 (insignificant).

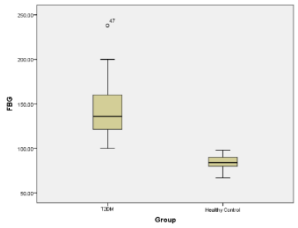


**Figure (5):** Distribution of the mean FBG among controls and cases.

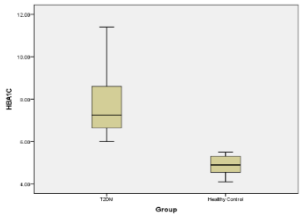


**Figure (6):** Distribution of the mean HbA1c (%) among controls and cases

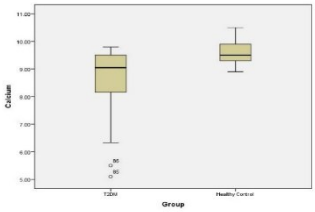


**Figure (7):** Distribution of the mean Calcium among controls and cases.

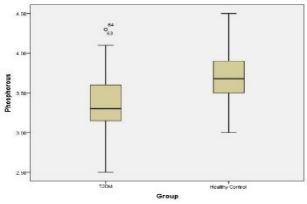


**Figure (8):** Distribution of the mean Phosphorus among controls and cases

Table 5 shows a comparison of T2DM and the control group according to serum levels of Vitamin D, VDBP, and VDR. As shown in the table, serum 25(OH)D (ng/ml) was significantly decreased in T2DM patients compared to the control group (17.23±6.24 vs. 43.86±9.11) (t = 15.73, P =< 0.01) as illustrated in (Figure 9).

The mean serum level of VDBP in T2DM patients was (0.96±0.91mg/dl), compared to the mean of VDBP in the control group was (1.44±1.11 mg/dl) (t = - 1.83, P = 0.070), as shown in (Table 5) and illustrated in (Figure 10).

As shown in Table 5, the mean serum VDR level was significantly lower in T2DM patients (15.10±5.99 mg/dl) compared with the control group (38.22 ± 8.48 mg/dl) (t = -14.75, P < 0.01), as illustrated in Figure 11.

**Table (5):** Comparison between the studied groups according to serum levels of Vitamin D, VDBP, and VDR.

| Parameter | Group | N | Range | Mean± SD | Median | t-value | p-value |
|---|---|---|---|---|---|---|---|
| 25(OH)D (ng/ml) | T2DM | 44 | 6.48-36.00 | 17.23±6.24 | 17.00 | -15.73 | <0.01 |
| | Control | 44 | 33.00-70.00 | 43.86±9.11 | 43.00 | | |
| VDBP (ng/ml) | T2DM | 44 | 0.02-3.40 | 0.91±0.90 | 0.59 | -1.83 | 0.070 |
| | Control | 44 | 0.03-3.86 | 1.31±1.11 | 0.91 | | |
| VDR (pg/ml) | T2DM | 44 | 3.00-30.39 | 15.10±5.99 | 14.00 | -14.75 | <0.01 |
| | Control | 44 | 22.00-67.00 | 38.22±8.48 | 37.00 | | |

P1 is the difference between the T2DM group and the control group, P < 0.05 (significant), P > 0.05 (insignificant). Figures 9, 10, and 11 present box plots showing the distribution of serum marker levels in the study groups, including median, minimum, and maximum values. The boxes represent the interquartile range in different investigations (first and third quartiles). The x-axis indicates the study groups, while the y-axis represents biomarker concentration: (A) 25-hydroxyVitamin D, (B) VDBP, (C) VDR. The figures demonstrate significant differences among the study groups for all measured markers, except for VDBP, which did not show statistically significant variation.

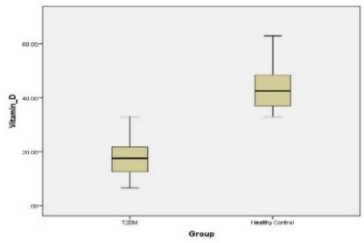


**Figure (9):** Box plots 25(OH)D of DM cases and control groups.

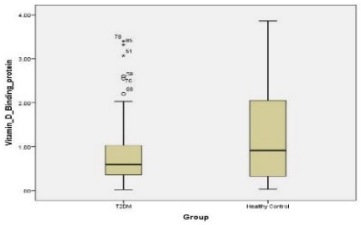


**Figure (10):** Box plots of VDBP cases and control groups

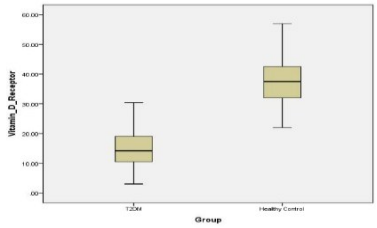


**Figure (11):** Box plots of VDR in DM cases and control groups

**Diagnostic Performance of the studied markers**:

Further analysis was conducted using the Receiver Operating Characteristic (ROC) curves to evaluate the diagnostic performance of the studied markers in distinguishing T2DM patients from controls. The corresponding area under the curve AUC, optimal cut-off values, specificity, sensitivity, as well as PPV (positive predictive values) and NPV (negative predictive values) are presented in Figure 12 and Table 6**.**

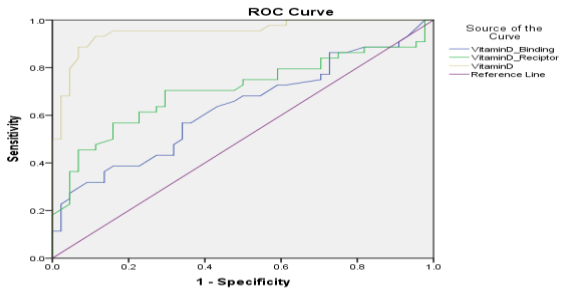


**Figure (12):** The diagnostic performance of the studied markers in T2DM patients vs. control subjects.

**Table 6:** ROC Analysis presenting Cut-off, Sensitivity, Specificity, AUC, PPV, and NPV of the Studied Markers.

| Parameter | 25(OH)D (ng/ml) | VDBP (ng/ml) | VDR (pg/ml) |
|---|---|---|---|
| Cut off value | 28 | 0.66 | 14 |
| Sensitivity (%) | 92 | 56 | 66 |
| Specificity (%) | 89 | 62 | 70 |
| Positive predictive value (%) | 88 | 75 | 72 |
| Negative predictive value(%) | 93 | 41 | 63 |
| Area Under the ROC | 0.95 | 0.63 | 0.70 |

**Diagnostic Performance of the studied markers:**

Analysis of the ROC curve data, as presented in Table 6, revealed the cut-off value for 25(OH)D to differentiate T2DM from healthy control subjects of 20.44ng/ml. This analysis yielded a sensitivity of 92%, specificity of 89 %, positive predictive value PPV of 88 %, and negative predictive value NPV of 93 %, with an area under the ROC curve (AUROC) of 0.95.

In contrast, at a cut-off value of 0.66 ng/ml, serum VDBP showed a sensitivity of 56 %, specificity of 62 %. The PPV of 75 % and the NPV of 41%. The AUROC was 0.63. Moreover, at 14 ng/ml optimal cutoff serum VDR concentration had 66% sensitivity and 70 % specificity. Positive Predictive Value PPV 72 %, NPV 63%and AUROC was 0.70. Table 7 presents the correlation between 25(OH)D levels with the studied parameters. 25(OH)D levels showed a statistically significant negative correlation with age (r = -0.359, P = < 0.05), BMI (r = -0.290, P = < 0.05), cholesterol (r = -0.260, P =< 0.05), TG (r = -0.657, P =< 0.05), LDL (r = -0.489, P =< 0.05), FBG (r = -0.690, P = < 0.05), Hb1Ac (r = -0.737, P = < 0.05).

25(OH)D levels showed a significant positive correlation which is significant with HDL (r = 0.385, P = < 0.05), as presented in Table 7. Additionally, 25(OH)D was positively and significantly correlated with Calcium (r = 0.521, p < 0.05) and Phosphorus (r = 0.482, P< 0.05).

Table 7 summarizes the correlation between VDBP levels and the studied parameters. VDBP showed a statistically significant negative correlation with total cholesterol (r = -0.219, P =< 0.013), TG (r = -0.283, P =< 0.05), LDL (r = -0.270, P =< 0.05), FBG (r = -0.250, P = < 0.05), Hb1Ac (r = -0.190, P = 0.032). In contrast, VDBP exhibited a non-significant positive correlation with HDL (r = 0.111, P = 0.211), and Phosphorous (r = 0.124, P = 0.163).

VDBP showed a non-significant negative correlation with age (r = -0.086, P = 0.423). In contrast, VDBP demonstrated significant positive correlation with BMI (r = 0630, P = < 0.05), Calcium (r = 0.255, P = 0.05).

VDR showed a significant positive correlation with HDL (r = 0.421, P = < 0.05), and Calcium (r = 0.519, P< 0.05),) and Phosphorous (r = 0.901, P = P< 0.05), as presented in (Table 7)

Table 7 presents the correlation between VDR levels with the studied parameters. VDBP showed statistically significant negative correlation with age (r = -0.345, P = < 0.05), total cholesterol (r = -0.327, P =< 0.05), TG (r = -0.714, P =< 0.05), LDL (r = -0.589, P =< 0.05), FBG (r = -0.707, P = < 0.05), Hb1Ac (r = -0.696, P = < 0.05).

**Table (7):** Correlation of Studied Markers with Other Parameters in the T2DM Group.

| Parameter | 25(OH)D | | VDBP | | | VDR |
|---|---|---|---|---|---|---|
| | r | P | R | p | r | p |
| Age | -0.359 | < 0.05 | -0.086 | 0.423 | -0.345 | < 0.05 |
| BMI | -0.290 | < 0.05 | 0.630 | < 0.05 | 0.901 | < 0.05 |
| FBG | -0.690 | < 0.05 | -0.250 | < 0.05 | -0.707 | < 0.05 |
| Hb1Ac | -0.737 | < 0.05 | -0.190 | 0.032 | -0.696 | < 0.05 |
| Cholesterol | -0.260 | < 0.05 | -0.219 | 0.013 | -0.327 | < 0.05 |
| Triglycerides | -0.657 | < 0.05 | -0.283 | < 0.05 | -0.714 | < 0.05 |
| HDL | 0.385 | < 0.05 | 0.111 | 0.211 | 0.421 | < 0.05 |
| LDL | -0.489 | < 0.05 | -0.270 | < 0.05 | -0.589 | < 0.05 |
| Calcium | 0.521 | < 0.05 | 0.255 | < 0.05 | 0.519 | < 0.05 |
| Phosphorous | 0.482 | < 0.05 | 0.124 | 0.163 | 0.285 | < 0.05 |

Body mass index; **FBG:** fasting blood glucose**; HbA1c:** Hemoglobin A1c; **HDL-C:** high-density lipoprotein cholesterol; **LDL-C:** low-density lipoprotein cholesterol; & **r:** Pearson correlation. *P-value significant at p ≤ 0.05.

As shown in Table 8 25(OH)D had a strong, statistically significant positive correlation with VDR (r = 0.979, P = < 0.05; Figure 12). In contrast, 25(OH)D showed a weak and non-significant correlation with VDBP (r = 0.140, P = 0.194; Figure 14).

Similarly, VDR showed a weak, non-significant correlation with VDR (r = 0.140, P = 0.193; Figure 13). These results show a very strong association between Vitamin D levels and VDR, while VDBP appears largely independent of either parameter.

**Table 8:** Correlations between the studied makers in the T2DM Group.

| Parameter | | 25(OH)D | VDBP | VDR |
|---|---|---|---|---|
| Vitamin D | r | 1 | 0.140 | 0.979 |
| | p | / | 0.194 | < 0.05 |
| VDBP | r | 0.140 | 1 | 0.140 |
| | p | 0.194 | / | 0.193 |
| VDR | r | 0.979 | 0.140 | 1 |
| | p | < 0.05 | 0.193 | / |

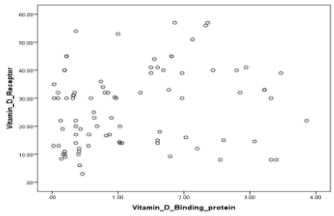


**Figure 13:** Correlation between VDR and VDBP

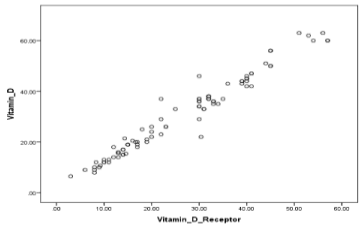


**Figure 14:** Correlation between 25(OH)D and VDR

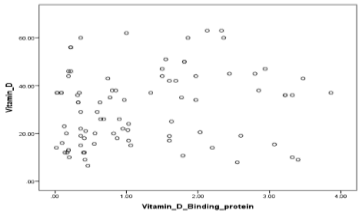


**Figure 15:** Correlation between 25(OH)D and VDBP

**Discussion:**

Type 2 diabetes mellitus (T2DM) is a chronic metabolic disorder that is increasing at an alarming rate worldwide. It is primarily characterized by insulin resistance and pancreatic β-cell dysfunction [21]. 25(OH)D deficiency has been associated with insulin resistance, impaired insulin secretion, and disrupted glucose homeostasis, thereby contributing to an increased risk of developing T2DM [22]. Therefore, this study aimed to investigate the association between vitamin D, VDBP, and VDR levels in type 2 diabetic patients compared to healthy control subjects in Benghazi, Libya.

However, type 2 diabetes most commonly occurs in middle-aged and older adults. The risk is particularly elevated in individuals aged 45 years or older, those with a family history of diabetes, and individuals who are overweight or obese.

Based on the laboratory findings of this study, patients with T2DM exhibited significantly higher serum cholesterol levels compared with the healthy control group ($p<0.01$). This observation is consistent with previous reports that have documented dyslipidemia as a common metabolic abnormality in diabetes. [23]

In this study, serum 25(OH)D concentration was measured using the sandwich ELISA technique in T2DM Libyan patients and healthy controls., The results demonstrated that 25(OH)D levels were significantly reduced in patients with T2DM compared with controls (P < 0.01)., ROC curve analysis

yielded an AUC of 0.95, sensitivity of 92.00%, specificity of 89.0%, PPV of 88.00%, NPV of 92.17%, and optimal cutoff value 28.00ng/ ml.

These results were in agreement with[24] [25] .They found a significant difference in serum 25(OH)D concentration in T2DM patients as compared to patients with control subjects, and they found a high Area under the curve of (0.75), sensitivity reaching 65.5 % and specificity of 89.1 % at a cut-off (29.7ng/ ml).

Furthermore, our results are in agreement with [26], who reported that 25(OH)D concentrations were significantly lower in diabetic patients compared to healthy individuals. Similarly, [27] in a cross-sectional study, the same association. In addition, [28] observed that the mean serum concentration of 25(OH)D was markedly lower in diabetic patients than the non-diabetic subjects. These findings support the growing body of evidence that 25(OH)D deficiency contributes to the pathogenesis of T2DM by promoting insulin resistance, increased hepatic glucose production, and impaired pancreatic β-cell function, ultimately leading to diminished insulin secretion, meaning that poor glycemic control and its associated lifestyle factors (e.g., less outdoor activity) lead to lower Vitamin D [29]. The intracellular metabolism of 25(OH)D is largely dependent on Vitamin D Binding Protein DBP, the primary plasma carrier protein for vitamin D sterols [30]. Consequently, alterations in DBP levels may influence 25(OH)D bioavailability and activity, which in turn can impact insulin secretion, β-cell function, and glucose metabolism [11].

In this work, level VDBP did not differ significantly from patients with T2DM when compared with healthy controls (P=0.070), while the AUC for serum VDBP was 0.63with sensitivity 56%, specificity 62%, PPV 75%, and NPV 41.0% and optimal cutoff value 0.66 ng/ml.

As regards the mean serum VDBP level, this research study found that it was not significantly different among patients with T2DM versus healthy controls (P=0.070), while the AUC for serum VDBP was 0.63with sensitivity 56%, specificity 62%, PPV 75%, and NPV 41.0% and an optimal cutoff value of 0.66 ng/ml. These findings are consistent with previous work by [24] Those who reported VDBP serum levels did not vary significantly between the groups (P = 0.857). Also, these results are consistent with (Subber et al.2021)[31], who found no significant variation in serum VDBP concentration in T2DM patients as compared to the control group.

This result was not consistent with [25] , who reported that patients with T2MD had significantly higher serum and urine VDBP levels compared to the controls. They explained this finding by explaining that increased production of serum VDBP may represent a compensatory mechanism for its enhanced urinary loss, which is indicative of tubular dysfunction in diabetes.

There is insufficient evidence in the association of serum VDBP and diabetes, with data being focused more on the association between VDBP polymorphisms and increased risk for T2DM.

We found no significant difference in serum VDBP levels between T2DM patients and controls. This could be because VDBP levels are influenced more by genetic differences than by diabetes itself, and its many roles beyond carrying vitamin D may help keep levels relatively stable even in people with established T2DM. Our modest sample size may also have made it harder to detect smaller differences. Additionally, the AUC of 0.63 suggests that VDBP has limited value as a diagnostic marker in this group. These findings should therefore be interpreted with caution, and larger studies that also consider VDBP gene variants are needed to better understand its role in type 2 diabetes.

25(OH)D exerts its biological effects by binding to the 25(OH)D receptor (VDR), a cytosolic/nuclear receptor belonging to the steroid–thyroid hormone receptor family, which functions as a transcriptional activator of numerous genes. VDR is widely expressed in various tissues, including those critical for glucose metabolism, such as skeletal muscle and pancreatic βcells. [32],[33],[34] Upon binding to 25(OH)D and subsequent specific phosphorylation via kinase cascades, VDR undergoes a conformational change that facilitates its heterodimerization with the retinoid X receptor[35].

Notably, there are no studies in the literature concerning the associations between serum VDR concentration and T2DM in Libya, Benghazi city. Several studies have reported an association between VDR gene polymorphisms and type 2 diabetes, although the results vary across different populations worldwide [36].

In this study, the measurement of serum VDR concentration showed a significantly decreased level in patients with T2DM than in controls (P< 0.01). This result, in contrast to that reported by [24], who found no significant difference in serum VDR concentration in T2DM patients as compared to controls.

We found that circulating VDR levels were significantly lower in T2DM patients compared with controls. Measuring serum VDR is still quite unconventional, and what it truly reflects in the body isn't fully understood. It may not directly show tissue-level VDR activity in organs like the pancreas or muscles, but it could give a rough idea of overall Vitamin D signaling. The very strong correlation we observed between 25(OH)D and VDR suggests that lower VDR levels may partly reflect the reduced Vitamin D status in these patients. Still, we can't rule out that assay limitations or other unknown factors

are influencing these results. More research is needed to clarify what circulating VDR really represents and how it relates to Vitamin D function and T2DM.

In this study was negatively correlated 25(OH)D with age (r = -0.359, P<0.05), BMI (r = -0.290, P<0.05), FBG (r = -0.690, P<0.05), Hb1AC (r = -0.737, P<0.05), Cholesterols (r = -0.260, P<0.05), Triglycerides (r = -0.657, P<0.05), LDL (r = -0.489, P<0.05). The significant positive correlation in 25(OH)D with HDL (r = 0.385, P<0.05), Calcium (r = -0.521, P<0.05), Phosphorous (r = 0.482, P<0.05), and VDR (r = 0.979, P<0.05).

The correlation between 25(OH)D and VDR (r = 0.979) was unusually strong. Both were measured using validated ELISA kits under consistent laboratory conditions, and we confirmed that no data entry errors occurred. Nevertheless, this high correlation could partly reflect the characteristics of the assays or the fact that circulating VDR is still an experimental marker, so these results should be interpreted with caution.

The concentration of 25(OH)D was not correlated with VDBP (r = -0.140, P<0.194) in the study. Our study has several important limitations. BMI was significantly higher in T2DM patients compared with controls, which could have affected our results; even though we adjusted for BMI in multivariate analyses, some remaining confounding may remain. The relatively small sample size may have limited our ability to detect smaller differences, particularly for VDBP. Additionally, circulating VDR is not yet a well-established biomarker, and our measurements may not fully reflect tissue VDR activity. Finally, because this is a cross-sectional study, we cannot establish causality. While we found strong associations between 25(OH)D, VDR levels, and glycemic parameters, it is also possible that T2DM or lifestyle factors related to the disease such as reduced outdoor activity or dietary changes, contribute to lower Vitamin D levels rather than the other way around. Longitudinal studies are needed to clarify these relationships

**Conclusions:**

In conclusion, our findings demonstrated that serum 25(OH)D concentrations are significantly lower in diabetic patients compared to non-diabetic individuals. We also observed a significant negative correlation between 25(OH)D level and Hb1C, FBG, TG, and LDL, while no significant correlation was found between VDBP level and 25(OH)D.

Given the high prevalence of 25(OH)D deficiency in this population, supplementation with 25(OH)D may be beneficial and appears to be warranted.

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