ORIGINAL ARTICLE

CONTENTS

Hyperhomocysteinemia – an early marker of retinal damage in patients with type 2 diabetes mellitus

Yelyzaveta S. Sirchak, Aliona V. Stoika, Erica S. Dobryanska (Opalenyk), Nelli V. Bedey, Ivan I. Pushkash, Yuliia V. Holosh

UZHHOROD NATIONAL UNIVERSITY, UZHHOROD, UKRAINE

ABSTRACT

Aim: To investigate the features of changes in serum homocysteine (Hcy) levels in patients with T2DM depending on the presence or absence of retinal damage.

Materials and Methods: We observed 77 patients with T2DM. Patients were divided into two groups: group 1 included 45 patients with T2DM and diabetic retinopathy (DR), group 2 included 32 patients with T2DM without DR. The level of Hcy concentration and B vitamins (vitamin B1, B6, B9, B12) was determined in blood serum.

Results: Proliferative and preproliferative stages of DR were more frequently detected in the examined patients with T2DM (in 44.4% and 26.7% of patients, respectively). Proliferative retinopathy with complications was diagnosed in 17.8% of patients, while nonproliferative retinopathy with maculopathy was diagnosed only 11.1% of cases. In patients of both groups, a decrease in the levels of B vitamins in the blood serum was detected, which was detected against the background of an increase in the level of homocysteine in the blood serum in these patients.

Conclusions: In patients with type 2 diabetes and DR, a decrease in the level of B vitamins was found against the background of an increased concentration of homocysteine (43.5±0.9 mkmol/l – $p<0.001$) in the blood serum, which proportionally increases with the progression of retinal damage in these patients.

KEY WORDS: diabetes mellitus type 2, homocysteine, B vitamins, retinal damage, body mass index, obesity

Wiad Lek. 2025;78(10):2095-2100. doi: 10.36740/WLek/213600 DOI

INTRODUCTION

The prevalence of type 2 diabetes mellitus (T2DM) continues to rise worldwide. T2DM is a complex disease in terms of pathogenesis, resulting from the interaction of a number of factors, such as genetic or environmental, each of which has a different impact on the onset of the disease. Many cultural, dietary, behavioural, contextual and lifestyle factors are also key determinants of T2DM risk. Given the significant healthcare costs, it is extremely important to identify modifiable factors for diabetes prevention [1]. An estimated 451 million people worldwide had diabetes in 2017, a figure expected to rise to 693 million by 2045 [2].

Diabetic retinopathy (DR) is the leading cause of vision loss in working-age adults in Western countries. It is the most common microvascular complication of diabetes. Diabetic retinopathy can lead to vision-threatening retinal damage and ultimately blindness [3]. Diabetic retinopathy affects people with diagnosed or undiagnosed diabetes. The susceptibility to developing DR is directly proportional to the patient's age and duration of diabetes, as well as poor glycemic control and blood pressure fluctuations [4].

According to epidemiological studies published by the American Academy of Ophthalmology, ninety-three million people worldwide suffer from diabetic retinopathy. The prevalence of DR is 77.3% in patients with type 1 diabetes and 25.1% in patients with T2DM, of whom approximately 25-30% may develop vision-threatening diabetic macular edema. Between 5% and 8% of patients with diabetic retinopathy will require laser treatment, and up to 5% of patients will require vitrectomy surgery [4, 5].

Morphological changes observed in the small retinal vessels in DR include early pericyte loss, thickening of the basement membrane, loss of endothelial cells, increased vascular permeability, platelet aggregation, leukostasis, and capillary atrophy [6, 7]. Diabetic retinopathy affects not only the retinal microvessels but also Müller cells, which are the main glial cells of the retina. The functions of Müller cells are to maintain the structural integrity of the retina, regulate the blood-

retinal barrier and retinal blood flow, absorb and recycle various neurotransmitters, retinoic acid compounds, and ions (such as potassium), and regulate metabolism and nutrient delivery to the retina [8].

Therefore, the assessment of markers that enable the evaluation of endothelial dysfunction and indicate the development of DR is extremely important.

AIM

The aim to investigate the features of changes in serum homocysteine (Hcy) levels in patients with T2DM depending on the presence or absence of retinal damage.

MATERIALS AND METHODS

At the clinical base of the Department of Propaedeutics of Internal Disease, we observed 77 patients with T2DM, who were undergoing inpatient treatment in the endocrinology and gastroenterology departments of the Andriy Novak Transcarpathian Regional Clinical Hospital, as well as undergoing outpatient observation at their place of residence with a family doctor, gastroenterologistor endocrinologist.

The patient cohort consisted of 47 female participants (61.0%) with a mean age of 51.6±5.9 years and 30 male participants (39.0%) with a mean age of 50.9±6.2 years. The control cohort comprised 30 healthy subjects, including 18 women (60.0%) and 12 men (40.0%). Female control participants had a mean age of 50.5±4.7 years, while male controls averaged 52.3±5.1 years.

All clinical assessments were performed following patient consent (written informed consent for applicable diagnostic and treatment procedures was obtained from all patients and control subjects), with comprehensive measures implemented to ensure the confidentiality of all data collected. The study methodology complied with the Helsinki Declaration of 1975 regarding Human Rights and its 1983 revisions, the Council of Europe's Convention for the Protection of Human Rights and Dignity of the Human Being with regard to the Application of Biology and Medicine, and Ukrainian legislative standards.

The exclusion criteria were: age under 18 and over 75; type 1 diabetes mellitus; systemic autoimmune diseases; open form of pulmonary tuberculosis; mental illnesses that prevent adequate assessment of the patient's health and signing of informed consent for diagnosis and treatment; pregnancy and lactation; cancer, AIDS.

All study participants received thorough evaluation including clinical examination, body measurements, diagnostic instrumentation, and laboratory analysis. Diagnostic verification was achieved through comprehensive recording of presenting complaints and patient history. Standardized abdominal ultrasound examination was performed on all participants, particularly targeting hepatobiliary system and bile duct structures. All patients were consulted by an ophthalmologist.

The diagnosis of type 2 diabetes was established in accordance with the IDF recommendations (2005), as well as taking into account the criteria of the unified clinical protocol (Order of the Ministry of Health of Ukraine dated 21.12.2012 No. 1118) and the criteria of the European Association for the Study of Diabetes (EASD), ADA, 2019. To determine the degree of compensation of carbohydrate metabolism, the level of glycosylated hemoglobin (HbA1c, %) was studied.

Anthropometric evaluation included the measurement of body height, weight, and waist diameter, followed by body mass index (BMI) computation. Participants were categorized using World Health Organization criteria into BMI classifications: extreme underweight (BMI ≤16.0); underweight (16.0-18.5); normal range (18.5-24.9); overweight (25.0-29.9); Class I obesity (30.0-34.9); Class II obesity (35.0-39.9); and Class III obesity (BMI ≥40.0).

Patients with type 2 diabetes were divided into two groups: group 1 included 45 patients with T2DM and diabetic retinopathy, group 2 included 32 patients with T2DM without retinal damage.

All examined patients underwent determination of serum Hcy concentration using the Cobas 8000 test system (Roche Dianostics), as well as B vitamins: vitamin B1 (thiamine) and vitamin B6 (pyridoxine) using high-performance liquid chromatography using test systems (Recipe complet Kit, Germany); vitamin B9 (folic acid) - using the immunochemical method with electrochemiluminescent detection, using the Roche Diagnostics test system (Switzerland), and vitamin B12 (cyanocobalamin) - using immunochemical chemiluminescent detection, using the Abbot Diagnostics test system (USA).

Patient data analysis and result processing were accomplished using STATISTICA 10.0 statistical software (StatSoft Inc, USA), applying parametric and non-parametric methods for statistical assessment of outcomes.

FRAMEWORK

The study was performed within the framework of the scientific topics "Clinical and Pathogenetic Features of Polymorbid Diseases in the Digestive System and Development of Differentiated Therapy Scheme in the Conditions of the COVID-19 Pandemic" (state registration number 0121U110177) researched by the Department of Propedeutics of Internal Diseases of State University "Uzhhorod National University".

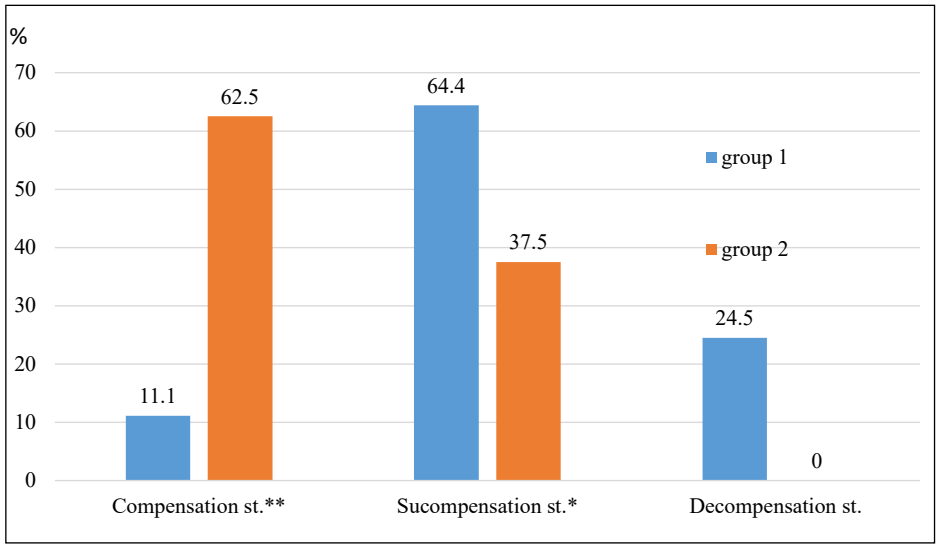

Fig. 1. Severity levels of type 2 diabetes
Note: the difference between the indicators in patients is significant: * - p<0.01; ** - p<0.001
Picture taken by the authors

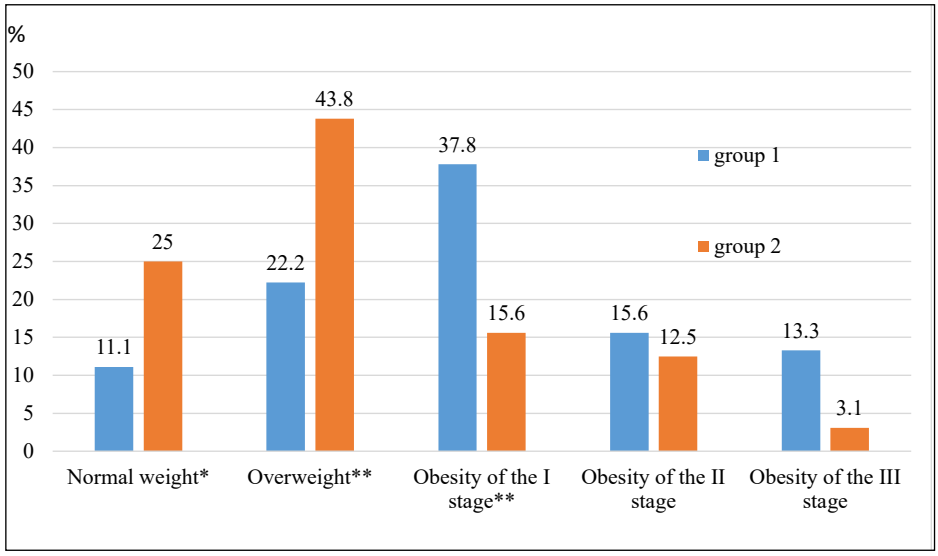

Fig. 2. Changes in BMI in patients with T2DM
Note: the difference between the indicators in patients is significant: * - p<0.05; ** - p<0.01
Picture taken by the authors

RESULTS

Patients with type 2 diabetes are distributed according to the severity of the disease (Fig. 1).

The majority of patients in group 1 had sub- and decompensated stage od diabetes mellitus, while patients in group 2 more often had a compensated stage of the disease.

The distribution of examined patients was carried out depending on the change in BMI (Fig. 2).

Among patients in group 1, people with 1 stage of obesity were more often found (37.8% of cases - p<0.01), while among patients in group 2, people with overweight were more often diagnosed (43.8% of patients, respectively - p<0.01).

Patients in group 1 were divided depending on the severity of retinopathy (Fig. 3).

Proliferative and preproliferative stages of DR were more frequently detected in the examined patients with T2DM (in 44.4% and 26.7% of patients, respectively). Proliferative retinopathy with complications was diagnosed in 17.8% of patients, while nonproliferative retinopathy with maculopathy was diagnosed only 11.1% of cases.

The level of B vitamins and homocysteine in the blood serum of the examined patients with diabetes was assessed (Table 1).

In patients of both groups, a decrease in the levels of B vitamins in the blood serum was detected, which

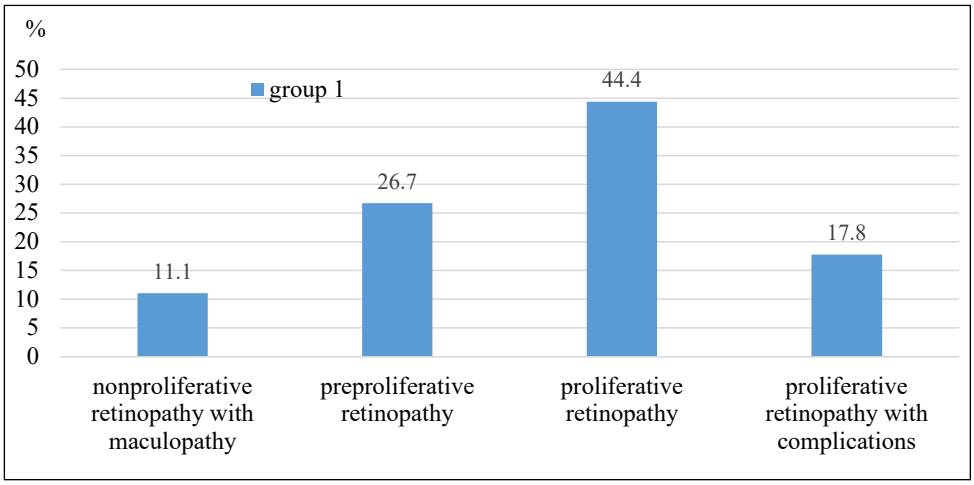

Fig. 3. Stage of DR at patients with T2DM of group 1
Picture taken by the authors

Table 1. Serum levels of B vitamins and homocysteine in the examined persons

Indicator	Reference values	Examined persons		
		Control group (n=30)	group 1 (n= 45)	2 group (n= 32)
Vitamin B1, mkg/l	More than 49.0	89.6±1.3	22.1±1.1++	37.5±0.6+,*
Vitamin B6, mkg/l	8.7-27.2	19.4±0.8	5.4±0.8+	7.6±0.4+,*
Vitamin B9, ng/ml	4.6-18.7	13.1±0,5	1.7±0.3++	3.1±0.6+,**
Vitamin B12, pg/ml	197.0-771.0	463.5±7.7	122.8±4.4++	173.2±5.2+,*
Hcy, mkmoll/l	no more 15.0	8.2±0.4	43.5±0.9++	27.1±0.4+**

Note: the differences between the indicators of the control group and the examined patients are significant: + – $p<0.01$; ++ – $p<0.001$; the difference between the indicators of patients of groups 1 and groups 2 is statistically significant: * – $p<0.05$; ** – $p<0.01$
Source: compiled by the authors of this study

Table 2. Changes in homocysteine levels depending on severity of DR

Stage of retinopathy	Examined persons, group 1 (n= 45)
	Level of Hcy, mkmoll/l
Nonproliferative retinopathy with maculopathy	32.8±1.1
Preproliferative retinopathy	39.9±0.7+
Proliferative retinopathy	51.7±1.5+
Proliferative retinopathy with complications	64.1±2.0+

Note: the differences between the differets stage of DR: + – $p<0.05$
Source: compiled by the authors of this study

was detected against the background of an increase in the level of homocysteine in the blood serum in these patients. It should be noted that more pronounced reliable changes in the examined indicators were mainly in patients of group 1 (patients with T2DM and DR).

For a clearer understanding of the role of homocysteine in the formation of retinal lesions in diabetes, we analyzed its level depending on the phase of DR (Table 2).

The obtained data indicate a progressive increase in serum homocysteine levels depending on the severity and severity of DR of patients with T2DM. It is noteworthy that in patients with nonproliferative retinopathy with maculopathy, the serum homocysteine level is significantly different from that in patients of group 2 without retinal damage ($p<0.05$).

Therefore, the data obtained by us allow us to state the feasibility of assessing serum homocysteine in patients with diabetes for predicting vascular lesions, including DR.

DISCUSSION

Elevated homocysteine (Hcy) levels have a well-established and clear causal relationship with epithelial

damage, leading to ischaemic heart disease. In addition, Hcy is closely related to other components of metabolic syndrome, such as arterial hypertension, which correlates with T2DM [1, 9-11].

DR is becoming an increasingly important cause of visual impairment due to the increasing number of diabetic patients. There is currently no known means to prevent the development of DR. However, there are various known risk factors that influence the progression of the disease. Among the various risk factors known to cause the progression of retinopathy, some are modifiable and others are non-modifiable. Hyperhomocysteinemia is considered a potential risk factor for the development and progression of DR. Homocysteine , an intermediate molecule in methionine metabolism, has attracted considerable interest as a risk factor for cardiovascular disease and other vaso-occlusive diseases, including retinal vascular occlusion. High blood Hcy levels are toxic to the vascular endothelium through the generation of free radicals. Free radicals disrupt the integrity of the endothelium, leading to platelet activation, hypercoagulability, and thrombus formation [12]

Homocysteine and folate metabolism plays an important role in nucleic acid synthesis, amino acid homeostasis, epigenetic maintenance, redox protection, and methylation. This metabolic process is also known as the methionine cycle. When methionine is deficient, Hcy undergoes remethylation. This metabolic pathway requires folate as a methyl group donor for methionine regeneration. The enzyme that plays a key role in this Hcy metabolic pathway is cystathionine β-synthase (CBS), an enzyme that requires vitamin B6 as a cofactor to catalyze the reaction of serine with Hcy to form cystathionine. If the remethylation and/or transsulfuration pathways are impaired, Hcy accumulates in cells and hyperhomocysteinemia develops [13].

Only a few studies have been conducted worldwide to investigate the role of hyperhomocysteinemia in the development of diabetic retinopathy. Vitamin B12 and folate deficiency are associated with increased serum Hcy levels. Therefore, hyperhomocysteinemia may be a potentially modifiable risk factor for the development of diabetic retinopathy [12].

The study by Tawfik A et al. (2019) indicates a correlation between elevated serum level of Hcy and DR in both humans with diabetes and animal models of diabetes. The results obtained by the authors indicate a relationship between elevated serum Hcy levels and increased severity of retinopathy. Therefore, Hcy may be a useful diagnostic marker for screening to predict the incidence and severity of retinal damage in patients with diabetes. In addition, increasing Hcy clearance through pharmacological or genetic manipulations may be a future preventive/therapeutic strategy in the fight against diabetic retinopathy [14].

Our results also indicate high levels of homocysteine in serum in DR at patients with T2DM. At the same time, the analysis conducted indicates the dynamics of homocysteine level depending on the stage of progression and retinal damage in these patients, which allows us to conclude that it is possible to determine the level of homocysteine in these patients to predict the progression or formation of DR for its timely correction.

CONCLUSIONS

In patients with type 2 diabetes and DR, a decrease in the level of B vitamins was found against the background of an increased concentration of homocysteine (43.5±0.9 mkmol/l – $p<0.001$) in the blood serum, which proportionally increases with the progression of retinal damage in these patients.

REFERENCES

1. Platt DE, Hariri E, Salameh P et al. Type II diabetes mellitus and hyperhomocysteinemia: a complex interaction. Diabetol Metab Syndr. 2017;9:19. doi: 10.1186/s13098-017-0218-0. DOI
2. Cho NH, Shaw JE, Karuranga S et al. IDF Diabetes Atlas: Global estimates of diabetes prevalence for 2017 and projections for 2045. Diabetes Res Clin Pract. 2018;138:271–81. doi: 10.1016/j.diabres.2018.02.023. DOI
3. Shukla UV, Tripathy K. Diabetic Retinopathy. Treasure Island (FL): StatPearls Publishing. 2025. https://www.ncbi.nlm.nih.gov/books/NBK560805/ [Accessed 27 June 2025]
4. Wang Y, Lin Z, Zhai G et al. Prevalence of and Risk Factors for Diabetic Retinopathy and Diabetic Macular Edema in Patients with Early- and Late-Onset Diabetes Mellitus. Ophthalmic Res. 2022;65(3):293-299. doi: 10.1159/000508335. DOI
5. Moutray T, Evans JR, Lois N et al. Different lasers and techniques for proliferative diabetic retinopathy. Cochrane Database Syst Rev. 2018;3(3):CD012314. doi: 10.1002/14651858.CD012314.pub2. DOI
6. Kern TS, Antonetti DA, Smith LEH. Pathophysiology of Diabetic Retinopathy: Contribution and Limitations of Laboratory Research. Ophthalmic Res. 2019;62(4):196-202. doi: 10.1159/000500026. DOI
7. Wang W, Lo ACY. Diabetic Retinopathy: Pathophysiology and Treatments. Int J Mol Sci. 2018;19(6):1816. doi: 10.3390/ijms19061816. DOI

8. Coughlin BA, Feenstra DJ, Mohr S. Müller cells and diabetic retinopathy. Vision Res. 2017;139:93-100. doi: 10.1016/j.visres.2017.03.013. DOI
9. Kundi H, Kiziltunc E, Ates I et al. Association between plasma homocysteine levels and end-organ damage in newly diagnosed type 2 diabetes mellitus patients. Endocr Res. 2017;42(1):36-41. doi: 10.3109/07435800.2016.1171235. DOI
10. Real JT, Folgado J, Molina Mendez M et al. Homocisteína plasmática, Lp(a) y marcadores de estrés oxidativo en la vasculopatía periférica del paciente con diabetes tipo 2 [Plasma homocysteine, Lp(a), and oxidative stress markers in peripheral macroangiopathy in patients with type 2 diabetes mellitus]. Clin Investig Arterioscler. 2016;28(4):188-194. doi: 10.1016/j.arteri.2016.05.007. (Spanish) DOI
11. Arroyo-Jousse V, García-Díaz DF, Pérez-Bravo F. La metilación global del ADN y los niveles de homocisteína en plasma se encuentran disminuidos en pacientes con diabetes mellitus tipo 1 [Global DNA methylation and homocysteine levels are lower in type 1 diabetes patients]. Rev Med Chil. 2015;143(5):562-8. doi: 10.4067/S0034-98872015000500002. (Spanish) DOI
12. Gupta P, John D, Rebekah G, John SS. Role of hyperhomocysteinemia in proliferative diabetic retinopathy: A case-control study. Indian J Ophthalmol. 2018;66(10):1435-1440. doi: 10.4103/ijo.IJO_350_18. DOI
13. Cheng Y, Wang C, Zhang X et al. Circulating homocysteine and folate concentrations and risk of type 2 diabetes: A retrospective observational study in Chinese adults and a Mendelian randomization analysis. Front Cardiovasc Med. 2022;9:978998. doi: 10.3389/fcvm.2022.978998. DOI
14. Tawfik A, Mohamed R, Elsherbiny NM et al. Homocysteine: A Potential Biomarker for Diabetic Retinopathy. *J Clin Med*. 2019;8(1):121. doi: 10.3390/jcm8010121. DOI

CONFLICT OF INTEREST

The Authors declare no conflict of interest

CORRESPONDING AUTHOR

Yelyzaveta S. Sirchak
Uzhhorod national university
1 Narodna Sqr., 88000 Uzhhorod, Ukraine
e-mail: sirchakliza777@gmail.com

ORCID AND CONTRIBUTIONSHIP

Yelyzaveta S. Sirchak: 0000-0001-6738-0843 A F
Aliona V. Stoika: 0009-0006-3031-685X B C D
Erica S. Dobryanska (Opalenyk): 0000-0001-6014-1110 D
Nelli V. Bedey: 0000-0002-8885-0258 B C
Ivan I. Pushkash: 0000-0003-4651-4608 B
Yuliia V. Holosh: 0000-0001-8516-0545 B

A – Work concept and design, B – Data collection and analysis, C – Responsibility for statistical analysis, D – Writing the article, E – Critical review, F – Final approval of the article

RECEIVED: 11.05.2025
ACCEPTED: 20.09.2025