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Impact of Diabetic Retinopathy on Vulnerability of Atherosclerosis in Type 1 Diabetes: the Role of Lipid Ratios as a Metabolic Biomarker

Walid Hassene Hamri[1,2], Mustapha Diaf [1,*], Noria Harir[1,2]

[1] Department of Biology, Faculty of Natural and Life Sciences, Djillali Liabes University of Sidi-bel-Abbes, Algeria;[2] Laboratory of Molecular Microbiology, Proteomics and Health, Department of Biology, Faculty of Natural and Life Sciences, Djillali Liabes University of Sidi-bel-Abbes, Algeria.

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Abstract

This study aims to examine the correlation between diabetic retinopathy (DR) and early atherosclerotic changes in type 1 diabetics. We conducted a cross-sectional and observational study of 293 type 1 diabetic patients who attended the Diabetes Center from January 2015 to December 2019 in Sidi-bel-Abbès province, northwest Algeria. The patients were segregated into the following groups: patients with DR and patients without DR. Our study revealed a definite preponderance of males (52.60%). The mean age was 28.26±10.52 years, whereas the diabetes duration was 10.15±9.06 years. The overall prevalence of DR was 32.1%. TC/HDL ratio was a strong indicator for atherosclerotic disease (sensitivity of 66.0%, a specificity of 64.8%, and diagnostic accuracy of 0.653). Our results reported that the 4th quartiles (OR= 2.15 [1.08-4.27]; p=<0.02) of TC/HDL ratio was markedly greater in DR patients. Likewise, the fourth quartile of TG/HDL ratio was significantly greater in DR patients (OR= 2.06 [1.03-4.13]; p=0.03). In contrast to females who developed DR, lipid ratios and lipid profiles were significantly greater in male patients. Patients who develop DR are characterized by dyslipidemia and a higher risk of atherosclerotic disease. Thus, early detection of DR accompanied by a more careful cardiovascular assessment in these patients is crucial to prevent premature cardiovascular morbidity and mortality.

Keywords: Diabetic retinopathy, type 1 diabetes, atherosclerosis, dyslipidemia.

1. Introduction

Endothelial dysfunction and increased arterial stiffness are linked to the development of atherosclerosis in type 1 diabetes (T1D), both of which may contribute to an increase in cardiovascular (CV) mortality. (Jenkins *et al*., 2019). Cardiovascular disease (CVD) caused by atherosclerosis is considered a significant cause of morbimortality in patients with T1D. Among the risk factors linked with the development of CV risk in diabetics are age, smoking, proteinuria, glomerular dysfunction, deteriorated glycemic control, obesity, hypertension, lipid values and the duration of diabetes (de Ferranti *et al*., 2014). Even in patients who maintain acceptable glycemic control, the risk of mortality from CVD remains higher than that of non-diabetic people (Lind *et al*., 2014). Patients with T1D usually do have higher incidences of hypertension and hypercholesterolemia compared with the overall population (Livingstone *et al*., 2012). Thus, the underlying pathways following the escalated risk of mortality from CVD among patients with T1D who have good glycemic control are not well explained. Recently, it has been reported that there is a shared background for the progression of endothelial complications of T1D related to mutual pathogenic mechanisms in all types of complications (Garofolo *et al*., 2019). Diabetic retinopathy (DR) remains a prevalent and distinct microvascular complication of diabetes leading to vision impairment and blindness globally, with an estimated prevalence of 30% among people with T1D (Cheung *et al*., 2010). The association of hyperglycemia, hypertension, dyslipidemia and albuminuria with DR is well known (Long and Dagogo-Jack, 2011). Several of these risk factors of DR have also been shown to be linked with atherosclerosis (Jenkins *et al*., 2019; Long and Dagogo-Jack, 2011). Many studies have shown that DR is an independent indicator of CV accidents and all-cause of death among diabetic patients. (Kramer *et al*., 2011). Many possible mechanisms have been suggested to be implicated in the pathological link between diabetic micro- and macroangiopathy. These comprise oxidative and glycemic stress, chronic low-grade inflammation and dysfunctional endothelial restoration mechanisms (Garofolo *et al*., 2019). In the action to regulate CV risk in T1D, the severity of DR has been proven to be linked with the risk of incidental CV events in patients with T1D (Carbonell *et al*., 2018). In patients with T1D, severe types of DR have been stated to be associated with the existence of coronary artery calcification (Almeida *et al*., 2011), along with developed carotid intima-media thickness (Rema *et al*., 2004). While there is a scarcity of data on atherosclerotic plaques in T1D patients, limited evidence exists on whether the atherosclerotic disease and DR in patients with T1D are

* Corresponding author. e-mail: diafmustapha@gmail.com.

related. Therefore, the purpose of this study was to determine the relationship between the presence of DR and elevated risk of atherosclerosis in patients with T1D, whose free of any CVD, by evaluating blood lipid ratios.

2. Patients and Methods

2.1. Study design

This was a retrospective study reporting data from January 1, 2015, to December 30, 2019, on type 1 diabetics who visited the Diabetes Center in Sidi-Bel-Abbes, northwestern Algeria. Our study included 293 type 1 diabetics (52.60%) diagnosed in their pubertal period (according to the WHO guideline) who were over 13 years old during this study. All the medical records of the participants were revised for the following: status of the diabetic disease, biochemical parameters, other associated diseases and complications. Inclusion criteria were defined as: (1) type 1 diabetic patient aged between (13-65 years), (2) performed a proper full eye examination by direct ophthalmoscopy, (3) underwent routine examinations at the Diabetes Center at least one time a year, (4) free from any cardiovascular disease. On this basis, according to retinal function, patients with retinal dysfunction were included in the DR group, and those with normal retinal function were included in the without DR group. Exclusion criteria: (1) history of cardiovascular disease or taking cardiovascular-related drugs, (2) missing medical record on the disease status, (3) incomplete clinical data, (4) missing informed consent.

2.2. Study protocol

2.2.1. Anthropometric parameters

For all patients, the anthropometric measures including body height, weight, waist circumference and body mass index (BMI) were taken from the patient's medical record. BMI was calculated as weight (kg)/Height^2 (m), and the patients were classified into the following categories: underweight (BMIs of <18.5 kg/m^2), normal (18.5–24.9 kg/m^2), overweight (25–29.9 kg/m^2), and obese (BMIs ≥30 kg/m^2).

2.2.2. Blood pressure

A sphygmomanometer was used to measure blood pressure in the supine position, followed by a standing measurement (after a few minutes). A systolic blood pressure (SBP) of 140 mmHg and diastolic blood pressure (DBP) of 90 mmHg or more are considered hypertension.

2.2.3. Hematological parameters

The latest biochemical measurement including fasting blood glucose, glycated hemoglobin (HbA1c), Hemoglobin (Hb), high-sensitivity C-reactive protein (hs-CRP), urea, urinary albumin excretion rate (UAER), serum creatinine, and lipid parameters, namely high-density lipoprotein cholesterol (HDL), low-density lipoprotein cholesterol (LDL), total cholesterol (TC), and triglycerides (TG), were available on the patients' medical records.

2.2.4. Complication assessment

The presence and severity of DR were assessed by retinal photography using a wide-angle (45°) mydriatic camera (Aldington *et al.*, 1995), and the eye with the critical retinal condition including prior photocoagulation or surgical approach was used for DR staging (absent, mild, moderate, or severe non-proliferative, proliferative) according to the Global DR Project Group criteria (Wilkinson *et al.*, 2003). For additional classification, patients with a non-proliferative, mild, or moderate stage were categorized as nonadvanced DR while those with severe non-proliferative, proliferative DR, or blindness were assembled into the advanced, sight-threatening DR category. Furthermore, lipid ratios (TC/HDL, LDL/HDL, and TG/HDL) were measured as indicators of atherogenic risk.

2.3. Ethical approval

The Ethics Committee of Diabetic Center granted approval AAU-3/2019 of this research and since its retrospective research, ethical approval was acquired from the center in which the research was conducted.

2.4. Statistical analysis

Data are shown as mean ± SD with its respective 95% CI for continuous variables, whereas percentages (%) with its relative frequencies for categorical variables. The differences between groups with and without DR were analyzed by the Chi-square test for qualitative variables, and the Student *t*-test was used for quantitative variables. After adjusting for quartiles of lipid ratios, multivariate logistic regression analysis was utilized to measure odds ratios (OR) and 95% CI for lipid ratios to examine the correlation between DR and atherosclerotic disease. Receiver operator characteristics (ROC) curves were applied to identify the predictive values of lipid ratios for atherosclerosis. A *p*-value of 0.05 or less ($p \leq 0.05$) was maintained as statistically significant. SPSS software package (SPSS 22.0; IBM Corporation; Chicago, IL, USA. August 2013) was employed for statistical analyses of data.

3. Results

The baseline characteristics of the enrolled participants are summarized in Table 1. A total of 293 type 1 diabetics (52.60% males and 47.40% females) were included in this research. Purposely, participants were segregated into two groups according to the existence of DR. Of the 293 diabetics, 94 (32.10%) of them had DR. The mean age of the patients was 28.26±10.52 years, whereas the mean of diabetes duration was 10.15±9.06 years. The mean age of DR patients was statistically greater than in those without DR (34.76±11.30 years *vs.* 25.19±8.59 years, $p<10^{-3}$; respectively). Likewise, the mean duration of T1D was statistically greater among those who had DR (17.21±8.74 years *vs.* 6.82±7.10 years, $p<10^{-3}$; respectively) (Table 2). The most affected age group was the "30-39 years" (36.20%). Conversely, the least affected age group was the "≥ 60 years". In addition, smoking history was strongly correlated with the occurrence of DR (23.40%, $p=0.02$) while there were no statistical differences between the various weight classes (Table 1).

In our study, the most commonly known complications in the two groups were low visual acuity (33.80%), followed by Anemia (28.30%). However, Ketosis on diabetes (60.60%, $p=<10^{-3}$), diabetic foot (37.20%, $p=<10^{-3}$), hypertension (28.70%, $p=<10^{-3}$), diabetic nephropathy (26.60%, $p=<10^{-3}$), and dyslipidemia (6.40%, $p=10^{-3}$) were all significantly higher in DR patients (Table 1).

Table 1. Basic characteristics of the participants

Variables	All Patients, n=293 Number (%)	Without DR, n=199 Number (%)	With DR, n=94 Number (%)	*p*-value
Gender				
Male	154 (52.60)	101 (50.80)	53 (56.40)	0.369
Female	139 (47.40)	98 (49.20)	41 (43.60)	
Age groups, years				
[13-19]	68 (23.20)	62 (31.20)	6 (6.40)	
[20-29]	110 (37.60)	83 (41.70)	27 (28.70)	
[30-39]	73 (24.90)	39 (19.60)	34 (36.20)	$<10^{-3}$*
[40-49]	28 (9.60)	14 (7.00)	14 (14.90)	
[50-59]	13 (4.40)	1 (0.50)	12 (12.90)	
≥ 60	1 (0.30)	0 (0.00)	1 (1.10)	
Smoking history				
Male	48 (16.40)	26 (13.10)	22 (23.40)	0.02*
Female	--	--	--	--
Prevalence of weight categories,				
Underweight, BMI <18.5 Kg/m²	84 (28.70)	63 (31.60)	21 (22.30)	
Normal weight, BMI=18.5–24.9Kg/m²	166 (56.70)	112 (56.30)	54 (57.50)	0.06
Overweight, BMI=25.0–29.9Kg/m²	30 (10.20)	18 (9.00)	12 (12.80)	
Obesity, BMI ≥30 Kg/m²	13 (4.40)	6 (3.00)	7 (7.40)	
Other associated complications				
Low visual acuity	99 (33.80)	15 (7.50)	84 (89.40)	$<10^{-3}$*
Diabetic nephropathy	25 (8.50)	0 (0.00)	25 (26.60)	$<10^{-3}$*
Hypertension	29 (9.90)	2 (1.00)	27 (28.70)	$<10^{-3}$*
Hypothyroidism	20 (6.80)	11 (5.50)	9 (9.60)	0.20
Hyperthyroidism	4 (1.40)	2 (1.00)	2 (2.10)	0.44
Anemia	83 (28.30)	47 (23.60)	36 (38.30)	0.009*
Dyslipidemia	6 (2.00)	0 (0.00)	6 (6.40)	$<10^{-3}$*
Diabetic foot	44 (15.00)	9 (4.50)	35 (37.20)	$<10^{-3}$*
Ketosis on diabetes	79 (27.00)	22 (11.10)	57 (60.60)	$<10^{-3}$*
Symptoms and signs				
Weight loss	159 (54.30)	123 (61.80)	36 (38.30)	$<10^{-3}$*
Polyuria-Polydipsia	273 (93.20)	190 (95.50)	83 (88.30)	0.02*
Asthenia	117 (39.90)	89 (44.70)	28 (29.80)	0.07
Overeating	153 (52.20)	101 (50.70)	52 (55.30)	0.14

(*) percentages were compared with Chi-square test, p≤0.05 was considered as significant; DR: Diabetic retinopathy; BMI: body mass index.

Table 2: Comparison of clinical characteristics between patients with and without diabetic retinopathy

Variables	All Patients, n=293		Without DR , n=199		With DR , n=94		p-value
	Mean±SD	95% CI	Mean±SD	95% CI	Mean±SD	95%CI	
Mean age (years)	28.26 ± 10.52	27.05-29.47	25.19 ± 8.59	23.99-6.39	34.76 ± 11.30	32.44-37.07	$<10^{-3}$*
Diabetes duration (years)	10.15 ± 9.06	9.11-11.20	6.82 ± 7.10	5.82-7.81	17.21 ± 8.74	15.42-19.00	$<10^{-3}$*
Age at 1st diagnosis (years)	18.17 ± 8.59	17.19-19.16	18.46 ± 8.39	17.29-9.64	17.56 ± 9.02	15.72-19.41	0.40
Body height (m)	1.68 ± 0.08	1.67-1.69	1.68 ± 0.09	1.67-1.69	1.67 ± 0.08	1.66-1.69	0.67
Body weight (Kg)	58.94 ± 11.23	57.65-60.24	58.06 ±10.76	56.55-9.56	60.88 ± 12.02	58.38-63.39	0.04*
BMI (Kg/m²)	20.82 ± 3.71	20.39-21.25	20.50 ± 3.66	19.98-1.01	21.54 ± 3.75	20.76-22.32	0.02*
Waist circumference (cm)	80.66 ± 9.70	78.62-82.69	79.02 ± 9.36	76.68-1.36	84.69 ± 9.52	80.85-88.54	0.01*
SBP (mmHg)	112.8 ± 12.9	111.4-114.3	110.1 ± 11.0	108.76-111.6	118.7 ± 14.7	115.7-121.7	$<10^{-3}$*
DBP (mmHg)	66.7 ± 8.7	65.7-67.7	65.5 ± 7.7	64.4-66.6	69.4 ± 10.0	67.3-71.4	$<10^{-3}$*
Fasting plasma glucose g/L)	2.98 ± 0.92	2.88-3.09	2.87 ± 0.86	2.75-2.99	3.23 ± 1.00	3.02-3.43	0.002*
HbA1c (%)	9.63 ± 2.11	9.38-9.87	9.25 ± 1.95	8.97-9.52	10.45 ± 2.24	9.98-10.92	$<10^{-3}$*
Hb (g/L)	12.63 ± 2.01	12.37-12.90	13.01 ± 1.88	12.71-3.31	11.80 ± 2.03	11.31-12.28	$<10^{-3}$*
hs CRP (mg/L)	35.73 ± 50.18	18.99-52.46	7.99 ± 6.38	4.13-11.84	50.75 ± 57.01	26.68-74.83	0.01*
Total cholesterol (g/L)	1.57 ± 0.36	1.53-1.61	1.56 ± 0.35	1.51-1.61	1.58 ± 0.38	1.50-1.66	0.73
HDL-c (g/L)	0.42 ± 0.11	0.41-0.43	0.43 ± 0.10	0.42-0.45	0.40 ± 0.11	0.38-0.43	0.02*
LDL-c (g/L)	0.90 ± 0.28	0.86-0.93	0.90 ± 0.28	0.86-0.94	0.89 ± 0.29	0.83-0.95	0.84
Triglycerides (g/L)	0.95 ± 0.57	0.88-1.02	0.91 ± 0.52	0.84-0.99	1.03 ± 0.67	0.89-1.17	0.12
TC/HDL-c	3.88 ± 1.17	3.74-4.02	3.75 ± 1.09	3.60-3.91	4.15 ± 1.30	3.88-4.41	0.008*
LDL/HDL-c	2.25 ± 0.90	2.15-2.36	2.20 ± 0.90	2.07-2.32	2.37 ± 0.91	2.18-2.56	0.13
TG/HDL-c	2.48 ± 1.88	2.26-2.69	2.31 ± 1.71	2.07-2.55	2.82 ± 2.17	2.38-3.27	0.03*
Creatinine (mg/L)	11.65 ± 11.50	9.73-13.57	7.32 ± 1.76	6.92-7.72	16.95 ± 15.52	13.04-20.86	$<10^{-3}$*
Urea (g/L)	0.38 ± 0.33	0.32-0.44	0.23 ± 0.07	0.21-0.25	0.56 ± 0.43	0.45-0.67	$<10^{-3}$*
UAER (mg/24h)	130.69±371.75	70.92-90.47	7.71 ± 6.16	6.31-9.11	258.66±501.38	142.50-74.82	$<10^{-3}$*

(*) means were compared with independent sample Student's t-test, a p<0.05was considered as significant; DR: Diabetic retinopathy; SD: standard deviation; CI: confidence interval; HbA1c: glycosylated hemoglobin; BMI: body mass index; SBP: systolic blood pressure; DBP: diastolic blood pressure; Hb: Hemoglobin; hs-CRP: high-sensitivity C-reactive protein; TC: total cholesterol; HDL-c: high-density lipoprotein cholesterol; LDL-c: low-density lipoprotein cholesterol; TG: triglycerides; UAER: Urinary albumin excretion rate.

The clinic characteristics and laboratory indexes are described in Table 2. Regarding the anthropometric measurements on admission, significant differences were highlighted in body weight (p=0.04), waist circumference (p=<0.01) and BMI (p=<0.02), whereas there was no significant difference in body height. Furthermore, in respect to blood pressure, significant differences were revealed in diabetics who developed DR (SBP, p=$<10^{-3}$; DBP, p=$<10^{-3}$) (Table 2).

Remarkably, fasting plasma glucose and HbA1c levels were statistically increased in DR patients (p=<0.002, p=$<10^{-3}$; respectively). A significant difference was maintained in terms of hemoglobin (p=$<10^{-3}$). Concerning renal function, patients who developed DR had higher plasma values of creatinine and urea and microalbuminuria (16.95±15.52 mg/L, p=$<10^{-3}$; 0.56±0.43 g/L, p=$<10^{-3}$; and 258.66±501.38 mg/24h, p=$<10^{-3}$; respectively).

Interestingly, hs-CRP was significantly increased in DR patients (patients with DR: 50.75±57.01 mg/L *vs.* patients without DR: 7.99±6.38 mg/L, p=0.01; respectively).

In terms of lipid values, as described in Table 2, HDL-c values statistically differed among the two groups (p=<0.02). Moreover, it was revealed that lipid ratios (TC/HDL-c and TG/HDL-c) were statistically higher in the DR group (p=0.008, p=0.008).

The multivariate regression was utilized to identify independent determinants of atherosclerosis according to lipid ratios quartiles. The results showed that the 4th quartile (OR= 2.15 [1.08-4.27]; p=<0.02) of TC/HDL ratio was statistically greater in DR patients as shown in Table 3. Likewise, the 4th quartile of TG/HDL ratio was significantly greater in DR group (OR= 2.06 [1.03-4.13]; p=0.03). ROC curve for lipid ratios showed strong discriminatory power for detecting atherosclerotic disease. TC/HDL ratio was a strong indicator with an optimal cut-off level of ≥ 4.0 (sensitivity of 66.0%, specificity of 64.8%, positive predictive value "PPV" of 64.5%, and negative predictive value "NPV" of 58.7% with a diagnostic accuracy of 0.653). On the other hand, TG/HDL ratio was a moderate indicator for atherosclerotic disease. The optimal cut-off level was ≥ 3.0 (sensitivity of 65.5%, specificity of 62.3%, PPV of 72.0%, and NPV of 62.7% with a diagnostic accuracy of 0.619) (Figure 1B, 1D).

As displayed in Figure 2A-2F, In contrast to females who developed DR, when comparing all lipid parameters (lipid ratios, and conventional lipid parameters) between the two genders, superior values were found in male patients with DR.

Table 3: Crude "Odds Ratio" of blood lipid ratios quartiles associated with diabetic retinopathy presence

Variables	Without DR, n=199 Number (%)	With DR, n=94 Number (%)	Odds ratio (95% CI OR)	p-value
TC/HDL ratio				
1st quartile (1.56-3.01)	52 (26.1)	21 (22.3)	Reference	---
2nd quartile (3.02-3.69)	56 (28.1)	17 (18.1)	0.75 [0.35-1.58]	0.45
3rd quartile (3.70-4.56)	52 (26.1)	22 (23.4)	1.04 [0.51-2.13]	0.89
4th quartile (4.57-8.10)	39 (19.6)	34 (36.2)	2.15 [1.08-4.27]	0.02*
LDL/HDL ratio				
1st quartile (0.35-1.53)	54 (27.1)	20 (21.3)	Reference	---
2nd quartile (1.54-2.12)	55 (27.6)	18 (19.1)	0.88 [0.42-1.85]	0.74
3rd quartile (2.13-2.75)	46 (23.1)	27 (28.7)	1.58 [0.78-3.18]	0.19
4th quartile (2.76-5.93)	44 (22.1)	29 (30.9)	1.78 [0.88-3.56]	0.10
TG/HDL ratio				
1st quartile (0.22-1.28)	53 (26.6)	20 (21.3)	Reference	---
2nd quartile (1.29-1.97)	56 (28.1)	17 (18.1)	0.80 [0.38-1.69]	0.56
3rd quartile (1.98-2.98)	49 (24.6)	25 (26.6)	1.35 [0.66-2.73]	0.40
4th quartile (2.99-8.10)	41 (20.6)	32 (34.0)	2.06 [1.03-4.13]	0.03*

(*) multivariate logistic regression significant at p = 0.05; DR: Diabetic retinopathy; CI, confidence interval; OR, Odd ratio; TC: total cholesterol (g/L); LDL: low-density lipoprotein cholesterol (g/L); HDL: high-density lipoprotein cholesterol (g/L); TG: triglycerides (g/L).

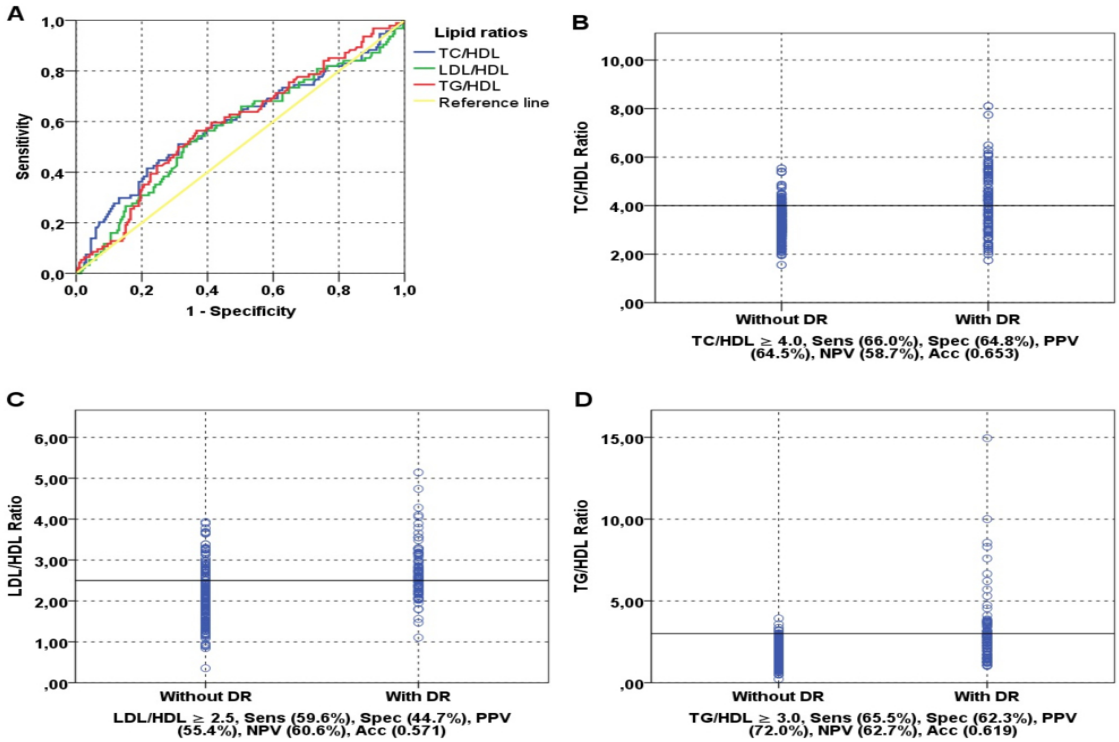

Figure 1. Receiver operating characteristic curve to define the best cut-off lipid ratios to detect atherosclerosis

DR: Diabetic retinopathy; Sens: Sensitivity; Spec: Specificity; PPV: Negative Predictive Value; NPV: Positive Predictive Value; Acc: Accuracy; TC: total cholesterol; LDL: low-density lipoprotein cholesterol; HDL: high-density lipoprotein cholesterol; TG: triglycerides

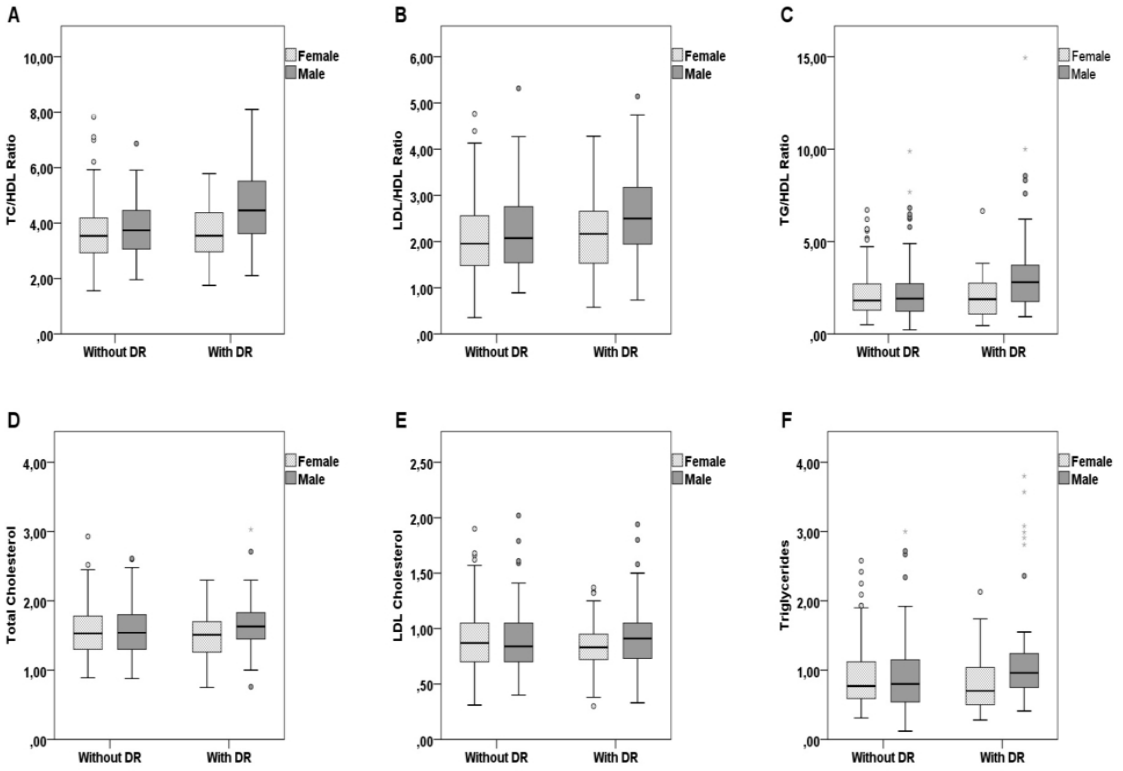

Figure 2. Comparison of lipid ratios levels between patients with and without diabetic retinopathy according to their gender

DR: Diabetic retinopathy; TC: total cholesterol; LDL: low-density lipoprotein cholesterol; HDL: high-density lipoprotein cholesterol; TG: triglycerides

4. Discussion

The current study assessed the potential role of biological and clinical features in diabetics with and without DR in order to establish the factors related with the existence of DR in this population. Surprisingly, in addition to several well-known CV risk factors, this study supports the idea that microvascular disease such as DR may promote the formation of the atherosclerotic plaques in type 1 diabetics. Thus, we examine the link between DR and atherosclerosis by comparing two groups of diabetics based on the existence of DR (patients with and without DR). We revealed that the distribution of diabetics by gender was inequitable, emphasizing a clear male preponderance over females (52.60% - 47.40%) with a sex ratio of 1.10. Compared to patients who developed DR, the proportion of men was higher than that of women among type 1 diabetics. A prospective study conducted by Rajalakshmi *et al*., (2014), demonstrated that 80 patients with T1D had DR; of those, 48 (60.0%) were men, whereas, in the Araszkiewicz's study group, 39 (44.8%) had DR; of those, 22 (56.4%) were men (Araszkiewicz *et al*., 2011). Our findings showed significant effects of age and duration of diabetes on the presence of DR. The findings concord with Matuszewski *et al.* who found that aged patients (≥ 30 years) with long-lasting diabetes (≥ 15 years) had higher risk to develop more severe DR (Matuszewski *et al*., 2020). The results are also consistent with previous studies in the literature (Carbonell *et al*., 2018; Rajalakshmi *et al*., 2014; Araszkiewicz *et al*., 2011). In addition, our study also indicates that tobacco strongly advanced the progression of DR. A similar conclusion was found by Uruska *et al.,* (2014) regarding patients with T1D. Other authors also established a causal link between smoking and the incidence of DR (Matuszewski *et al*., 2020; Campagna *et al*., 2019). Interestingly, body corpulence elevated the probability of DR in our study population. Authors studied the impact of BMI on the development of DR and concluded that DR is associated to BMI only in its correlation with HbA1c, cholesterol concentration and hypertension (Carbonell *et al*., 2018; Kaštelan *et al*., 2014). Price *et al.,* (2014) hypothesized that obesity in subject with T1D may lead to the progression of DR and macrovascular complications. Our findings have shown strong and consistent correlations between diabetic nephropathy and DR. Statistically significant associations were highlighted in the Renin-Angiotensin-System investigation between DR and preclinical diabetic nephropathy in patients T1D (Klein *et al.,* 2005). Same observations were made by Kaštelan *et al.* (2014). In our study, hypertension was identified in 29 patients, and 27 (28.70%) of them have developed DR. Our results are in agreement with the previous conclusions which indicate that the prevalence of resistant hypertension escalates with advanced retinopathy and define hypertension as the most significant risk factor for DR (Carbonell *et al*., 2018; Rajalakshmi *et al*., 2014; Araszkiewicz *et al*., 2011; Kaštelan *et al.,* 2014). Notably, our study revealed an association of an increased prevalence of DR with low hemoglobin levels in patients with T1D. Our results are in line with the conclusions

made by Li *et al.,* (2020) who ascertained a similar correlation between an increased progression to DR and anemia. Our results demonstrated that ketosis on diabetes was associated with an increased incidence of DR, and this echoes with a former follow-up study of adults with T1D (Martin *et al.,* 2005). We also detected a sustained increase in HbA1c levels in DR patients, proposing an early interaction between these two conditions. Previous studies reported impaired microvascular function in the retina (retinal arteriolar dilation response to flickering light) was associated with higher levels of HbA1c (Carbonell *et al*., 2018; Araszkiewicz *et al*., 2011; Matuszewski *et al*., 2020; Kaštelan *et al.,* 2014). With regard to decreased glomerular filtration rate (known as diabetic nephropathy), we also noticed an incremental increase in UAER (≥ 30 mg/24h) in patients who developed DR, which may allow the findings to be more credible. Carbonell *et al.,* (2018) ascertained that in patients with a urine albumin/creatinine ratio higher than 4 mg/g, DR was observed significantly more often. Additionally, in concordance with several studies in the literature (Carbonell *et al*., 2018; Araszkiewicz *et al*., 2011; Miljanovic *et al.,* 2004), we further established that there were increased triglycerides concentration and decreased HDL-cholesterol concentration in patients who had developed DR compared to those who did not. Moreover, these findings suggest that implication of dyslipidemia may reflect underlying, as jet unidentified, mechanisms linked to vascular dysfunction (Chang *et al.,* 2013). According to Melo *et al.* (2019), in both males and females, several clinical risk factors and pathophysiological features are shared between cardiovascular disease and diabetic (type 1 or type 2 collectively) retinopathy. However, even after controlling for the major risk factors, patients with DR have a higher probability of presenting with CVD. A strong association between DR and the atherosclerosis risk (screened through lipid ratios and traditional lipid parameters) was disclosed in our male T1D patients comparing to females. In their review study, Ozawa *et al*., (2015) suggest that basically, the difference in sex seems to modify the risk of developing T1D and vision-threatening retinopathy and further cardiovascular complications. Indeed, the relationship between sex difference, DR and cardiovascular risk is not unidirectional. The causal relationship can be explained in several ways. For at least two decades, the findings that elevated serum lipid levels and also the TC/HDL ratio have been associated to an increased risk of progression of DR, thus suggest a relationship with disruption of the blood-retinal barrier. At the same time, controlling retinal complications in diabetic patients can help prevent the onset or even worsening of CVD (Miljanovic *et al.,* 2003).

To evaluate the CV risk linked with a microvascular complication, such as DR in diabetics, longitudinal studies are recommended, and to properly establish this risk, future inquiries should include type 1 diabetic subjects (with and without DR).

5. Conclusion

In conclusion, our data confirmed that type 1 diabetics with DR exhibit a higher risk of atherosclerotic disease, suggesting that dyslipidemia might play an important role in the development of macro-angiopathy in this population. Moreover, our results established the usefulness of the employment of lipid ratios in the evaluation of subclinical atherosclerosis risk in this population. Thus, our findings could have both therapeutic and investigational implications that encourage extensive monitoring accompanied by a more careful cardiovascular assessment in patients with DR, in order to prevent the development of major adverse CVD.

Conflict of interest

The authors declared no conflicts of interest.

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