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STUDY THE RELATIONS BETWEEN ANGIOPOIETIN-LIKE PROTEIN 4 (ANGPTL4) AND LIPOPROTEIN LIPASE AS PREDICTORS FOR ISCHEMIC HEART DISEASE IN PATIENTS WITH T2DM

Hend Ahmed ABASS[1]

Al- Nahrain University, Iraq

Mustafa Ridha SHIHAN[2]

Ahl Al Bayt University, Iraq

Zahraa Sabbar OMRAN[3]

Karbala University, Iraq

Abstract

Betatrophin production and blood lipid profile have been proven in previous research to have a correlation, both in the laboratory and in clinical settings. Investigations on the connection and contact behavior of lipoprotein lipase, betatrophin, and angiogenin- likeprotein3 (ANGPTL3) continue to vary from one another (LPL). This study aimed to evaluating the relationship between betatrophin, ANGPTL4, and LPL in addition to the severity of IHD in individuals with diabetes. There were sixty people with diabetes in the case group (30 with IHD and 30 without IHD). The control group included thirty people in total. While the serum concentration of ANGPTL4 was assessed using a double antibody sandwich ELISA, the serum concentrations of betatrophin, LPL, and ANGPTL4 were assessed using the enzyme-linked immunosorbent assay (ELISA). The levels of total cholesterol, fasting blood glucose, and triglycerides in each group were determined using an automated biochemical analyzer. The clinical baseline data for each patient group was incorporated into the analysis within the same time frame. Consequently, compared to diabetic patients without IHD and those in the control group, individuals with IHD had significantly higher levels of betatrophin, ANGPTL4 and LPL (p 0.05). With diabetic patients with IHD, the Gensini rating significantly and favorably correlated with TC, TG, LDL-C, betatrophin, LPL, and BMI (r = 0.201, 0.501, 0.045, 0.562, 0.611, and 0.431 and P = 0.041, 0.008, 0.038, 0.009, 0.002, and 0.039 respectively).

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[1] hendahmedabass80@gmail.com

[2] mrsheehan89@gmail.com

[3] Zahraa.sabbar@uokerbala.edu.iq

In all groups of gensini score with (P<0.05), there is a statistically significant difference found in the levels of betatrophin, ANGPTL4, and LPL. In patients with diabetes, the degree of IHD was associated with the betatrophin, ANGPTL4, and LPL parameters**.**

Keywords: *Angiopoietin-like protein 4, Lipoprotein lipase, Diabetes mellitus, Ischemic heart disease*.

Introduction

Hyperglycemia, often known as high blood sugar, is a characteristic of the chronic illness diabetes mellitus, which is simply referred to as diabetes from here on out. The excessive quantity of urination that occurs as a result of the condition is where the term diabetes got its start. The presence of sugar in a diabetic's urine is referred to as mellitus, which means "sweetened by honey" in Latin. The ancient Egyptians made use of its pleasant flavor as a diagnostic tool even back in their day. Thomas Willis noticed in 1674 that his diabetic patients' urine had a sweet taste. A century later, Matthew Dobson proved that blood glucose levels both preceded and followed the phenomenon of sweetness, with sugar being the cause. Dipstick tests are a frequent method for detecting the absorption of sugar into the urine. having undergone the fact that diabetes is characterized by elevated blood sugar levels, these tests are renowned for their simplicity and speed (1).

Since the turn of the twenty-first century, cardiovascular disease (CVD) has become the leading cause of death. One type of cardiovascular disease that affects 126 million people worldwide—roughly 1.72 percent of the world's population—is ischemic heart disease (IHD). IHD is also the primary cause of mortality in the population, and its prevalence has been steadily growing over the course of the last decade along with the fast aging of the population (2). Therefore, the focus that should be made on controlling the incidence of IHD events should be centered on screening persons who are at a high risk and implementing appropriate preventive measures (3). IHD is sometimes used interchangeably with coronary arteries, which are responsible for vascular blockage. Atherosclerotic plaque development and propagation characterize the degenerative process known as coronary artery disease (CAD) (4). The degree of myocardial ischemia that these plaques can cause varies, and this lowers the quantity of coronary blood flow that reaches the myocardium. Acute coronary syndrome (ACS), which occurs when there is an immediate destabilization of the plaque, and chronic coronary syndrome (CCS) are two of the clinical manifestations of IHD that may be associated with CAD (5). However, coronary artery disease is not the only factor that may contribute to myocardial ischemia (6). In point of fact, coronary angiography has shown that obstructive coronary artery abnormalities were not discovered in up to 70 percent of patients who have been diagnosed with angina and proven myocardial ischemia (7). Ischemia in non-obstructive coronary arteries (INOCA) is characterized by a mismatch between the myocardium's metabolic demand and the blood supply supplied by the coronary arteries, absent severe coronary blockage (8).

Angiopoietin-like proteins, also known as ANGPTLs, are a family of proteins that are secreted, and ANGPTL4 is a member of this family. It is proposed that they will inhibit the activity of the lipoprotein lipase (LPL) enzyme in a working condition in order to preserve metabolic homeostasis. This will result in an increase in the stockpile of fats as a fuel source since different tissues, such as the body is in afasted state. In addition, maintenance of metabolic balance during meals results in increased fat accumulation in adipose tissue (9). The interaction between ANGPTL3 and ANGPTL4 and ANGPTL8, which results in

hypertriglyceridemia, is responsible for regulating the regulation of LPL functioning by these two proteins. Aside from their function in cell function, human genetic investigations have shown that mutations that inactivate ANGPTL3, 4, and 8 are associated with a lower risk of hypertension and CAD (10). In addition, it is possible to stop the formation of atherosclerosis and slow its progression in mice by eliminating one or more of these ANGPTLs (11). They are linked to obesity, dyslipidemia, and insulin resistance, with increasing the risk of cardiovascular disease and atherosclerosis (12). Additionally, anti-ANGPTL4 supply fresh possibilities for therapeutic intervention in the treatment of lipids, obesity, atherosclerotic, and CAD, but their safety has to be further investigated before they can be used successfully in a clinical environment (13).

MATERIALS AND METHODS

Sample collection and preparation

This research is a case-controlled trial that includes a total of ninety samples, including sixty diabetic patients (30 with IHD and 30 without it) and thirty volunteers serving as a control group. During the time period of November 2021 through March 2022, samples of the patient were obtained from the department of Cardiology at AL-Hussain medical city in Karbala. Each patient underwent a clinical examination, and a doctor with expertise in diabetes diagnosed both clinically and analytically for each patient. Each participant is given the opportunity to answer questions about their age and their past experiences with diabetes. The individuals and the team serving as a control are both given information on the research. As the study's comparison group, we chose thirty participants who seemed to be in good health. The age ranges that they covered were similar to those of the patients. Each of these participants had a diagnosed case of diabetes.

The veins of both the controls and the patients were punctured in order to withdraw five milliliters of blood apiece. After the development of a clot, the plasma was separatedinto two pipes: one contained EDTA, which was used for hematological and moleculartesting, the other included serum, which was used for biochemical analysis, and thethird tube was allowed to sit at room temp for ten minutes. Using a macro-centrifuge,the plasma was spun at a speed of 3000 rotations per minute for 5 minutes. A serumwith a quantity of 2.5 ml that was yellow in color was isolated. The venom was utilizedfor the testing right away, and if it wasn't needed right away, it was stored frozen untilthe proper time to finish the remaining experiments. Enzyme linking ımmunosorbent assay (ELISA) testing kits were employed in order to determine the concentrations ofANGPTL4 and LPL found in the serum samples of patients as well as healthyvolunteers.

Statistical Analysis

The data analysis was performed using the SPSS application (version 25). The modifications and differences were considered significant when the likelihood (P) value was less than 0.05 (P0.05). When the likelihood value was less than 0.001 (P0.001), the changes

and differences were considered to be extremely significant. The mean and standard deviation were used to summarize the results, and an independent t-test was run to assess any differences between the patient and control groups. The relationships between the different subgroups of the measured parameters were assessed using the chi-square and ANOVA tests. To provide an evaluation of the degree of relationship between parameters, the Pearson's correlation coefficients (r) were calculated.

RESULTS

Distribution of Participated Patients

In this particular research (Figure.1), there were a total of 90 patients, 60 of the patients have diabetic, 30 of whom had IHD and the other 30 of whom did not and the last 30 of whom served as controls.

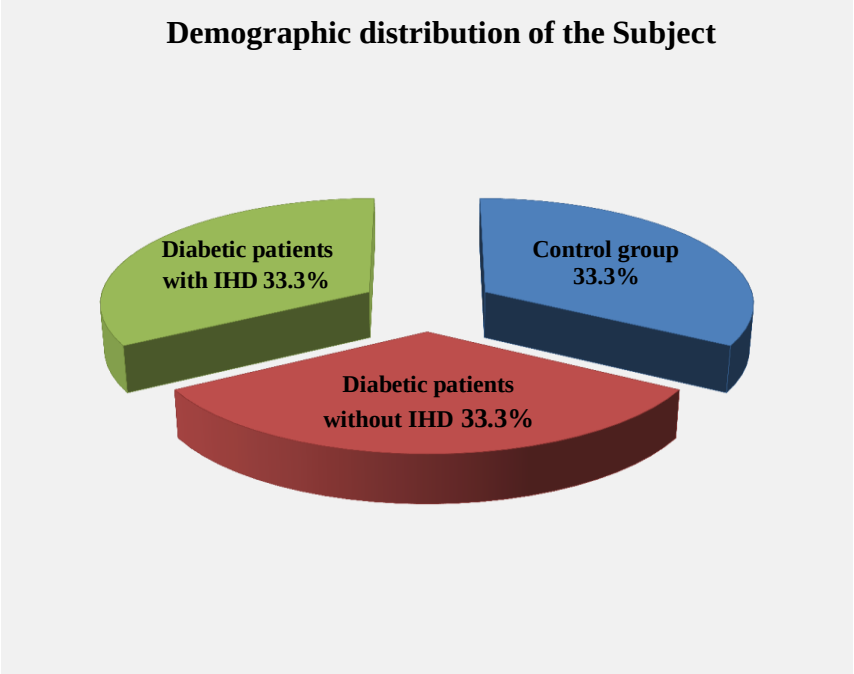

Table.1 Analyzing the clinical and biochemical characteristics of people with diabetes

Subjects	DM with IHD Mean ±SD	DM without IHD Mean ±SD	Control group (N=30) Mean ±SD	P-Value
Age (year)	54.33 ± 11.44	56.14 ± 10.15	55.73 ± 9.83	>0.05
Duration of diabetes (year)	9.11 ± 7.34	7.78 ± 5.13	**0**	>0.05
Systolic BP (mmHg)	137.46± 14.01	128.65± 13.8	104.03± 8.11	<0.05
Diastolic BP (mmHg)	95.36± 8.11	81.73± 31	70.71± 3.12	<0.05
Fasting plasma glucose (mg/dl)	138.13± 21.88	120.92± 21.12	83.17± 22.23	<0.05
Postprandial plasma glucose (mg/dl)	186.24± 11.43	174.78± 20.15	121.63± 4.83	<0.05
HbA1c (%)	13.5± 2.8	12.02± 2.21	6.9± 1.11	<0.05
Total cholesterol (mg/dl)	241.08± 25.98	210.96± 25.78	160.32± 0.92	<0.05
Triglyceride (mg/dl)	198.21± 17.14	178.88± 12.74	120.51± 10.62	<0.05
HDL- cholesterol (mg/dl)	39.22± 12.18	43.99± 10.9	58.82± 16.31	<0.05
LDL-cholesterol(mg/dl)	150.72± 20.21	142.60± 22.25	100.73± 10.7	<0.05
Serum creatinine (mg/dl)	1.46± 0.71	0.99± 0.12	0.4± 0.2	<0.05
Serum urea (mg/dl)	57.09± 15.24	45.77± 4.12	23.11± 2.61	<0.05
Microalbuminuria (mg/day)	221.18± 35.14	201.53± 6.88	10.14± 2.41	<0.05

Accepted is statistically significant. No. = Number of Persons if P<0.05. Regarding the levels of betatrophin, ANGPTL3, and LPL, there was a statistically significant difference (P 0.05) between the sick group and the normal control group. The result showed in this study is accepted with another previous study which that showed a statistically significant difference (P 0.05) between the not healthy group and the normal (healthy) controls group.

Table.2 Concentrations of the indicators in each of the different study groups and compared them using the Gensini score

Variables	Mean ±SD			
	Patients IHD (N=30)	Patients with (N=30)	Healthy group (N=30)	P.Value
ANGPTL4 (ng/mL)	27.54± 7.31	22.44± 3.11	9.21± 4.21	<0.05
LPL (ng/mL)	58.41± 6.32	43.21± 3.31	10.11± 4.11	<0.05
Betatrophin (pg/mL)	358.21± 4.54	232.21± 7.89	60.73± 1.54	<0.05

Blood sugar levels of ANGPTL3, LPL and betatrophin were statistically significantly higher in individuals who had severe coronary artery disease compared to those who had light to low and mild coronary artery disease (P 0.05). the three parameters were used in the table 2 light differentiation between them and showed acceptance with another previous study.

Table.3 The different research groups' levels analyzed using the Gensini Score

Groups	Mean ±SD			
	ANGPTL3 (ng/mL)	LPL (ng/mL)	Betatrophi (pg/mL)	P.Value
Gensini ≤25	10.33± 5.81	13.74 ± 8.81	63.20± 9.61	<0.05
Gensini 25-50	20.42± 4.87	41.76± 1.11	240.71± 8.31	<0.05
Gensini ≥50	29.94± 9.53	61.65± 8.19	396.70± 5.14	<0.05

In diabetic patients who were in the ischemic heart disease (IHD) group, there was a significant positive correlation (r = 0.201, 0.501, 0.45, 0.562 and 0.611, respectively) and (P = 0.041, 0.008, 0.038, 0.009, 0.002 and 0.039 respectively) between the Gensini score and the levels of total cholesterol, total fat, low-density lipoprotein cholesterol (LDL-C), betatrophin, and low-density lipoprotein (LPL). These results were accepted by another previous study that showed the same deference between the parameters used to diagnose the cause (14).

Table.4 Correlation between atherosclerotic risk variables

Parameters	TG (mmol/L)	TC (mmol/L)	Betatrophin (pg/ml)	LPL (ng/ml)	LDL (mmol/L)	BMI
r	0.501	0.201	0.611	0.562	0.045	0.431
P.Value	0.008	0.041	0.002	0.009	0.038	0.039

DISCUSSION

Although there is strong genetic evidence that ANGPTL4 affects risk, published clinical research has demonstrated that there are wide variations in ANGPTL4 levels in people with coronary artery disease (CAD). Research on animals and humans has shown us that ANGPTL4 may be impacted by a variety of conditions, including altered lipid statuses, feedback loops, and metabolism. Additionally, other members of the family of proteins, such as ANGPTL3 and ANGPTL8, are required for the role, making it difficult to detect changes in their levels in the serum. These factors include: the metabolism, altered lipid profiles, and feedback loops (15). Our research does have several drawbacks, the first of which is that the results may not be generalizable to the full population since there were only a limited number of participants. A further limitation of our research is that it is a cross-sectional study, which prevents us from having full and complete control over all of the possible confounding variables that are not yet known.

The current study's findings are noteworthy enough to require citation. More large-scale research analyzing the connection between iron overload and ANGPTL4 is needed to confirm the importance of our findings. To confirm the relationship between SUA and ANGPTL4, it would be most beneficial to conduct a follow-up study on patients receiving uric acid-lowering medication and having higher levels of SUA. Meanwhile, more mechanistic studies will eventually need to be carried out in order to confirm the cause-and-effect relationships between SUA and ANGPTL4. The results of this study suggest that the degree of coronary heart disease (CHD) may be related to the betatrophin, ANGPTL4, LPL pathway. Furthermore, the results showed that LPL and ANGPTL3 levels increased in people with CHD diagnoses, and the rising trends observed for both of these markers were consistent with the findings reported in (16). The functional difference between members of the ANGPTL family is attributed to the variation between their C-terminal and N-terminal domains, as demonstrated by the results of previous studies. The lack of a fibrinogen-like domain at the C terminus of betatrophin led to its identification as a novel member of the ANGPTL family, deviating from the typical composition of the family.

Nevertheless betatrophin and ANGPTL4 are closely related, and ANGPTL4 can indirectly decrease the effect of ANGTPL4 on LPL by stimulating the cleavage of ANGPTL3. Using ApoE (-/-) mice, some researchers were able to show that betatrophin is significantly produced

during the course of atherosclerosis-induced artery hardening and narrowing. Previously, it was believed that betatrophin functions as a regulator and plays a major role in metabolic diseases (17). They found that betatrophin encourages the conjunction of ANGPTL4 and LPL, and they confirmed their findings (18). The aforementioned research has shown that betatrophin and ANGPTL4 have a complementary role in the control of lipids and together they play an important part in the process. 201 CAD patients participated in an 8-year follow-up study as part of traditional clinical research conducted by others; the results showed that elevated levels of betatrophin had predictive value for the incidence of cardiovascular events (19). Moreover, a study discovered a correlation between elevated blood levels of betatrophin in patients with coronary heart disease and endothelial dysfunction, total cholesterol, and body mass index (BMI) (20). Furthermore, we discovered that the amount of betatrophin had a positive correlation with the degree to which coronary artery stenosis had developed. The blood levels of LPL and ANGPTL4, on the other hand, showed a constant rise, which was a significant departure from the findings of earlier investigations.

Conclusion

A positive link may be shown between TC, TG, LDL, LPL, Betatrophin, and BMI in individuals with IHD who are diabetic, as determined by the Gensini score. The severity of IHD in diabetic individuals was connected to the betatrophin, ANGPTL4, LPL pathways. The Gensini rating had a substantial and favorable connection with total cholesterol (TC), triglycerides (TG), LDL-C, betatrophin, LPL, and BMI in diabetic patients with IHD as shown in this study. The Betatrophin, ANGPTL4, and LPL levels give statistically significant differences in all groups of gensini score with ($P< 0.05$). The betatrophin, ANGPTL4, LPL parameters were linked to the severity of IHD in Diabetic patients.

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