Lipid Metabolism Biomarkers in Diabetic Retinopathy in Patients With Type 1 Diabetes Mellitus

Natalia Pălărie (npalarie@gmail.com)
Department of Ophthalmology, International Clinic for Diabetes, Nutrition and Metabolic Diseases

Ecaterina Pavlovschi
Department of Biochemistry and Clinical Biochemistry, Nicolae Testemitanu State University of Medicine and Pharmacy

Cornelia Lazăr
Department of Biochemistry and Clinical Biochemistry, Nicolae Testemitanu State University of Medicine and Pharmacy

Victor Pălărie
Department of Ophthalmology, International Clinic for Diabetes, Nutrition and Metabolic Diseases

Natalia Palii
Department of Ophthalmology, International Clinic for Diabetes, Nutrition and Metabolic Diseases

Irina Rotaru[3]
Department of Ophthalmology, Promed Clinic

Olga Tagadiuc
Department of Biochemistry and Clinical Biochemistry, Nicolae Testemitanu State University of Medicine and Pharmacy

Research Article

Keywords: type 1 diabetes mellitus, diabetic retinopathy, triglyceride, cholesterol, lipoprotein (a)

Posted Date: November 16th, 2022

DOI: https://doi.org/10.21203/rs.3.rs-2217300/v1

Abstract

Background. Diabetic retinopathy (DR) is the most common ocular complication of diabetes mellitus, as well as the most prevalent cause for irreversible vision loss in working-age population.

The aim of the study was to analyze the association between DR in patients with type 1 diabetes mellitus (T1DM), some lipid biomarkers changes, as well as to highlight the possible correlation.

Methods. A total of 72 T1DM patients were included in this study. The enrolled participants were assigned into three groups, based on the results of fundus photographs as following: 1st group - no DR, 2nd group - non-proliferative diabetic retinopathy (NPDR), and 3rd group - proliferative diabetic retinopathy (PDR). Serum lipids: triglycerides (TG), total cholesterol (TC) and lipoprotein (a) (Lp (a)) were measured at the baseline.

Results. The data revealed no difference in TG serum levels in patients of the 1st and 2nd groups. The increase of TG serum levels was identified with the progression of the DR in the 3rd group (+121%, p=0.018). TC also rose in patients in the 3rd study group (+19%, p>0.05 compared to the 1st and 2nd study groups). An increase of Lp (a) was highlighted with the evolution of DR: in the 2nd group (+73%, p>0.05) and in the 3rd group (by about 320%, p=0.019) compared to 1st study group. The correlation analysis revealed a weak positive correlation between the grade of DR and Lp (a) levels (r_s=0.319, p=0.006), and TG (r_s=0.239, p=0.043).

Conclusion. Our study exposed statistically significant modifications of the TG and Lp (a) levels correlated with the DR grade and a non-significant increase in TC level in T1DM persons. Our data indicate a likely involvement of lipid metabolism disorders in the progression of DR.

Introduction

Recent epidemiological data show an enhancement in the prevalence of type 1 diabetes mellitus (T1DM), which is characterized by autoimmune destruction by CD4+ and CD8+ T cells of insulin-producing β cells in the pancreas, with further infiltration of the pancreatic islets by the macrophages. T1DM accounts for about 10% of all cases of diabetes, affecting around 2 million of people in Europe and North America [1].

DR is the most common ocular complication of diabetes mellitus, as well as the most prevalent cause for irreversible vision loss in working-age population [2].

Vision-threatening DR is rarely present at the moment of T1DM diagnosis, but after 20 years, 99% of patients with T1DM develop DR having different complex symptoms. On the other hand, in diabetic patients with type 2 diabetes mellitus (T2DM), DR can be found at the time of diagnosis and 20 years afterwards, approx. 60% of patients having different manifestations of DR [3].

The pathophysiology of the DR is considered more or less known, being mostly described the role of hyperglycemia and altered metabolic pathways in the development of oxidative stress and neurodegeneration in the initial stage. As early hallmarks of non-proliferative diabetic retinopathy (NPDR) are remarked: vascular endothelial damage, the development of microaneurysms, and dot intraretinal hemorrhage. Continuously, under fundoscopy is observed the disruption of the blood–retinal barrier, with further leakage of multiple inflammatory cytokines and plasma proteins that explains the appearance of the hard exudates. Tortured capillaries and retinal ischemia are explained by the vasoconstriction and capillary occlusions. In the last stage of DR, marked hypoxia induce neovascularization, vitreous hemorrhage, and retinal detachment [2, 4].

Many different classifications have been proposed in order to simplify the approach to the diagnostic and treatment of DR. In order to ease the communication between doctors has been developed an International Clinical Disease Severity Scale, which is based on the findings of the Wisconsin Epidemiologic Study of Diabetic Retinopathy (WESDR) and the Early Treatment Diabetic Retinopathy Study (ETDRS). There are five stages of DR that are recognized [5].

An increase in the frequency of the disease and its complications, underlines the need of a better understanding of the pathophysiology of DR, in order to be able to identify it in the first stage, the high-risk patients and at the same time to assure a personalized tailored therapy with a minimization of further negative consequences [6].

Nowadays a series of biomarkers of DR related to the visualization of the retinal vasculature and measuring of glycemia, lipids, blood pressure, body weight are used for diagnostic and treatment purpose. Moreover, novel biomarkers and mediators of DR, as for example the one related to inflammation and angiogenesis, have influenced the development of extra therapeutics, especially for late-stage retinopathy, like intra-ocular corticosteroids and intravitreal vascular endothelial growth factor inhibitors ('anti-VEGFs') agents. Unfortunately, in spite of a range of treatments, a lot of patients still are underdiagnosed [7].

Many studies have demonstrated that intensive control of the risk factors for DR is essential in reducing the onset and progression of DR. At present, the relationship between lipid profile and DR remains unclear. But lipid metabolic abnormalities have emerged as potential risk factors for the onset and progression of diabetic complications, including DR [8, 9].

Enhanced lipid levels were incriminated to induce endothelial dysfunction (ED) as a result of reduced bioavailability of nitric oxide. Hence, it has been suggested that ED plays a role in retinal degeneration in DR [10]. However, a disturbance in metabolism of lipids cannot be fully responsible for all pathological changes that lead to the development of retinopathy, there is still a lot of evidence to be rechecked and examined, due to interesting new data that are arriving continuously. Large epidemiologic studies imply an inconsistent association between serum TG or major cholesterol species and the severity of DR, but certain specific lipoprotein species are proved to have a stronger association with DR severity, suggesting

a pathological role for lipoproteins, which are modified in the intraretinal environment, creating substantial local damage [11].

Nevertheless, there is a huge gap in the research of lipid markers, especially in DR at the patients with T1DM. Mostly all the recent data are based on T2DM population [12]. Also, only few studies have specifically explained the role of lipids and lipoproteins in different retinal diseases [13]. Therefore, we consider that it would be of much interest to search for how conventional biomarkers of lipid status as well as lipoproteins can contribute to the development and progression of DR.

The aim of the study was to analyze the association between DR in patients with T1DM, and lipid biomarkers changes, as well as to highlight possible existing correlations.

Material And Methods

Study design

This clinic-based observational study followed the tenets of the Declaration of Helsinki and was approved by the Research Ethics Committee (certificate nr.6 from 20.05.2021) of the *Nicolae Testemitanu* State University of Medicine and Pharmacy in Chisinau, the Republic of Moldova. A written informed consent was obtained from each participant. Patients were assessed in 2 centers in Moldova: International Clinic for Diabetes, Nutrition and Metabolic Diseases, Ophthalmology Department in Orhei and Promed Clinic, Ophthalmology Department in Chisinau.

Patient selection

For this study we recruited T1DM patients that came for a consult at one of the participating clinics. All patients undergone standardized ophthalmological examination, as well as retinal photography and optical coherence tomography of macula and optic nerve head. The enrolled participants were divided into three groups, as following: no DR group, non-proliferative diabetic retinopathy (NPDR) group, and proliferative diabetic retinopathy (PDR) group. Grading was performed by three experienced retina specialists according to ETDRS grading standards and based on the results of fundus photographs. Individual DR grade was based on the worse eye DR level.

From the study group were excluded patients on any medication that can interfere with the results of the research, pregnant women, as well as persons with the presence of severe diabetes complications and comorbidities.

Sample collection

Fasting (> 12 hours) venous blood samples (5 ml) were collected by vena cubitalis puncture at the day of eye examination at each participating center. The blood was centrifuged, with further serum storage in Eppendorf microtubes at –40°C, prior being tested.

Biochemical analysis

The level of the following serum lipid markers was measured: TG, TC and Lp (a). In order to determine the markers of interest were used commercially available standard kits, produced by DiaSys Diagnostic Systems GmbH (Germany).

TG was determined by colorimetric enzymatic test using glycerol-3-phosphate-oxidase (GPO) after enzymatic splitting with lipoprotein lipase. Quinonimine is the indicator, generated from 4-aminoantipyrine and 4-chlorophenol by hydrogen peroxide under the catalytic action of peroxidase (Triglycerides FS*).

TC level was assessed according to the “CHOD-PAP” enzymatic photometric test. The method is based on the determination of cholesterol after enzymatic hydrolysis and oxidation. The colorimetric indicator is quinonimine which is generated from 4-aminoantipyrine and phenol by hydrogen peroxide under the catalytic action of peroxidase (Trinder’s reaction) (Cholesterol FS*).

For the assessment of Lp (a) was used particle enhanced immunoturbidimetric test based on the photometric measurement of antigen-antibody-reaction between antibodies against Lp(a) bound to particles and Lp(a) present in the sample (Lp(a) 21 FS*).

Statistical analysis

The data were analysed using SPSS 23.0 software. Descriptive statistical methods (median, interquartile range (IQR)) were used. Normality tests (Shapiro-Wilk and Kolmogorov-Smirnov) were performed. Box plots were created to detect the outliers. The homogeneity of variance was assessed by Levene’s test. The comparison between study groups was accomplished using nonparametric Kruskal-Wallis test with Pairwise comparison of groups as a post-hoc test. Correlation analysis was done by Spearman correlation test. The $p<0.05$ value was considered statistically significant.

Results

A total of 72 T1DM patients met the selection criteria: 42 females and 30 males, were assigned into groups according to DR grade, after obtaining the eye fundus digital photos, and performance of the entire consult: I – no DR, (n=28), II – NPDR (n=24), III – PDR (n=20). The median age in the 1st group was 33 (IQR 12.8), in the 2nd – 36 (IQR 18.8) and in the 3rd – 37,5 (IQR 13.8), p=0.032. All patients had the BMI in the normal range.

Demographic characteristics of the patients included in this study is presented in Table 1.

Table 1 Demographic characteristics of patients included in the study

Characteristics	1st group (N = 28)	2nd group (N = 24)	3rd group (N = 20)
Age (years)	33 (IQR 12.8)	36 (IQR 18.8)	37.5 (IQR 13.8)
Male/Female	9/19	10/14	11/9
BMI (kg/m^2)	24 (IQR 4.8)	23.5 (IQR 4.0)	23.5 (IQR 6.5)
Diabetes duration (years)	13 (IQR 10.3)	18.5 (IQR 13.3)	23 (IQR 9.0)
HbA1c	6.85 (IQR 1.8)	8.2 (IQR 2.0)	9 (IQR 1.6)

Note. BMI – body mass index, HbA1c – glycated hemoglobin A1c

The results of serum lipid assessment: TG, TC and Lp (a) are presented in Table 2.

Table 2 Changes of serum triglycerides, total cholesterol and Lipoprotein (a) levels in diabetes mellitus type 1 patients with different degree of diabetic retinopathy

Study group	Triglycerides (mmol/L)	Total cholesterol (mmol/L)	Lipoprotein (a) (g/L)
1st group	0.85 (IQR 0.72)	4.785 (IQR 1.64)	**0.060** (IQR 0.177) #
2nd group	0.785 (IQR 0.85) *	4.73 (IQR 1.58)	**0.104** (IQR 0.286)
3rd group	1.735 (IQR 1.7) *	5.69 (IQR 2.09)	**0.252** (IQR 0.311)-#
p-value (Kruskal-Wallis test)	**0.015**	0.382	**0.024**

Note. Data are presented as median and IQR; p-value after Pairwise comparisons of groups: * – statistically significant difference between 2nd and 3rd group, p<0.05; # – statistically significant difference between 1st and 3rd group, p<0.05.

The data revealed no difference in TG serum levels in patients of 1st and 2nd group (p>0.05). The increase of the TG serum levels was identified with the progression of DR to PDR level, i.e. in the 3rd group (+121%, p=0.018**,** compared to 2nd group).

The research determined insignificant change of TC in patients in the 2nd group compared to those in the 1st group (p>0.05). The amounts rose in patients in the 3rd group (+19%, p>0.05 compared to 1st and 2nd group).

We have identified an increase of Lp (a) with the evolution of DR: statistically insignificant in the 2nd group (+73%, p>0.05) compared to the 1st group, and a significant increase in the 3rd group by about 320%, p=0.019, compared to the 1st study group.

The correlation analysis revealed a weak positive correlation between the grade of DR and Lp (a) level (r_s=0.319, p=0.006), as well as between the DR grade and TG (r_s=0.239, p=0.043). There was no correlation between the severity of DR and TC (rs=0.107, p=0.372) (Table 3).

Table 3 Correlation of serum triglycerides, total cholesterol and lipoprotein (a) levels with DR grade in patients with DM 1

		Triglycerides	Total cholesterol	Lipoprotein (a)
Diabetic Retinopathy	Correlation coefficient	0.239*	0,107	0,319**
	Statistical significance, 2-tailed (p)	**0.043**	0.372	**0.006**

Note: differences between study groups and p-value: *<0.05; **<0.01

Discussion

DR is a multifactorial pathology, and many different researchers have proven that its pathogenesis is certainly more complicated than just taking into account the level of blood glucose. The first studies in 90s of Miccoli R. et al. (1987) established that the TC levels increased along with the severity of DR [14]. Later, a series of various studies elucidate the implication of disturbances in the metabolism of lipids and the degenerative alterations in DR, high serum lipid levels being even proposed as a risk factor [10]. The complexity of our study lies in the fact that only patients with T1DM were selected. Less studies are available under this condition, with conflicting reports in the literature regarding the effect of lipid profile on the advancement of DR.

The results of our study showed a number of lipid biomarker abnormalities in diabetic retinal disease, some of which are consistent with the findings of other researchers. Similar to our results (statistically significant increase in TG in the PDR and correlation with DR) in the Miljanovic et al (2003) and Lyons Tj et al (2004) articles, was reported a statistically significant association of TG with progression of DR [15,16]. Moreover, in an article presented by Tolonen N. et al. (2013), patients with PDR had higher TC levels, as well as increased TG and apolipoprotein B concentrations than patients without DR or with NPDR [17].

Resembled to our results were highlighted and regarding Lp (a). We attested an enhancement of Lp (a) level through the groups, once the DR advanced. This result is supported by the findings of the research conducted by Malaguarnera G. et al (2013), that proved that Lp (a) levels are increased in a significant proportion of patients with retinopathy compared to diabetic patients without retinopathy [18] and Gazzaruso C. et al. (1998) that proved that among patients with retinopathy, those with PDR had higher Lp (a) levels than those with NPDR [19]. On the contrary, the conclusion of Lyons TJ et al. (2004) is reversed, assuring no associations identified with Lp(a) [16].

Conclusion

There is a lack of new research regarding the role of dyslipidemia in the development and progression of DR, and the existing one offers contradictory data. Our study exposed statistically significant modifications of the TG and Lp (a) levels correlated with the DR degree and a non-significant increase in TC level in T1DM patients. Our data indicate the possible involvement of lipid metabolism disorders in the pathogenesis of DR endorsing the results of other researchers. The results and the analyzed existing data attest the necessity for a future more complexed research with the extension of the study groups and of markers spectrum, in order to be able to establish with certainty the role of dyslipidemia in the development and evolution of DR.

Abbreviations

T1DM – type 1 diabetes mellitus;

T2DM – type 2 diabetes mellitus;

DR – diabetic retinopathy;

ED – endothelial dysfunction;

Lp(a) – lipoprotein (a);

ETDRS - Early Treatment Diabetic Retinopathy Study;

NPDR – non-proliferative diabetic retinopathy;

PDR - proliferative diabetic retinopathy;

TC – total cholesterol;

TG – triglycerides;

VEGF – vascular endothelial growth factor;

Declarations

Ethics approval and consent to participate

The study followed the tenets of the Declaration of Helsinki and was approved by the Research Ethics Committee (certificate nr.6 from 20.05.2021) of the *Nicolae Testemitanu* State University of Medicine and Pharmacy in Chisinau, the Republic of Moldova. A written informed consent to participate was obtained from each participant.

Consent to publish

A written informed consent to publish was obtained from each participant.

Availability of data and materials

The datasets analyzed during this study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

Funding

Study was carried out with the support of the project 22.80015.8007.257T "Early diagnosis of the dyslipidemic profile in the prevention of the development and progression of complications associated with microangiopathy (retinopathy and periodontitis) in type 1 diabetes" (2022), project leader: PĂLĂRIE Victor, PhD., financing authority: National Agency for Research and Development of Republic of Moldova.

Acknowledgements

Not applicable

Author's contribution

N.Palarie – study design, consult of the patients, data acquisition, participation in the collection of biological samples for biochemical investigations, wrote the manuscript with support of O.T.

C.L. – study design, data analysis and interpretation, biochemical analysis, final approval of the manuscript.

E.P. – study design, data analysis and interpretation, biochemical analysis, final approval of the manuscript.

V.P. - consult of the patients, data acquisition, participation in the collection of biological samples for biochemical investigations.

N.Palii - consult of the patients, data acquisition, participation in the collection of biological samples for biochemical investigations.

I.R. - consult of the patients, data acquisition, participation in the collection of biological samples for biochemical investigations.

O.T. – study design, biochemical analysis, data analysis and interpretation, critical revision, final approval of the version to be published.

References

1. Gillespie KM. Type 1 diabetes: pathogenesis and prevention. CMAJ. 2006;18;175(2):165-70.
2. Lin KY, Hsih WH, Lin YB, Wen CY, Chang TJ. Update in the epidemiology, risk factors, screening, and treatment of diabetic retinopathy. J Diabetes Investig. 2021;12(8):1322-1325.
3. Guidelines for Diabetic Retinopathy Management. Available online: https://www.nhmrc.gov.au/_files_nhmrc/publications/attachments/di15.pdf (accessed on 12 October 2022).
4. Katsarou A, Gudbjörnsdottir S, Rawshani A, Dabelea D, Bonifacio E, Anderson BJ, Jacobsen LM, Schatz DA, Lernmark Å. Type 1 diabetes mellitus. Nat Rev Dis Primers. 2017;30; 3:17016.
5. Wu L, Fernandez-Loaiza P, Sauma J, Hernandez-Bogantes E, Masis M. Classification of diabetic retinopathy and diabetic macular edema. World J Diabetes. 2013;15;4(6):290-4.
6. Hommer N, Kallab M, Schlatter A, Howorka K, Werkmeister RM, Schmidl D, Schmetterer L, Gerhard G. Retinal Oxygen Metabolism in Patients with Type 1I Diabetes and Different Stages of Diabetic Retinopathy. Diabetes 2022; db220219.
7. Jenkins AJ, Joglekar MV, Hardikar AA, Keech AC, O'Neal DN, Januszewski AS. Biomarkers in Diabetic Retinopathy. Rev Diabet Stud. 2015;12(1-2):159-95.
8. Busik JV. Lipid metabolism dysregulation in diabetic retinopathy. J Lipid Res. 2021; 62:100017.
9. Eid S, Sas KM, Abcouwer SF, et al. New insights into the mechanisms of diabetic complications: role of lipids and lipid metabolism. Diabetologia. 2019;62, 1539–1549.
10. Landmesser U, Hornig B, Drexler H. Endothelial dysfunction in hypercholesterolemia: mechanisms, pathophysiological importance, and therapeutic interventions. Semin Thromb Hemost. 2000;26(5):529–537.
11. Modjtahedi BS, Bose N, Papakostas TD, Morse L, Vavvas DG, Kishan AU. Lipids and Diabetic Retinopathy, Seminars in Ophthalmology. 2016;31;1-2:10-18.
12. Chou Y, Ma J, Su X et al. Emerging insights into the relationship between hyperlipidemia and the risk of diabetic retinopathy. Lipids Health Dis. 2020;19, 241.
13. Pavlovschi E, Borovic D, Pantea V, Lîsîi L, Tagadiuc, O. Biomarkers of Lipid Status and Metabolism in Retinal Hypertensive Disorder. Journal of Biosciences and Medicines, 2021;9, 77-89.
14. Miccoli R, Odello G, Giampietro O, Marchetti P, Cristofani R, Penno G, Meucci G, Navalesi R. Circulating lipid levels and severity of diabetic retinopathy in type 1 diabetes mellitus. Ophthalmic Res. 1987;19(1):52-6.
15. Miljanovic BM, Glynn RJ, Nathan DM, Manson JE, Schaumberg DA. Serum Lipid Levels and Diabetic Retinopathy in Type 1 Diabetes Mellitus. Invest. Ophthalmol. Vis. Sci. 2003;44(13):3099.

16. Lyons TJ, Jenkins AJ, Zheng D, Lackland DT, McGee D, Garvey WT, Klein RL. Diabetic retinopathy and serum lipoprotein subclasses in the DCCT/EDIC cohort. Invest Ophthalmol Vis Sci. 2004 Mar;45(3):910-8.
17. Tolonen N, Hietala K, Forsblom C, Harjutsalo V, Mäkinen VP, Kytö J, Summanen PA, Thorn LM, Wadén J, Gordin D, Taskinen MR, Groop PH. FinnDiane Study Group. Associations and interactions between lipid profiles, retinopathy and nephropathy in patients with type 1 diabetes: the FinnDiane Study. J Intern Med. 2013;274(5):469-79.
18. Malaguarnera G, Gagliano C, Bucolo C, Vacante M, Salomone S, Malaguarnera M, Leonardi DG, Motta M, Drago F, Avitabile T. Lipoprotein(a) serum levels in diabetic patients with retinopathy. Biomed Res Int. 2013; 2013:943505.
19. Gazzaruso C, Garzaniti A, Buscaglia P, D'Annunzio G, Porta A, Vandelli G, Lorini R, Finardi G, Fratino P, Geroldi D. Lipoprotein(a) levels and apolipoprotein(a) polymorphism in type 1 diabetes mellitus: relationships to microvascular and neurological complications. Acta Diabetol. 1998;35(1):13-8.