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Higher insulin resistance is associated with decreased expression of the vitamin D receptor gene in obese type II diabetic patients with vitamin D deficiency

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KEYWORDS

VDR-gene, insulin-resistance, obesity, vitamin D, T2DM.

ABSTRACT

The Background: The etiology of numerous medical conditions, such as metabolic irregularities, is interconnected with the insufficiency of vitamin D. Furthermore, a theoretical association between the resistance to insulin and the insufficiency of vitamin D has been postulated. This concept is substantiated by empirical evidence obtained through various investigations. The objective of this investigation is to analyze and investigate the significance and influence of vitamin D and the expression of the vitamin D receptor (VDR) gene in the progression of insulin resistance in individuals with T2DM.

Methods: The study was carried out 100 type II diabetic patients and 100 healthy controls. Both groups had anthropometry examinations to determine their weights, heights, hip and waist circumferences. Fasting glucose, insulin, insulin resistance (HOMA-IR), and vitamin D levels were all measured. Quantitative real-time PCR was used to analyze the expression of VDR genes in both groups. The folding change was computed using the conventional $2^{-(\Delta\Delta ct)}$ technique.

Results: T2DM patients had higher levels of all anthropological measurements, and biochemical parameters compared to controls. Patients had lower levels VDR folding change and vitamin D levels. The diagnostic accuracy of anthropological assessments and biochemical parameters in prediction disease showed significant results. VDR gene expression has a highly significant negative linear association with insulin resistance in obese with vitamin D deficiency patients. Also, negative linear correlation noted between insulin resistance and serum vitamin D levels without significant variation.

Conclusion: VDR gene expression and insulin resistance were connected in obese patients T2DM with vitamin D insufficiency..

1. Introduction

The Type II Diabetes Mellitus (T2DM), a chronic metabolic circumstance that involves high blood sugar levels is widespread recognized as one of the most rapidly expanding global health crises, presenting a serious risk to public health worldwide. With its prevalence steadily increasing over the years, there is a growing concern that diabetes will eventually surpass all other factors contributing to illness and death, thereby becoming the primary factor causing mortality and morbidity worldwide in the not-too-distant future [1].The rise of T2DM and its associated comorbidities, which are influenced through an interaction of genetic factors, way of life choices, and conditions linked to environment, poses a significant challenge for healthcare systems in the wake of the ongoing pandemic [2].

Both metabolic syndrome and insulin resistance are highly influential risk factors that significantly contribute to the advancement and manifestation of T2DM as well as cardiovascular disease, thereby underscoring their clinical significance in the field of medicine. The pathogenesis of T2D ensues when an individual with insulin resistance fails to produce adequate levels of insulin to rectify this underlying

deficiency, thereby leading to the onset of the disease. Insulin resistance, a pivotal characteristic of T2D, can be characterized as the incapacity of insulin to effectively stimulate the elimination of glucose within the body [3]. On the other hand, metabolic syndrome is a multifaceted disorder that manifests because of the simultaneous occurrence and interaction of various risk factors, thus creating a complex interplay between these different components. This intricate relationship between metabolic syndrome and insulin resistance provides clinicians and researchers with a comprehensive understanding of the fundamental processes that lead to the development T2D and cardiovascular disease, thereby guiding them in the implementation of effective preventive measures and therapeutic interventions [4].

Currently, there is a prevalent occurrence of vitamin D insufficiency, which is associated with the pathophysiology of various disorders, including metabolic anomalies [5]. Furthermore, it has been postulated that there exists a relationship between insulin resistance and vitamin D insufficiency [6]. Several clinical investigations have provided evidence to support this notion, demonstrating that the administration of vitamin D supplements leads to improvements in insulin resistance as measured by the HOMA-IR index, as well as the levels of important metabolic markers like total cholesterol (TC), low-density lipoprotein (LDL), triglyceride (TG), and glycated hemoglobin (HbA1c) among people suffering from (T2DM). These findings underscore the potential therapeutic benefits of vitamin D supplements in managing insulin resistance and metabolic abnormalities in T2DM individuals [7-11].

A growing body of research is providing support for the hypothesis that deficiencies in vitamin D are associated with a higher risk of T2DM [12]. Numerous cross-sectional studies have consistently found negative associations between the levels of 25(OH) vitamin D in the serum and both insulin sensitivity and the occurrence of type 2 diabetes. Furthermore, research conducted on a cohort of 700 individuals who were at risk acquiring a T2DM and/or metabolic disorder demonstrated that the serum 25(OH)D level was independently correlated with both insulin sensitivity and beta-cell function [13-17]. In addition to these findings, a recent investigation involving a group of healthy individuals with normal glucose tolerance who participated in hyperglycemic euglycemic clamp trials reaffirmed these results, providing further evidence for the relationship between vitamin D levels and insulin sensitivity [18].

It is unquestionably evident that the multitude of genetic variables exert an undeniably profound and irrefutable influence on the intricate and delicate balance of serum 25(OH)-D concentrations, thereby substantiating the crucial role played by these hereditary factors in the regulation and maintenance of this essential biomarker in the human body [19-22]. The main aim of this study is to explore the role and impact of vitamin D and the gene expression of the vitamin D receptor (VDR) in the advancement and development of insulin resistance within individuals diagnosed with T2DM, a chronic metabolic circumstance that involves high blood sugar levels and impaired insulin function

2. Methodology

1.1. Subjects:

The study was carried out 100 T2DM individuals and 100 healthy controls. The sample size was calculated using http://www.raosoft.com/samplesize.html website at a 95% level of confidence, a 9.78 % margin of error, and keeping a population size of 20000.

2.2. Inclusion Criteria: Lack of malignancies, liver, kidney or bone illnesses or any history of using insulin, anticonvulsants, calcium and vitamin D, over the previous three months, as well as a HbA1c <8%.

2.3. Exclusion Criteria: Post-menopause, pregnancy, breastfeeding, and using oral contraceptives. Patients suffering from diseases mainly alter lipid metabolism such as thyroid problems, pancreatitis, uremia, obstructed liver disease, renal failure, and untreated diabetes.

2.4. RNA extraction gene expression:

2.4.1. RNA extraction and purification

Utilizing a Qiagen QIAamp RNA Blood Mini Kit (Catalog number: 52304), entire RNA was isolated from blood samples, and then a Nanodropper 2000 served to verify the purity of the collected RNA. (ThermoScientific).

2.4.2. cDNA synthesis

Reversing RNA into complementary DNA (cDNA) is accomplished by using the Invitrogen cDNA Reverse Transcription Kit.

2.4.3. Quantitative real-time PCR:

Maxima SYBER Green Q PCR Master Mix, cDNA, specific primers, and a Bio-Rad Cycler were used to conduct quantitative real-time PCR. The PCR involved 40 cycles of denaturation, annealing, and extension. Normalization was carried out using the activity level of the internal regulating gene beta-actin and specific primer sequences were used for beta-actin and Vitamin D [Table 1] [23].

The common $2^{-(\Delta\Delta ct)}$ procedure is used to assess the amount of variation in mRNA expression, and the threshold cycle (Ct) values derived from triplicate amplified processes are averaged to estimate the relative mRNA expression of the candidate gene [24].

TABLE 1. Primer sequence for target gene and housekeeping gene:

Parameters	Primer sequence
VDR gene (Target gene) Human ensemble gene ID ENSG00000111424	Forward primer 5′ CATGCATT TGTCTTTGTAATGTCAC-3′ Reverse primer 5′-AGGAGTTC CCCGAAGAAGG-3′
beta-actin (housekeeping gene)	Forward primer 5′: TGTATGAAGGCTTTTGG TCTCC-3′ Reverse primer 5′ CTGGTCTCAAGTCAGTG TACAGGT-3′

2.4.4. Bioinformatic analysis for primer sequences:

The primer sequence was subjected to a blasting procedure, which involved utilizing the online tool provided by the National Center for Biotechnology Information (NCBI) known as Blast. This web-based application, accessible at the URL https://blast.ncbi.nlm.nih.gov/Blast.cgi, was employed to conduct an in-depth evaluation of the primer sequence. By utilizing the Blast program, the primer sequence was extensively examined and analyzed within the vast gene bank database, to gain a comprehensive understanding of its specific characteristics and potential implications.

2.5. Biochemical analysis:

Measurement of serum glucose was conducted utilizing a kit from Bio-System, Spain, while the measures of insulin level were done using an ELISA kit from Demeditec, Germany. The estimation of insulin resistance was performed using the homeostasis model assessment of insulin resistance (HOMA-IR), with IR calculated as fasting insulin multiplied by fasting glucose divided by 22.5.

Furthermore, using the Roche Cobas e411 system from Roche Diagnostics, Switzerland, baseline blood 25-hydroxyvitamin D values were determined and categorized as adequate, inadequate, or deficient according to the Institute of Medicine's Food and Nutrition Board standards [25].

Hemoglobin A1c (HbA1c) was measured using Crystal Chem's Hemoglobin A1c (HbA1c) kit, with a normal range of 4% to 5.6%; levels between 5.7% and 6.4% indicate prediabetes, while levels of 6.5% or higher indicate diabetes.

2.6. Anthropometry assessment:

Anthropometric assessment was conducted on both obese and control females, where measurements of body weight, height, waist circumference, and hip circumference were taken in accordance with the International Biological Program Bodyweight standards [26]. Body mass index (BMI) was subsequently calculated by dividing body weight by height squared (kg/m2).

2.7. Statistical analysis:

The data underwent analysis using SPSS software version 17. The accurate measurement of VDR gene expression, insulin resistance, fasting blood sugar, and fasting insulin concentrations were evaluated using ROC curve analysis, with the best cutoff values selected based on the most potential predictive sensitivity and specificity. The standards for qualifying the area under the curve (AUC) were as follows: 0.90–1 = excellent, 0.80–0.90 = good, 0.70–0.80 = fair, 0.60–0.70 = poor and 0.50–0.60 = fail. All tests were two-sided, and a p-value below 0.05 was considered significant. Independent t-tests were employed to compare two groups, while the Chi-square test was utilized to compare two or more groups of categorical data; in addition, a One-way (ANOVA) test was utilized with numerous analyses comparing multiple groups, and a Person correlation coefficient test was applied to investigate the linear relationship among several continuous variables, where (+) shows a positive correlation and (−) shows a negative one

3. Result and Discussion

1. General characteristics for study groups

The research study was comprised of a cohort of one hundred patients diagnosed with T2DM, with an average age of 39.29 ± 8.04 years. In contrast, the control group includes one hundred healthy participants, whose average age was 34.26 ± 7.4 years. In terms of gender distribution, the patient group includes 44 males and 56 females, and the control sample includes 46 males and 54 females. To further categorize the patients, their body mass index (BMI) was taken into consideration, resulting in three distinct categories: T2DM patients with obesity accounting for 64% of the cohort, T2DM patients with overweight constituting 23% of the group, and T2DM patients with normal weight representing 13% of the patients. Additionally, the patients were classified based on their vitamin D levels, resulting in three groups: T2DM patients with adequate vitamin D levels comprising 13% of the cohort, T2DM patients with inadequate vitamin D levels accounting for 48% of the patients, and T2DM patients with deficient vitamin D levels making up 39% of the group. It is worth noting that when compared to the healthy controls, the T2DM patients exhibited increased serum vitamin D levels, shown in [Table 2].

Whereas T2DM displayed an elevated magnitude across all anthropological measurements, as well as biochemical parameters, as explicitly outlined in the extensive [Table 2], it is noteworthy to mention that individuals afflicted with this metabolic disorder exhibited diminished vitamin D receptor values

(VDR) folding change compared to the control group, as evidenced by the data elucidated in the visually compelling [Figure 1].

TABLE 2. Clinical and laboratory characteristics of both patients and controls.

Variables	T2DM patients N=100	Healthy controls N=100	P-Value
Age (year) (mean ± SD)	39.29 ± 8.04	34.26 ± 7.4	0.872
Gender Male / Female (N/%)	44 (44%) 56 (56%)	46(46%) 54(54%)	NA
Height (cm) (mean ± SD)	168.2±7.3	167.9±5.6	0.810
Weight (Kg) (mean ± SD)	96.6±6.3	68.2±4.9	0.001*
BMI (Kg/m^2) (mean ± SD)	34.09±4.4	24.18±4.6	0.001*
Waist circumferences (cm) (mean ± SD)	117.68±9.3	94.6±4.78	0.001*
Hip circumferences (cm) (mean ± SD)	104.22±7.5	81.22±5.77	0.001*
Classification of subjects according to obesity Obese (N/%) Overweight (N/%) Normal weight (N/%)	 64(64%) 23(23%) 13(13%)	 12 (12%) 13(13%) 75(75%)	 0.001* 0.005** 0.001*
Classification of subjects according to vitamin D levels Adequate (> 20 ng/ml) (N/%) Inadequate (12-20 ng/ml) (N/%) - Deficient (<12 ng/ml) (N/%)	 13(13%) 48(48%) 39(39%)	 25(25%) 50(50%) 25(50%)	 0.001* 0.834 0.005**
HbA1C (%) (mean ± SD)	9.155±2.2	4.86±0.86	0.001*
Fasting glucose (mmoL/L) (mean ± SD)	179.62 ± 3.9	79.62 ± 3.2	0.001*
Fasting insulin (mlu/ L) (mean ± SD)	20.93±4.1	13.5±3.9	0.005**
Insulin resistance (HOMA-IR) (mean ± SD)	167.5±3.78	47.3±6.3	0.001*
Serum vitamin D level (ng/ml) (mean ± SD)	13.2±4.3	21.65±6.3	0.001*

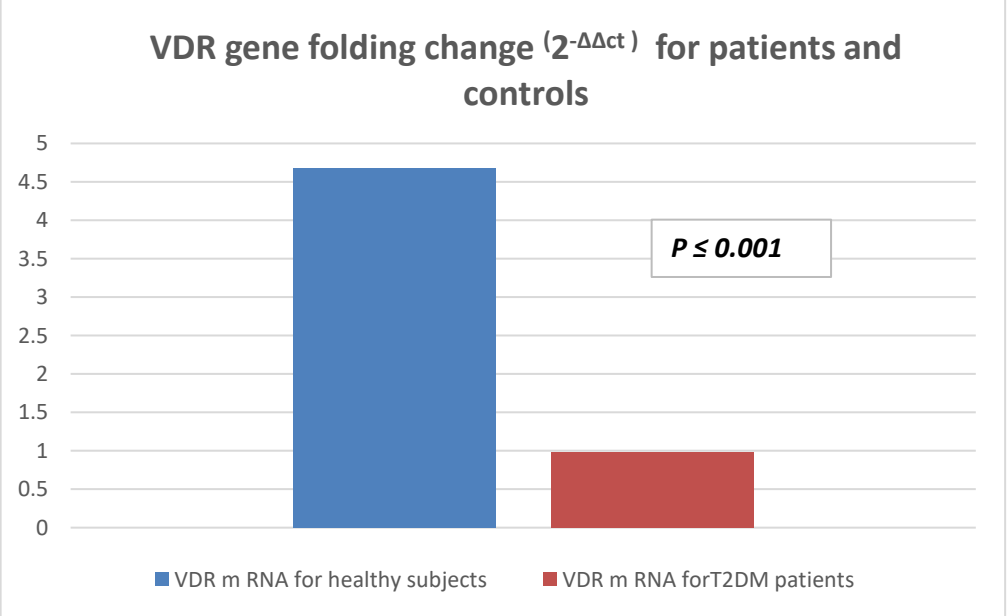


FIGURE 1. The difference in VDR gene folding change (2-ΔΔct) for patients and controls

Analysis of the anthropological and biochemical parameters as predictors for the disease.

The results of the anthropological assessments in the prediction of disease demonstrated a significant level of diagnostic accuracy, as evidenced by the obtained values for BMI, waist circumferences, and hip circumferences, which were found to be 0.945, 0.813, and 0.811 respectively in terms of the area under the curve (AUC). This indicates a high level of predictive power for all three parameters. Furthermore, the statistical analysis revealed that the obtained p-values for all three parameters were 0.001, indicating a highly significant relationship between these anthropometric measures and the prediction of disease.

Moreover, when considering the diagnostic accuracy of BMI, the confidence interval was found to be 91.3% - 97.6% at a cutoff value of 28.95. At this threshold, the sensitivity was determined to be 93.1%, indicating the ability of BMI to recognize accurately affected individuals, while the specificity was 86.1%, suggesting the ability of BMI to accurately determine those who are disease-free.

Similarly, the diagnostic accuracy of waist circumferences was evaluated, and the confidence interval was determined to be 75.4% - 87.2% at a cutoff value of 103.5. At this value, the sensitivity was found to be 79.2%, indicating the ability of waist circumference to recognize accurately affected individuals, while the specificity was 73.3%, suggesting the ability of waist circumference to accurately determine those who are disease-free.

Lastly, the diagnostic accuracy of hip circumferences was also assessed, and the confidence interval was determined to be 75.2% - 87% at a cutoff value of 82.5. At this threshold, the sensitivity was found to be 92.1%, indicating the ability of hip circumference to recognize accurately affected individuals, while the specificity was 59.4%, suggesting the ability of hip circumference to accurately determine those who are disease-free.

The diagnostic accuracy for the biochemical parameters was found to exhibit a significant association in the prediction of disease, except for the assessments of vitamin D and VDR gene expression. The area under the curve (AUC) values and the associated confidence intervals were determined for several parameters, including insulin resistance, fasting glucose, fasting insulin, and HbA1C. The AUC values for these parameters were found to be 0.943 (with a confidence interval of 91.1% to 97.4%), 0.986 (with a confidence interval of 96.6% to 100%), 0.74 (with a confidence interval of 67.2% to 80.9%), and 0.993 (with a confidence interval of 98.6% to 100%), respectively. Moreover, the sensitivity and specificity for insulin resistance, fasting glucose, fasting insulin, and HbA1C were also assessed. It was noted that the sensitivity and specificity metrics were 98% and 82.2%, respectively, at a cutoff value of 76.55 for insulin resistance. Similarly, for fasting glucose, the sensitivity and specificity metrics were 100% and 96%, respectively, at a cutoff value of 131. For fasting insulin, the sensitivity and specificity values were 88.8% and 52.1%, respectively, at a cutoff value of 12.8. Lastly, for HbA1C, the sensitivity and specificity values were determined to be 93.1% and 100%, respectively, at a cutoff value of 6.7. However, in the case of vitamin D and VDR gene expression, the AUC results were considerably lower, measuring at 0.137 and 0.177, respectively, as illustrated in [Figure 2].

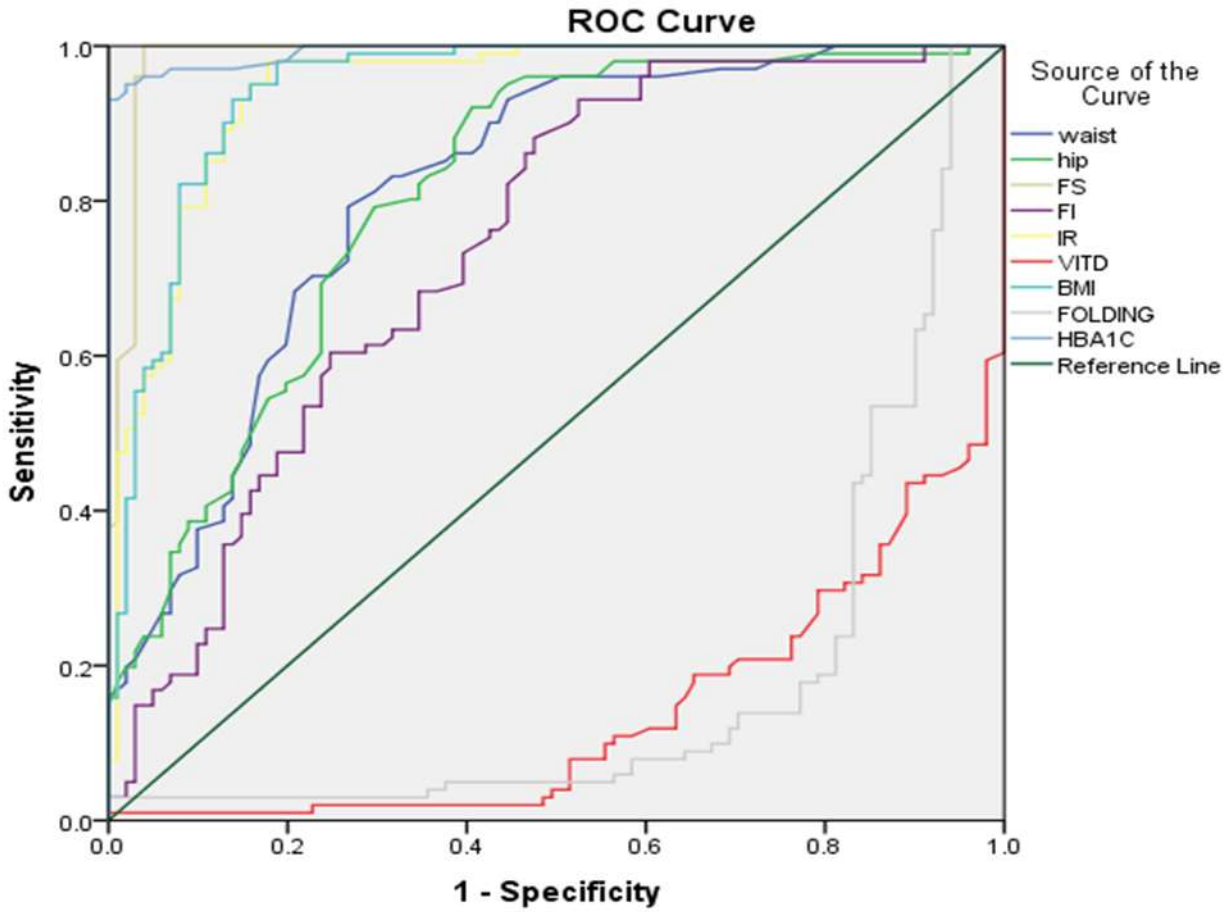


FIGURE 2. ROC curve analysis for biochemical and anthropological parameters.

Association between anthropological and biochemical variables and insulin resistance

T2DM patients exhibit remarkably positive simple linear correlations of substantial significance between insulin resistance and various biochemical as well as anthropological parameters. These parameters include fasting insulin, fasting glucose, hip circumferences, waist circumferences, and BMI. The correlation coefficients for these relationships are 0.742, 0.670, 0.625, 0.670, and 0.620 respectively) p-values = 0.001(. Furthermore, a relatively moderate but still significant positive simple linear correlation exists between insulin resistance and HbA1C, with a correlation coefficient of 0.500 and a p-value of 0.005. Interestingly, a highly significant negative linear correlation is observed between vitamin D, VDR gene expression, and insulin resistance. The correlation coefficients for these associations are -0.627 and -0.657 respectively, for both (p-values = 0.001([Table 3].

TABLE 3. Correlation between insulin resistance anthropological and biochemical parameters for T2DM patients.

Parameters	T2DM patients N=100	
	R^2	P-Value
Insulin resistance and fasting insulin	0.551	0.001*
Insulin resistance and fasting glucose	0.450	0.001*
Insulin resistance and HbA1C	0.251	0.005**
Insulin resistance and Hip circumferences	0.391	0.001*

Insulin resistance and Waist circumferences	0.450	0.001*
Insulin resistance and BMI	0.385	0.001*
Insulin resistance and VDR folding change	0.432	0.001*
Insulin resistance and Serum vitamin D	0.394	0.001*

N: number, %: percentage, SD: standard deviation, *: highly significant. P≤0.001, **: mild significant differences P≤0.005, BMI: Body mass index, and HbA1C: Hemoglobin A1

Analysis of the anthropological and biochemical parameters for patients according to gender:

The outcomes obtained from the process of dividing individuals with type 2 diabetes mellitus (T2DM) based on their sex, to highlight the disparities in anthropological and biochemical criteria about sex, as well as evaluating the extent of divergence between the two groups, yielded various outcomes. These outcomes, when analyzed, revealed a statistically significant dissimilarity in certain factors such as waist circumferences (P = 0.003), fasting blood sugar levels (P = 0.004), insulin resistance (P = 0.005), vitamin D levels (P = 0.005), and VDR folding change (P = 0.003). Conversely, no statistically significant variance was observed in other parameters. [Table 4].

TABLE 4. Variation between male and female in biochemical and anthropological parameters for T2DM patients.

Variables	Male patients N=44	Female patients N=56	P-Value
BMI (Kg/m^2) (mean ± SD)	35.5±4.6	32.5±3.8	0.563
Waist circumferences (cm) (mean ± SD)	124±8.3	112 ±9.3	0.003**
Hip circumferences (cm) (mean ± SD)	109±8.3	100±7.5	0.014
HbA1C (%) (mean ± SD)	9.2±2.4	9±2	0.594
Fasting glucose (mmoL/L) (mean ± SD)	186± 4.3	174± 3.9	0.004**
Fasting insulin (mlu/ L) (mean ± SD)	23±4.6	19±4	0.018
Insulin resistance (HOMA-IR) (mean ± SD)	190±2.65	146±3.9	0.005**
Serum vitamin D level (ng/ml) (mean ± SD)	15±4.8	10±4.2	0.005**
VDR folding change $(2^{-\Delta\Delta ct})$ (mean ± SD)	1.3±0.36	0.66±0.034	0.003**

N: number, %: percentage, SD: standard deviation, VDR: vitamin D receptor, HOMA-IR: homeostasis model assessment of insulin resistance, *: highly significant. P≤0.001, **: mild significant differences P≤0.005, BMI: Body mass index, and HbA1C: Hemoglobin A1 C.

Association between anthropological and biochemical variables and insulin resistance in accordance with patient's gender.

For the male individuals, a highly significant positive linear correlation was found within insulin resistance and various parameters in the study. These parameters include fasting insulin, fasting glucose, HbA1C, hip circumferences, waist circumferences, and BMI. The correlation coefficients for these relationships were 0.980, 0.642, 0.723, 0.559, 0.641, and 0.518, respectively. The corresponding p-values were all found to be significant at 0.001 levels. Additionally, we found a negative linear correlation observed within vitamin D and insulin resistance, although this relationship did not show any significant variation. The correlation coefficient in this case was -0.275, with a p-value of 0.035 [Table 5A]. Comparatively, a negative correlation founded within VDR gene expression and insulin

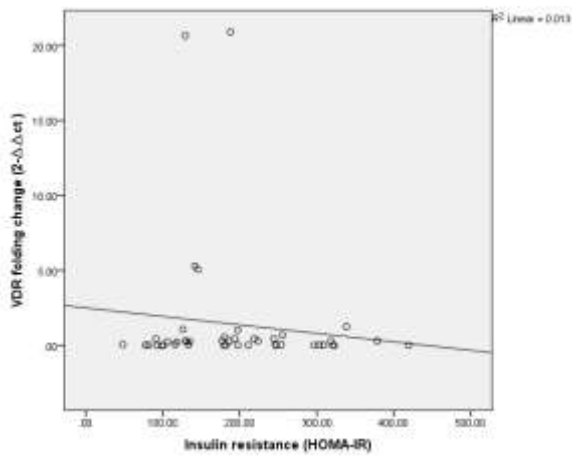
resistance, but again, this relationship did not reach statistical significance. The correlation coefficient for this association was -0.114, with a p-value of 0.230 [Figure 3 A].

For the female patients, highly significant positive simple linear correlations were discovered between insulin resistance and fasting insulin, as well as between insulin resistance and fasting glucose, HbA1C, hip circumferences, waist circumferences, and BMI (with correlation coefficients of r = 0.977 and P = 0.001, r = 0.633 and P = 0.001, r = 0.694 and P = 0.001, r = 0.653 and P = 0.001, r = 0.634 and P = 0.001, and r = 0.624 and P = 0.001, respectively) as shown in [Table 5 B]. Additionally, there was a negative linear correlation observed within insulin resistance, VDR gene expression, and vitamin D (with correlation coefficients of r = -0.139 and P = 0.151 as depicted in [Figure 3 B], and r = -0.265 and P = 0.261 as depicted in [Figure 3 C], respectively).

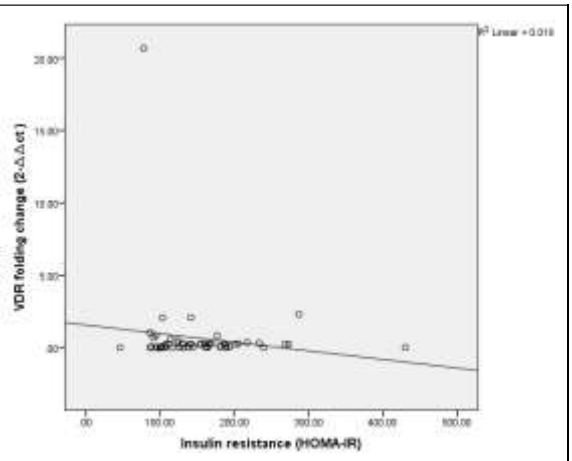
TABLE 5. Correlation between insulin resistance anthropological and biochemical parameters for male and female patients.

Parameters	A. Male N= 44	
	R^2	P-Value
Insulin resistance and fasting insulin	0.961	0.001*
Insulin resistance and fasting glucose	0.412	0.001*
Insulin resistance and HbA1C	0.523	0.001*
Insulin resistance and Hip circumferences	0.313	0.001*
Insulin resistance and Serum vitamin D	0.076	0.035
Insulin resistance and Waist circumferences	0.412	0.001*
Insulin resistance and BMI	0.269	0.001*
Parameters	B. Female N =56	
	R^2	P-Value
Insulin resistance and fasting insulin	0.956	0.001*
Insulin resistance and fasting glucose	0.401	0.001*
Insulin resistance and HbA1C	0.483	0.001*
Insulin resistance and Hip circumferences	0.427	0.001*
Insulin resistance and Waist circumferences	0.403	0.001*
Insulin resistance and BMI	0.390	0.001*

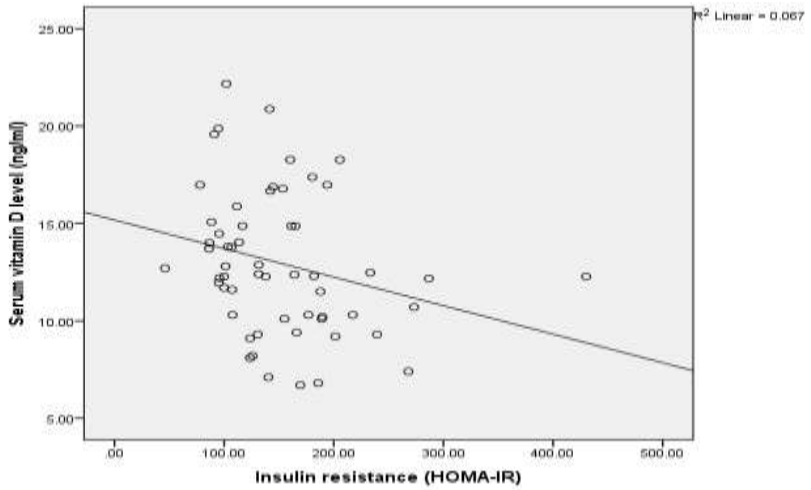
N: number, %: percentage, SD: standard deviation, *: highly significant. P≤0.001, BMI: Body mass index, and HbA1C: Hemoglobin A1 C.



A) Negative linear correlation between VDR gene expression and insulin resistance for male patients.



B) Negative linear correlation between VDR gene expression and insulin resistance for female patients.



B) Negative linear correlation between serum vitamin D level and insulin resistance for female patients.

FIGURE 3. Correlations between Insulin Resistance, VDR gene expression, and vitamin D level in male and female patient.

Analysis of the anthropological and biochemical parameters for patients according to BMI:

Classification of patients based on their BMI is an essential step in understanding the various weight categories, namely obese, overweight, and normal weight. It is worth noting that the obese patients exhibited significantly elevated levels of BMI, Waist circumferences, Hip circumferences, HbA1C, and Insulin Resistance. The statistical analysis revealed a statistically significant difference (P= 0.001, 0.001, 0.001, 0.005, 0.005, and 0.005, respectively) between these parameters in obese patients compared to both overweight and normal-weight individuals. However, when it comes to fasting insulin, the absence of statistical significance was found among obese, overweight, and normal-weight patients (P = 0.019). Interestingly, the normal-weight patients demonstrated increased expression of

the VDR gene and vitamin D concentrations when compared to their obese and overweight counterparts. It was determined that these variations were of statistical significance (P = 0.005, 0.002, respectively) [Table 6].

TABLE 6. Variation in biochemical and anthropological parameters for T2DM patients depends on the patient's BMI.

Variables	Obese patients N=64	Overweight patients N=23	Normal weight patients N = 13	P-Value
BMI (Kg/m^2) (mean ± SD)	35.82±4.1	31.72±3.2	25.32±1.9	0.001*
Waist circumferences (cm) (mean ± SD)	123.06±3.9	110.052±5.9	104.32±4.6	0.001*
Hip circumferences (cm) (mean ± SD)	109.3±3.7	98.04±2.8	91.32±4.8	0.001*
HbA1C (%) (mean ± SD)	10.5±2.1	8.6±1.9	8.7±1.3	0.005**
Fasting glucose (mmoL/L) (mean ± SD)	185±6.3	172±8.4	163±9.3	0.005**
Fasting insulin (mlu/ L) (mean ± SD)	22.25±5.2	19.23±3.2	16.15±2.9	0.019
Insulin resistance (HOMA-IR) (mean ± SD)	182.41±6.9	1451.56±8.1	115.23±4.3	0.005**
Serum vitamin D level (ng/ml) (mean ± SD)	8.9.23±3.1	12.22±4.3	14.93±2.9	0.005**
VDR folding change $(2^{-\Delta\Delta ct})$ (mean ± SD)	0.6±0.21	0.9±0.31	1.3±0.025	0.002**

N: number, %: percentage, SD: standard deviation, VDR: vitamin D receptor, HOMA-IR: homeostasis model assessment of insulin resistance, *: highly significant. P≤0.001, **: mild significant differences P≤0.005, BMI: Body mass index, and HbA1C: Hemoglobin A1 C.

Association between anthropological and biochemical variables and insulin resistance among obese patients

Several highly significant positive simple linear correlations were discovered among insulin resistance fasting insulin, fasting glucose, hip circumferences, waist circumferences, and BMI for individuals who were obese. The correlation coefficient for insulin resistance and fasting insulin was 0.975 (p-value = 0.001). In the same way, the correlation coefficient for insulin resistance and fasting glucose was 0.789 (p-value = 0.001). Additionally, significant positive linear correlations were observed between insulin resistance and hip circumferences (r = 0.522; P = 0.001), waist circumferences (r = 0.549; P = 0.001), and BMI (r = 0.871; P = 0.001). These findings, as presented in [Table 7], indicate a strong relationship between these variables in obese patients. However, when examining the association within HbA1C and insulin resistance, a positive linear correlation was observed, but it did not exhibit significant variation. Conversely, a negative linear correlation was detected between vitamin D and insulin resistance, but no significant variation was identified (r = -0.044; P = 0.083) [Table 7]. Similarly, the correlation within VDR gene expression and insulin resistance was negative but lacked significant variation (r = -0.161; P = 0.101), as depicted in [Figure 4].

TABLE 7. Correlation between insulin resistance, anthropological, and biochemical parameters for obese patients.

Parameters	Obese patients N=64

	R^2	P-Value
Insulin resistance and fasting insulin	0.952	0.001*
Insulin resistance and fasting glucose	0.623	0.001*
Insulin resistance and HbA1C	0.005	0.074
Insulin resistance and Hip circumferences	0.273	0.001*
Insulin resistance and Serum vitamin D	0.002	0.083
Insulin resistance and Waist circumferences	0.302	0.001*
Insulin resistance and BMI	0.376	0.001*

N: number, %: percentage, SD: standard deviation, *: highly significant. P≤0.001, BMI: Body mass index, and HbA1C: Hemoglobin A1 C.

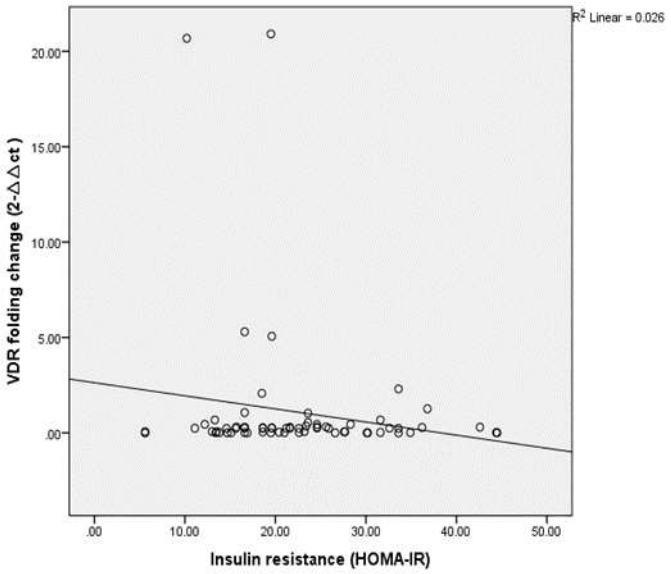


FIGURE 4. Negative linear correlation between VDR gene expression and insulin resistance for obese patients.

Analysis of the anthropological and biochemical parameters for patients according to BMI:

The categorization of diabetic individuals through different groups according to their vitamin D levels is a common practice. In this study, the researchers divided the patients into three groups: those with deficient vitamin D, those with inadequate vitamin D, and those with adequate vitamin D. This classification's objective was to compare the anthropological and biochemical parameters among these groups and identify any significant variations. Surprisingly, the results revealed no significant variations in most parameters except vitamin D concentration and VDR gene expression. These two factors showed a significant statistical variance among groups (P = 0.001, 0.001, respectively). Additionally, a mild but significant variation was observed in insulin resistance among the groups. Patients with low levels of vitamin D had higher insulin resistance (P=0.005) [Table 8]. Interestingly,

a negative linear correlation was also found between insulin resistance and vitamin D levels in diabetic patients with a deficiency of vitamin D. However, this correlation did not reach statistical significance (r= -0.234; P=0.076) [Figure 5].

There exists a direct and positive relationship of a linear nature between the resistance of the hormone insulin and VDR gene expression, in the context of individuals diagnosed with T2DM and are also deficient in vitamin D. Notably, this correlation has been observed to be constant and not significantly varying among this specific group of patients, as indicated by the statistical analysis results of the correlation coefficient (r = 0.026; p-value = 0.437), which were depicted in [Figure 6].

TABLE 8. Variation in biochemical and anthropological parameters for T2DM patients depends on the patient's vitamin D levels.

Variables	Serum vitamin D level Deficient N=39	Serum vitamin D level Inadequate N=48	Serum vitamin D level Adequate N = 13	P-Value
BMI (Kg/m^2) (mean ± SD)	34±3.6	34±2.8	33±2.55	0.832
Waist circumferences (cm) (mean ± SD)	117±3.5	118±2.8	114±4.3	0.786
Hip circumferences (cm) (mean ± SD)	103±3.44	105±4.55	101±2.64	0.699
HbA1C (%) (mean ± SD)	8±1.5	9±1.8	9±2.3	0.275
Fasting glucose (mmoL/L) (mean ± SD)	179±4.1	180±5.3	130±3.9	0.795
Fasting insulin (mlu/ L) (mean ± SD)	21±4.66	20±3.88	20±2.44	0.974
Insulin resistance (HOMA-IR) (mean ± SD)	167±2.7	160±2.9	142.2±3.6	0.005**
Serum vitamin D level (ng/ml) (mean ± SD)	8 ±3.12	12±2.88	16±3.15	0.001*
VDR folding change $(2^{-\Delta\Delta ct})$ (mean ± SD)	0.5±0.052	0.7±0.35	1.5±0.64	0.001*

N: number, %: percentage, SD: standard deviation, VDR: vitamin D receptor, HOMA-IR: homeostasis model assessment of insulin resistance,*: highly significant. P≤0.001, **: mild significant differences P≤0.005, BMI: Body mass index, and HbA1C: Hemoglobin A1 C.

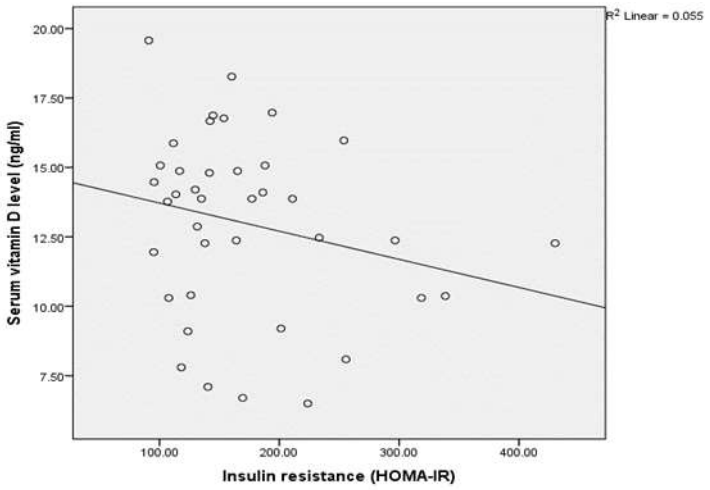


FIGURE 5. Negative linear correlation between vitamin D level and insulin resistance for diabetic

patients with Serum vitamin D level Deficient.

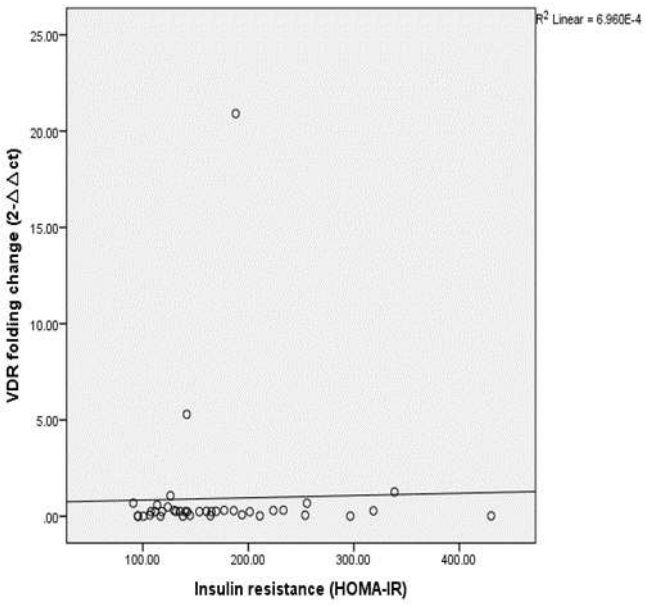


FIGURE 6. Correlation between VDR gene expression and insulin resistance for diabetic patients with Serum vitamin D level Deficiency.

Analysis of the impact of obesity and vitamin D deficiency on insulin resistance.

In the majority of the previous findings within this particular investigation, it has been duly acknowledged that there exists an inverse relationship between insulin resistance, vitamin D rates, and VDR gene expression. This implies that a decline in vitamin D rates may be linked to an elevation in insulin resistance. Additionally, it has been observed that there is a positive and direct correlation between insulin resistance and obesity. Consequently, we made the deliberate decision to specifically select patients who were classified as obese and subsequently categorized them according to their respective levels of vitamin D. The main purpose of this process intended to illuminate all potential risk factors that could potentially contribute to the incidence of insulin resistance. As a result, the obese patients were effectively divided into three distinct groups, which consisted of individuals who were obese with a deficiency in vitamin D, those who were obese with insufficient vitamin D rates, and those who were obese with adequate levels of vitamin D. No noteworthy deviation was observed among the three distinct clusters in terms of measurements of waist circumferences, hip circumferences, fasting blood sugar, and fasting insulin concentrations. Nevertheless, a markedly significant variation has been identified in terms of insulin resistance, vitamin D levels, and the expression of the VDR gene (P = 0.001 for all aforementioned variables) [see Table 9].

A remarkably substantial adverse linear correlation has been observed within the gene expression of the Vitamin D receptor (VDR) and insulin resistance, with a correlation coefficient (r) of -0.672 (p-value = 0.001), as evidenced by the data presented in [Figure 7]. Furthermore, it is also worth noting that a negative linear correlation was detected between insulin resistance and serum vitamin D levels, although the variations were not found to be statistically significant. The correlation coefficient (r) for this relationship was -0.327, while the coefficient of determination (R2) was 0.107, and the p-value (P) was 0.042

TABLE 9. Variation in biochemical and anthropological parameters for obese T2DM patients according to vitamin D levels.

Variables	Obese with vitamin D deficiency N=29	Obese with Inadequate levels of vitamin D N=27	Obese with Adequate levels of vitamin D N = 8	P-Value
Waist circumferences (cm) (mean ± SD)	114±2.5	123±3.1	114±1.9	0.217
Hip circumferences (cm) (mean ± SD)	99±5.1	111±7.2	99±6.3	0.060
Fasting glucose (mmoL/L) (mean ± SD)	176±6.2	185±8.1	176±6.8	0.217
Fasting insulin (mlu/ L) (mean ± SD)	20±3.6	19±4.2	15±4.3	0.216
Insulin resistance (HOMA-IR) (mean ± SD)	156±4.1	156±3.4	117±2.66	0.001*
Serum vitamin D level (ng/ml) (mean ± SD)	8.6±2.1	12.3±3.4	15.5±3.8	0.001*
VDR folding change $(2^{-\Delta\Delta ct})$ (mean ± SD)	0.5±0.03	0.6±0.45	1.9±0.6.3	0.001*

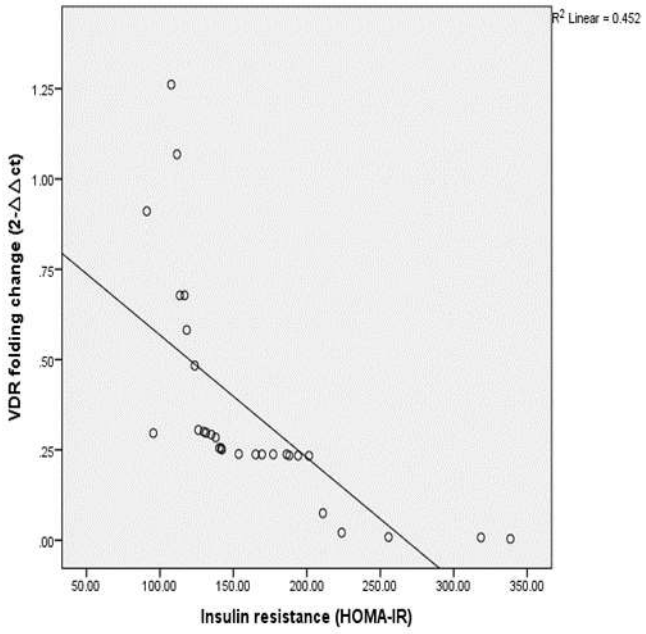


FIGURE 7. Negative linear correlation between VDR gene expression and insulin resistance for diabetic obese patients with Serum vitamin D level Deficient

Discussion:

Our study offers a thorough and all-encompassing evaluation of the pivotal role played by vitamin D and the expression of the VDR gene in the intricate process of insulin resistance development in T2DM individuals within our specific population. It is important to note that, to the best of our knowledge, this is a pioneering study that endeavors to shed light on the impact of the VDR gene on the progression of insulin resistance. Moreover, this investigation endeavors to elucidate additional factors, such as anthropological and biochemical aspects, that contribute to the intricate process of insulin resistance development. Therefore, this study provides a comprehensive and holistic understanding of the multifaceted nature of insulin resistance development in individuals with T2DM, considering various genetic, environmental, and physiological factors.

In the present investigation, it was determined that individuals classified as healthy controls exhibited elevated serum vitamin D rates compared to patients diagnosed with Type 2 Diabetes Mellitus (T2DM). These observations are congruent with the outcomes of a study carried out by Nasr et al. within the Arabian Gulf region. Through their investigation, it was ascertained that individuals without diabetes presented with higher levels of vitamin D when contrasted with patients diagnosed with diabetes. Furthermore, their findings revealed the presence of a statistically significant correlation between the deficiency of vitamin D and the occurrence of T2DM [27]. A further investigation was conducted in the Northern region of India by Daga and colleagues, wherein an interesting finding emerged. It was discovered that a staggering 91.1% of individuals diagnosed with diabetes exhibited insufficient levels of vitamin D. The researchers meticulously analyzed the data, observing that patients with diabetes presented vitamin D concentrations of 7.88 ± 1.2. In stark contrast, those without diabetes displayed significantly higher concentrations of the vitamin, measuring 16.64 ± 7.83 [28]. A subsequent examination conducted by Lee et al. unveiled a noteworthy revelation, namely that a staggering 89% of the patients afflicted with T2DM exhibited a pronounced deficiency in the essential micronutrient known as vitamin D [29]. According to the research conducted by Gagnon et al., it was observed that individuals who were diagnosed with diabetes exhibited average levels of serum vitamin D concentrations that were comparatively lower when compared to the individuals who did not have diabetes [30]. In agreement with the study conducted by Taheri, it was observed that individuals who do not have diabetes exhibited a mean level of vitamin D in their serum, which was measured to be approximately 22.22 ± 16.03 units, whereas diabetic patients demonstrated a relatively lower mean level of vitamin D in their serum, measuring around 20.6 ± 11.4 units [31].

Furthermore, in our study, we made an interesting discovery regarding the disparity in vitamin D levels between male and female diabetic patients. Specifically, our findings revealed that male individuals with diabetes exhibited significantly higher levels of vitamin D in comparison with female counterparts. This observation aligns perfectly with the research conducted by Bayani et al., which also demonstrated that vitamin D levels were consistently lower in females when compared to males. Therefore, our study not only corroborates the findings of Bayani et al. but also provides additional evidence to support the notion that there exists a gender-based difference in vitamin D levels among diabetic individuals [32]. Further, the findings are in line with the investigations carried out by Bajaj et al., which stated that in comparison to less than fifty percent of male diabetic patients, approximately seventy-five percent of female diabetic patients exhibited a deficiency in vitamin D. Noteworthy is the fact that our results align with those of Bajaj et al., providing further evidence to support the notion that a significant gender disparity exists in the prevalence of vitamin D deficiency among individuals diagnosed with diabetes [33]. This gender-specific variation in vitamin D levels among diabetic patients may have implications for the management and treatment of the disease, highlighting the need for targeted interventions to address this disparity and improve health outcomes for female diabetic patients. Healthcare professionals must be aware of this gender difference in vitamin D deficiency rates and consider screening and supplementation strategies tailored to the specific needs of female patients with diabetes. Furthermore, these findings emphasize the importance of more investigation in this area in order to comprehend the underlying factors contributing to this gender disparity and explore potential mechanisms through which vitamin D deficiency may impact diabetes outcomes differently in males and females.

The potential explanation for the disparity observed between individuals with diabetes and those without, along with the distinction between males and females, may be associated to multitude factors, such as the duration of sun exposure, variations in dietary habits, prolonged utilization of protective garments, application of sunscreen, advancing age, as well as the absence of food sources containing ergocalciferol and the presence of malabsorption syndrome [34,35].

In the present study, it was observed that obese individuals who were diagnosed with diabetes exhibited decreased levels of vitamin D when compared to those who were either overweight or of normal weight. This noteworthy finding aligns with previous research investigations, which have consistently demonstrated that the accumulation of calcidiol in adipose tissue leads to a decline in 25(OH) D concentrations as the percentage of body fat escalates [36-38].

Recently, there has been a demonstration of the fact that individuals who are obese possess adipose tissue that exhibits reduced concentrations of vitamin D as a result of modifications in the functioning of enzymes responsible for metabolizing vitamin D and a decline in the release of 25-hydroxyvitamin-D3-induced by catecholamine in these patients [39].

According to the results obtained from our research, it has been observed that the levels of VDR folding change exhibited by the patients were comparatively lower in comparison to the controls. Moreover, our investigation revealed a significant correlation between obesity and reduced levels of VDR gene expression among the patients. Furthermore, we also discovered that females tended to have lower levels of VDR gene expression. This valuable evidence further corroborates the earlier research conducted by Fayez and his esteemed research team, wherein they successfully demonstrated that patients suffering from diabetic nephropathy showcased lower levels of VDR gene expression as compared to the healthy individuals in the control group [40]. The gene expression of the VDR gene has been observed to exhibit a lower level of activity in individuals diagnosed with T2DM, as concluded by a separate study conducted by Yi et al. In further support of this finding, Sayl et al. also reported comparable results in their investigation. These consistent findings across multiple studies provide compelling evidence for the association between reduced VDR gene expression and the presence of T2DM [41]. The observed reduction in the expression of the Vitamin D receptor (VDR) gene among individuals afflicted with diabetes mellitus could potentially account for the comparatively diminished levels of Vitamin D in this patient population.

In the present study, we founded an exceedingly notable inverse linear correlation was ascertained between the levels of vitamin D, the expression of the vitamin D receptor (VDR) gene, and the manifestation of insulin resistance within the entire cohort of T2DM individuals. Findings obtained in our study concur with numerous previously conducted research endeavors that have reported on the criticality of a dearth in vitamin D and VDR gene expression as pivotal determinants in the pathogenesis of insulin resistance. Numerous investigations have corroborated the notion that the insufficiency of vitamin D and the diminished expression of the VDR gene has a crucial position in the etiopathogenesis of insulin resistance, thereby emphasizing the significance of our results [42-45].

Our findings also demonstrated that individuals who are obese and have a deficiency in the vitamin D category of patients exhibit an exceedingly significant negative linear connection between the expression of the VDR gene and the state of insulin resistance. These were consistent with many researches that found a strong correlation between insulin resistance and extreme obesity and low vitamin D levels [46-50].

We can explain the role of vitamin D deficiency and VDR gene lower expression in occurrence of insulin resistance by understanding the process of insulin secretion. The release of insulin by pancreatic β-cells is a consequence of high blood glucose levels, as glucose enters the β-cells through the glucose transporter 2 (GLUT-2) and is metabolized to produce ATP, which then inhibits the ATP-sensitive K+ channel, leading to β-cell membrane depolarization and activation of L-type voltage-operated channels that generate localized Ca2+ pulses necessary for insulin secretion [51]. The alteration of calcium signaling, which involves a reduction in the expression of L-type Ca2+ channels, is believed to be caused by 1,25(OH)2D3, leading to a decline in intracellular Ca2+ concentration [52]. The elevation in intracellular calcium concentration responsible for triggering the release of insulin in pancreatic

beta-cells was subsequently found to be induced by rapid, nongenomic actions of 1,25(OH)2D3, a form of vitamin D, involving the regulation of signaling pathways including PKA and PLC synthesis [53-56].

The findings derived from our meticulous research on insulin resistance and anthropological parameters are of utmost importance and should not be disregarded, which provide a significant positive linear correlation between waist, BMI, and hip circumferences, and insulin resistance among all patient groups, including individuals with T2DM, T2DM patients with obesity, and varying gender distributions. This correlation aligns with the conclusions drawn from several other studies in the field [57-60]. Therefore, it is crucial to recognize and take into account the implications of these well-established connections in future medical and anthropological investigations.

4. Conclusion and future scope

T2DM patients exhibited elevated anthropological measurements and biochemical parameters in comparison to controls, along with decreased VDR folding change and vitamin D levels. Anthropological assessments and biochemical parameters demonstrated significant diagnostic accuracy in disease prediction, while a highly significant negative linear correlation founded within VDR gene expression and insulin resistance in obese individuals with vitamin D deficiency. Furthermore, a negative linear correlation founded within insulin resistance and serum vitamin D levels, although no significant variation was noted.

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Competing interests

The authors have no relevant financial or non-financial interests to disclose.

Author contributions

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by [Ahmed Ibrahim Abd Elneam], [Awatef A. Msolly] and [Ali Ismail A. Abdul Rahim]. The first draft of the manuscript was written by [Zafer S. Alshehri], [Sharif H. Alhajlah], [Faez F. Alshehri], [Alhomidi Almotiri] and [Essam M. Elmahdi] and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Data Availability

The datasets generated during and/or analysed during the current study are not publicly available due to [we do not have permission from the participants to do so] but are available from the corresponding

author on reasonable request.

Ethics approval

This study was performed in line with the principles of the Declaration of Helsinki. The project was approved by the Shaqra University Ethics Committee and is registered under the agreement number ERC_SU_F_202300013.

Consent to participate

Written informed consent was obtained from all participants who had comprehensive health histories including details on their demographics, health status of T2DM, and use of medications.

Consent to publish

The authors affirm that human research participants provided informed consent for publication.

Potential for Future Development

The healthcare data.

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Conflict of Interest

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