RESEARCH PAPER

Association between Serum Vitamin D levels and the Atherogenic index of plasma, among patients with Type II Diabetes Mellitus, A Cross Sectional Study

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ABSTRACT

Introduction: The Vitamin D deficiency and insufficiency is a wide spread global health issue, highly prevalent, regardless of age, gender, and country of origin. Vitamin D is known to regulate insulin secretion and synthesis by beta cells of pancreas, thus deficiency contributes to development of Diabetes Mellitus. Apart from glycaemic control, vitamin D is also involved in lipid metabolism. The low levels of vitamin D, enables vascular inflammation, endothelial dysfunction, foam cell formation and smooth muscle proliferation. Increasing circulating vitamin D levels, has been found to reduce the risk of hypertension, stroke, and myocardial infarction effectively. Dyslipidaemia, an important public health problem, is an independent risk factor for cardiovascular disease. The vitamin D has an inverse association with LDL cholesterol, contributing to the development of dyslipidaemia in deficiency.

AIM: To evaluate the association between serum vitamin D levels and the Atherogenic index of plasma (AIP), among patients with Type II Diabetes Mellitus.

Materials and methods: This analytical cross-sectional study was conducted in the Department of Biochemistry, Mamata Academy of Medical Sciences, Bachupally, Hyderabad, Telangana, from January 2026 to June 2026. A total of 152 subjects were included in the study, comprising 76 individuals with Type 2 Diabetes Mellitus (T2DM) and 76 healthy controls. Glycemic control was assessed by measuring glycosylated hemoglobin (HbA1c) by high performance liquid chromatography (HPLC), while serum vitamin D levels were estimated using the chemiluminescent immunoassay (CLIA) method. Dyslipidaemia indices were calculated from lipid profile parameters, including total cholesterol (TC), triglycerides (TG), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), and very-low-density lipoprotein cholesterol (VLDL-C). Statistical analyses were performed using RStudio version 4.5.1 (2025-06-13 ucrt). The differences in HbA1c, serum vitamin D levels, and dyslipidaemia indices between the study groups were assessed using the Mann–Whitney U test. The associations between serum vitamin D, HbA1c, and dyslipidaemia indices were evaluated using Spearman's rank correlation analysis.

Conclusion: Patients with Type 2 Diabetes Mellitus (T2DM) and poor glycaemic control may have an increased atherogenic risk, suggesting that HbA1c may serve as a useful marker for assessing cardiovascular risk in this population. The vitamin D deficiency is associated with poor glycemic control and atherogenic dyslipidemia. Therefore, improving vitamin D status as an important indicator may alleviate AIP as a surrogate marker for predicting the risk of cardiovascular events in healthy population.

Keywords: T2DM, dyslipidemia, LDL-C, HDL-C, TG, Glycemic control, atherogenic indices: AIP, CRI I, CRI II, cardiovascular,TG.

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INTRODUCTION

Diabetes mellitus (DM) is the most common endocrine disorder and is considered as one of the most growing chronic diseases worldwide. The hallmarks of Type 2 Diabetes Mellitus (T2DM) include chronic hyperglycaemia, insulin resistance, and progressive insulin deficiency. Insulin resistance plays a central role in the development of the characteristic dyslipidaemia associated with T2DM, contributing to abnormalities in lipid metabolism and an increased risk of cardiovascular complications. Disturbances in lipid metabolism appear to be an early feature in the pathogenesis of Type 2 Diabetes Mellitus (T2DM) and may precede the clinical onset of the disease by several years. These metabolic alterations may contribute to the development of insulin resistance and the subsequent dyslipidaemia commonly observed in individuals with

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T2DM [1]. The characteristic features of diabetic dyslipidaemia include elevated plasma triglyceride levels, reduced high-density lipoprotein cholesterol (HDL-C) concentrations, and an increased proportion of small, dense low-density lipoprotein cholesterol (LDL-C) particles. These lipid abnormalities are primarily associated with insulin resistance, which promotes increased flux of free fatty acids into the circulation and contributes to impaired lipid metabolism in individuals with diabetes mellitus [2].

Dyslipidemia, is one of the most important recognized risk factor of CVD events apart from smoking, physical inactivity, genetic susceptibility, stress, hypertension, diabetes mellitus. Hence, individual lipid risk factors (ILRF), (TG, TC, LDL-C, HDL-C) and non-HDL-C have been proposed as valuable tools in the prediction of development of cardiovascular risk [3]. Besides, the various constructed lipid indices such as, Framingham risk score (FRS), castelli risk index (CRI) I (TC/HDL ratio), and castelli risk index II (LDL/HDL ratio), atherogenic index of plasma (AIP) (TG/HDL ratio), etc., are considered as the better predictive of CVD events than ILFR [4]. FRS estimates the probability of CVD event in an individual within a specified period of time (10 years) [5]. Similarly, CRI I and II have been used as screening tools to identify increased CV risk, CRI I >4 - reflects the formation of coronary plaques and CRI II>3 - predicts acute myocardial infarction and insulin resistance. Conversely, Atherogenic index of plasma, has been shown to be high with normal TG and HDL-C, CV risk parameters. A higher AIP indicates higher risk of CVD due to atherosclerosis. An AIP with -0.3 to 0.11 indicates low risk, 0.11 to 0.24 indicates an intermediate risk and ≥0.24 indicates high risk of CVD [6].

The Vitamin D deficiency is a wide spread health issue, highly prevalent and present in approximately 30-50% of the world population. The vitamin D besides its association primarily with bone health, it is also required for optimal function of many organs and tissues throughout the body, including cardiovascular system. The vitamin D exerts its function through vitamin D receptors (VDRs), present on large variety of cell types like myocytes, cardiomyocytes, pancreatic beta cells, vascular endothelial cells, neurons, immune cells and osteoblasts [7]. The vitamin D is involved in calcium metabolism, lipid metabolism, glycaemic control, insulin secretion, and sensitivity. Apart from this, vitamin D has also several antihypertensive properties like renin angiotensin aldosterone system suppression, reno protective effects, direct effect on endothelial cells. Thus, vitamin D deficiency enables vascular inflammation, foam cell formation, smooth muscle proliferation, favouring atherosclerosis [8]. The vitamin D affects lipid metabolism by various factors such as genetic, non-genetic and also at the level of synthesis. The vitamin D is synthesized from 7-dehydrocholesterol (7DHC) by UV-B irradiation in the skin. This 7DHC, by the action of the enzyme 7-dehydrocholesterol reductase (DHCR7), is also converted to cholesterol. Thus, increased levels of vitamin D lead to a rapid decrease in DHCR7 activity and result in a lipid decrease, representing a fundamental correlation between vitamin D and lipids [9].

A couple of studies revealed correlation between polymorphism of VDRs with higher LDL-C and lower vitamin D levels, and also proposed that VDRs regulates bile acid synthesis at genetic level, and determines cholesterol level [10,11]. In this context, the authors sought to investigate the association between serum vitamin D levels and the Atherogenic Index of Plasma (AIP) in patients with Type 2 Diabetes Mellitus (T2DM). The study aimed to determine the correlations between serum vitamin D, HbA1c, dyslipidaemia indices such as AIP, Castelli Risk Index I (CRI-I), and Castelli Risk Index II (CRI-II) in healthy controls and type II diabetic subjects.

MATERIALS AND METHODS

An analytical cross-sectional study was carried out in Mamata Academy of Medical sciences, Bachupally, Hyderabad, Telangana, between January 2026 to June 2026, involving 152 subjects aged 30-60 years.

Ethical considerations: This study obtained ethical clearance from Institutional Ethics committee of Mamata academy of Medical Sciences, Bachupally, Hyderabad. (IEC reg no IEC/MAMS/2026/304).

Inclusion Criteria: A total of 152 subjects were enrolled in the study. The inclusion criteria include

1) Willingness to participate in the study and sign the informed consent,
2) The presence of type 2 diabetes at least for one year
3) patients receive either diet therapy or diet therapy with a combination of oral anti-diabetic medications,
4) No history of myocardial infarction, stroke, cardiovascular disease, active cancer, liver, kidney, and thyroid dysfunction, and infectious diseases,
5) No history of high blood pressure,

Exclusion criteria:

1) Willingness to participate in the study and sign the informed consent,
2) No history of diabetes mellitus,
3) No history of myocardial infarction, stroke, cardiovascular disease, active cancer, liver, kidney, and thyroid dysfunction, and infectious diseases,
4) No history of high blood pressure,

Study Procedure:

After 12 hours overnight fasting, about 3 ml of venous blood was drawn from the study subjects from the median cubital vein under aseptic precautions. 1 mL was collected in Ethylenediamine Tetra acetic acid (EDTA) for glycosylated haemoglobin, and 2 mL was collected in plain vial. The serum was separated by centrifugation for about 20 minutes at 3000 rpm and was used for the analysis of vitamin D by

Chemiluminescence immunoassay (CLIA), and HbA1c by High performance liquid chromatography (HPLC) method and for lipid profile, the serum total cholesterol (TC) estimated by **Cholesterol oxidase** and **Phenol + 4-Aminoantipyrine**(CHOD-PAP)method, Triglycerides (TG) by Glycerol Phosphate Oxidase (GPO) Trinder method, HDL level by Phosphotungstic acid method end point, and LDL, VLDL values were calculated by applying Friedwald's equation, [VLDL = TG/5 and LDL = TC – (VLDL + HDL)].

$$\text{CRI- I} = \frac{\text{Total Cholesterol}}{\text{HDL-Cholesterol}}$$

$$\text{CRI - II} = \frac{\text{LDL-C}}{\text{HDL-Cholesterol}}$$

$$\text{AIP} = \log_{10}\left(\frac{\text{Triglyceride}}{\text{HDL-Cholesterol}}\right)$$

The biochemical parameters reference ranges used for the study include: FBG=70-110 mg/dL(12), HbA1c: 5.7%- normal, 5.7-6.5% - prediabetes range, >6.5% - diabetes (13), vitamin D: toxicity >150 ng/mL, sufficiency ≥30 ng/mL, relative insufficiency 21-29 ng/mL, deficiency ≤20 ng/mL [14]. The glycaemic control **was classified based on HbA1c levels,** as good for HbA_{1c} <7%, fair for ≥7% to <8%, poor for ≥8% to <10%, and very poor for ≥10% [15].

Statistical Analysis:

Statistical analyses were performed using RStudio version 4.5.1 (2025-06-13 ucrt). Continuous variables were assessed for normality using the Shapiro-Wilk test. Quantitative variables were summarised using mean and standard deviation or median with interquartile range as per the skewness of the variable. Categorical variables are expressed as frequency and percentage.

Comparisons between case and control groups were performed using Welch's t-test for normally distributed continuous variables, Mann-Whitney U test for non-normally distributed continuous variables, and Chi-square test for categorical variables. Comparisons across vitamin D status groups were performed using one-way analysis of variance (ANOVA) or the Kruskal-Wallis test, as appropriate.

Associations between continuous variables were assessed using Spearman's rank correlation coefficient (ρ). Partial correlation analyses, adjusted for age and sex, were performed to evaluate the independent relationships between vitamin D and cardiometabolic risk markers. Factors associated with the Atherogenic Index of Plasma (AIP) were evaluated using multivariable linear regression analysis, with regression coefficients (β), 95% confidence intervals (CI), and p-values reported. A two-sided p-value < 0.05 was considered statistically significant.

RESULTS

A total of 152 subjects participated in the present study, comprising 76 individuals with Type 2 Diabetes Mellitus (T2DM) and 76 healthy controls. The age-wise distribution revealed a median age of 45 years among individuals with T2DM compared with 55 years among healthy controls. This difference in median age between the two groups was statistically significant. Gender-wise distribution revealed a male preponderance among the T2DM group, with 43 males, whereas females were predominant among the control group. However, the observed difference in gender distribution between the two groups was not statistically significant. [Table 1]

Intergroup comparison of lipid profile parameters was performed between T2DM subjects with poor glycaemic control and healthy controls. The concentrations of high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C) were calculated by mean ± SD, whose values were significantly higher among the T2DM subjects compared with healthy controls, with the observed differences being statistically significant ($p < 0.001$). The median concentrations of total cholesterol (TC), very-low-density lipoprotein cholesterol (VLDL-C), and triglycerides (TG) were significantly higher among the T2DM subjects compared with healthy controls, with the observed differences being statistically significant ($p < 0.001$). [Table 2]

Assessment of glycaemic control, serum vitamin D levels, and dyslipidaemia indices was performed among cases and controls. The median concentrations of fasting blood glucose (FBG), glycosylated hemoglobin (HbA1c) and dyslipidaemia indices, namely Atherogenic Index of Plasma (AIP), Castelli Risk Index I (CRI-I), and Castelli Risk Index II (CRI-II), were significantly higher among cases compared with controls. In contrast, serum vitamin D levels showed an inverse association with dyslipidaemia indices. A significantly lower median serum vitamin D concentration was observed among cases compared with controls. The difference between these study groups was statistically highly significant ($p<0.001$). (These findings suggest an inverse relationship between serum vitamin D status and atherogenic lipid indices, indicating that lower vitamin D levels may be associated with a more adverse lipid-related cardiovascular risk profile in individuals with Type 2 Diabetes Mellitus.) [Table 3].

The spearman correlation was done among study subjects which revealed a strong negative correlation between vitamin D and all parameters FBG ($r=-0.675$, $p< 0.001$*), HbA1c ($r=-0.397$, $p<0.001$*), AIP ($r=-0.691$, $p< 0.001$*), CRI I ($r=-0.833$, $p< 0.001$*), CRI II ($r=-0.772$, $p< 0.001$*), and a strong positive correlation was observed between HbA1c and FBG ($r=0.663$, $p<$

0.001*), HbA1c and dyslipidaemia indices such as AIP (r=0.394, p< 0.001*), CRI I (r=0.391, p< 0.001*), CRI II (r=0.402, p< 0.001*). [Table 4]

Demographic Details	Case (N=76)	Control (N=76)	Total (N=152)	p-value
Age (years)	45.00 (35.00, 59.50)	55.50 (44.00, 60.00)	50.00 (36.00, 60.00)	0.038*
Gender				
Male	43 (56.6%)	37 (48.7%)	80 (52.6%)	0.417
Female	33 (43.4%)	39 (51.3%)	72 (47.4%)	

Table 1: Intergroup comparison of demographic characteristics of the study participants between case and controls. Data are presented as median (IQR) for numerical variables or n (%) for categorial variables. Comparisons were performed using Mann-Whitney U test or Chi-square test, as appropriate. P < 0.05* was considered statistically significant.

Variables	**Case (N=76)**	**Control (N=76)**	**Total (N=152)**	**p-value**
Lipid Profile				
Total Cholesterol (mg/dL)	267.00 (244.75, 296.25)	177.00 (166.75, 188.25)	212.50 (177.00, 272.00)	<0.001*
HDL Cholesterol (mg/dL)	37.30 ± 9.47	55.29 ± 9.90	46.30 ± 13.22	<0.001*
LDL Cholesterol (mg/dL)	129.88 ± 24.73	98.50 ± 23.40	114.19 ± 28.70	<0.001*
VLDL Cholesterol (mg/dL)	35.00 (25.25, 44.00)	23.00 (19.00, 29.00)	29.00 (20.00, 39.00)	<0.001*
Triglycerides (mg/dL)	195.00 (180.00, 228.00)	124.00 (110.00, 157.00)	168.50 (122.75, 197.25)	<0.001*

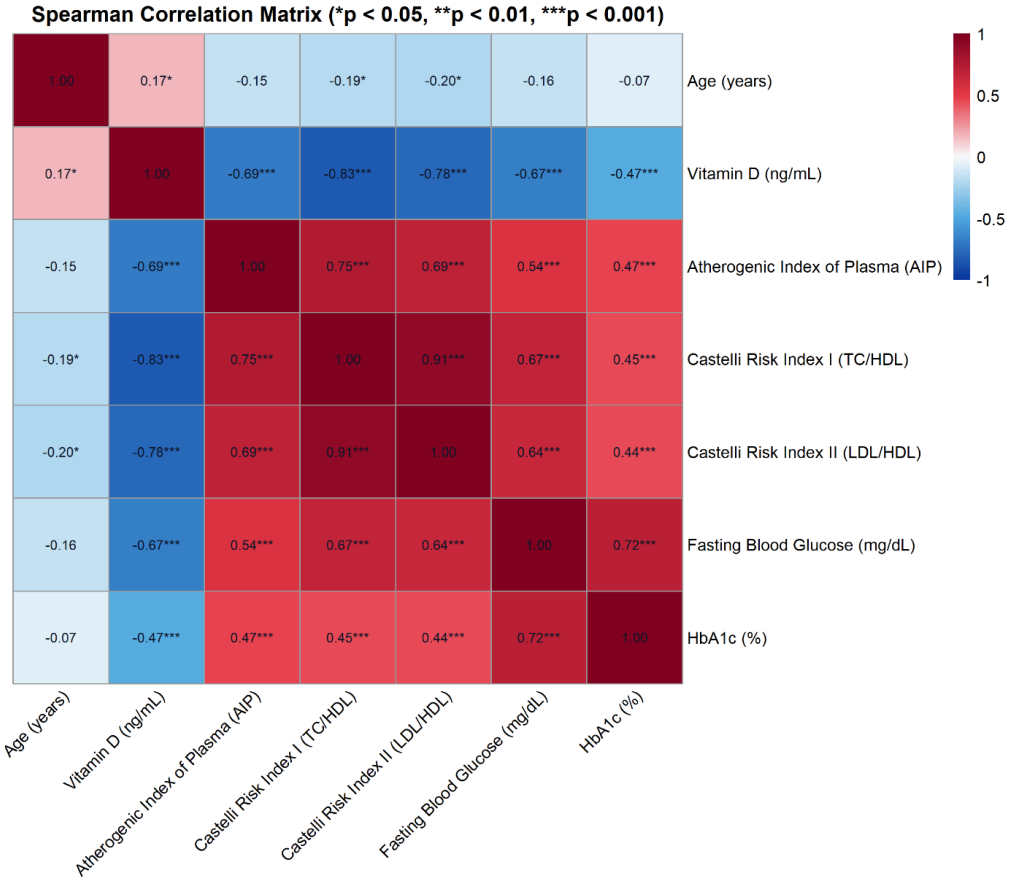
Table 2: Intergroup comparison of lipid profile. Data are presented as median (IQR) / mean ± SD for numerical variables or n (%) for categorial variables. Comparisons were performed using Mann-Whitney U test or Chi-square test, as appropriate. P < 0.05* was considered statistically significant.

Variables	**Case (N=76)**	**Control (N=76)**	**Total (N=152)**	**p-value**
Vitamin D (ng/mL)	17.50 (12.00, 22.00)	46.50 (36.00, 54.25)	29.50 (17.75, 47.00)	<0.001*
Fasting Blood Glucose (mg/dL)	150.00 (142.25, 159.25)	77.00 (69.00, 88.00)	98.00 (76.00, 150.00)	<0.001*
HbA1c	7.01 (6.50, 7.40)	5.60 (5.40, 5.70)	5.85 (5.60, 7.20)	<0.001*
Atherogenic Index of Plasma (AIP)	0.39 (0.32, 0.48)	0.06 (0.02, 0.13)	0.26 (0.06, 0.39)	<0.001*
Castelli Risk Index I (TC/HDL)	7.51 (6.49, 8.89)	3.24 (2.93, 3.57)	3.87 (3.23, 7.51)	<0.001*
Castelli Risk Index II (LDL/HDL)	3.63 (3.00, 4.44)	1.76 (1.51, 2.07)	2.26 (1.74, 3.65)	<0.001*

Table 3: Intergroup comparison of glucose parameters and cardiovascular risk indices of the study participants between case and controls. Data are presented as median (IQR) for numerical variables. Comparisons were performed using Mann-Whitney U test. P < 0.05* was considered statistically significant.

Correlation between	**Cases (N=76)**		**Control (N=76)**		**Total (N=152)**	
	r-value	**p-value**	**r-value**	**p-value**	**r-value**	**p-value**
Vitamin D vs AIP	-0.41	< 0.001*	-0.02	0.862	-0.691	< 0.001*
Vitamin D vs CRI I	-0.489	< 0.001*	-0.485	< 0.001*	-0.833	< 0.001*
Vitamin D vs CRI II	-0.485	< 0.001*	-0.369	0.001*	-0.772	< 0.001*
Vitamin D vs HbA1c	0.052	0.658	0.393	< 0.001*	-0.397	< 0.001*
HbA1c vs AIP	-0.037	0.753	0.149	0.199	0.394	< 0.001*
HbA1c vs CRI I	0.069	0.551	-0.269	0.019*	0.391	< 0.001*
HbA1c vs CRI II	0.075	0.519	-0.285	0.013*	0.402	< 0.001*
HbA1c vs FBG	0.625	< 0.001*	0.234	0.042*	0.663	< 0.001*

Table 4: Spearman correlation analyses for cases, controls and all study participants. r-value denotes Spearman's rank correlation. P-value<0.05* is statistically significant.



DISCUSSION

The present cross-sectional study demonstrated that fasting blood glucose (FBG) and glycosylated hemoglobin (HbA1c) levels were markedly elevated in individuals with Type 2 Diabetes Mellitus (T2DM) compared with healthy controls. Furthermore, a strong positive correlation was observed between glycaemic control parameters (FBG and HbA1c) and dyslipidaemia indices, including Atherogenic Index of Plasma (AIP), Castelli Risk Index I (CRI-I), and Castelli Risk Index II (CRI-II). Our findings are consistent with those reported by Mabrouk A et al., [16].

The HbA1c levels reflect the average blood glucose concentration over the preceding 3-4 months and therefore serve as an important indicator of long-term glycaemic control. Patel MB et al. reported that poor glycaemic control in individuals with Type 2 Diabetes Mellitus (T2DM) is associated with progressive deterioration of insulin secretion over time, primarily due to pancreatic β-cell dysfunction and failure. Progressive β-cell impairment contributes to worsening hyperglycaemia and may further aggravate the metabolic abnormalities associated with T2DM. [17]

Poor glycaemic control in individuals with Type 2 Diabetes Mellitus (T2DM) is associated with characteristic lipoprotein abnormalities, including hypertriglyceridemia, reduced plasma high-density lipoprotein cholesterol (HDL-C), increased concentrations of small, dense low-density lipoprotein (LDL) particles, and elevated levels of triglyceride-rich very-low-density lipoprotein (VLDL). Small, dense LDL particles are particularly atherogenic and contribute to the increased cardiovascular risk associated with T2DM. [18] [19]. The present study also demonstrated that individuals with poor glycaemic control, as indicated by HbA1c levels ≥7%, had significantly higher concentrations of triglycerides (TG), low-density lipoprotein cholesterol (LDL-C), and total cholesterol (TC) compared with those with HbA1c levels <7%. The differences were statistically significant ($p < 0.001$*).These findings were in accordance with the study done by Ghimire MR et al. [20]

A marked significant decrease in the median concentration of vitamin D was noted in T2DM subjects with poor glycaemic control, when compared to healthy controls, indicating an association of vitamin D with beta cell function, insulin resistance. Mathieu C et al., in their study revealed that Vitamin D has an important role in the functional regulation of the endocrine pancreas, particularly pancreatic β-cells. The vitamin D signalling pathway is also associated with the presence of the vitamin D-dependent calcium-binding protein, calbindin-D28k, which is expressed in pancreatic β-cells and may contribute to the regulation of intracellular calcium homeostasis. The active form of vitamin D, 1,25-dihydroxyvitamin D_3 [$1,25(OH)_2D_3$], has been suggested to influence insulin secretion

through modulation of intracellular calcium concentrations, as an increase in cytosolic Ca^{2+} levels has been observed following $1,25(OH)_2D_3$-stimulated insulin secretion from pancreatic islet cells. Vitamin D deficiency may adversely affect β-cell function and glucose homeostasis, potentially contributing to glucose intolerance and an increased susceptibility to Type 2 Diabetes Mellitus. [21]

In the present study, a significant negative correlation was observed between serum vitamin D levels and dyslipidaemia indices, including Atherogenic Index of Plasma (AIP), Castelli Risk Index I (CRI-I), and Castelli Risk Index II (CRI-II). Individuals with Type 2 Diabetes Mellitus (T2DM), particularly those with poor glycaemic control, demonstrated significantly lower serum vitamin D levels accompanied by higher values of all three dyslipidaemia indices. These findings indicate that reduced vitamin D status may be associated with a more atherogenic lipid profile in patients with T2DM. Our results are consistent with the findings of Mahmoodi MR et al., who reported a significant reverse association between AIP and serum vitamin D and low serum level of vitamin D is associated with atherogenic dyslipidaemia [22]. A study by Hu T et al. reported a significant negative correlation between serum vitamin D levels and the Atherogenic Index of Plasma (AIP), suggesting that higher vitamin D levels may be associated with a more favourable atherogenic profile [23].

Surdu AM et al., and Lemire JM in their study reported that Vitamin D has been proposed to exert anti-inflammatory effects that may contribute to its potential protective role against atherosclerosis. It may modulate the inflammatory response by suppressing pro-inflammatory cytokines, including interleukin (IL)-12, IL-6, IL-8, interferon-γ (IFN-γ), and tumour necrosis factor-α (TNF-α), while promoting the production of anti-inflammatory cytokines such as IL-4, IL-5, and IL-10 [24] [25]. A study done by Kassi E et al., revealed that the vitamin D may also exert protective effects against several systemic metabolic and vascular abnormalities that contribute to the development of atherosclerosis. These include insulin resistance, pancreatic β-cell dysfunction, dyslipidaemia, activation of the renin–angiotensin–aldosterone system (RAAS), and subsequent hypertension. Through its potential effects on these interconnected pathways, adequate vitamin D status may contribute to reducing the overall atherogenic and cardiovascular risk [26].

CONCLUSION

The Type 2 Diabetes Mellitus (T2DM) patients with poor glycaemic control may have an increased atherogenic risk, suggesting that HbA1c may serve as a useful predictor of cardiovascular risk in this population. A significant inverse association was observed between serum vitamin D levels and glycaemic control parameters as well as atherogenic indices. Lower vitamin D levels may adversely affect glucose regulation and contribute to an increased cardiovascular risk among T2DM individuals with poor glycaemic control. These findings suggest a potential protective role of adequate vitamin D status against atherosclerosis and cardiovascular disease. Therefore, screening for and appropriate management of vitamin D deficiency may potentially contribute to reducing cardiovascular morbidity and mortality, although further prospective and interventional studies are required to establish a causal relationship.

Limitations:

The present study has several limitations. It was a hospital-based, single-centre study with a relatively small sample size, which may limit the generalizability of the findings. Several potential confounding factors that may contribute to dyslipidaemia, including body mass index (BMI), smoking, alcohol consumption, hypothyroidism, hypertension, and the use of different antidiabetic medications, were not evaluated or adequately controlled in the present study. Therefore, the findings should be interpreted with caution and may not be generalizable to the wider population. Future multicentre studies with larger sample sizes and appropriate adjustment for potential confounding factors are warranted.

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