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LIPID PROFILE VARIABLES AND PREDICTION OF THE SEVERITY OF CORONARY ARTERY DISEASE IN TUNISIAN TYPE 2 DIABETIC PATIENTS

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ABSTRACT: DM is characterized by insulin resistance and dyslipidemia, in particular, high levels of total cholesterol, triglycerides and LDL, and low levels of HDL-C, wich confer increased risk for CAD. Our study aimed to evaluate lipid profile in type 2 diabetic (T2DM) patients with coronary artery disease (CAD) in Tunisian population. Plasma levels of lipid profile variables were determined in 148 CAD patients with T2DM and in 159 patients with only CAD. Severity of CAD was quantified according to the prevalence of multivessel disease and the degree of coronary stenosis. Our results didn't show any significant differences between diabetic and non diabetic patients in lipid profile variables. We didn't found significant differences between the number of vessel disease and the extent of coronary stenosis, and lipid variables levels potentially reflecting the absence of a relationship between lipid profile and the severity of CAD. Using the receiver operating characteristic (ROC) curve that included all these variables, TG was found to be the single most powerful predictor of the severity of coronary artery disease in T2DM.

INTRODUCTION: Coronary artery disease (CAD) is one of the main causes of mortality and morbidity in Tunisia. Predispositions of patients to cardiovascular risk factors stimulate the progression of main and conditional CAD risk factors that cause CAD. Diabetes mellitus (DM), a well known risk factor of atherosclerosis, is responsible for a three to four fold increase in cardiovascular events.

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It is known that hyperglycemia stimulates the production of advanced glycosylated end products, activates protein kinase C, and enhances the polyol pathway leading to increased superoxide anion formation [1]. Dyslipoproteinemia with high levels of total cholesterol, triglycerides and LDL and low levels of HDL and family history of early CAD have been demonstrated to be predisposing factors of early CAD [2]. Increased TG and decreased HDL-c are characteristic dyslipidemia associated with the metabolic syndrome and T2DM [3].

Recent studies have suggested that determination of HDL function may be more informative than its concentration in predicting its protective role in coronary artery disease [1].

With regard to CVD, previous study showed that TG did not provide meaningful information about CAD risk when performing secondary analyses of data from the Multiple Risk Factor Intervention Trial, the Lipid Research Clinics Coronary Primary Prevention Trial, and the Lipid Research Clinics Prevalence and Mortality Follow-up Study [3]. It is fundamental to explore the features of T2DM patients with concomitant CAD that distinguishes this population from CAD patients that did not develop the disease. The objective of this work is to study the relationship between different serum lipid profiles and the severity of coronary artery disease in T2DM, carried out on a representative sample of the Tunisian population.

MATERIAL AND METHODS:

Population study: A total of 307 patients with CAD admitted to the Department of Cardiology University Hospital, Monastir were prospectively studied. T2DM was diagnosed according to the American Diabetes Association criteria. CAD was diagnosed as the presence of a luminal diameter stenosis >50% in at least one major coronary artery (left main coronary artery (LMCA), left anterior descending (LAD), left circumflex (LCx) or right coronary artery (RCA) or their major branches) by angiography. Multi-vessel CAD was defined as a disease stage in which at least two of the major coronary arteries were involved with atherosclerosis of significant severity.

Study subjects were divided into two groups: patients with CAD only (n=159) and patients with T2DM and CAD (n=148). Exclusion criteria were renal failure, malignant diseases, autoimmune disorders, and inflammatory diseases. This study was approved by the Ethics Committee of Medical University, and informed consent was obtained from all participants.

Plasma collection and storage: Total cholesterol (TC) and triglycerides (TG) and HDL- cholesterol were measured by enzymatic colorimetric methods, LDL-cholsesterol was estimated by the Friedewald equation. The assays were performed in the biochemistry laboratory of the University Hospital, Farhat Hached Sousse.

Coronary angiography and assessment of CAD severity: Coronary angiography was performed using standard techniques and all coronary angiograms were reported and reviewed by experienced cardiologists who were blinded to procedural and clinical data. In cases of disagreement, the final decision was reached by consensus.

CAD was defined as >50% luminal narrowing of at least one major epicardial vessel. We attempted to quantify the ‘‘severity of CAD’’ by ascertaining the prevalence of multivessel disease, extent of CAD [one, multi vessel disease stenosis (>50%)]. According to the number of diseased arteries, patients were categorized as having no disease, or one, multi vessel disease. Degree of coronary stenosis was classified according to previously published guidelines [4]: moderate: 50–70% stenosis and severe: >70% stenosis.

Statistical analysis: All statistical analyses were performed using SPSS (Statistical Package for the Social Sciences). Continuous data are reported as mean ± standard deviation and categorical data as percentages. All statistical tests were two-sided and P values of less than 0.05 were considered statistically significant. A receiver-operating characteristic (ROC) curve analysis was drawn to identify the best parameter for predicting CAD in T2DM. The area under the curve (AUC) value was calculated to determine accuracy of the test.

RESULTS:

Clinical Characteristics: Among 307 subjects with CAD, 148 had T2DM. The group of DM patients comprised 94 (63.5%) men at a mean age of 60.9 ± 9.4 years. Clinical characteristics and parameters of the two groups and significant differences are shown in **Table 1**. The DM group had significant higher incidence of dyslipidemia, smoking, hypertension, obesity, and a lower rate of statin drugs use than the non-DM group. In all patients, 72.6 % on artery disease involved the left anterior descending (LAD) as the culprit vessel, 50.7 % the left circumflex (LCx), 48.4% the right coronary artery (RCA). 61.1% of DM patients had multivessel disease and 66.1% had severe stenosis >70%.

TABLE 1: RISK FACTORS AND CLINICAL CHARACTERISTICS OF THE STUDY POPULATION

Characteristics	All Patients N=307	NDM N=159	DM N=148	p
Age(years)	60.3±11.0	59.7±12.4	60.9±9.4	0.377
Male, n (%)	230(74.3)	136(85.5)	94(63.5)	<0.001
BMI(Kg/m²)	27.5±4.3	27.3±4.2	27.7±4.4	0.462
Smoker (%)	88.2	61.9	38.1	0.002
Obesity (%)	22.6	20.4	24.7	0.595
Hypertension (%)	47.1	37.7	57.4	0.001
Dyslipidemia (%)	23.5	16.5	31.1	0.003
Personal history of CAD (%)	33.2	28.9	37.8	0.169
Vessel involved (%)				
LMCA	7.0	10.3	3.7	0.058
LCx	50.7	45.8	55.6	0.152
LAD	72.6	67.3	77.8	0.085
RCA	48.4	44.9	51.9	0.305
Stenose				
<50%	5.8	4.5	6.8	
[50-70] %	27.2	27.3	27.1	0.890
>70%	67.0	68.2	66.1	
Vessel disease (stenose > 70%), (%)				
One vessel	43.5	49.1	38.9	0.087
Multivessel	56.5	50.9	61.1	
SBP (mmHg)	13.1±2.2	13.0±2.2	13.2±2.2	0.510
DBP (mmHg)	7.5±1.3	7.5±1.5	7.4±1.1	0.435
Statines use (%)	62.6	52.6	47.4	0.109

DM: diabetes mellitus, **NDM:** non-diabetes mellitus**, BMI:** body mass index, **CAD:** coronary artery diseases, **LMCA:** Left main coronary artery, **LCx:** left circumflex, **LAD:** left anterior descending, **RCA:** right coronary artery, **SBP:** Systolic Blood Pressure, **DBP:** Diastolic Blood Pressure. Values are expressed as the mean ± SD. P < 0.05.

Differences in lipid profile between DM and NDM patients were evaluated. Mean TC, TG, LDL-c, HDL-c, levels between these groups did not show significant differences. Levels were similar in diabetic and no diabetic patients (**Table 2**).

TABLE 2: LIPID PROFILE OF THE STUDY POPULATION

Characteristics	All Patients N=307	NDM N=159	DM N=148	P
TC (mmol/l)	4.3±1.4	4.3±1.3	4.4±1.6	0.743
TG (mmol/l)	1.7±1.3	1.5±1.2	1.8±1.4	0.053
HDL-c (mmol/l)	1.0±0.7	1.0±0.9	1.0±0.3	0.524
LDL-c (mmol/l)	2.6±1.4	2.6±1.4	2.6±1.3	0.968

DM: diabetes mellitus, **NDM:** non-diabetes mellitus, **TC:** total cholesterol, **TG:** triglycerides, **HDL- c:** high density cholesterol**, LDL-c:** light density cholesterol. Values are expressed as the mean ± SD. P < 0.05.

Lipid variables were similar in diabetic and no diabetic patients categorized according to the number of vessel disease. Any significant differences were shown in these groups (**Table 3**).

TABLE 3: LIPID PROFILE IN DIABETIC PATIENTS ACCORDING TO THE NUMBER OF VESSEL DISEASE

Biochemical parameters	One Vessel		Multi Vessel		p
	NDM	DM	NDM	DM	
TC (mmol/l)	4.45±1.34	4.24±1.57	4.19±1.26	4.36±1.42	0.725
HDL-c (mmol/l)	0.88±0.34	1.01±0.39	0.94±0.43	0.89±0.34	0.718
LDL-c (mmol/l)	2.77±1.05	2.58±1.45	2.64±1.14	2.67±1.19	0.926
TG (mmol/l)	1.58±1.72	1.63±1.15	1.47±0.72	1.73±0.89	0.909
Mediane	1.23	1.36	1.28	1.51	

DM: diabetes mellitus, **NDM:** non-diabetes mellitus, **TC:** total cholesterol, **TG:** triglycerides, **HDL- c:** high density cholesterol**, LDL-c:** light density cholesterol. Values are expressed as the mean ± SD. P < 0.05.

The same results were shown in these groups when patients had been categorized according to the severity of stenosis, any significant differences between diabetic and non diabetic patients, and between moderate and severe stenosis (p>0.05) (**Table 4).**

TABLE 4: LIPID PROFILE IN DIABETIC AND NON-DIABETIC PATIENTS WITH SIGNIFICANT STENOSIS

Biochemical parameters	Stenosis [50-70]%		Stenosis >70 %		P
	NDM	DM	NDM	DM	
CT (mmol/l)	3.9±1.1	4.2±1.6	4.7±1.2	4.5±1.3	0.127
HDL-c (mmol/l)	1.0±0.2	1.0±0.3	1.0±0.4	0.8±0.3	0.104
LDL-c (mmol/l)	2.2±1.0	2.6±1.1	3.0±1.0	2.9±1.1	0.048
TG (mmol/l)	1.5±1.0	1.7±1.0	1.3±0.6	1.8±1.1	0.821

DM: diabetes mellitus, **NDM:** non-diabetes mellitus, **TC:** total cholesterol, **TG:** triglycerides, **HDL-c:** high density lipoprotein cholesterol, **LDL-c:** low density lipoprotein cholesterol. Values are expressed as the mean ± SD. P < 0.05.

ROC Curve for ApoB/ApoA-1 ratio in predicting CAD in T2DM: The area under the ROC curve for TC, TG, LDL-c, HDL-c, in patients with T2DM was (TC: 0.515±0.039; (95% CI: 0.439, 0.591), p=0.698) ; (HDL-c: 0.519±0.039 ; (95% CI: 0.439, 0.591), p=0.698); (LDL-c: 0.478±0.039 ; (95% CI: 0.403, 0.554), p=0.573) ; (TG: 0.595±0.038; 95% CI: 0.521, 0.669; p=0.013). TC, LDL-c, HDL-c didn't display significant value, but TG can be useful in predicting the severity of CAD in patients with T2DM (**Fig. 1**).

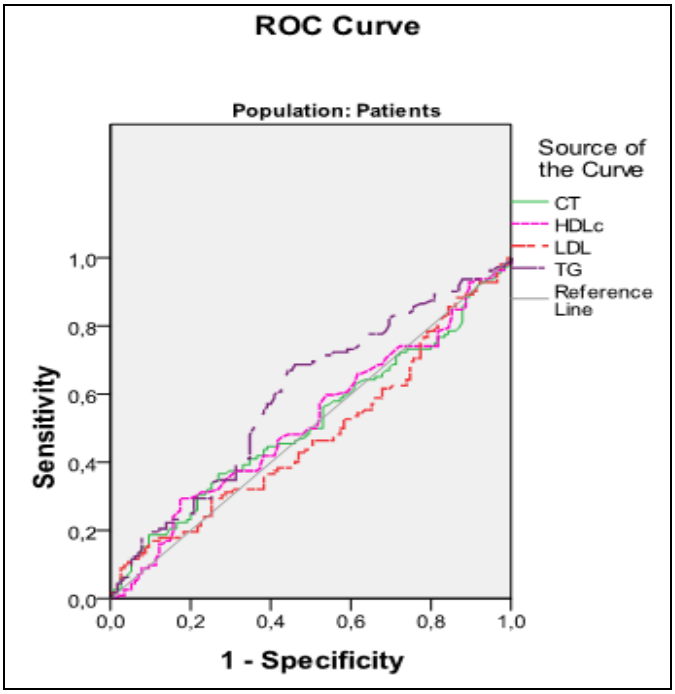

FIG. 1: RECEIVER-OPERATING CHARACTERISTIC (ROC) CURVE IN PREDICTING CORONARY ARTERY DISEASE IN PATIENTS WITH T2DM. Area under the receiver-operating characteristic curve: (TC: 0.515±0.039; (95% CI: 0.439, 0.591), p=0.698) ; (HDL-c: 0.519±0.039 ; (95% CI: 0.439, 0.591), p=0.698) ; (LDL-c: 0.478±0.039 ; (95% CI: 0.403 , 0.554) , p=0.573) ; (TG: 0.595±0.038 ; 95% CI: 0.521 , 0.669 ; p=0.013).

DISCUSSION: In this study, it was particularly evident that the lipid profile wasn't significantly higher in diabetic patients than those without diabete mellitus. In addition, our results show that the study of lipid profile seems to be no correlated with the severity of CAD as defined by the observation of vessel disease and the degree of stenosis in patients with DM. As shown in ROC curves, the fact that the serum levels of TC, LDL-c, HDL-c are not clearly predictive in T2DM in CAD patients, and the absence of significant differences in the degree of stenosis and vessel disease between diabetic and non diabetic patients, could be due to other factors as the obesity and the use of lipid lowring drugs. In contrast, the area under the receiver-operating characteristic curve of TG was the only significant value in predicting severity in CAD patients with T2DM and this can be explained by high fat diet and inactivity of the Tunisian population.

According to prior investigations, low HDL-c has emerged as an independent and modifiable cardiovascular risk factor, as demonstrated in several follow-up European and North-American countries, as well as TG in certain specific groups, suggesting that targeting the whole lipid profile may have a positive impact on cardiovascular prognosis [5]. The atherogenic link between high triglycerides and HDL-c is due to the higher plasma concentration of triglyceride-rich, very low-density lipoprotein that generates small, dense LDL during lipid exchange and lipolysis. These LDL particles accumulate in the circulation and form small, dense HDL particles, which undergo accelerated catabolism, thus closing the atherogenic circle [6].

In fact, lipid abnormalities may be the result of the unbalanced metabolic state of diabetes as hyperglycemia and insulin resistance. Improved control of hyperglycemia does moderate diabetes associated dyslipidemia, but even if ideal glycemic control is achieved, elevated cholesterol levels persist and need to be specifically treated [7]. The Copenhagen Male Study showed triglycerides on their own to be a strong risk factor, but it found that stratifying triglyceride levels by HDL-c levels led to more accurate detection of increased risk of coronary disease [6]. Sierra-Johnson and al [8] support the notion that lipoprotein abnormalities that are part of the metabolic-risk-factor clustering are significantly associated with insulin resistance and that they may provide additional mechanistic information on the complex metabolic syndrome.

Thereby, lipid profile variables are strongly associated with insulin resistance beyond the association explained by traditional risk factors, metabolic syndrome components, and inflammatory risk factors. Their findings were be explained by the fact that the basis for the metabolic-risk-factor clustering concept is that insulin resistance is the primary underlying pathophysiological disturbance that clusters along with atherogenic dyslipidaemia, elevated blood pressure, elevated plasma glucose, a prothrombotic state, and a proinflammatory state that ultimately may lead to increase cardiovascular risk [8].

Oxidation of lipoproteins involves the attack on many constituents, including cholesterol, fatty acids, antioxidants [9]. The presence of small dense LDL with persistent hyperglycemia increases susceptibility of LDL to undergo glycation. Beneficial HDL can be degenerated to a dysfunctional HDL with pro-atherosclerotic and pro-inflammatory properties using oxidation and glycation [10]. The athero-protective properties of HDL that have been an area of active research include its anti-oxidant, anti-inflammatory, and antithrombotic role. Of particular interest is the small dense HDL, which is considered to have the highest anti-oxidant and antiinflammatory potential and is the most potent promoter of cholesterol efflux [11]. HDLs increase insulin synthesis and secretion in pancreatic β cells [12]. In pre-diabetic patients, reduced HDL-c has been reported to be associated with progression to T2DM [10]. Elevated plasma LDL-c and triacylglycerol, and reduced HDL-cholesterol contribute to accelerate atherogenesis in T2DM [13]. LDLs modify the antithrombotic properties of the vascular endothelium and change vessel contractility by reducing the availability of endothelial nitric oxide and activating proinflammatory signaling pathways. LDL entering affected vessels undergo modifications including glycation and conversion to small dense form.

These modifications potentiate its atherogenic properties [14]. The formation of small dense LDL is closely associated with insulin resistance and hypertriglyceridemia. However, small dense LDL was also observed in patients with type 2 diabetes and insulin resistance with close to normal triglyceride levels. This might be explained by increased hepatic lipase activity commonly seen in patients with type 2 diabetes [15].

In the World Health Organization Study on Vascular Disease in Diabetes, TG is a significant risk factor for CAD, and this association is even stronger than the association with serum total cholesterol (TC). In another study, TG is the only significant predictor for CAD mortality in men with impaired glucose tolerance or diabetes [3]. Similar to our results, these studies suggest that TG can be a very important risk factor for CAD in either diabetic or non-diabetic subjects. TG is inversely correlated with the size and density of LDL and HDL particles, which may be the real culprit of atherosclerosis. Besides, high TG levels are also associated with increased concentrations of factor VII and plasminogen activator inhibitor, increased insulin resistance, and increased blood leukocyte counts. All of these may contribute to the atherosclerosis and thrombosis associated with high TG levels [3].

Althougt our results didn't demonsrate significant differences between diabetic and no diabetic patients in lipid profiles, it is tempting to hypothesis that abnormalities in the lipid profiles, might account for the increased risk for development of type 2 diabetes. In fact, a possible explanation that the majority of advances in the treatment of dyslipidaemia and in the primary or secondary prevention of CVDs have come from the introduction of cholesterol-lowering drugs [1].

Numerous lifestyle aspects with diet, environmental factors and genetic predisposition affect the outcome and development of atherosclerosis and thrombosis [2]. It has been shown that LDL-lowering drugs are the most effective therapy against atherothrombotic cardiovascular disease [9]. In contrast, the overall risk of cardiovascular disease cannot be explained purely on the basis of abnormal conventional lipid parameters because many times CAD occurs in patients who are normolipidemic [16].

In our study, although high levels of lipid profile variables didn't show any significant differences between diabetic and no diabetic patients, the AUC following the ROC analysis indicates that only triglycerides was the most predictive marker, to identify the severity of CAD effectively in T2DM. Nevertheless, the determination of triglycerides may be a valuable tool in the atherogen risk assessment of Tunisian diabetic patients and the severity of coronary stenosis.

There are some limitations to our study that deserve, as sample size is limited, the current study population might be unable to represent diabetic Tunisian population. Moreover, we should compare the usefulness of lipid profile variables for predicting the severity of CAD in diabetic patients without other cardiovascular risk factor as dyslipidemia, which need lipid-lowering drugs.

CONCLUSION: Nearly all routinely assessed lipid variables were not associated with the extent of coronary disease in type 2 diabetic patients, but only triglycerides levels were robustly associated with disease extent. Elevation in TG was the single most powerful predictor of extensive coronary heart disease among all the lipid variables examined in T2DM.

CONFLICT OF INTERESTS: The authors declare that they have no competing interests.

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