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ORIGINAL ARTICLE

Study of Serum Vitamin D Levels, Atherogenic Index of Plasma, and Cardiovascular Risk in Type 2 Diabetes Mellitus

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Abstract
Background: Patients with type 2 diabetes mellitus (T2DM) have an increased risk of cardiovascular disease, largely driven by atherogenic dyslipidaemia. Vitamin D deficiency is highly prevalent in this population and has been implicated in adverse cardiometabolic profiles. The relationship between serum vitamin D levels and composite lipid risk markers such as the atherogenic index of plasma (AIP) remains insufficiently explored, particularly in Indian clinical settings. **Objectives:** To assess serum vitamin D levels and lipid profile parameters in patients with T2DM and to evaluate the association between vitamin D status and the atherogenic index of plasma. **Methods:** This prospective observational study was conducted in the Department of General Medicine, ACS Medical College and Hospital, Chennai, from December 2018 to March 2019. A total of 120 patients with T2DM were enrolled using consecutive sampling. Fasting blood samples were analyzed for lipid profile using enzymatic methods on a semi-autoanalyzer, and serum vitamin D levels were estimated by ELISA. The atherogenic index of plasma was calculated as $\log_{10}$(triglycerides/HDL cholesterol). Associations were assessed using Pearson correlation analysis. **Results:** The mean serum vitamin D level was 20.0 ± 7.8 ng/mL. Serum vitamin D showed significant inverse correlations with triglycerides ($r = -0.246$, $p = 0.007$), VLDL cholesterol ($r = -0.244$, $p = 0.007$), and the atherogenic index ($r = -0.278$, $p = 0.002$). No significant correlations were observed between vitamin D levels and total cholesterol, HDL cholesterol, LDL cholesterol, fasting blood glucose, or post-prandial blood glucose. **Conclusion:** Lower serum vitamin D levels were associated with higher atherogenic index values in patients with T2DM, suggesting a link between vitamin D status and lipid-related cardiovascular risk. Evaluation of serum vitamin D alongside composite lipid indices may aid cardiovascular risk stratification in this population.

Keywords: Type 2 diabetes mellitus, Vitamin D deficiency, Atherogenic index of plasma, Dyslipidaemia, Cardiovascular risk

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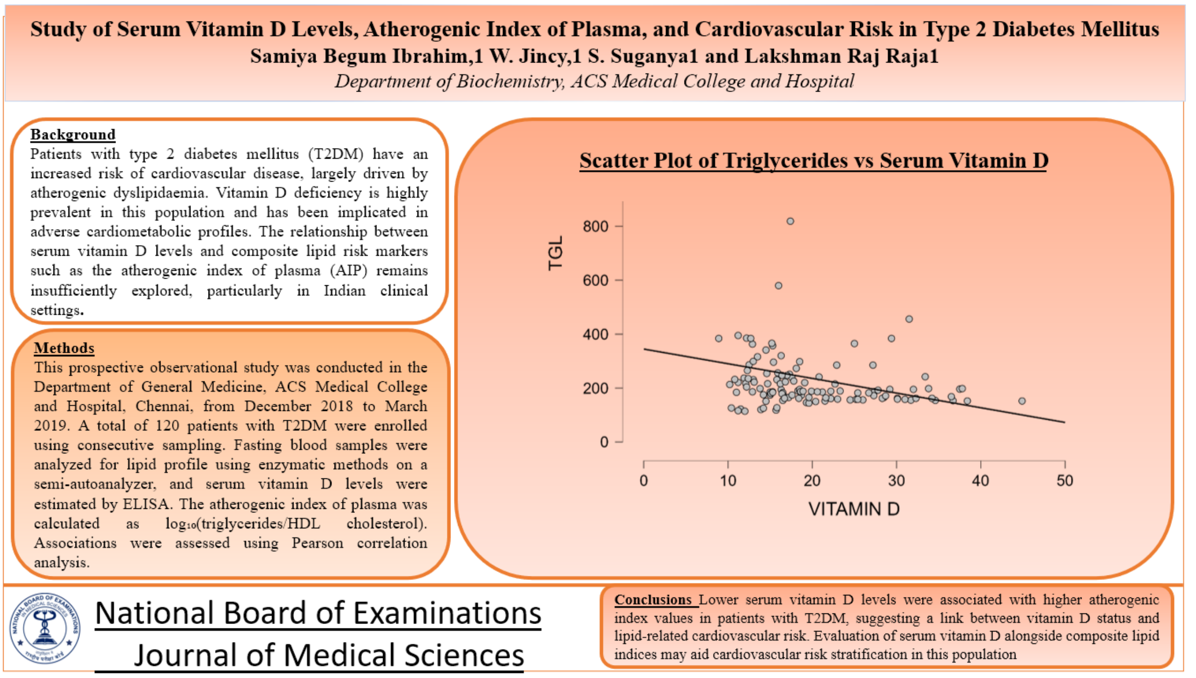
Graphical Abstract



Introduction

Many people around the world are affected by type 2 diabetes mellitus (T2DM); it is one of the most common non-communicable diseases [1,2]. Due to increasing numbers of older adults, urbanization, sedentary lifestyles, and changes in diets, this prevalence of T2DM is increasing [1,2]. The majority of morbidity and mortality from cardiovascular disease (CVD) occurs in people suffering from T2DM, leading to a large proportion of premature deaths and long-term disabilities [3]. A recent meta-analysis of prospective studies indicates that people with T2DM have almost twice the risk of developing coronary artery disease and stroke than people without diabetes, after controlling for all established cardiovascular risk factors [3]. While there have been considerable advances made in glucose-lowering therapies, this excess cardiovascular risk remains, highlighting the need for better risk stratification using clinically accessible markers.

Type 2 Diabetes Mellitus (T2DM) is a major component of the global diabetes burden and is projected to substantially increase over the next several decades [1,2]. Many Indians with T2DM have an abnormal lipid profile that includes elevated triglyceride levels and decreased levels of high-density lipoprotein (HDL) cholesterol along with qualitative abnormalities in low-density lipoprotein (LDL) particles; these characteristics accelerate the rate of atherosclerosis and promote earlier onset of CVD [5]. Although traditional lipid measures can be informative, they do not fully represent the atherogenic lipid environment that is commonly observed in T2DM, especially in populations at high baseline cardiometabolic risk.

The atherogenic index of plasma (AIP), defined as the log ratio of triglycerides to HDL-cholesterol, has emerged as a useful composite measure of atherogenic dyslipidemia [6]. Studies have demonstrated that AIP correlates with lipoprotein particle size, insulin resistance and cardiovascular risk and therefore provides a more complete evaluation of lipid-related risk than measurement of single lipid fractions [7]. Because AIP uses only lipid parameters that are routinely measured, it has the potential to be a highly useful tool in resource-poor clinical settings for the evaluation of cardiovascular risk in T2DM.

Vitamin D traditionally has been recognized for its role in maintaining skeletal health. It is now increasingly being recognized for its potential to influence other physiological systems, including glucose metabolism, immune system function, endothelial cell function, and cardiovascular health [4]. Receptors for Vitamin D have been identified in pancreatic beta-cells, vascular smooth-muscle cells, and endothelial cells, thereby suggesting a biologically plausible relationship between Vitamin D status and cardiometabolic health. Vitamin D deficiency is present worldwide, but is particularly prevalent in India; despite India's abundance of sunlight, Vitamin D deficiency remains widespread in India due to a variety of reasons including, but not limited to, limited sun exposure, skin pigmentation, inadequate nutrition, and an urban lifestyle [8,9].

Evidence supporting relationships between Vitamin D deficiency and adverse lipid profiles, insulin resistance, and increased cardiovascular risk is provided by observational and interventional studies; however, findings have been inconsistent across different populations [10]. Meta-analyses of randomized-controlled trials in patients with T2DM have shown small improvements in some lipid measures with Vitamin D supplementation, while effects on HDL-cholesterol and total cardiovascular endpoints have varied [10,13]. Moreover, many of the previous studies have focused on individual lipid fractions rather than composite indices that are more representative of atherogenic risk.

There is limited data available concerning the relationship between serum levels of Vitamin D and atherogenic indices, such as AIP, in patients with T2DM. Some studies have demonstrated that Vitamin D status inversely correlates with atherogenic risk markers in diabetic populations, implying that lower Vitamin D levels are associated with more adverse lipid profiles [11]. Associations similar to those described above have been reported in population-based studies evaluating the relationship between serum Vitamin D concentrations and cardiometabolic risk markers, including atherogenic indices [12]. However, there is a paucity of information regarding how Vitamin D relates to atherogenic indices in clinical settings in India, where both the prevalence of T2DM and Vitamin D deficiency are high.

Therefore, we investigated the relationship between serum Vitamin D levels and the atherogenic index in patients with T2DM to gain clinically relevant information about cardiovascular risk assessment using easily obtainable laboratory values. Although the prevalence of both T2DM and vitamin D deficiency are high in India, data specifically addressing the relationship between serum vitamin D levels and composite atherogenic indices such as AIP in an Indian clinical cohort remain limited. Therefore, we hypothesized that lower serum vitamin D levels were independently associated with higher AIP values in patients with T2DM. Therefore, this study was conducted to evaluate whether there exists an association between serum Vitamin D levels and the atherogenic index of plasma as a marker of cardiovascular risk in patients with type 2 diabetes mellitus.

Materials and Methods

Study Design

This was a prospective observational study.

Study Period

The study was conducted from December 2018 to March 2019.

Study Setting

The study was carried out in the Department of Biochemistry and Department of General Medicine, ACS Medical College and Hospital, Chennai, Tamil Nadu, India. Both outpatients and inpatients attending the department during the study period were screened for eligibility.

Study Population and Sample Size

The sample size was determined based on the ability to detect a statistically significant correlation between serum vitamin D levels and the atherogenic index of plasma. The required sample size for correlation analysis was estimated using **Fisher's z transformation**, according to the formula:

$$n = \left(\frac{Z_{1-\alpha/2} + Z_{1-\beta}}{0.5 \times \ln\left(\frac{1+r}{1-r}\right)} \right)^2 + 3$$

where r represents the anticipated correlation coefficient, α is the type I error, and β is the type II error.

With a **two-sided α of 0.05** and **80% power**, a sample size of **120 participants** was sufficient to detect a minimum correlation coefficient of approximately **r = 0.25** between serum vitamin D levels and the atherogenic index. With **90% power**, the same sample size corresponded to the detection of a correlation coefficient of approximately **r = 0.29**. Accordingly, a total of **120 patients** were included in the study.

A total of **120 patients** diagnosed with **type 2 diabetes mellitus** were included in the study.

Sampling Method

Patients were enrolled using **consecutive sampling**.

Inclusion Criteria

- Adults diagnosed with type 2 diabetes mellitus
- Patients attending the outpatient or inpatient services of the Department of General Medicine during the study period
- Patients who provided informed consent for participation

Exclusion Criteria

- Patients with known **chronic kidney disease**
- Patients with documented **coronary heart disease**
- Patients with acute or chronic illnesses likely to influence lipid metabolism or vitamin D status

Data Collection and Clinical Assessment

After obtaining informed consent, demographic details and clinical data were recorded. Anthropometric measurements including height, weight, and waist circumference were obtained using standard techniques. Blood pressure was measured using a calibrated sphygmomanometer with the patient in a seated position.

Laboratory Investigations

After an overnight fast, venous blood samples were collected under aseptic precautions.

- Fasting blood glucose and lipid profile parameters (total cholesterol, triglycerides, HDL cholesterol, LDL cholesterol, and VLDL cholesterol) were analyzed using enzymatic methods on a semi-autoanalyzer (Microlab 300).
- Post-prandial blood glucose samples were collected two hours after a standard meal.
- Serum vitamin D levels were estimated using the enzyme-linked immunosorbent assay (ELISA) method.

Statistical Analysis

Data were entered and analyzed using JASP (0.95.4). Continuous variables were expressed as mean ± standard deviation. Prior to correlation analyses, normality of continuous variables was assessed using the Shapiro–Wilk test. For variables that deviated significantly from normality (triglycerides and VLDL cholesterol), Spearman's rank correlation was performed as a supplementary analysis; results were concordant with those from Pearson's analysis. The primary association between serum vitamin D levels, lipid parameters, glycaemic parameters, and the atherogenic index was assessed using Pearson correlation analysis. A p-value < 0.05 was considered statistically significant.

Results

The study population consisted of 120 men and women who averaged 50.1 years old and weighed an average of 70.6 kilograms. The population's mean body mass index (BMI) was 28.9 and ranged from 17 to 43 kg/m². The mean waist circumference of the participants was 108.3 centimeters and ranged from 61 to 153 cm.

Participants' mean systolic blood pressures were 133.7 mmHg, with a range of 90-200 mmHg. Participants' mean diastolic blood pressures were 87.5 mmHg, with a range of 60-140 mmHg. Participants' mean fasting blood glucose levels were 168.5 milligrams per deciliter (mg/dl). Blood glucose values ranged from 47 to 305 mg/dl.

Participants' mean total cholesterol levels were 183.4 mg/dl. Total cholesterol levels ranged from 85 to 275 mg/dl. Mean triglyceride levels among the participants were 233.6 mg/dl. Triglyceride levels ranged from 12 to 1137 mg/dl.

Mean high-density lipoprotein (HDL) cholesterol levels among the participants were 37.9 mg/dl. HDL levels ranged from 14 to 88 mg/dl. Mean low-density lipoprotein (LDL) cholesterol levels among the participants were 100.9 mg/dl. LDL levels ranged from 25 to 206 mg/dl. Mean very-low-density lipoprotein (VLDL) cholesterol levels among the participants were 46.5 mg/dl. VLDL levels ranged from 8 to 163 mg/dl.

Participants' mean vitamin D levels were 20.0 nanograms per milliliter (ng/mL) and ranged from less than 10 to greater than 150 ng/mL. Participants' mean atherogenic indices were 0.74. Atherogenic indices ranged from 0.04 to 3.36. The majority of the study population were men (78 men, 42 women; 65%). This is similar to the mean HDL cholesterol of 37.9 mg/dl found, since male sex is independently related to lower HDL levels; lipid parameter means by gender are provided in Table 5.

There were statistically significant relationships between the participants' serum vitamin D levels and their serum triglycerides (r=-.246, p=0.007), and VLDL cholesterol (r=-.244, p=.007), but no statistically significant relationships between serum vitamin D levels and total cholesterol or HDL cholesterol.

Serum vitamin D levels were inversely correlated with participants' atherogenic indices (r = -.278, p = .002); however, there were no statistically significant relationships between participants' fasting blood glucose or postprandial blood glucose and atherogenic indices.

Table 1. Baseline demographic, anthropometric, and clinical characteristics of the study population

Parameter	**Mean ± SD**
Age (years)	50.1 ± 10.5
Sex (Male/Female)	78/42
Body mass index (kg/m²)	28.9 ± 3.1
Waist circumference (cm)	108.3 ± 8.9
Systolic blood pressure (mmHg)	133.7 ± 16.7
Diastolic blood pressure (mmHg)	87.5 ± 10.1
Fasting blood glucose (mg/dL)	168.5 ± 55.3
Post-prandial blood glucose (mg/dL)	252.9 ± 78.6

Table 2. Lipid profile parameters, serum vitamin D levels, and atherogenic index (n = 120)

Parameter	**Mean ± SD**
Total cholesterol (mg/dL)	183.4 ± 33.9
Triglycerides (mg/dL)	233.6 ± 168.3
HDL cholesterol (mg/dL)	37.9 ± 4.2
LDL cholesterol (mg/dL)	100.9 ± 35.2
VLDL cholesterol (mg/dL)	46.5 ± 33.7
Serum vitamin D (ng/mL)	20.0 ± 7.8
Atherogenic index	0.74 ± 0.21

Table 3. Correlation between serum vitamin D levels and lipid profile parameters

Lipid parameter	**Pearson's r**	**p-value**
Triglycerides	−0.246	**0.007**
Total cholesterol	−0.104	0.260
HDL cholesterol	0.169	0.065
LDL cholesterol	0.061	0.510
VLDL cholesterol	−0.244	**0.007**

Table 4. Correlation of serum vitamin D and glycaemic parameters with atherogenic index

Parameter	**Pearson's r**	**p-value**
Serum vitamin D	−0.278	**0.002**
Fasting blood glucose	0.028	0.764
Post-prandial blood glucose	0.088	0.339

Table 5. Sex-stratified lipid profile parameters, serum vitamin D levels, and atherogenic index

Parameter	**Male (n=78)**	**Female (n=42)**
Total cholesterol (mg/dL)	181.2 ± 34.6	187.4 ± 32.5
Triglycerides (mg/dL)	241.3 ± 176.4	219.8 ± 152.7
HDL cholesterol (mg/dL)	36.8 ± 4.0	39.9 ± 4.1
LDL cholesterol (mg/dL)	99.1 ± 36.3	104.1 ± 33.4
VLDL cholesterol (mg/dL)	48.3 ± 35.3	43.0 ± 30.5
Serum vitamin D (ng/mL)	19.6 ± 7.9	20.8 ± 7.6
Atherogenic index	0.77 ± 0.22	0.68 ± 0.19

Discussion

This prospective observational study of individuals with type 2 diabetes mellitus (T2DM) found serum vitamin D levels to be inversely associated with lipid-derived cardiovascular risk factors. The population studied had low mean serum vitamin D levels along with a dyslipidemic profile consisting of high triglycerides and low HDL cholesterol. Statistically significant inverse correlations were noted between serum vitamin D and triglycerides, very low-density lipoprotein (VLDL) cholesterol, and the atherogenic index of plasma (AIP). No statistically significant relationships were identified for total cholesterol, low-density lipoprotein (LDL) cholesterol, or glycemic variables. Thus, these results support the hypothesis that the vitamin D status in T2DM is correlated with atherogenic lipid profiles rather than current measures of glycemic control (Figures 1 and 2).

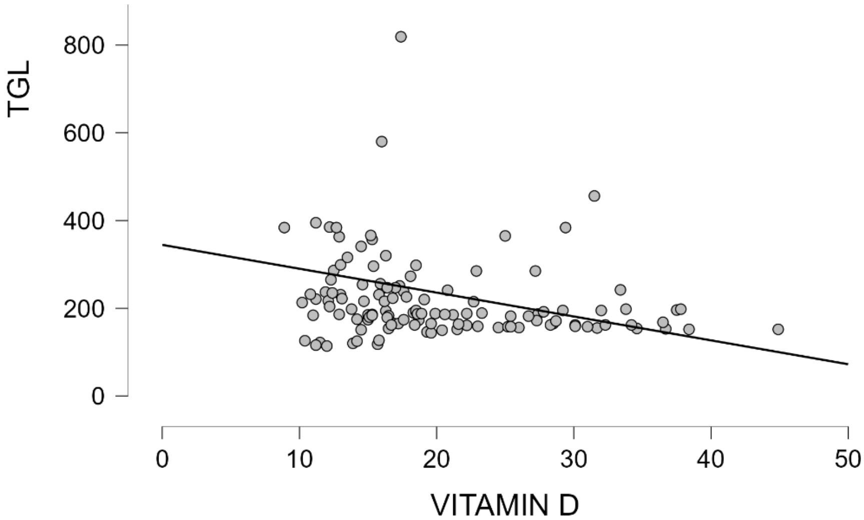


Figure 1. Scatter Plot of Triglycerides vs Serum Vitamin D

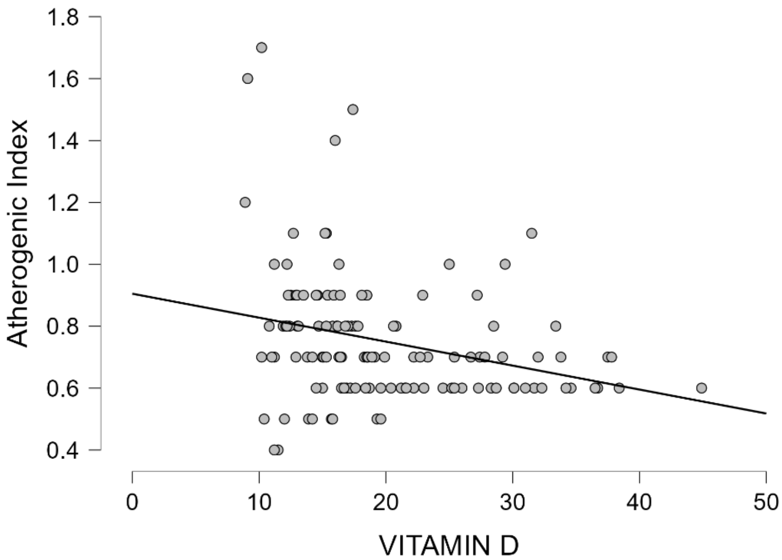


Figure 2. Scatter Plot of Atherogenic Index vs Serum Vitamin D

Comparative Studies with Indian Data

Vitamin D deficiency is widespread in India among individuals with metabolic disease, and has been documented consistently through community and hospital based studies [8,9]. The low mean serum vitamin D levels observed in this study are therefore consistent with prior reports of vitamin D deficiency in India. Dyslipidemia characterized by high triglycerides and low HDL cholesterol is a well-documented feature of Indian patients with T2DM and contributes to a substantial increase in their cardiovascular risk [5].

There are limited Indian studies that have examined the relationship between vitamin D status and composite lipid risk markers in individuals with T2DM. Pokhrel et al. evaluated the relationship between vitamin D deficiency and cardiovascular risk in individuals with diabetes and found that vitamin D deficient subjects had adverse lipid parameters and higher atherogenic indexes compared to non-deficient individuals [11]. While there are methodologic differences in the two studies, the results of Pokhrel et al. demonstrate a similar inverse relationship between serum vitamin D levels and AIP in Indian patients with T2DM.

Comparative Studies with International Data

International data also document a relationship between vitamin D status and cardiometabolic risk; however, the magnitude of the relationship varies between studies. The KERCADR study demonstrated significant inverse relationships between serum vitamin D levels and atherogenic indices in patients with diabetes, and the results of that study are consistent with the inverse relationship we observed between serum vitamin D and AIP in our study [12]. Pokhrel et al. reported higher atherogenic indexes in vitamin D deficient individuals with T2DM, indicating a similar relationship between vitamin D status and atherogenic indices in multiple populations [11].

Meta-analyses of vitamin D supplementation in T2DM have shown modest reductions in triglycerides and total cholesterol, but inconsistent effects on HDL cholesterol [10,13]. Our results support the findings of meta-analyses in that we observed significant relationships between serum vitamin D levels and triglyceride rich lipoproteins, but not with HDL or LDL cholesterol. Collectively, these findings indicate that vitamin D status may affect certain aspects of lipid metabolism, but does not uniformly impact all lipid fractions.

Pathophysiologic Considerations

Several pathophysiologic mechanisms may explain the observed association between low serum vitamin D levels and higher AIP. Vitamin D receptors are present in the liver, adipose tissue, vascular smooth muscle cells, and endothelial cells, suggesting that vitamin D may play a role in lipid metabolism and vascular homeostasis [4]. Deficiency in vitamin D has been associated with increased hepatic production of triglyceride rich lipoproteins and decreased activity of lipoprotein lipase resulting in increased circulating triglycerides.

In addition, insulin resistance common in T2DM can further contribute to these effects by promoting VLDL production and decreasing HDL cholesterol levels. Since AIP is calculated from triglyceride and HDL cholesterol concentrations, the metabolic changes that occur as a result of low vitamin D status and insulin resistance will directly alter its value [6,7]. Moreover, low vitamin D status has been associated with low grade inflammation and endothelial dysfunction, and this may contribute to the atherogenic potential of triglyceride rich lipoproteins. The lack of a significant association between vitamin D levels and glycemic parameters in this study indicates that the relationship between vitamin D levels and lipid related cardiovascular risk may be greater than the relationship between vitamin D levels and short term glycemic control.

Implications for Clinical Practice

The findings of this study have several implications for clinical practice. First, the significant inverse association between serum vitamin D levels and AIP indicates that vitamin D status may be used as an adjunctive marker in assessing cardiovascular risk in individuals with T2DM. Both serum vitamin D measurement and AIP calculation can be performed with standard laboratory tests and thus, this approach is feasible and practical for use in everyday clinical practice.

Second, the lack of association between glycemic parameters and AIP underscores the need to assess cardiovascular risk beyond glycemic control. Individuals who have adequate glycemic control may still carry significant atherogenic risk due to dyslipidemia and other micronutrient deficiencies. Therefore, incorporation of vitamin D assessment in metabolic evaluation may help identify individuals at higher cardiovascular risk. It should be noted, however, that the present study was not designed to validate specific clinical cut-off combinations; while published literature has proposed an AIP value of >0.11 as indicating moderate-to-high cardiovascular risk and vitamin D sufficiency is generally defined as ≥ 20 ng/mL, these thresholds require prospective evaluation before clinical implementation can be recommended.

Limitations

Several limitations of this study should be acknowledged. First, the cross-sectional, observational design precludes any inference of causality between vitamin D status and atherogenic dyslipidaemia. Second, this was a single-centre study conducted at a tertiary care hospital in Chennai, Tamil Nadu, which may limit the generalisability of findings to other geographic regions, ethnicities, or healthcare settings. Third, dietary vitamin D intake and individual sun exposure were not assessed, both of which are important determinants of serum vitamin D levels in the Indian population. Fourth, data on potentially important confounding variables including HbA1c, duration of diabetes, and concurrent use of statins, fibrates, or vitamin D supplements were not systematically collected; their influence on the observed associations cannot therefore be excluded. Fifth, a formal sex-stratified correlation analysis was not performed, and hormonal influences on lipid metabolism and vitamin D status in female participants warrant further investigation. Collectively, these limitations underscore the need for larger, multicentre, prospective studies with comprehensive covariate assessment to confirm and extend the findings of this study.

Conclusion

In this prospective observational study of patients with type 2 diabetes mellitus, lower serum vitamin D levels were significantly associated with higher atherogenic index values, reflecting an unfavorable lipid-related cardiovascular risk profile. Serum vitamin D demonstrated inverse associations with triglycerides, VLDL cholesterol, and the atherogenic index, while no significant relationships were observed with glycaemic parameters. These findings suggest that vitamin D status may be linked to atherogenic dyslipidaemia in patients with type 2 diabetes mellitus. Assessment of serum vitamin D levels alongside composite lipid indices may serve as a hypothesis-generating observation warranting prospective validation of clinically actionable thresholds. Further longitudinal and interventional studies with comprehensive covariate adjustment are needed to clarify the clinical implications and to determine whether targeting vitamin D deficiency may reduce lipid-related cardiovascular risk in this population.

Ethical Considerations

The study was conducted after obtaining approval from the Institutional Ethics Committee of ACS Medical College and Hospital. Written informed consent was obtained from all participants prior to enrollment.

Statements and Declarations

Conflicts of interest

The authors declare that they do not have conflict of interest.

Funding

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