Original Article

The significance of ANGPTL3 and ANGPTL4 proteins in the development of dyslipidemia in Type 2 diabetes mellitus

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Abstract

Background: Dyslipidemia is the leading cause of cardiovascular disease (CVD) in Type 2 diabetes mellitus patients. As a result, it is critical to target and manage the level of atherogenic lipids. Angiopoietin-like proteins 3 and 4 (ANGPTL 3 and ANGPTL 4) play an important role in the intravascular lipolysis of triglyceride-rich lipoproteins by blocking the enzyme lipoprotein lipase. This study aimed to determine the amounts of these angiopoietin-like proteins in T2DM and find their association with dyslipidemia in T2DM. **Material and Methods:** Sixty-one T2DM patients of age group 25–65 years and 27 healthy age-matched control participants were enrolled in the study. Glycemic status (FBS, PPBS, HbA1C), serum lipid parameters (cholesterol, TG, LDL, VLDL, HDL, Tc/HDL ratio), free fatty acid, serum insulin, and ANGPTL3, 4 were measured. A correlation was found between the ANGPTLs and the above parameters in T2DM patients. **Results:** Serum ANGPTL3 ($P < 0.05$) and ANGPTL4 ($P < 0.001$) were significantly decreased in T2DM. ANGPTL4 was also negatively correlated to PPBS (0.03), HbA1C ($P = 0.05$), and IR ($P = 0.04$). However, no such correlation was observed with ANGPTL 3. It was observed that lipid parameters were correlated with ANGPTL3 (LDL ($P = 0.03$), TC/HDL ($P = 0.02$)). There was a significant relationship between ANGPTL3 and 4 with FFA ($P = 0.001$ and $P = 0.03$, respectively). **Conclusion:** This study shows that ANGPTL 3,4 may be associated with dyslipidemia in T2DM. ANGPTL4 is more correlated with glycemic status.

Keywords: ANGPTL 3,4, DKD, dyslipidemia, FFA, glycemic status, insulin resistance, lipid profile, T2DM

Introduction

Type 2 diabetes mellitus (T2DM) is a chronic disorder that occurs when either the pancreas does not produce enough insulin or when the body cannot effectively use because of insulin resistance.

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In 2019, T2DM was the direct cause of 1.5 million deaths being the 9th leading cause of deaths worldwide with 48% of all deaths before the age of 70 years.[1] By 2030 and 2045, their numbers are predicted to reach 643 million and 783 million, respectively.[2] In India, the incidence of diabetes has increased from 7.1% in 2009 to 8.9% in 2019. With 77 million people suffering from T2DM, India comes in second place in the world's diabetes epidemic.[3]

72–85% of T2DM patients present with dyslipidaemia. As dyslipidaemia plays a significant role in genesis and progression

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of atherosclerosis, there is substantially increased incidence of CVD among T2DM patients with dyslipidaemia. High plasma triglyceride levels, low HDL, and high small dense LDL cholesterol particles are the hallmarks of diabetic dyslipidaemia.[4] Furthermore, low-density lipoproteins (LDL) are transformed into smaller, potentially more atherogenic lipoproteins known as small dense LDL. In contrast to type 1 diabetes, this phenotype is seldom totally rectified with glycemic management. This dyslipidaemia is commonly identified in prediabetics or people with insulin resistance but normal plasma glucose levels. As a result, this lipid anomaly is linked to insulin action defects rather than hyperglycemia itself. The primary source of these alterations is increased free fatty acid flow caused by insulin resistance, which is compounded by an increase in inflammatory adipokines. Tracer kinetic investigations in humans have shown that ApoB synthesis from the liver, which is a key component of LDL and VLDL, increases in T2DM. Dietary modifications do not appear to significantly impact the ApoB gene. Lipid attachment inhibits decomposition of ApoB. Insulin resistance in T2DM further causes increased lipolysis in adipocytes, which results in higher FFA release. There is greater FFA transport to the liver, which causes an increase in VLDL secretion. Thus, FA has a crucial role in ApoB levels, and insulin lack or IR enhances ApoB function. In addition, insulin regulates Apo CIII levels, which are responsible for an increase in VLDL by blocking LPL (Lipoprotein Lipase) activity and limiting lipoprotein absorption via LDL receptor related protein (LRP). Even in insulin shortage, hepatic lipase activity diminishes, which affects postprandial lipoprotein clearance.[5]

LPL is the primary enzyme responsible for the conversion of lipoprotein TG to FFA. LPL activity is reduced in T2DM. Diabetes may affect several processes in the synthesis of physiologically active LPL, including its cellular production as well as its transit to and interaction with endothelial cells. Studies have shown that insulin, a key regulator of lipoprotein lipase, causes an increase in the activity of lipoprotein lipase mRNA in adipose tissue while decreasing that in skeletal muscle upon active insulin infusion.[6] Insulin resistance increases postprandial hypertriglyceridemia in T2DM by increasing free fatty acid levels, increased VLDL from the liver, and decreased triglyceride clearance. These lipid abnormalities are all very atherogenic and influence cardiovascular disease (CVD). With associated risk factors like smoking, dyslipidaemia, and hypertension as well as metabolic abnormalities like oxidative stress, low NO generation, and chronic inflammation, diabetes patients are predisposed to high risk of CVD.[7]

The ANGPTL protein family shares structural similarities with angiopoietin proteins, thus the name. Although ANGPTL proteins may not bind to the receptor tyrosine kinase Tie2, they may still play a role in angiogenesis. The family presently includes eight known members, including ANGPTL1-8, which has pleiotropic activities like lipid metabolism, inflammation, stem cell activity, and promoting metastatic invasion of cancer cells.[8,9] ANGPTL3 is produced in the liver and circulates in the circulation. It is considered as the primary regulator of lipoprotein metabolism and an effective mediator of insulin sensitivity with a significant impact in regulating lipid and glucose metabolism.[10] The physiological evidence suggests that circulating ANGPTL3 interacts with LPL in the vasculature, limiting lipase activity and lowering clearance of TG-rich lipoprotein particles. Without ANGPTL3, LPL remains active, leading to increased clearance of VLDL and chylomicrons. Research suggests that ANGPTL3 may be a potential target for the development of therapies aimed at managing dyslipidemia and reducing the risk of CVD.[11]

ANGPTL-4 is synthesized from hepatocytes, adipose tissue, and intestine and expressed in small amounts in glomerular podocytes. Synergistic activation of lipid-sensing peroxisome proliferator-activated receptor (PPARs) α, β, and γ regulates the expression of ANGPTL-4.

In addition, angiogenesis and lipoprotein metabolism are both impacted by ANGPTL-4. It functions as a lipoprotein lipase inhibitor, limiting the breakdown of triglycerides from chylomicrons and VLDL containing apolipoprotein B, which increases nonesterified fatty acid levels.[12] Chronic low-grade inflammation in T2DM results in increased ANGPTL-4 levels.[13] Loss of function variants of ANGPTL-4 are associated with improved glucose homeostasis and lower odds ratio of type 2 diabetes mellitus compared to controls. ANGPTL4 loss-of-function mutations in humans lead to reduced plasma triglycerides. ANGPTL4 deficiency in mice leads to decreased plasma triglycerides, whereas overexpression raises triglycerides.[13]

Though ANGPTLs are now widely considered as therapy targets, the evidence for their function in lipid metabolism and T2DM pathophysiology remains mixed. With this backdrop, the purpose of this study was to determine the levels of ANGPTL 3, 4 in T2DM and better understand their relationship in causing dyslipidaemia in T2DM.

Materials and Methods

Study design and duration

This was a hospital-based case–control study done in the Department of Biochemistry in collaboration with the Department of Endocrinology at Kalinga Institute of Medical Sciences for a period from March 2021 to Jan 2023.

Prior approval from the institutional research committee and ethical committee was taken (KIIT/KIMS/IEC/407/2020) and progressed by taking written informed consent including in local language from each study participant.

Study population

Sixty-one T2DM patients of age group 25–65 years and 27 healthy

age matched control participants were enrolled for the study purpose. ADA criteria-2019 were used to diagnose T2DM cases.[14]

Clinical examination and biochemical analysis

Thorough history was taken and detailed relevant physical examination and laboratory investigation was done in all subjects as par the proforma. Anthropometric measurements and demographic characteristics were noted, and BMI was calculated. Three measurements of SBP and DBP were done, and the mean was taken in participants in a stable and sitting position.

Under all aseptic measures, 5 ml of fasting venous blood sample was collected in fluoride vial for FBS, EDTA vial for HbA1c, and in plain vacutainer for analysis of serum cholesterol, triglycerides, LDL, HDL, ANGPTL-3, ANGPTL-4, insulin, and FFA. Two hours post, prandial sample was collected in fluoride vial for PPBS estimation. The aliquots containing plasma, and serum were labelled properly and were stored at -80°C, when and if estimations were not done within 8 hours of collection.

Routine biochemical parameters were analysed in autoanalyzer (Vitros 5600 by OCD) in Biochemistry section of central laboratory. HbA1C was measured by HPLC (BioRad).

Serum insulin was measured by direct solid phase enzyme immunoassay using Insulin ELISA kit [Lablisa Human INS by Labreon, LAB4963, 96T]. Insulin resistance was calculated by the using

HOMA – IR = Fasting Insulin (microU/L)×FBS (nmol/L) ÷ 22.5.

Biocodon Sandwich ELISA kit was used to measure the concentrations of human ANGPTL-3 and ANGPTL-4 and FFA[Catalog #BC-EH100244, 96 Wells, RUO, #BC-EH100245, 96 Wells, RUO and Catalog #BC-EH101416, 96 Wells, RUO, respectively].

ELISA was done in ELISA reader by BioRad.

Statistical analysis

The Kolmogorov–Smirnov test was used for normality. Continuous variables, following Gaussian distribution, were expressed as Mean ± Standard Deviation and were compared by Student *t*-test (two groups) or one-way ANOVA (more than two groups). Continuous variables, which were not following normal distribution, were expressed as Median (inter-quartile range) and were compared by Mann–Whitney U test (two groups) or Kruskal–Wallis test (more than two groups) and correlated by Spearman Rank correlation. Categorical variables were compared by the chi Square test. All the statistical analyses were carried out by IBM SPSS 25.0. *P* value lesser than 0.05 was taken as statistically significant value.

Results

Demographic and anthropometric characteristics of the subjects who were included in the study are shown in Table 1a. In our study group, the cases and controls were age-matched. SBP was significantly elevated in T2DM patients. There was no significant difference in BMI among T2DM and control groups [Table 1b]; FBS, PPBS, and HbA1C were significantly increased in T2DM. While comparing the serum lipid profile among the study population, serum cholesterol was found to be significantly increased among T2DM cases compared to the healthy controls ($P > 0.001$). Significant difference was also seen in TG levels among the study groups. LDL and cholesterol/HDL ratio were also significantly increased among T2DM compared to the healthy controls ($P = 0.002$ and $P = 0.002$, respectively). FFA was increased in T2DM compared to healthy controls [Table 1c]. ANGPTL3 ($P = 0.007$) and

Table 1a: Comparison of demographic and anthropometric parameters between T2DM and healthy participants

Parameters	T2DM (61)	Healthy (27)	*P*
Age	58 (48-65.5)	60 (54-64)	0.426
Height (m)	1.70 (1.59-1.755)	1.58 (1.56-1.76)	0.022*
Weight (Kg)	72.62±9.02	66.96±9.01	0.009*
BMI (kg/m^2)	26.04±2.46	25.42±3.47	0.350
HR	88 (82.5-92)	78 (75-87)	<0.001**
SBP	140 (130-147)	125 (120-136)	0.002*
DBP	85 (80-90)	83 (80-90)	0.455

*Correlation is significant at the level of <0.05. **Correlation is significant at the level of <0.001. BMI: Body Mass Index, HR: Heart Rate, SBP: Systolic Blood Pressure, DBP: Diastolic Blood Pressure

Table 1b: Comparison of biochemical parameters between T2DM and healthy group

Parameters	T2DM (61)	Healthy (27)	*P*
FBS (mg/dl)	131 (109.5-157.5)	90 (85-93)	<0.001**
PPBS (mg/dl)	211 (185-277)	127 (114-135)	<0.001**
HbA1c	7.2 (6.55-8.75)	5.8 (5.5-6.0)	<0.001**
T Chol (mg/dl)	194 (155.72-240.23)	142 (119.92-185)	<0.001**
TG (mg/dl)	183.60 (118.87-252.60)	130 (95-203)	0.05*
HDL (mg/dl)	42.34 (37-49.05)	45.74 (41.00-54.62)	0.215
LDL (mg/dl)	104.33 (69.50-140.64)	64.50 (43.47-103.13)	0.002*
VLDL (mg/dl)	30.72 (23.77-46.22)	26 (19-40.6)	0.097
TC/HDL	4.39 (3.38-5.55)	3.29 (2.21-4.23)	0.002*

*Correlation is significant at the level of <0.05. **Correlation is significant at the level of <0.001. FBS: Fasting Blood Sugar, PPBS: Post Prandial Blood Sugar, T Chol: Total Cholesterol, TG: Triglycerides, HDL: High Density Lipoproteins; LDL: Low Density Lipoproteins, VLDL: Very Low Density Lipoproteins, TD/HDL: Total Cholesterol and HDL Ratio

Table 1c: Comparison of special parameters between T2DM and healthy group

Parameters	T2DM (61)	Healthy (27)	*P*
ANGPTL4	65.886 (55.546-97.846)	98.74 (70.6363-427.4288)	0.002
ANGPTL3	954.36 (833.62-1147.74)	1300 (1027.1777-3690.428)	0.004
FFA	565.49 (485.87-647.81)	625.6 (500.08-2007.851)	0.050
Insulin	11.59 (5.28-22.30)	10.545 (7.31-16.9935)	0.582
IR	4.29 (1.80-7.76)	2.317 (1.624-3.734)	0.016

ANGPTL3 : Angiopoeitin like protein 3, ANGPTL4 : Angiopoeitin like protein 4, FFA: Free Fatty Acid, IR: Insulin Resistance

ANGPTL4 ($P < 0.001$) were significantly decreased in T2DM. There was increase in IR significantly in T2DM patients (4.86) (1.956–8.009) [Table 2]. When levels of these proteins were compared in T2DM patients, it showed that ANGPTL3 and ANGPTL4 were significantly decreased among overweight and obesity compared to lean-normal group. Females had decreased levels of ANGPTL3 and 4 compared to males. [Table 3] The results of the correlation coefficient between the evaluated parameters and the levels of ANGPTL4 and ANGPTL3 showed that there was a significant negative correlation between ANGPTL 4 with BMI, SBP, and HR, but no such relationship was observed with ANGPTL3. ANGPTL4 was also negatively correlated to PPBS (0.03), HbA1C ($P = 0.05$), and IR ($P = 0.04$). However, no such correlation was observed of ANGPTL 3 with glycaemic parameters. It was observed that lipid parameters were more correlated with ANGPTL 3 (LDL ($P = 0.03$) and TC/HDL ($P = 0.02$)). There was a significant relationship between ANGPTL3 and 4 with FFA ($P = 0.001$ and $P = 0.03$, respectively).

Discussion

Plasma lipoproteins in T2DM fluctuate as a result of insulin resistance and hyperglycaemia.

Table 2: ANGPTL3 and ANGPTL4 in different BMI and gender in T2DM patients

Parameters	ANGPTL3	ANGPTL4
BMI		
BMI (18.5-24.9) (*n*=23)	2315.30±1104.17	155.16±118.03
BMI (25.0-29.9 and ≥30) (*n*=38)	1235.30±987.60	105.26±98.09
P	<0.001**	<0.05*
Gender		
Males (*n*=38)	1692.29±1758.32	120.23±106.17
Females (*n*=23)	906.53±336.08	56.72±20.66
P	<0.05*	<0.05*

BMI: Body Mass Index ANGPTL3: Angiopoeitin Like Protein 3: ANGPTL4: Angiopoeitin Like Protein 4, *Correlation is significant at the level of <0.05. **Correlation is significant at the level of <0.001

Causative factors for this dyslipidaemia are linked to the effect of insulin on the formation of apoproteins in the liver, regulation of lipoprotein lipase, the actions of cholesteryl ester transfer protein (CETP), and the peripheral actions of insulin on adipose tissue and muscle.[15] However, all T2DM patients do not present with dyslipidaemia which indicates the need for understanding factors responsible for these variations. In the present study, the serum level of ANGPTL 3 and 4 in T2DM was studied to add to the prevailing research works.

ANGPTL3 and ANGPTL4 in study population

ANGPTL3 and ANGPTL4 were named *per se* because of their structural resemblance to angiopoietins. ANGPTL4 is widely expressed, with a high level of expression in adipose tissue, ANGTPL3 is nearly solely generated and released into the systemic circulation by the liver. In coordination with one another, ANGPLT3 and ANGPLT4 control lipid metabolism, primarily through inhibiting LPL in response to alterations in nutritional status.[15]

In our study, ANGPTL3 and ANGPTL4 levels were significantly decreased in T2DM compared to controls. While comparing the levels among different BMI groups, levels were lower in overweight obese group compared to the individuals with normal BMI. Because of the small sample size we have not divided overweight and obese in different groups. Our findings are in concordance with the study done by Xu A *et al.*[16] and Harada *M et al.*[17] These studies found that newly diagnosed T2DM patients have lower levels of ANGPTL3 than the control group. *Xu et al.*[16] discovered a comparable drop in ANGPTL4 levels and proposed that this decrease might be a major cause of diabetes dyslipidaemia.[16]

Controversial studies exist where they suggested that obesity has a profound effect on levels of these proteins irrespective of T2DM.[13,18] As in the study done by Cinkajzlova A *et al.,*[13] they

Table 3: Correlation among parameters in T2DM

Parameters	ρ	*P*	Parameters	ρ	*P*
ANGPTL4 & BMI	-0.61	0.005*	ANGPTL3 & BMI	0.112	0.408
ANGPTL4 & SBP	-0.369	0.005*	ANGPTL3 & SBP	-0.119	0.379
ANGPTL4 & DBP	-0.381	0.003*	ANGPTL3 & DBP	-0.125	0.356
ANGPTL4 & HR	-0.282	0.033*	ANGPTL3 & HR	-0.134	0.322
ANGPTL4 & FBS	-0.094	0.489	ANGPTL3 & FBS	-0.011	0.936
ANGPTL4 & PPBS	-0.284	0.032*	ANGPTL3 & PPBS	-0.173	0.197
ANGPTL4 & HbA1c	-0.326	0.054*	ANGPTL3 & HbA1c	0.094	0.493
ANGPTL4 & TC	-0.166	0.218	ANGPTL3 & TC	-0.162	0.230
ANGPTL4 & TG	-0.158	0.242	ANGPTL3 & TG	-0.087	0.522
ANGPTL4 & HDL	0.060	0.656	ANGPTL3 & HDL	-0.023	0.866
ANGPTL4 & LDL	-0.081	0.548	ANGPTL3 & LDL	-0.32	0.034*
ANGPTL4 & VLDL	-0.178	0.186	ANGPTL3 & VLDL	-0.120	0.375
ANGPTL4 & TC/HDL	-0.226	0.091	ANGPTL3 & TC/HDL	-0.347	0.027*
ANGPTL4 & IR	0.354	0.042	ANGPTL3 & IR	0.033	0.808
ANGPTL4 & FFA	0.283	0.033*	ANGPTL3 & FFA	0.547	<0.001**

ANGPTL3: Angiopoeitin Like Protein 3; ANGPTL4: Angiopoeitin Like Protein 4. BMI: Body Mass Index, SBP: Systolic Blood Pressure, DBP: Diastolic Blood Pressure. HR: Heart Rate, FBS: Fasting Blood Sugar, PPBS: Post Prandial Blood Sugar, T Chol: Total Cholesterol, TG: Triglycerides, HDL: High Density Lipoproteins, LDL: Low Density Lipoproteins, VLDL: Very Low Density Lipoproteins, TD/HDL: Total Cholesterol and HDL Ratio. *Correlation is significant at the level of <0.05. **Correlation is significant at the level of <0.001

observed increased ANGPTL4 in obese with or without T2DM, while ANGPTL3 levels exhibited an inverse trend. Although, in another investigation, ANGPTL3 showed a comparable rise in obesity.[13,19] Few studies have mentioned higher levels of ANGPTL3 in young and obese individuals compared to lean fasting ones and was positively correlated with BMI.[20,21] Studies have shown statistically significant weak correlation between ANGPTL3 and BMI. Even by multiple regression analysis, they have shown BMI as an independent predictor of ANGPTL4 levels.[22] Because of the small sample size, we were not able to perform regression analysis and studied the relationship via correlation. Our study also implicated a significant relationship of ANGPTL4 with BMI. However, no such correlation was seen with ANGPTL3. Hence, it shows that these proteins are influenced by metabolic disturbances associated with obesity.

In the present study, females exhibited significantly lower levels of ANGPTL 3 and 4 compared to males. Morinaga *et al.*[23] in their work found that men had larger levels of ANGPTL 4, whereas females had higher levels of ANGPTL3. The study has also implicated that ANGPTL3 levels have increased in nondiabetic females and nondiabetic obese men which is not in conjunction with our study but in accordance with our study they also found an increase in these proteins in T2DM.[23]

Relationship of ANGPTL 3, 4 with the glycaemic status of the study participants

ANGPTL4 levels were negatively correlated with FBS and PPBS in T2DM. ANGPTL4 was likewise displayed to be negatively correlated with HbA1C; however, there was no apparent relationship between it and ANGPTL3 levels. Our study is in congruence with the study done by Xu *et al.*[16] In their study, ANGPTL4 levels were significantly lower in T2DM (243.5 ± 12.6 ng/ml) compared to healthy subjects (345.04 ± 10.83 ng/ml), and there was an inverse correlation with plasma ANGPTL4 concentrations and HOMA-IR. They contend that ANGPTL4's ability to regulate blood sugar levels may be attributed to its direct interaction with hepatocytes. There was also no apparent link identified between ANGPTL3 and glycaemic status markers (HbA1C, FBS, and HOMA-IR). Although ANGPTL4 was shown to be higher in patients with obesity and impaired glucose tolerance.[17]

Similarly, in an investigation, ANGPTL4 was observed to decrease in T2DM, but its level increased with the duration of T2DM, and no relationship between ANGPTL4 and the glycemic state in T2DM was identified.[24] Few other studies have found a substantial rise in the level of ANGPTL3 in T2DM, which correlates positively with HOMA-IR.[25,26] Hence, an accurate depiction of the involvement of ANGPTL 3, 4 and their effects on glucose metabolism and insulin sensitivity remains elusive. Obesity, duration of T2DM, and the presence of other metabolic diseases may have a significant impact on ANGPTL 3 and 4 levels. The observed gap might be due to differences in the metabolic features of people included in various studies.

Relationship of ANGPTL3, 4 with dyslipidaemia in the study participants

Serum cholesterol, LDLc, and the TC/HDL ratio were all considerably higher in T2DM patients, whereas HDLc and FFA were significantly lower. A significant negative relationship was observed among ANGPTL3 and LDLc, and total cholesterol/HDL in the study's population. Both ANGPTL3 and ANGPTL4 showed a significant positive relationship with FFA ($P < 0.001$). Various population-based studies have demonstrated that loss of function of either ANGPTL 3,4 is related to a decrease in plasma triglycerides, indicating a lower risk for CVD. Loss of function mutation in ANGPTL3 was found to decrease in TG, LDLc, and HDLc, but still no positive correlation between altered lipid metabolism and ANGPTL3 levels was found.[16] Harada *et al.*[17] showed a positive correlation with HDL c levels attributed to inhibition of endothelial lipase. ANGPTL4 was negatively correlated with HDLc levels. A study also found that ANGPTL4 levels were considerably lower in T2DM patients with dyslipidaemia, although there was no significant link between ANGPTL4 levels and serum TG or TC, but a positive correlation was seen between ANGPTL4 and TG in T2DM patients with metabolic syndrome.[24] This depicts that studies on angiopoietin-like proteins have demonstrated inconsistent results in insulin-resistant conditions such as obesity and T2DM. The relationship between ANGPTL3 and 4 is an important regulator of TG trafficking during feeding and fasting. They primarily function as LPL and EL inhibitors. These are extracellular enzymes that hydrolyses the TG transported by chylomicrons and VLDL, allowing for cellular replenishment of FFA and blood TG clearance. ANGPTL3 primarily operates in response to feeding by transferring energy to storage or oxidative tissues. ANGPTL4 is mostly increased during fasting and works as an LPL inhibitor, transforming active LPL homodimers into inactive monomers. Aside from lipid metabolism, ANGPTLs have a variety of actions, including the development of insulin resistance.[27] Vupanorsen, an antisense oligonucleotide of ANGPTL3, was administered in a phase III study in T2DM, and preliminary findings indicated no significant change in HOMA-IR. Only a reduction in circulating TGs and non-HDL cholesterol was seen.[27] Various studies tying it to T2DM also has exhibited varied findings. These contradictory and disparate findings relating T2DM and lipid status show that the amounts of these proteins are influenced by energy intake and prior metabolic states. This was further validated by investigating the amounts of these proteins in severe nutritional conditions. Hence, no standard has been internationally certified till now. Thus, more research is required to determine the repeatability of these findings.

Our study had several limitations. Maximum patients with dyslipidaemia were under treatment and medication history (hypolipidemic) along with their effect was not investigated. The duration of T2DM and diabetic treatment history was not taken into consideration during analysis. The sample size was too small to do a regression analysis hence results

are based upon correlation only. Based on the current findings, a population-based investigation is planned to assist establish the results further.

Conclusion

In our study, levels of ANGPTL 3 and 4 were considerably reduced and linked with glycemic and lipid parameters in T2DM. The current study adds to the prior findings indicating ANGPTL3 and 4 have a role to play in dyslipidaemia in T2DM and requires future investigations to establish their relevance by taking into account various criteria, including individual's nutritional status, metabolic profile, and methods for testing ANGPTL 3 4, to identify the pathogenic importance and therapeutic potential of these proteins in T2DM patients.

Institutional review board statement

The study procedures followed in the study were conducted in agreement with the Helsinki-II Declaration and the protocol was approved by the Institutional Research and Ethical Committee of Kalinga Institute of Medical Sciences (Permission No. KIIT/KIMS/IEC/407/2020). Written informed consent was obtained from recruited patients.

CRediT author statement

Rik Swarnakar; Investigation, Resources, Data Curation, Software, Methodology. Debadyuti Sahu; Formal analysis, Software, Data Curation, Review and Editing original draft. Jyotirmayee Bahinipati; Conceptualization, Methodology, Validation, Writing original draft, Review and Editing, visualization, Project administration. Tapaswini Pradhan; Supervision, Project Administration. Dayanidhi Meher; Methodology, Project administration. Rajlaxmi Sarangi; Methodology, Visualization. Srikrushna Mahapatra; Conceptualization, Validation, Supervision.

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Conflicts of interest

There are no conflicts of interest.

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