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RESEARCH ARTICLE

The Role of Vitamin D Pathway Components in Determining Disease Severity and Cardiovascular Risk in Type 2 Diabetes Mellitus: A Precision Medicine Approach

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ABSTRACT

Objectives: This study evaluates the vitamin D pathway and atherogenic markers in T2DM patients, develops composite indices to quantify disease severity and cardiovascular risk, and tests their predictive utility using precision modeling.

Material and Methods: Serum levels of vitamin D (Vit D), vitamin D receptor (VDR), binding protein, and lipid parameters were measured in 50 T2DM patients and 25 healthy controls. Atherogenic indices: AIP, Castelli Risk Index I (CAST-1), and Castelli Risk Index II (CAST-2) were calculated. Principal component analysis (PCA), multivariate regression, neural networks, and partial least squares (PLS) modeling were performed.

Results: Patients with T2DM exhibited significantly lower Vit D and VDR levels, higher cholesterol, triglycerides, LDL, VLDL, and atherogenic indices, with lower HDL. Composite PCA scores confirmed increased disease severity and cardiovascular risk. Insulin resistance strongly predicted atherogenic indices, whereas Vit D levels predicted both disease severity and cardiovascular risk.

Conclusion: Vitamin D pathway disruption significantly correlates with insulin resistance, metabolic severity, and cardiovascular risk in T2DM, underscoring its potential as a personalized therapeutic target.

Keywords: Atherogenicity; Cardiovascular; Diabetes Mellitus; Insulin resistance; Vitamin D.

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1. Introduction

Type 2 diabetes mellitus (T2DM) represents a significant global health issue, impacting over 500 million individuals and constituting around 10.5% of the worldwide population [1]. The prevalence is increasing significantly, especially in low- and middle-income areas, primarily due to rising obesity rates, physical inactivity, and nutritional imbalances [2]. T2DM ranks as the ninth leading cause of mortality globally [3, 4], presenting a significant burden on both individuals and healthcare systems. This highlights the necessity for a comprehensive understanding of its biological mechanisms to develop more effective prevention and intervention strategies.

Insulin resistance (IR) is central to the pathophysiology of T2DM, defined by diminished responsiveness of peripheral tissues to insulin signaling [5]. Initially, hyperinsulinemia compensates for IR, but over time, IR leads to pancreatic β-cell exhaustion and sustained

hyperglycemia [6]. In addition to its metabolic function, IR independently increases cardiovascular risk by initiating a series of interconnected abnormalities, such as dyslipidemia, hypertension, and chronic inflammation, all of which contribute to the acceleration of atherosclerosis [5, 7, 8]. Atherogenic dyslipidemia, characterized by elevated triglyceride levels, decreased HDL-C, and an increase in small dense LDL particles, is increasingly acknowledged as a defining lipid profile in T2DM. Clinical indices, including the Atherogenic Index of Plasma (AIP) and Castelli Risk (CAST) Indices 1 and 2, function as effective and readily available indicators of cardiovascular risk [9, 10].

Accumulating evidence indicates that vitamin D (Vit D) and its pathways play a role in modulating glucose and lipid metabolism, inflammation, and vascular function [11, 12]. Vit D functions via the vitamin D receptor (VDR), a nuclear receptor found in key metabolic tissues, including pancreatic β-cells, adipose tissue, and vascular endothelium [13]. The Vit D binding protein regulates the transport and availability of Vit D metabolites in circulation [14]. Evidence indicates that lower serum levels of 25-hydroxyvitamin D [25(OH)D] correlate with reduced insulin sensitivity, increased inflammation, and elevated cardiovascular risk in individuals with T2DM [13, 15, 16].

While considerable advancements have been made in comprehending individual risk factors, most studies conducted thus far have analyzed these components independently. There is a distinct necessity for integrative analyses that can elucidate the interactions among Vit D pathway components, insulin resistance, and atherogenic risk. Conventional statistical methods frequently fail to clarify these intricate interactions.

Therefore, the objectives of the present study are: (1) examine serum Vit D, VDR, Vit binding protein levels, alongside cardiovascular risk markers including lipid profiles and indexes derived from it such as AIP, CAST-1 and CAST-2 in individuals with T2DM; (2) developing composite indices that represent vitamin D pathway activity, T2DM severity, and cardiovascular risk via principal component analysis (PCA); and (3) evaluating the predictive value of these markers using multivariate regression, neural network models and pathway analysis. We hypothesized that disruptions in the vitamin D pathway are closely associated with insulin resistance and atherogenic lipid profiles, and that integrative modelling will provide strong indicators of overall disease burden.

2. Materials and Methods

2.1. Subjects

The study included 75 participants, comprising 50 individuals diagnosed with T2DM and 25 HCs. All diagnoses of T2DM were validated by a senior endocrinologist in accordance with the criteria established by the American Diabetes Association (ADA) [17]. Patients were recruited from the Diabetes and Endocrine Centre at Al-Sadr Teaching Hospital in Najaf, Iraq, during the period from December 2024to February 2025. HCs were selected from the same population, comprising hospital staff, relatives, and acquaintances of the patients.

Individuals with concurrent chronic inflammatory conditions, including rheumatoid arthritis, inflammatory bowel disease (such as Crohn's disease and ulcerative colitis), cancer, thyroid disorders, or bone diseases associated with vitamin D deficiency (e.g., osteoporosis, rickets, osteomalacia) were excluded from the study. Individuals with serum C-reactive protein (CRP) levels below 6 mg/L were included to confirm the absence of systemic inflammation. Several patients received insufficient prescriptions for medications, including Galvus (50/850 mg and 50/1000 mg), glimepiride (4 mg), Glucophage (500 mg) and aflibercept (Eylea, 2 mg) for diabetic retinopathy.

The research adhered to national and international ethical standards, including the Declaration of Helsinki. Informed consent was obtained in writing from all participants or their legally authorised representatives. The

Institutional Review Board of the Kufa institute, Al-furat Al-awsat Technical University, granted ethical approval (approval number 10981/2023).

2.2. Biochemical and Clinical Evaluations

Fasting blood samples (5 mL) were collected on the day of clinical evaluation between 8:00 and 10:00 a.m. using sterile syringes and disposable needles. Participants underwent a fasting period of no less than 8 hours. Samples were obtained using EDTA tubes for HbA1c analysis and serum gel tubes for the other assays. Following a 15-minute incubation at room temperature, samples underwent centrifugation at 3500 rpm for a duration of 10 minutes. Aliquots were preserved at –80°C prior to analysis.

Serum CRP was quantified utilizing a latex agglutination assay (Linear Cromatest, Spain). Fasting blood glucose and HbA1c were assessed using enzymatic colorimetric methods with kits obtained from LINEAR CHEMICALS, Spain. Commercial ELISA kits from Elabscience, Inc. (CA, USA) were utilized for the quantification of insulin, vitamin D, vitamin D receptor (VDR), and vitamin D binding protein. ELISA readings were acquired utilising a microplate reader (Human, Germany).

The components of the lipid profile, including triglycerides (TG), total cholesterol, and HDL-C, were evaluated using colorimetric kits (Q-SLAP, Egypt). Low-density lipoprotein cholesterol (LDL-C) and very low-density lipoprotein cholesterol (VLDL-C) were determined using the Friedewald equation: LDL-C is calculated as Total Cholesterol minus HDL-C and the quotient of triglycerides divided by five. VLDL-C is calculated as TG divided by 5 [18, 19]. Renal function marker (creatinine) was evaluated using the colorimetric kit (Q-SLAP, Egypt). Atherogenic indices were calculated as follows: AIP is defined as the logarithm of the ratio of triglycerides to HDL cholesterol. CAST-1 is calculated as the ratio of Total Cholesterol to HDL-C. CAST-2 is calculated as LDL-C divided by HDL-C [20]. Insulin resistance indices were determined utilizing the HOMA2 calculator from the University of Oxford, yielding values of insulin resistance (HOMA2-IR) based on fasting insulin and glucose levels. The Body Mass Index (BMI) was calculated in kg/m^2, and smoking status was documented. Socio-demographic data for patients was gathered by trained paramedical personnel, whereas one of the authors (A.K.A.) collected this information for the control group.

2.3. Statistical analysis

All statistics were conducted via SPSS V 30, Windows. The Kolmogorov–Smirnov test was employed to assess data distribution. Due to the deviation of all variables from normality, both non-parametric and adjusted parametric methods were employed. Continuous variable differences between groups were assessed through one-way ANOVA and ANCOVA, with adjustments for age, sex, and BMI. Categorical variables were analyzed through the χ^2 test. Pairwise Pearson correlation was utilized to evaluate the relationships among biomarkers.

Multivariate regression analyses were conducted to determine predictors of AIP, CAST-1, CAST-2, composite scores for disease severity (PC-severity), vitamin D status (PC-VitD), and cardiovascular risk (PC-CVD), incorporating age, sex, and BMI as covariates. Further models were utilized to examine predictors of HbA1c and HOMA-IR. Stepwise regression was conducted with an entry p-value of 0.05 and a removal p-value of 0.1. The evaluation of model quality was conducted using F-statistics, R^2, standardized beta coefficients, along with their respective t- and p-values. Variance inflation factors (VIF) and tolerance values were evaluated to identify multicollinearity. Heteroscedasticity was assessed utilizing White and modified Breusch–Pagan tests.

Principal component analysis (PCA) was utilized to aggregate related variables into composite indices. A principal component was retained when it accounted for over 50% of the variance, with all loadings surpassing 0.7. The adequacy of factor analysis was evaluated through the Kaiser–Meyer–Olkin (KMO)

measure (>0.6) and Bartlett's test of sphericity ($p < 0.05$). The anti-image matrix was reviewed to verify the factorability of the matrix.

Partial least squares (PLS) path analysis using SmartPLS was utilized to examine the structural relationships among vitamin D status, disease severity, and cardiovascular risk. Variables were conceptualized as latent constructs or as individual indicators. The quality of the model was validated through: (a) convergent and construct validity, evidenced by Cronbach's α exceeding 0.7, composite reliability above 0.8, rho_A greater than 0.8, and indicator loadings surpassing 0.7 at $p < 0.0001$; (b) an acceptable model fit, as indicated by a standardized root mean square residual (SRMR) below 0.08.

A priori power analysis using G*Power 3.1.9.7 indicated that a minimum sample size of 55 participants is necessary to identify a significant R^2 in multiple linear regression (effect size = 0.27, power = 0.95, $\alpha = 0.05$, 4 predictors).

3. Results

3.1. Demographic and Clinical Characteristics Between T2DM patients and HCs

Table 1 presents a summary of the demographic and clinical differences between patients with T2DM and HCs. Participants with T2DM exhibited significantly greater age and BMI in comparison to the control group. In the T2DM group, key metabolic parameters such as fasting blood sugar (FBS), HbA1c, insulin levels, and HOMA-IR were significantly elevated. Patients with T2DM demonstrated significantly elevated levels of LDL, triglycerides (TG), VLDL, and total cholesterol (CHO), accompanied by a notable reduction in HDL levels.

Atherogenic indices such as the AIP, CAST-1, and CAST-2 were significantly elevated in participants with T2DM. Serum levels of Vit D and VDR were significantly reduced in the T2DM group, while vitamin D binding protein levels showed no significant difference between the groups. Composite principal component scores indicative of disease severity (PC-Severity), cardiovascular risk (PC-CVD), and Vit D pathway integrity (PC-VitD) demonstrated significant differences between groups.

Table 1. Demographic and clinical characteristics of Type 2 diabetes mellitus (T2DM) and healthy controls (HC).

Variable	T2DM (n=50)	HC (n=25)	F (df=1/70)	p-value
Age (years)	48.14(13.74)	28.60(8.25)	42.634	<0.001
Gender (M/F)	24/12	26/13	-	NS
BMI (Kg/m^2)	26.73(4.35)	22.91(1.51)	17.993	<0.001
FBS (mg/dL)	263.14 (78.73)	94.96(8.13)	64.18	<0.001
HbA1c	7.90(1.31)	4.95(0.263)	65.92	<0.001
Insulin (mU/L)	19.04(6.36)	9.22(2.36)	28.53	<0.001
HOMA-IR	11.97(4.61)	2.12(0.430)	51.76	<0.001
HDL (mg/dL)	36.71(2.37)	48.56(4.55)	128.22	<0.001
LDL (mg/dL)	147.64(40.20)	60.23(26.33)	56.46	<0.001
TG (mmol/L)	2.24(0.096)	2.06(0.084)	41.27	<0.001
VLDL (mg/dL)	1.54(0.096)	1.36(0.084)	41.27	<0.001
CHO (mg/dL)	220.39(47.60)	132.31(24.83)	44.13	<0.001
AIP	0.680(0.095)	0.377(0.093)	104.09	<0.001
CAST-1	6.01(1.26)	2.74(0.586)	84.70	<0.001
CAST-2	4.02(1.08)	1.25(0.592)	79.98	<0.001

Creatinine (mg/dL)	0.700(0.179)	0.566(0.117)	4.14	0.045
Vit D (nmole/L)	28.13(8.98)	43.86(13.83)	28.99	<0.001
Vit D receptor (pg/mL)	85.39(8.59)	90.31(8.86)	5.54	0.021
Vit D binding proteins (ng/mL)	178.11(9.29)	173.06(8.92)	1.46	0.231
PC Severity	0.619(0.576)	-1.23(0.094)	129.29	<0.001
PC CVD Risk	0.593(0.623)	-1.18(0.289)	106.33	<0.001
PC VitD pathway	-.396(0.796)	.792(0.898)	24.46	<0.001

All the clinical findings are controlled for age, gender, and BMI. M: Male, F: Female, BMI: Body mass index, FBS: Fasting blood glucose, HbA1c: Glycated hemoglobin, HOMA-IR: Homeostatic Model Assessment for Insulin Resistance, HDL: High-density lipoprotein, LDL: Low-density lipoprotein, TG: Triglyceride, VLDL: Very-low density lipoprotein, CHO: Cholesterol, AIP: Atherogenic index of plasma, CAST: Castelli index, Vit: Vitamin D, CVD: Cardiovascular disease, PC: Principal component. PC Severity: Principal component was extracted from FBS, HbA1c, Insulin, and HOMA-IR. PC CVD Risk: Principal component was extracted from AIP, CAST-1, and CAST-2. PC Vit D pathway: Principal component was extracted from Vit D, Vit D receptor, and Vit D binding protein.

3.2. Correlation Analysis

Figure 1 illustrates an inverse correlation between serum Vit D concentrations and AIP, CAST-1, CAST-2, PC-Severity, and PC-CVD scores. VDR levels exhibited negative correlations with both PC-Severity and PC-CVD. Conversely, HOMA-IR, insulin, FBS, and HbA1c demonstrated significant positive correlations with all cardiovascular indices and composite severity scores. Lipid components such as LDL, TG, CHO, and VLDL exhibited a positive association with PC-Severity and PC-CVD, whereas HDL demonstrated an inverse correlation with both conditions.

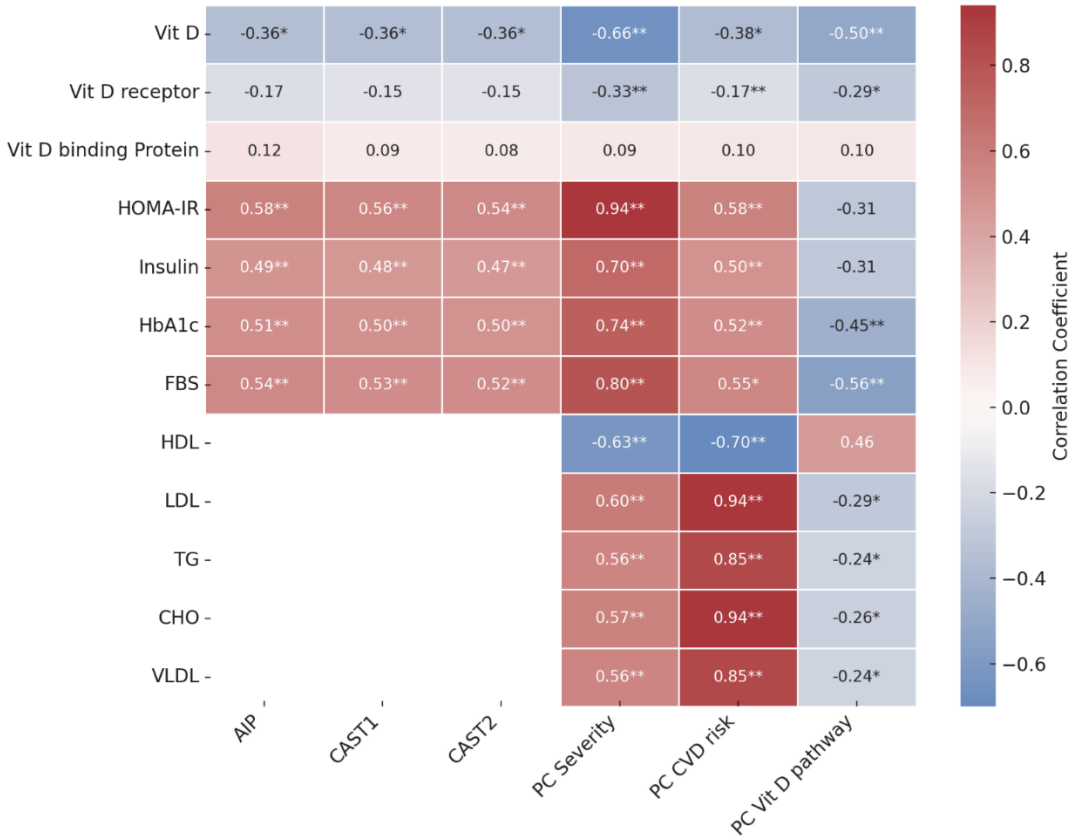

Figure 1. Heatmap representing the results of the Pearson correlation coefficients

3.3. Multivariate regression analyses

Table 2 presents the results of multivariate regression analysis that have been adjusted for age, sex, and BMI. HOMA-IR has been identified as a reliable predictor. Regression #1 indicated that HOMA-IR was a significant predictor of AIP, explaining 48.8% of its variance. Regression #2 showed that 46.6% of the variance in CAST-1 could be explained by HOMA-IR. Part of the variance (45.3%) in CAST-2 as shown in regression #3 explained by HOMA-IR. Regression analysis #4 revealed that CAST-1 and vitamin D collectively accounted for 43.5% of the variance in HbA1c levels. In Regression #5, HDL, creatinine, and LDL collectively explained 54.9% of the variance in HbA1c. Regression analysis indicated that insulin, AIP, creatinine, vitamin D, age, and VDR accounted for 80.3% of the variance in HOMA-IR. Regression analysis indicated that AIP, vitamin D, creatinine, age, and VDR were significant predictors of PC-Severity ($R^2 = 0.804$). In Regression #9, age and vitamin D emerged as significant predictors of PC-CVD, with a R^2 value of 0.344. FBS (Regression #10) and HOMA-IR (Regression #11) accounted for 38.5% and 30.1% of the variance in PC-VitD, respectively. All models demonstrated statistical significance.

Table 2. Results of multiple regression analysis with the cardiovascular and diabetic indices along with principal component for T2DM severity, cardiovascular disease risk and vitamin D pathway as dependent variables and different biomarkers as independent variables.

Dependent Variables	Explanatory Variables	Coefficient statistics			Model statistics			
		β	t	p	R^2	F	df	p
#1. AIP	Model				0.484	70.51	1/73	<0.001
	HOMA-IR	0.701	8.39	<0.001				
#2. CAST-1	Model				0.466	65.70	1/73	<0.001
	HOMA-IR	0.688	8.10	<0.001				
#3. CAST-2	Model				0.453	62.23	1/73	<0.001
	HOMA-IR	0.678	7.88	<0.001				
#4. HBA1c	Model				0.435	29.45	2/72	<0.001
	CAST-1	0.524	5.384	<0.001				
	Vit-D	-0.248	-2.553	0.013				
#5. HBA1c	Model				0.549	31.06	3/71	<0.001
	HDL	-0.425	-4.137	<0.001				
	Creatinine	0.309	3.837	<0.001				
	LDL	0.250	2.432	0.018				
#6. HOMA-2	Model				0.803	51.146	6/68	<0.001
	Insulin	0.441	6.091	<0.001				
	AIP	0.221	3.176	0.002				
	Creatinine	0.138	2.474	0.016				
	Vit-D	-0.191	-2.999	0.004				
	Age	0.166	2.698	0.009				
	Vit-D Receptor	-0.120	-2.269	0.026				
#7. PC-Severity	Model				0.804	61.61	5/69	<0.001
	AIP	0.438	6.825	<0.001				
	Vit-D	-0.330	-5.655	<0.001				
	Creatinine	0.214	3.899	<0.001				
	Age	0.218	3.576	<0.001				
	Vit-D Receptor	-0.128	-2.420	0.018				
#9. PC-CVD	Model				0.344	20.42	2/72	<0.001
	Age	0.415	4.229	<0.001				
	Vit-D	-0.335	-3.416	0.001				
#10. PC-Vit-D	Model				0.385	47.32	1/73	<0.001
	FBS	-0.627	-6.879	<0.001				
#11. PC-Vit-D	Model				0.301	32.81	1/73	<0.001
	HOMA-IR	-0.557	-5.728	<0.001				

3.4. Results of Neural Networks Analysis

Table 3 shows the results of neural network analysis. Two multilayer perceptron neural network models were developed to evaluate the predictive capabilities of chosen biomarkers. Model NN#1 (**Figure 2**) utilized four input variables (vitamin D, age, creatinine, VDR) to predict PC-Severity as output variable. The network

architecture comprised two hidden layers containing three and two nodes, respectively, along with an output layer featuring two units. The activation functions utilized were hyperbolic tangent for the hidden layers and identity for the output layer. The relative errors remained consistent across training (0.282), testing (0.286), and holdout (0.288) sets, suggesting robust model generalization. The model accounted for 71.5% of the variance in PC-Severity. The primary predictors, listed in order of significance, were vitamin D, age, creatinine, and VDR.

Table 3. Results of neural networks (NN) with the PC-Severity and PC-CVD as output variables and different biomarkers as input data

	Models	NN#1	NN#2
Input Layer	Number of units	4	4
Hidden layers	Number of hidden layers	2	2
	Activation function	Hyperbolic tangent	Hyperbolic tangent
	Number of units in hidden layer 1	3	3
	Number of units in hidden layer 2	2	2
Output layer	Dependent variable	PC-Severity	PC-CVD
	Number of units	2	2
	Activation function	Identity	Identity
Training	Error term	7.757	6.774
	%incorrect or relative error	0.282	0.301
Testing	Sum of Squares error	1.445	2.551
	% incorrect or relative error	0.286	0.301
Holdout	%incorrect or relative error	0.288	0.315
	R2	0.715	0.717

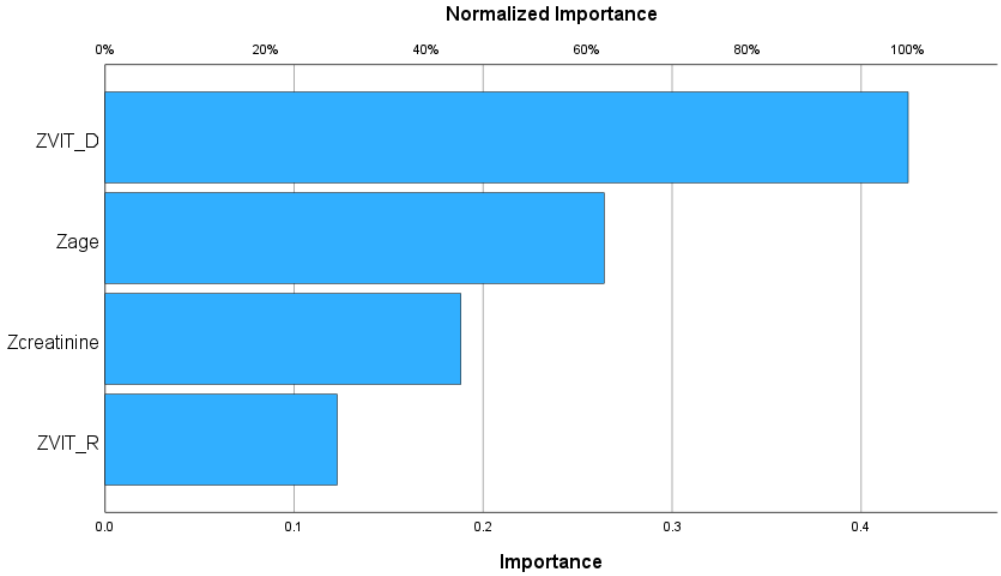

Figure 2. Results of neural network analysis demonstrate the importance chart. The output variables PC-Severity.

Model NN#2 as shown in **Figure 2** utilized PC-Severity, age, vitamin D, and BMI as predictors for PC-CVD. The architecture was the same as NN#1. Relative error values were stable across datasets (training = 0.301, testing = 0.301, holdout = 0.315), indicating strong model stability. This model accounted for 71.7% of the variance in PC-CVD. The importance of predictors, listed in descending order, was as follows: PC-Severity, age, vitamin D, and BMI (see Figure 2).

3.5. Partial Least Squares Analysis

Partial Least Squares (PLS) analysis was performed to assess latent constructs and their predictors, utilizing 5000 bootstrapped samples. As exhibited in **Figure 4**, the model incorporated PC-Severity, PC-CVD, and PC-VitD as latent variables. The model fit was deemed acceptable (SRMR < 0.08), and all latent variables demonstrated high construct reliability (Cronbach's α > 0.80). PC-Severity exhibited a Cronbach's α of 0.879, a composite reliability of 0.941, and an AVE exceeding 0.70. All factor loadings were greater than 0.70 ($p < 0.001$), thereby confirming strong convergent validity. Confirmatory Tetrad Analysis validated the reflective characteristics of all measurement models. Cross-validated redundancy values ($Q^2 > 0.30$) demonstrated substantial predictive relevance. The model accounted for 62.1% of the variance in PC-Severity and 59.6% in PC-CVD. Lower vitamin D pathway activity, advanced age, and elevated BMI were significantly correlated with increased severity of T2DM, which subsequently predicted a heightened cardiovascular risk. The vitamin D pathway exhibited a significant indirect effect on cardiovascular risk, mediated by PC-Severity ($t = 3.09$, $p < 0.001$). The impact of PC-Severity on PC-CVD was significant ($t = 4.13$, $p < 0.001$) (Figure 3).

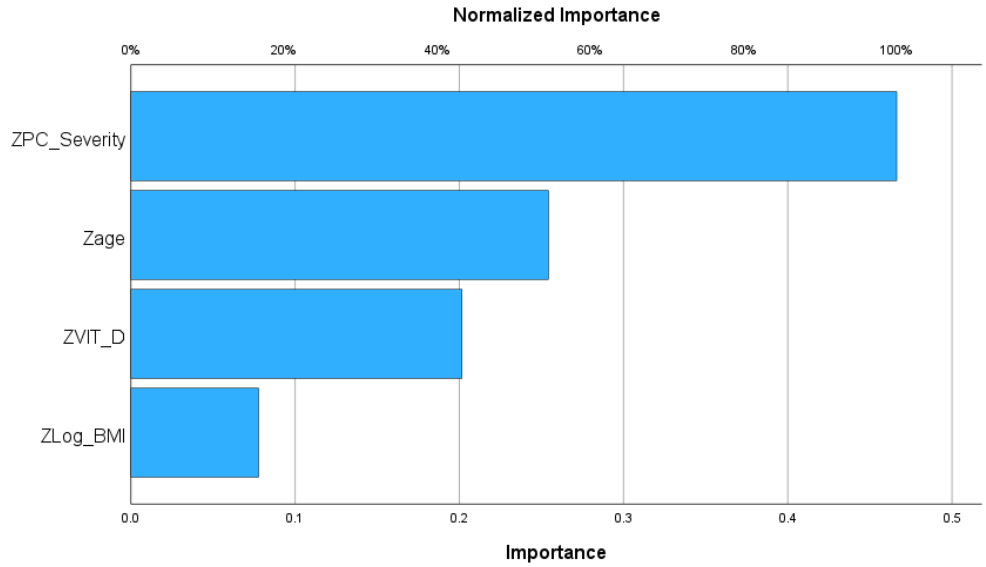

Figure 3. Results of neural network analysis demonstrate the importance chart. The output variables PC-CVD.

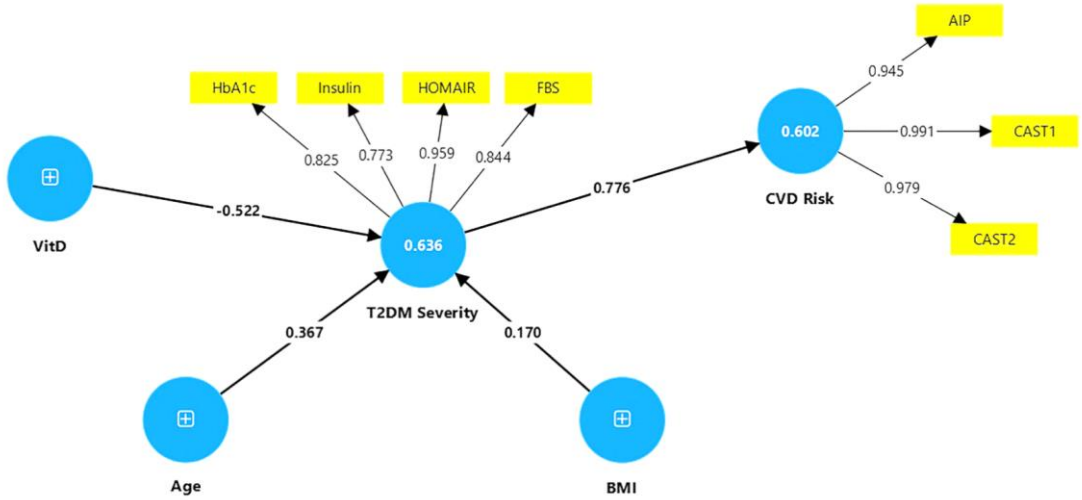

Figure 4. Results of partial least squares (PLS)–SEM analysis with the cardiovascular (CVD) risk as the output variable. The latter was entered as a latent vector (blue circle) extracted from three domains (yellow shapes). The phenome latent vector was predicted by T2DM severity and the latter is a latent vector extracted from four domains and predicted by Vit D, age, and BMI.

4. Discussion

4.1. Abnormal Vitamin D Pathway Function in T2DM

The first key finding of our study indicated significantly lower serum levels of Vit D and VDR in patients with T2DM relative to healthy controls, with no observed difference in vitamin D binding protein. The results are consistent with earlier studies indicating reduced Vit D levels T2DM populations [21, 22]. Our data indicated decreased VDR concentrations; however, prior studies have reported mixed findings. Arafat et al. (2020) found no significant differences in VDR or vitamin D binding proteins between T2DM patients and healthy individuals, which partially supports our observation of unchanged vitamin D binding protein levels [21]. In contrast, Fawzy and Al Beladi (2018) reported increased concentrations of Vitamin D binding protein in T2DM, indicating persistent inconsistencies that require additional investigation [23].

The current regression analysis confirmed that reduced levels of Vit D and VDR significantly predict increased severity of T2DM and demonstrate an inverse correlation with clinical outcomes. Our neural network analysis highlighted the predictive value of Vit D, accounting for approximately 71.7% of the variance in T2DM disease severity. Furthermore, increased FBS and insulin resistance (assessed by HOMA-IR) are significant predictors of disruptions in the Vit D pathway. The findings indicate a potential mechanistic connection in which hyperglycemia and insulin resistance may worsen Vit D dysregulation. This aligns with the work of Wang et al. (2019), who demonstrated that Vit D deficiency could increase insulin resistance through inflammatory signaling mediated by NF-κB pathways [24].

Through comprehensive meta-analysis, we identified associations between specific single nucleotide polymorphisms (SNPs) in the VDR gene, namely FokI, BsmI, and TaqI, and increased susceptibility to T2DM among certain ethnic groups [25] indicating possible molecular basis for its dysfunction. Vit D has significant regulatory effects on immune function, particularly by reducing pro-inflammatory cytokines such as tumor necrosis factor (TNF)-α, interleukin (IL)-6, IL-8, and monocyte chemoattractant protein (MCP)-1, while enhancing anti-inflammatory mediators like IL-10 [24, 26]. This process enhances antioxidative defenses by increasing the activities of glutathione (GSH) and superoxide dismutase (SOD), thus reducing inflammation-induced cellular damage [26]. Previous research indicated increased levels of pro-inflammatory cytokines, MCP-1, and IL-8 in patients with T2DM, implying a chronic inflammatory condition [27] which could be attributed to Vit D deficiency [26]. Recent evidence highlights the clinical potential of Vit D supplementation in mitigating neuropathic complications and inflammation in patients with T2DM [28].

Vit D may mimic insulin action by activating transcription factors related to glucose metabolism and insulin receptor function, while also modulating intracellular calcium dynamics essential for insulin signaling[29]. Animal models indicate that Vit D regulates insulin/AKT/GSK-3β and Wnt/β-catenin signalling pathways, suggesting broader therapeutic implications beyond glycemic control, including neuroprotective and cognitive benefits [30]. Clinically, lower serum Vit D levels are significantly associated with higher fasting insulin levels, increased insulin resistance, and decreased insulin sensitivity indices (QUICK-I and McAuley's index), especially in postmenopausal women with T2DM [31]. Higher Vit D intake is associated with reduced insulin resistance in T2DM, although this association is not consistently observed in latent autoimmune diabetes in adults (LADA), indicating a disease-specific effect of Vit D status on insulin regulation [32].

4.2. Atherogenic Indices and Cardiovascular Risk in T2DM

The second key finding of our study identified significantly elevated atherogenic indices, including the AIP and Castelli risk indices (CAST-1, CAST-2), in patients with T2DM. This was accompanied by an increased risk of CVD, as demonstrated through principal component analysis derived from these biomarkers. Regression analyses indicated that insulin resistance (HOMA-IR) serves as a primary explanatory factor for lipid abnormalities, suggesting a significant role of hepatic insulin resistance in the development of

dyslipidemia. This is consistent with foundational experimental studies by Avramoglu et al. (2006), which indicated that hepatic insulin resistance promotes increased VLDL-triglyceride secretion [33], and Biddinger et al. (2008), who demonstrated that deficits in insulin signaling within hepatic tissue elevate LDL cholesterol and foster vascular lipid deposition [34].

Our findings also revealed significant inverse correlations between serum Vit D levels and atherogenic markers (AIP, CAST-1, CAST-2), the severity of T2DM, and the risk of CVD. These relationships indicate that Vit D deficiency may significantly exacerbate diabetic complications. However, our PLS analysis reveals that the influence of low Vit D on cardiovascular risk, as measured by AIP, CAST-1, and CAST-2 indices, is primarily mediated by the severity of T2DM. This suggests an indirect role of Vit D in lipid metabolism. Neural network analyses further confirmed this indirect influence, highlighting Vit D deficiency as the most significant predictor of T2DM severity, with subsequent implications for cardiovascular risk. Our findings align with previous research indicating that Vit D deficiency significantly elevates the risk of atherosclerotic cardiovascular disease, especially among diabetic populations [35].

Vit D deficiency is consistently linked to unfavorable lipid profiles, characterized by increased total cholesterol, LDL-C, non-HDL-C, triglycerides, VLDL-C, remnant lipoproteins, and small dense LDL-C, along with reduced HDL-C levels [36-39]. Furthermore, reduced Vit D levels are positively associated with elevated values of several atherogenic indices, including the AIP, atherogenic coefficient (AC), Castelli risk indices, and lipid accumulation product (LAP) [40-42]. A clear dose-response relationship is evident between decreasing Vit D levels and an increased risk of dyslipidemia, particularly in young adults and females [39]. Vit D likely influences lipid homeostasis, endothelial cell function, inflammatory processes, and vascular smooth muscle cell proliferation, all of which contribute to atherogenesis [38]. Also, creatinine emerged as a significant predictor in multiple regression models consistent with the well-established relationship between impaired renal function, vitamin D metabolism, and cardiovascular risk in T2DM [43, 44]. The kidney is the primary site of 1α-hydroxylation of 25(OH)D to its active form, and declining renal function reduces this conversion, compounding vitamin D deficiency and its downstream atherogenic effects [45].

Elevated inflammatory markers, including neutrophil-to-lymphocyte ratio (NLR), platelet-to-lymphocyte ratio (PLR), systemic immune-inflammation index (SII), and monocyte-to-HDL ratio (MHR), are linked to Vit D deficiency and increased atherogenic risk [46-49]. Furthermore, insufficient Vit D levels contribute to a pro-inflammatory monocyte phenotype, heightened monocyte-platelet aggregation, and enhanced endothelial cell adhesion, which are critical processes in the initial stages of atherosclerotic plaque development [46-48]. Vit D deficiency leads to endothelial activation and dysfunction characterized by heightened expression of adhesion molecules and reduced nitric oxide bioavailability, which contribute to vascular inflammation and the initiation of plaque formation [38, 50, 51]. Vit D deficiency is associated with increased oxidative stress, which further intensifies vascular inflammation and facilitates atherogenic processes [38, 47, 51].

Overall, the present study highlights the intricate associations between vitamin D deficiency, insulin resistance, and atherogenic lipid profiles, emphasizing their collective role in determining T2DM severity and cardiovascular risk.

5. Limitations

This study presents several limitations that warrant acknowledgement. The cross-sectional design restricts the capacity to determine causal relationships among Vit D pathway, insulin resistance, and cardiovascular risk in patients with T2DM. Future longitudinal studies are essential to elucidate temporal and causal relationships. Secondly, while several biomarkers were examined, unmeasured confounding variables such as dietary habits, lifestyle factors, seasonal variation in Vit D levels, and genetic background beyond the studied polymorphisms may have impacted our findings. Age differences between groups and pharmacological

heterogeneity within the T2DM group may have introduced residual confounding despite ANCOVA adjustment; future studies should employ age-matched designs and stratify by medication class. While power analysis results showed adequate power, small sample size may reduce the statistical power and generalizability of our findings, highlighting the necessity for replication in larger cohorts. Finally, the generalizability of our findings may be limited by the specific demographic and ethnic characteristics of our study cohort, necessitating validation in varied populations.

6. Conclusion

In summary, our results emphasize the significant relationship between Vit D pathway dysfunction, insulin resistance, and atherogenic dyslipidemia in T2DM. Reduced serum Vit D and VDR levels are significant predictors of disease severity and indirectly contribute to increased cardiovascular risk. These findings suggest the significance of regular Vit D status evaluation and personalized supplementation as essential elements of T2DM management strategies, with the goal of mitigating metabolic complications and cardiovascular morbidity. Further research, especially intervention and longitudinal studies, is necessary to validate these relationships and elucidate the therapeutic potential of Vit D optimization in T2DM.

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Ethical approval and consent to participate.

The Ethics Committee of the Institutional Review Board of the College of Medicine, University of Kufa, granted ethical approval (approval number 347/2019). Written informed consent was obtained from all patients and control participants, and all procedures were conducted in accordance with Iraqi and international ethical standards.

Declaration of interest

No conflicts of interest to be declared by authors.

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Author's contributions

AKA supervised blood sample collection and all patient-related procedures. Serum biomarker quantification was performed by AKA, AFA, HAM, and AAA. Statistical analysis was carried out by AFA. The initial manuscript draft was prepared by AFA and subsequently reviewed and revised by AKA, AFA, HAM, and AAA. All authors approved the final version of the manuscript.

Availability of data

The corresponding author (AFA) will provide access to the dataset supporting this study upon formal request and completion of a thorough data evaluation process.

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