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Research Article

Correlation Between Vitamin D3, Atherogenic Indices of Plasma and Cardiometabolic Biomarkers in Diabetic Patients

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Abstract
Background: Cardiometabolic disease is a cluster of interconnected cardiovascular and metabolic disorders. These include obesity, insulin resistance, hypertension, heart disease and type 2 diabetes. In this study, focus was to identify correlation between serum vitamin D3 levels along with Atherogenic Indices of Plasma (AIP) and cardio metabolic biomarkers with a purpose to assess as potential indicators of the cardio metabolic disease risk in the type 2 diabetes mellitus (T2DM) patients.
Methods: The study was a cross-sectional investigation conducted in a total of 105 patients with diabetes mellitus in Islamabad region of Pakistan. Participants were selected randomly with an age range of 45-79 years. The clinical data sets were analyzed using Pearson correlation, Kolmogorov-Smirnov and independent t-test.
Results: Vitamin D3 showed a strong inverse correlation with the total cholesterol ($r=-0.483$, $p<0.01$) and low-density lipoprotein cholesterol (LDL-C, $r=-0.471$, $p < 0.01$). This indicated that higher vitamin D3 levels are associated with the lower levels of these lipids. Additionally, there was a positive correlation with high-density lipoprotein cholesterol (HDL-C) levels. Significant inverse relationship was noticed between vitamin D3 and triglycerides ($r=-0.595$, $p<0.01$). Correlation with systolic blood pressure (SBP) and diastolic blood pressure (DBP) was not found to be significant. Vitamin D3 showed a strong negative correlation with AIP such as CRI-I ($r=-0.595$, $p<0.01$) and CRI-II ($r=-0.582$, $p<0.01$) indicating the role in reducing cardiovascular risk. An inverse relationship between vitamin D3 levels and AIP in diabetics was found. Lower levels of serum vitamin D3 were associated with higher AIP, suggesting a link between vitamin D3 deficiency and atherogenic dyslipidemia in the population.
Conclusion: Higher vitamin D3 levels are associated with favorable lipid profile, including lower total cholesterol, LDL-C, AIP along with higher HDL-C levels. Lower vitamin D3 levels were also linked to increased atherogenic dyslipidemia and adverse cardiometabolic biomarkers. Findings suggest that vitamin D3 may play a protective role in reducing the cardiovascular risk in individuals with diabetes.
Key Words: Vitamin D3, Atherogenic indices, Cardio metabolic biomarkers, Type 2 diabetes mellitus

INTRODUCTION

Hyperglycemia resulting from an absolute deficiency in insulin synthesis characterizes the broad group of illnesses known as diabetes mellitus (DM) [1]. Hyperglycemia may result from insulin resistance, impaired insulin secretion, or increased glucagon production [2]. Polyuria, polydipsia, polyphagia, weight loss, exhaustion, blurred eyesight and other physiological dysfunctions are the characteristics of diabetes [3].

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DM is a common chronic illness in almost every country, is becoming more prevalent due to changing lifestyles leading to less physical activity and increase in obesity. Additionally, type 2 DM (T2DM) causes poorer blood glucose management brought on by dysfunctional pancreatic beta cells [4-7].
Vitamin D is a lipid-soluble steroid prohormone. Numerous organs have been shown to express vitamin D receptors. The pancreatic β-cells express vitamin D receptors VDR. Sufficient level of vitamin D support optimal functioning of β cells, aiding in insulin and effective glucose metabolism [8, 9]. The circulating form of vitamin D in humans, known as 25-hydroxyvitamin D (25(OH) D), is primarily created by the body, however it can be found in a few natural

food sources [10]. Numerous studies have been published, revealing that there is a strong connection between the amount of sun exposure an individual receives and their vitamin D status, as well as insulin resistance [11]. Diabetes is both an inflammatory disorder and a failure to appropriately manage glucose levels. Vitamin D has anti-inflammatory qualities, and it also improves islet cell activity, increases insulin release and reduces insulin resistance [12]. Diabetes is associated with increased rates of hypovitaminosis D in individuals with diabetes [13].

Active metabolites known as 1α, 25-dihydroxyvitamin D3 can stimulate pancreatic β-cells and insulin production. Additionally, it has the potential to impact insulin sensitivity through various mechanisms. Mechanistic and experimental research provides evidence in glucose metabolism and insulin sensitivity. There is a correlation between low levels of 25(OH) D3 and an altered lipid profile [14, 15]. Higher levels of triglycerides and low-density lipoprotein (LDL-C) in blood along with simultaneous decrease in levels of high-density lipoprotein (HDL-C) are hallmarks of atherogenic dyslipidemia [16, 17]. Atherogenic index is the logarithm (log) of plasma concentration ratio TG/HDL-C. It is thought that the Atherogenic Index of Plasma (AIP) is a powerful marker. It has been observed that dyslipidemia is a key factor in the progression of micro- and macro vascular problems in individuals with DM [18]. According to research reports, AIP and the visceral fat region in T2DM patients may be related. Cardio-metabolic biomarkers are biological indicators that help in diagnosing cardiovascular disease, metabolic disorders, diabetes and obesity [19]. Cardio metabolic biomarkers include height, weight, blood pressure, lipid profile and glucose level [20]. Serum lipid levels and AIP were shown strongly associated with serum vitamin D levels. According to the data, vitamin D insufficiency may raise the dyslipidemias, particularly in males [21]. Moreover, the relationship between vitamin D levels and blood lipids may differ in women and men.

The purpose of this study was to look at the relationship between T2DM patients with their serum vitamin D3 levels and AIP level. Although a possible correlation between vitamin D3 and AIP has been proposed, it is unknown whether type 2 diabetics are affected by this relationship.

METHODS

Samples Collection

Patients were selected from Al-khidmat Raazi Diagnostics Islamabad. Initial confirmatory tests (BSF, and HbA1C) were performed and 105 patients were recruited who fulfill our inclusion criteria (Age over 45 years, diabetes at least for 1 year) and exclusion criteria (MI, Stroke, Cancer, Liver diseases, Renal diseases, Cardiac abnormalities). After informed consent, serum vitamin D3, AIP and cardio metabolic markers were analyzed.

Clinical and Biochemical Examinations

At the study baseline, samples of blood were taken into EDTA tubes. Venipuncture was used to obtain a blood sample after an 8–12 hour fast. A fully automated Cobas c111 (Roche Diagnostics), and Micro Lab 300 (Vital Scientific) chemistry analyzers were used to measure the biochemical parameters, such as Fasting Plasma Glucose (FPG) and lipid profile. D10 HbA1c completely automated analyzer (Bio-Rad) was used to measure HbA1c levels. A fully automated Cobase411 (Roche Diagnostics), Mindray i1000 was used to assess vitamin D3. While HbA1c was represented as a percentage (%) and vitamin D3 as ng/ml, all other biochemical parameters were expressed in mg/dL. For validating the test, external quality control was carried out every three months in addition to daily internal quality control. Vitamin D3 and HbA1c had intra- and inter-assay coefficients of variation (CVs) of 4.7 and 6.2%, respectively, to determine AIP, the formula (AIP) = log (TG/HDL-C) was utilized. Colorimetric assay was utilized to analyze blood sugar fasting and cardio metabolic biomarkers.

Data Analysis Procedure

Version 22.0 of IBM SPSS Statistics was used for statistical analysis. The distribution of biomarkers was examined by using the Kolmogorov-Smirnov test. An independent t-test was utilized in research to examine the average deviations in the subjects' plasma levels of atherogenic indices, cardio metabolic indicators and serum vitamin D3. To analyze relationship between atherogenic indices and vitamin D3 as well as between lipid and lipoprotein profiles, the Pearson correlation coefficient was calculated.

RESULTS

Overview of Study Population

A total of 105 subjects were included in this study, with a gender distribution in which 35 participants were male (33.3%) and 70 were female (66.7%). The average age of the participants was 56.06 ± 0.85 years. The results of the study reveal significant sex-dependent differences among the participants. The mean age of male participants was 59.0 ± 1.84 years, which was significantly higher compared to the female participants, whose mean age was 54.6 ± 0.88 years ($p = <0.001$). This showed a notable age difference between males and females in the study population (Figure 1).

Blood Glucose Levels

For glycemic control, HbA1c levels were 7.83 ± 0.20% in males and 8.18 ± 0.05% in females, with

the difference being statistically significant (p = <0.001) This indicates that females had higher HbA1c levels, suggesting comparatively poorer glycemic control than men.

Vitamin D3 Levels

Vitamin D3 levels were categorized into three groups as indicated *Deficiency* (≤ 20ng/ml), *Insufficiency* (21-29 ng/ml) and *Sufficiency* (>30ng/ml). Pairwise comparisons were made among these groups. Among the samples, 44 participants (41.9%) were vitamin D3 deficient, while 59 participants (56.2%) were insufficient. Only 2 participants (1.9%) had sufficient vitamin D3 levels. Overall, 98.1% of participants had suboptimal vitamin D3 status, indicating a high prevalence of deficiency and insufficiency in the study population (Figure 2). Distribution of vitamin D3 status among participants, stratified by gender, showed a higher prevalence of deficiency and insufficiency in females compared to males. Among males, 10 participants (28.6%) were classified as vitamin D3 deficient and 23 (65.7%) as insufficient, while 2 participants (5.7%) had sufficient levels. In contrast, among females, 34 participants (48.6%) were deficient and 36 (51.4%) were insufficient, with none achieving sufficient vitamin D3 levels. Overall, vitamin D3 deficiency and insufficiency were more prevalent in females than in males (Table 1). Pearson correlation coefficient was –0.508 indicating moderate negative correlation between vitamin D3 and HbA1c.

Atherogenic Indices and Vitamin D3 Levels

AIP showed a mean of 0.323 ± 0.050 in men and 0.369 ±0.026 in women. Difference was not statistically significant (p=.284), which indicates similar atherogenic risk profile among men and women of this group. Regarding the vitamin D3, levels in male (26.23±1.45 ng/ml) were significantly higher as compared to the females (20.64±0.45 ng/ml, p-value of <0.001), indicating male vitamin D3 status was better than the women. Total cholesterol levels were higher in males (220.20 ± 9.06 mg/dL) than females (197.39 ± 4.83 mg/dL), but this difference was not statistically significant (p = .720).

Cardio Metabolic Biomarkers

LDL-C levels were significantly diverse among males and females. Males had lower levels (132.20 ± 4.58 mg/dl) when compared to the females (141.19 ± 0.92 mg/dl, p-value of <0.001). This indicates that women had higher levels of LDL-C, which is a known risk factor for the cardiovascular disease. HDL-C levels were higher in the males (44.63 ± 1.52 mg/dL) as compared to the females (42.77 ± 0.61 mg/dL). The difference was statistically significant (p=<0.001) and the men were having better protective levels of HDL-C. Mean triglyceride levels were 212.94±19.72 mg/dL in the males and 217.24±6.97 mg/dL in the females. Difference was not statistically significant (p=.252), which pointed similar triglyceride levels among men and women included in the study population.

Correlations of Vitamin D3 and Cardio Metabolic Biomarkers

Correlation analysis was also performed to examine relationship between vitamin D3 levels, AIP, total cholesterol, LDL-C, HDL-C, and triglycerides in the 105 diabetic patients, Pearson correlation coefficients were calculated for each pair of factors. Correlation of the vitamin D3 levels with the various metabolic and cardio metabolic biomarkers revealed a significant association (Table 5). Vitamin D3 showed significant negative correlations with total cholesterol (r=-0.483, p<0.01) and LDL-C (-r=-0.471, p < 0.01). The findings indicated that higher vitamin D3 levels are associated with the lower levels of these lipids. There was also a positive correlation with the HDL-C levels. The inverse relationship with triglycerides (r=-0.564, p<0.01) further supported the potential beneficial role of the vitamin D3 in lipid metabolism. Correlation with systolic blood pressure and systolic blood pressure were not statistically significant. Vitamin D3 showed a strong negative correlation with the Atherogenic Indices such as CRI-I (r=-0.595, p<0.01) and CRI-II (r=-0.582, p<0.01).

Results revealed a significant, strong negative correlation between vitamin D3 and AIP (r = -0.640, p < 0.01). This, in turn, indicated that higher levels of vitamin D3 were associated with the lower levels of AIP were correlated. Further analysis also showed that AIP was significantly positively correlated with LDL-C (r=0.383, p<0.01), triglycerides (r = 0.904, p < 0.01) and total cholesterol (r = 0.565, p < 0.01). This data showed that higher AIP values are associated with the high levels of the lipid profile components. In contrast, strong negative correlation was observed between AIP and HDL-C (r=-0.705, p<0.01). This indicated that higher AIP values were associated with the lower HDL-C levels. LDL-C exhibited a significant, moderate negative correlation with HDL-C (r = -0.500, p < 0.01) and a weak positive correlation with total cholesterol (r = 0.206, p < 0.05) and triglycerides is (r = 0.262, p<0.01). HDL-C also showed significant, moderate negative correlation with the total cholesterol (r=-0.413, p<0.01) along with a strong negative correlation with the triglycerides (r=-0.550, p<0.01). Lastly, a significant, moderate positive correlation was noticed between total cholesterol and the triglycerides (r=0.598, p<0.01) and indicated that higher levels of total cholesterol were associated with higher levels of triglyceride.

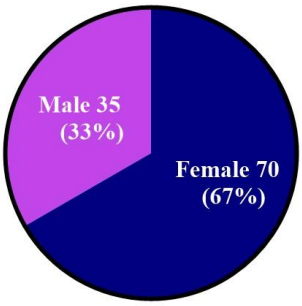

Figure 1: Gender distribution of participants

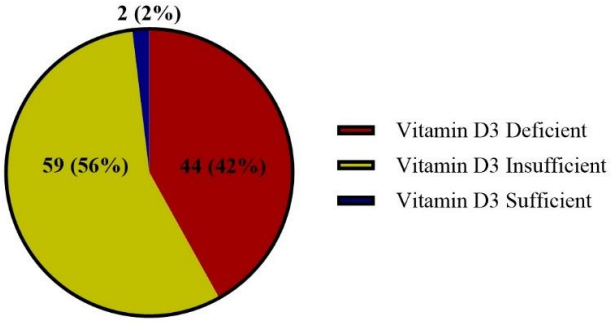

Figure 2. Vitamin D3 levels in the participants.

Table 1. Vitamin D3 levels in the participants

Vitamin D3 status	Male n (%)	Female n (%)
Deficient	10 (28.6%)	34 (48.6%)
Insufficient	23 (65.7%)	36 (51.4%)
Sufficient	2 (5.7%)	0 (0.0%)

Table 2. Correlation between vitamin D3 and HbA1c.

HbA1c (%)		
Pearson Correlation	1	-0.508**
Sig. (2-tailed)		<0.001
N	105	105
Vitamin D3 (ng/mL)		
Pearson Correlation	-0.508**	1
Sig. (2-tailed)	<0.001	
N	105	105

** Correlation is significant at the 0.01 level (2-tailed)

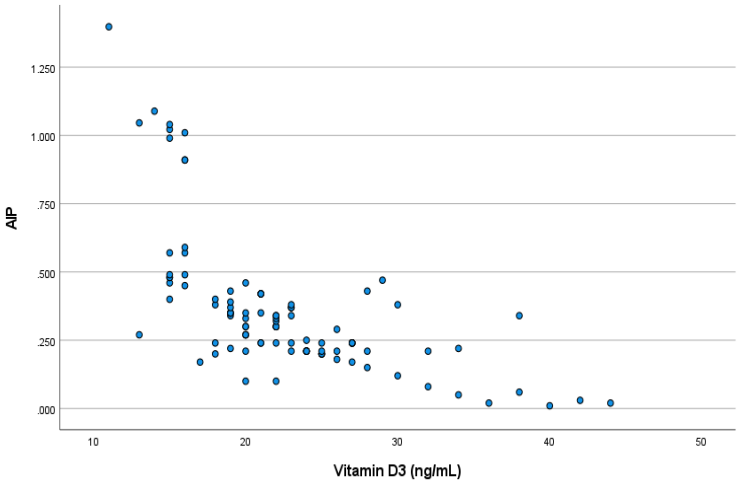

Figure 3. Scatter plot showing correlation between vitamin D3 and Atherogenic Index of Plasma (AIP)

Table 3. Mean ± SD of Vitamin D3 and Atherogenic indices. AIP: Atherogenic Index of Plasma; CRI-I: Castelli Risk Index I; CRI-II: Castelli Risk Index II; AC: Atherogenic Coefficient. AIP=log (TG/HDL-C); CRI-I=TC/HDL-C; CRI-II=LDL-C/HDL-C; AC=(TC-HDL-C)/HDL-C.

Parameter	Gender	N	Mean	Std. Deviation	P-value
Vitamin D3 (ng/mL)	Male	35	26.23	8.555	<0.001
	Female	70	20.64	3.780	
AIP	Male	35	0.32274	0.297737	0.284
	Female	70	0.36899	0.219644	
CRI I	Male	35	5.2344	2.03720	0.093
	Female	70	4.7370	1.34540	
CRI II	Male	35	3.1366	1.06756	<0.001
	Female	70	3.3570	0.50824	
AC	Male	35	4.2344	2.03720	0.093
	Female	70	3.7370	1.34540	

Table 4. Mean ± SE of cardiometabolic biomarkers of participants according to gender.

Biomarker	Male (n=35)	Female (n=70)	P-value
Age (years)	59.0 ± 1.84	54.6 ± 0.88	<0.001
HbA1c (%)	7.83 ± 0.20	8.18 ± 0.05	<0.001
AIP	0.323 ±0.050	0.369 ± 0.026	.284
Vitamin D3 (ng/mL)	26.23 ± 1.45	20.64 ± 0.45	<0.001
Total Cholesterol (mg/dL)	220.20 ± 9.06	197.39 ± 4.83	.720
LDL-C (mg/dL)	132.20 ± 4.58	141.19 ± 0.92	<0.001
HDL-C (mg/dL)	44.63 ± 1.52	42.77 ± 0.61	<0.001
Triglycerides (mg/dL)	212.94 ± 19.72	217.24 ± 6.97	.252

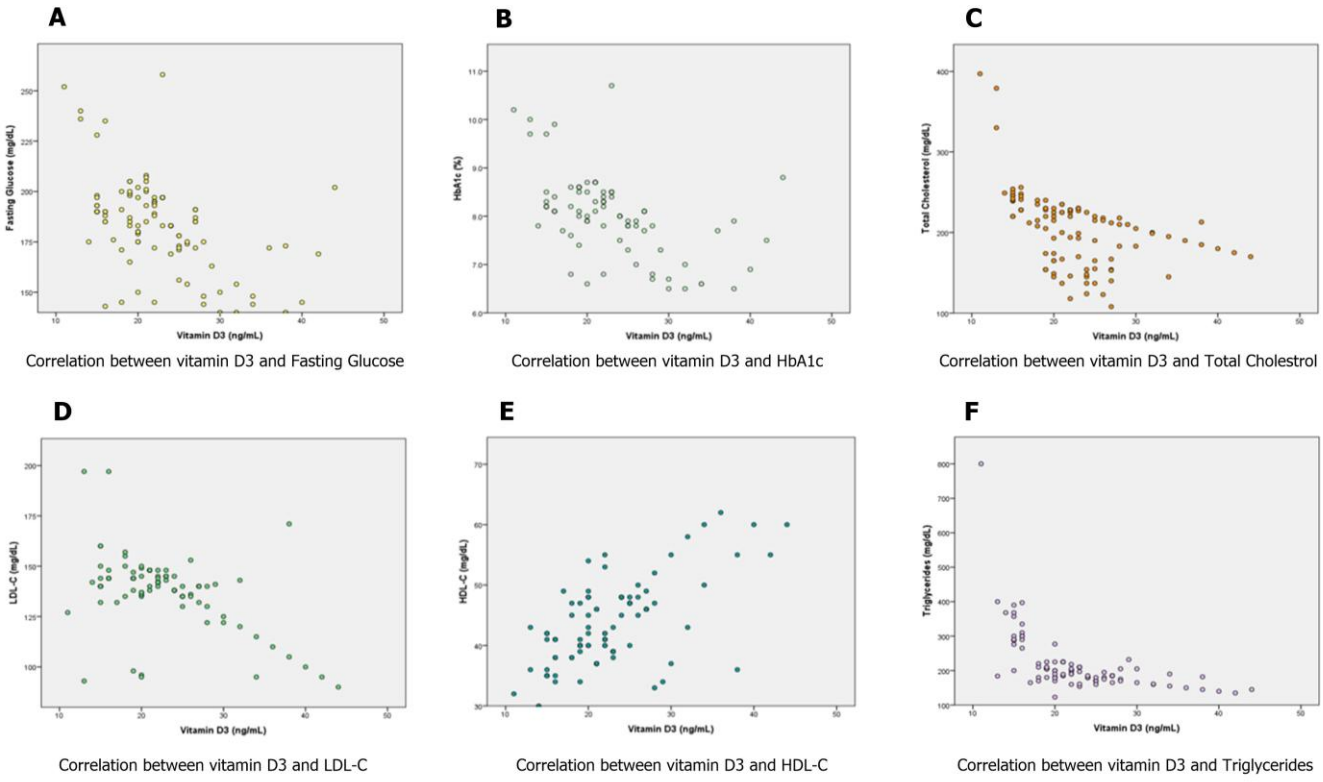

Figure 4. Scatter plots show correlation between vitamin D3, metabolic markers and lipid profile.

Table 5. Correlations of vitamin D3 and cardio metabolic biomarkers

	Vit. D3	HbA1c	F. Glu	SBP	DBP	T. Chol	Tri	LDL-C	HDL-C	AIP	BMI
Vit. D3	1	- 0.508**	- 0.504**	- 0.113	- 0.063	- 0.483**	- 0.564**	- 0.471**	0.625**	- 0.640**	- 0.129
P. Cor	1	- 0.508**	- 0.504**	- 0.113	- 0.063	- 0.483**	- 0.564**	- 0.471**	0.625**	- 0.640**	- 0.129
Sig. (2-t)		<0.001	<0.001	0.250	0.522	<0.001	<0.001	<0.001	<0.001	<0.001	0.190
N	105	105	105	105	105	105	105	105	105	105	105

Vit. D3: Vitamin D3, **F. Glu:** Fasting Glucose, **T. Chol:** Total Cholesterol, **Tri:** Triglyceride

DISCUSSION

This study was aimed at evaluating the correlation between serum vitamin D3 levels, AIP along with the cardiometabolic biomarkers in diabetic patients. Findings demonstrated significant inverse association among serum vitamin D3 levels and AIP as well as adverse lipid parameters which included triglycerides, LDL-C and total cholesterol. A positive association was noticed among vitamin D3 and HDL-C in males only. These findings indicate that lower vitamin D3 levels are associated with the unfavorable cardiometabolic profile in diabetic individuals.

Vitamin D is a steroid hormone primarily synthesized in the skin upon exposure to ultraviolet radiation and subsequently converted in liver to 25-hydroxyvitamin D [25(OH)D], the major circulating form used to assess vitamin D status. Previous studies have reported that vitamin D3 deficiency is associated with insulin resistance, which may contribute to impaired glucose metabolism and poor glycemic control in diabetic patients [22]. This may partially explain the inverse relationship observed in the present study between vitamin D3 levels and fasting blood glucose as well as HbA1c.

The present findings are consistent with earlier studies that have demonstrated that individuals with vitamin D deficiency tend to have higher levels of total cholesterol, LDL-C, and triglycerides compared to those with sufficient vitamin D levels [23]. Furthermore, it has been reported that individuals with dyslipidemia often exhibit lower serum vitamin D concentrations, supporting the association between vitamin D deficiency and adverse lipid profiles observed in this study [24]. A decreasing trend in the prevalence of dyslipidemia with increasing vitamin D levels has also been described, suggesting a potential dose response relationship between vitamin D status and lipid abnormalities [17]. In terms of gender differences, females in present study exhibited lower vitamin D levels compared to males, which may be attributed to differences in sun exposure, dietary intake, or hormonal factors. Additionally, females showed comparatively higher LDL-C levels, indicating a potentially increased cardiovascular risk. These findings highlight the importance of considering sex-specific variations in assessment of cardiometabolic risk.

The scatter plot analyses further supported these findings, demonstrating inverse relationships between vitamin D levels and fasting blood glucose as well as HbA1c. Similarly, an inverse association was observed between vitamin D and lipid parameters, reinforcing the potential role of vitamin D in cardiometabolic regulation. These findings are supported by previous research indicating that the inverse relationship between vitamin D and lipid parameters remains significant even after adjusting for potential confounding variables, suggesting an independent association [25]. Low serum 25(OH)D levels have been linked to an increased risk of dyslipidemia, highlighting the potential clinical relevance of vitamin D in cardiovascular risk assessment [26].

Overall, the findings suggest that low vitamin D levels are associated with increased AIP and adverse cardiometabolic profiles in diabetic patients. These results highlight the potential importance of monitoring and managing vitamin D levels as part of strategies aimed at reducing cardiovascular risk. This study has some limitations. The cross-sectional design limits the ability to establish causality between vitamin D levels and cardiometabolic outcomes. Additionally, the relatively small sample size may affect the generalizability of the findings. Important confounding variables such as BMI, medication use, and lifestyle factors were not controlled, which may influence the observed associations. Despite these limitations, this study provides valuable insights into the relationship between vitamin D and cardiometabolic risk factors in a diabetic population. The inclusion of participants from multiple clinical settings adds strength to the findings. Further longitudinal studies are required to establish causality and evaluate the predictive role of vitamin D in cardiometabolic health.

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Author Contributions: UA: Conceptualization, methodology, study design, investigation, data curation, formal analysis, interpretation of results, writing original draft, writing review and editing, supervision, project administration. STM: Data collection, laboratory investigation. AR: Statistical analysis, data curation and writing review and editing. NHR: Literature review, data organization. UQ: Data verification. All authors reviewed and approved the final manuscript and agreed to be accountable for all aspects of the work.
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Data Availability: The datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request.