Research Square

Preprints are preliminary reports that have not undergone peer review.
They should not be considered conclusive, used to inform clinical practice,
or referenced by the media as validated information.

Plasma Homocysteine as a Biomarker of Diabetic Retinopathy in Type 1 Diabetes: A Case–Control Study

Marwa chiboub

marwa.chiboub@yahoo.com

National Institute of Nutrition and Food Technology , Tunis

Asma Ben Hamida

National Institute of Nutrition and Food Technology , Tunis

Meriem Adel

National Institute of Nutrition and Food Technology , Tunis

Kaouther Derouich

National Institute of Nutrition and Food Technology

Chiraz Amrouch

National Institute of Nutrition and Food Technology

Emna Talbi

National Institute of Nutrition and Food Technology

Houda Bouhajja

National Institute of Nutrition and Food Technology

Omar Fekih

National Institute of Nutrition and Food Technology

Manel Jemel

National Institute of Nutrition and Food Technology , Tunis

Ines Kammoun

National Institute of Nutrition and Food Technology , Tunis

Article

Keywords: Biomarker, diabetic retinopathy, glycated hemoglobin, homocysteine, type 1 diabetes mellitus

Posted Date: July 30th, 2026

DOI: https://doi.org/10.21203/rs.3.rs-10366737/v1

License: This work is licensed under a Creative Commons Attribution 4.0 International License.
Read Full License

Additional Declarations: No competing interests reported.

Abstract

Background.

Diabetic retinopathy (DR) is a major complication of diabetes mellitus and a leading cause of visual impairment in adults. The study aimed to evaluate Hcy as a potential biomarker for DR in type 1 diabetes mellitus (T1DM) and investigate its association with DR severity.

Methods.

A case–control study included adult patients with T1DM, stratified into two groups according to the presence (DR group, n = 60) or absence (non-DR group, n = 58) of DR. Plasma Hcy, folate, and vitamin B12, creatinine, urinary microalbumin, and glycated hemoglobin were measured using automated methods. Estimated glomerular filtration rate (eGFR) was calculated according to the CKD-EPI formula. Multivariate analyses were performed to determine independent predictors for DR and plasma Hcy.

Results.

Diabetes duration, HbA1c, microalbuminuria, and folate were higher, whereas eGFR was lower in the DR group. Plasma Hcy was higher in the DR group than in the non-DR group (12.6 ± 5.40 *vs.* 9.51 ± 3.58 µmol/L; p < 0.001), and hyperhomocysteinemia was more prevalent in the DR group than the non-DR group (25% *vs*. 10.3%; p = 0.037); mean Hcy did not differ between the NPDR and PDR subgroups, but HHC was more prevalent in the PDR subgroup than the NPDR subgroup (33.3% *vs*. 16.7%; p = 0.041). In multivariate analysis, Plasma Hcy was increased in the DR group independently of diabetes duration and control, and renal function [OR (95% CI), 1.13 (1.022–1.257); p = 0.018]. Glycated hemoglobin, microalbuminuria, and eGFR were the other independent predictors for DR in T1DM patients.

Conclusions.

The findings support plasma Hcy as a clinically relevant biomarker for DR screening and suggest that targeting its metabolism may be a therapeutic option. Prospective studies and clinical trials are needed to confirm the results.

Background

Diabetes mellitus is one of the most significant public health challenges of the 21st century. As of 2024, an estimated 589 million individuals aged 20 to 79 years, corresponding to 11.1% of the global adult population, are living with diabetes [1]. Projections from the International Diabetes Federation indicate that this figure will rise to approximately 852.5 million (13%) by 2050, underscoring the urgency of effective prevention and management of complications [1]. Type 1 diabetes mellitus (T1DM), which

accounts for 5–10% of all diabetes cases, results from autoimmune-mediated destruction of pancreatic beta cells [2]. Its clinical significance is largely attributable to microvascular and macrovascular complications, which develop insidiously and are frequently diagnosed at advanced stages. Among these, diabetic retinopathy (DR) stands out as a neurovascular complication specific to diabetes and the leading cause of blindness in individuals aged 20 to 74 years in high-income countries [3]. Contemporary studies report a prevalence of DR in patients with T1DM after 15–20 years of disease duration, ranging from 60% to 90%, depending on glycemic control and the population [4].

The global burden of DR is growing rapidly, from an estimated 103.12 million cases in 2020 to 129.84 million in 2030, and 160.5 million in 2045 [5]. In response to this burden, the American Diabetes Association (ADA) 2025 guidelines recommend that patients with T1DM undergo a comprehensive ophthalmological examination within five years of diagnosis, followed by annual monitoring in those without retinal disease [2]. The pathophysiology of DR is complex and multifactorial [6], involving well-established risk factors, such as chronic hyperglycemia, hypertension, hyperlipidemia, obesity, and diabetes duration [2]. However, some diabetics develop retinopathy, whereas others do not. Because known environmental factors do not fully explain this, growing attention has been directed toward emerging biomarkers, including C-reactive protein, interleukin-6, and homocysteine, as potential contributors to retinal microvascular injury [7].

Homocysteine (Hcy) is a non-proteinogenic sulfur-containing amino acid produced through demethylation of methionine. Its metabolism proceeds via two pathways: remethylation to methionine, which depends on folate and vitamin B12, and transsulfuration to cystathionine, which requires vitamin B6 [8]. Hyperhomocysteinemia (HHC) has been estimated to affect 5–10% of the general population and up to 30% of elderly individuals [9]. Its deleterious vascular effects are well established, encompassing endothelial injury, platelet activation, and promotion of a prothrombotic state [10]. Both thrombotic risk and cardiovascular risk [10] have been linked to elevated Hcy, and associations with neurodegenerative diseases such as Alzheimer's disease [11] and retinal microvascular injury [12] have also been described. These vascular properties provide a strong biological rationale for examining Hcy in DR, which is a microvascular complication driven by endothelial dysfunction and oxidative stress. Several studies have examined the relationship, showing higher Hcy concentrations in patients with retinopathy and associations with disease severity [13, 14]. A recent comprehensive review further delineated the multifaceted role of Hcy in disrupting the retinal neurovascular unit, encompassing endothelial cells, neurons, glial cells, and the retinal pigment epithelium, through a synergistic interplay of oxidative stress, inflammation, mitochondrial dysfunction, and epigenetic dysregulation [15]. The available evidence nevertheless remains inconsistent, and it is still unclear whether elevated Hcy actively contributes to retinal injury or merely reflects coexisting metabolic or renal dysfunction [16]. This uncertainty is compounded by the predominance of data from type 2 diabetes (T2DM) and from European, North American, and Asian cohorts. Studies focusing specifically on T1DM remain limited and have yielded conflicting results, in part due to the confounding effects of nephropathy [17–20]. Data on the Hcy–DR relationship in T1DM patients from African and Mediterranean populations are scarce. We therefore hypothesized that elevated plasma Hcy levels are associated with the presence and/or severity of DR in

patients with T1DM, independently of established risk factors and renal function. Accordingly, the primary objective of this study was to evaluate the association between plasma Hcy concentrations and DR in patients with T1DM. The secondary objective was to determine the optimal Hcy threshold for DR screening in T1DM patients.

Methods

Study design and setting

This was a single-centre, hospital-based case–control study conducted over two years (June 2023 to June 2025) in the Department of Endocrinology at the National Institute Zouhair Kallel of Nutrition and Food Technology, Tunis, Tunisia.

Study population

Outpatients with T1DM, diagnosed and treated in the department, with a minimum disease duration of 5 years, were eligible for inclusion. T1DM diagnosis was based on ADA 2025 criteria [2], including onset before age 35, body mass index (BMI) below 25 kg/m², history of unintentional weight loss, inaugural diabetic ketoacidosis, or plasma glucose exceeding 360 mg/dL (20 mmol/L) at diagnosis. Although pancreatic autoantibody testing is a strong predictor of T1DM, it was not routinely required owing to limited local availability. Non-inclusion criteria were pregnancy; systemic or ocular conditions susceptible to impact retinal vasculature or neural function (including arterial hypertension, glaucoma, age-related macular degeneration, peripheral neuropathy, and end-stage renal disease); prior ophthalmological or bariatric surgery; malabsorptive disorders; and use of medications known to interfere with Hcy or B-vitamin metabolism (including phenytoin, carbamazepine, nitrous oxide, metformin, methotrexate, cholestyramine, or vitamin supplements). Patients with incomplete investigation, inadequate cooperation, or withdrawn consent were excluded from the analysis. The patients were divided into two groups: those with confirmed DR on ophthalmological examination (DR group) and those with no evidence of DR (non-DR group). The two groups were matched one-to-one for sex and age. The local ethics committee approved the study protocol, and all participants provided written informed consent. The study adheres to the ethical principles of the Declaration of Helsinki for medical research involving human subjects.

Clinical and ophthalmological assessments

Data were collected through structured patient interviews and a review of medical records. A comprehensive ophthalmological examination was performed for all participants, including assessment of visual acuity, intraocular pressure, lens examination, and fundus examination of the retina, optic disc, macula, and blood vessels. Optical coherence tomography imaging was performed when clinically

indicated. This examination allowed classifying patients without DR (non-DR), and having non-proliferative DR (NPDR), or proliferative DR (PDR).

Blood sampling and analytical methods

Blood samples were collected in EDTA-containing tubes and anticoagulant-free tubes following a 12-hour overnight fast. The tubes were immediately placed on melting ice and then centrifuged at 4000 rpm on a refrigerated centrifuge. Aliquots of plasma and serum were stored at − 80°C for the assessment of Hcy, folate, and vitamin B12. Another aliquot of serum was used for routine biochemical analyses.

Plasma Hcy was measured using an enzymatic assay kit (Diazyme Laboratories, Inc., CA) on a Beckman Coulter AU480 analyzer (Beckman Coulter, Fullerton, CA). The inter-series CV of the method was 12.2%. HHC was defined as plasma Hcy above 15 µmol/L [9, 10]. Serum folate and vitamin B12 concentrations were determined by electrochemiluminescence immunoassay methods using the Cobas e411 autoanalyzer system and the respective reagent kits (Roche Diagnostics, GmbH, Mannheim, Germany). The inter-series CVs for the methods were below 12%. Metabolic parameters, including fasting plasma glucose, serum creatinine, urea, total cholesterol (TC), HDL cholesterol (HDL-C), triglycerides, and 24-hour urinary microalbumin, were measured on a DxC 700 AU (Beckman Coulter, Fullerton, CA). Glycated hemoglobin (HbA1c) was measured in whole blood by capillary electrophoresis using the Capillarys 3 Tera system (Sebia, Lisses, France). All analyses were performed according to standard laboratory procedures, with daily internal quality control and monthly external quality assessment. The analytical performance of the methods fits international standards. LDL cholesterol (LDL-C) was calculated using the Friedewald formula [21]. The estimated glomerular filtration rate (eGFR) was calculated according to the CKD-EPI formula [22]. Diabetic nephropathy is defined as positive microalbuminuria, i.e., urinary microalbumin > 30 mg/24H.

Statistical analysis

Statistical analyses were performed using IBM SPSS Statistics, version 26 (SPSS, Inc., Chicago, IL), and figures were generated using GraphPad Prism 9 software (GraphPad Software, Boston, MA). Continuous variables were assessed for normal distribution using the Shapiro–Wilk test. The variables were compared using Student's independent t-test or the Mann–Whitney U test, as appropriate. Categorical variables were compared using Pearson's chi-square test or Fisher's exact test, as appropriate. Receiver operating characteristic (ROC) analysis was performed to explore the efficacy of plasma Hcy in discriminating DR and to determine its optimal cutoff value for DR. A binary logistic regression model with backward stepwise selection was applied to identify independent predictors of DR. The model was adjusted for age, diabetes duration, HbA1c, eGFR, urinary microalbumin, and plasma Hcy, vitamin B12, and folate concentrations as continuous variables, and sex (male/female) and tobacco smoking (yes/no) as categorical variables. Multi-adjusted odds ratios (ORs) with their 95% confidence intervals (CIs) were reported. A multiple linear regression model was applied to test whether the association between Hcy and DR is independent of confounding factors. The model comprised plasma Hcy as the

response variable, and sex, age, BMI, tobacco smoking, diabetes duration, HbA1c, positive microalbuminuria (> 30 mg/24H), eGFR, plasma folate and vitamin B12, and DR as covariates. A two-tailed p-value < 0.05 was considered significant.

Several measures were taken to limit potential sources of bias. Cases and controls were recruited from the same source population and matched one-to-one for age and sex. Conditions and medications known to interfere with homocysteine or B-vitamin metabolism were pre-specified non-inclusion criteria to limit confounding. Diabetic retinopathy was graded by ophthalmologists according to standardised clinical criteria.

The study is reported in accordance with the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement for case–control studies (Additional file 1).

Results

Of the 120 patients enrolled, two lacked biochemical assessment due to defective plasma samples and were excluded from the analysis. Finally, 118 patients were enrolled: 60 in the DR group and 58 in the non-DR group. Among the DR group, 30 patients had NPDR, and 30 others had PDR. No significant adverse events or protocol withdrawals were recorded beyond the above-mentioned exclusions. The main clinical and laboratory characteristics of both groups are presented in Table 1. There were no significant differences between the two groups regarding age, sex, body composition, plasma lipids, plasma vitamin B12, and smoking status. However, diabetes duration, HbA1c, urinary microalbumin, plasma folate, and the frequency of nephropathy were higher, whereas eGFR was lower in the DR group.

Table 1
Characteristics of patients with type 1 diabetes with and without diabetic retinopathy (n = 118).

Age, years	**Non-DR group (n = 58)**	**DR group (n = 60)**	**p-value**
	30.8 ± 8.12	**32.9 ± 7.80**	**0.153**
Female gender, count (%)	35 (60.3)	35 (58.3)	0.824
Tobacco smoking, count (%)	16 (27.6)	16 (26.7)	0.911
Diabetes duration, years	15.3 ± 4.32	17.1 ± 3.45	0.015
Body mass index, kg/m²	24.5 ± 4.68	23.8 ± 4.04	0.438
Systolic blood pressure, mm Hg	118 ± 10.1	117 ± 9.97	0.758
Diastolic blood pressure, mm Hg	70.3 ± 10.3	70.7 ± 8.21	0.851
Glycated hemoglobin, %	8.40 ± 0.94	8.89 ± 0.83	0.003
eGFR, mL/min/1.73 m²	119 ± 17.2	107 ± 20.1	< 0.001
Urinary microalbumin, mg/24H	5.00 (3.00–8.25)	19.0 (5.25–33.0)	< 0.001
Diabetic nephropathy [a], count (%)	4 (6.90%)	16 (26.7%)	0.004
Total cholesterol, mmol/L	4.52 ± 0.74	4.50 ± 0.90	0.920
Triglycerides, mmol/L	0.85 ± 0.35	0.89 ± 0.34	0.592
Folate, µg/L	7.14 ± 3.56	8.53 ± 3.67	0.039
Vitamin B12, pg/ml	378 ± 126	481 ± 435	0.087
Plasma homocysteine, µmol/L	9.51 ± 3.58	12.6 ± 5.40	< 0.001
Values were expressed as mean ± standard deviation, median (25th -75th percentile), or as count (percentage)			
eGFR, estimated glomerular filtration rate; DR, diabetic retinopathy; [a], urinary microalbumin > 30 mg/24 hours.			

Plasma Hcy concentrations were significantly higher in the DR group than in the non-DR group, whereas no significant difference was observed between the NPDR and PDR subgroups (Fig. 1). In contrast, the prevalence of HHC was higher in the DR group than in the non-DR group (25% *vs*. 10.3%; p = 0.037), and in the PDR subgroup than in the NPDR subgroup (33.3% *vs*. 16.7%; p = 0.041). Neither plasma Hcy nor HHC rate differed by nephropathy in all T1DM patients or in the DR group. ROC analysis identified a plasma Hcy concentration of 10.7 µmol/L as a threshold that differentiates DR patients from non-DR patients [AUC (95% CI), 0.685 (0.589–0.781); p = 0.001]. At this cutoff, the test demonstrated a sensitivity of 57% and a specificity of 77% (Fig. 2). A binary regression model adjusting for potential

confounders confirmed plasma Hcy as an independent predictor of DR [multi-adjusted OR (95% CI), 1.133 (1.022–1.257); p = 0.018]. In addition to plasma Hcy, eGFR, glycated hemoglobin, and urinary microalbumin were identified as independent risk factors for DR in T1DM patients (Table 2). The multiple linear regression model showed that plasma Hcy is associated with DR, independently of age, sex, body mass index, diabetes duration, tobacco smoking, HbA1c, eGFR, microalbuminuria, and vitamin B12 and folate concentrations. Besides DR, sex and eGFR were independent predictors of plasma Hcy in these patients (Table 3).

Table 2
Independent predictors of diabetic retinopathy in patients with type 1 diabetes (n = 118).

Homocysteine	**β coefficient**	**SE**	**P-value**	**OR (95% CI)[a]**
	0.125	**0.053**	**0.018**	**1.133 (1.022–1.257)**
Glycated hemoglobin	0.835	0.271	0.002	2.305 (1.354–3.923)
eGFR	-0.035	0.014	0.010	0.965 (0.939–0.992)
Urinary microalbumin	0.038	0.017	0.022	1.039 (1.006–1.074)
Constant	-5.203	2.649	0.050	-

Backward stepwise binary logistic regression. eGFR, estimated glomerular filtration rate; 95% CI, 95% confidence interval; OR, odds ratio; SE, standard error.

[a], multi-adjusted odds ratio (95% confidence interval). Covariates entered in the model at the first step were age, sex, body mass index, diabetes duration, tobacco smoking, glycated hemoglobin, eGFR, urinary microalbumin, plasma homocysteine, vitamin B12, and folate concentrations.

Table 3
Independent determinants of plasma homocysteine in patients with type 1 diabetes (n = 118).

Diabetic retinopathy	**β coefficient**	**Standard error**	**p-value**
	2.224	**0.891**	**0.014**
Sex	-3.191	0.865	< 0.001
Age	0.057	0.061	0.350
Body mass index	-0.166	0.099	0.097
Tobacco smoking	0.052	0.952	0.957
Diabetes duration	-0.014	0.113	0.903
Glycated hemoglobin	0.079	0.447	0.860
Estimated glomerular filtration rate	-0.066	0.023	0.005
Positive microalbuminuria	0.575	1.103	0.603
Folate	-0.148	0.115	0.201
Vitamin B12	-0.001	0.001	0.480
Constant	25.672	5.967	0.000

Discussion

The present study showed that plasma Hcy is increased in T1DM patients with DR. Although mean Hcy concentrations did not differ between the NPDR and PDR subgroups, hyperhomocysteinemia was twice as frequent in patients with proliferative disease, suggesting that the association with disease severity may be driven by the proportion of patients crossing the pathological threshold rather than by a graded increase in mean concentrations. The association between plasma Hcy and DR remained significant after adjusting for the main potential confounders, including diabetes duration and control, vitamin B status, and renal function. In multivariate analysis, each 1 µmol/L increase in plasma Hcy is associated with a 13% increase in DR risk in T1DM patients. These results support the growing body of evidence involving Hcy as a clinically meaningful biomarker and maybe a risk factor in diabetic microvascular disease.

Several studies examined the relationship between Hcy and DR, most of which focused on T2DM and often reported a positive association. The association of Hcy and DR in T1DM has been less investigated, and the findings are inconsistent. Li et al. [23] found an association between Hcy and DR risk that disappeared after adjustment for confounding factors. In T1DM patients of ≥ 10 years' duration, plasma Hcy levels were higher in those with PDR than those without DR, independent of confounders and coexisting conditions (i.e., cardiovascular disease, impaired renal function, vitamin status, and blood

pressure) [17]. Bulum et al. [18] found that plasma Hcy is independently associated with retinopathy in normo-albuminuric T1DM with normal eGFR. Agardh et al. [19] found no significant difference in Hcy levels between T1DM patients with and those without DR. In a multicentric study across 13 European countries, including 533 T1DM patients, DR was associated with higher Hcy levels, but the association was dependent on albuminuria and eGFR [20]. A meta-analysis of 31 studies including 6394 diabetic patients demonstrated a relationship between elevated Hcy levels and DR risk, which was stronger in T1DM [OR (95% CI) = 1.83 (1.28–2.62)] than T2DM [1.59 (0.72–3.51)] [13]. A second meta-analysis concluded that plasma Hcy is higher in T1DM patients with DR compared with those without complications [24]. Another meta-analysis concluded that no association exists between increased Hcy levels and increased risk of DR in T1DM patients [1.27 (0.93–1.75); p = 0.134] [14]. Discrepancies may result from heterogeneity of study populations, differences in methodological approaches, and confounders such as renal function, B vitamin status, and genetic background.

DR arises from chronic hyperglycemia-driven metabolic disturbances that progressively impair the structure and function of the retinal microvasculature. Key pathogenic pathways include activation of the polyol pathway, formation of advanced glycation end-products, activation of protein kinase C, and overload of the hexosamine pathway [23]. These mechanisms collectively promote oxidative stress and inflammation and disrupt the inner blood–retinal barrier. Concomitantly, hyperglycemia induces epigenetic modifications that sustain the expression of pro-inflammatory and pro-apoptotic genes [23], contributing to the dual phenotype of diabetic retinopathy- i.e., increased vascular permeability in the central retina and occlusive ischemia in the peripheral retina, leading to pathological neovascularization. Several mechanisms directly relevant to the microvasculature may explain homocysteine's association with DR. Beyond its recognized systemic vascular toxicity, elevated Hcy promotes oxidative stress, decreases nitric oxide bioavailability, and thereby impairs endothelial function, upregulates VEGF and favors neovascularization, and activates NF-κB signaling with downstream release of TNF-α and IL-6 [25]. It also fosters a prothrombotic state that can lead to microvascular occlusion [26]. More recently, mitochondrial dysfunction [27, 28] and epigenetic silencing of homocysteine-clearing enzymes such as CBS, CSE, and MTHFR [12] have been implicated, suggesting that Hcy may sustain a self-amplifying cycle of retinal injury. These mechanisms provide a biologically plausible framework, suggesting that HHC may contribute to DR.

While Hcy toxicity in the microvasculature is well established, the mechanisms underlying the increase in plasma Hcy in DR are poorly understood. Several conditions could disrupt Hcy breakdown and increase its circulating levels. These include genetic or acquired defects in enzymes such as methionine synthase (MS), cystathionine β-synthase (CBS), cystathionine-γ-lyase (CSE), and methylenetetrahydrofolate reductase (MTHFR), nutritional deficiencies in B vitamins, impaired renal function, illnesses like hypertension, obesity, hypothyroidism, and medications such as methotrexate and proton pump inhibitors [8]. It also established that Hcy levels are higher in males, smokers, and elderly people [8]. In the current study, these factors are unlikely to solely explain the plasma Hcy rise in DR. Indeed, sex, age, body composition, blood pressure, smoking rate, or plasma lipids did not differ between DR and non-DR groups. Furthermore, B-vitamin deficiency cannot account for the higher homocysteine levels observed

in the DR group: plasma folate was in fact significantly higher in this group, and vitamin B12 concentrations did not differ significantly between groups. Moreover, no patient suffered from hypertension, obesity, or overt hypothyroidism, or was taking drugs known to affect Hcy metabolism. Even though the two groups differed in diabetes duration, HbA1c, microalbuminuria, and eGFR, the two regression analyses applied in this study indicated that increased Hcy in DR is independent of these factors. In line with our findings, Brazionis et al. [29] found that the relationship between Hcy and DR in T2DM is independent of major risk factors for DR (i.e., duration of diabetes, HbA1c, and blood pressure) and determinants of higher Hcy (i.e., age, sex, renal function, and folate). This data suggests that factors other than those mentioned above may be driving plasma Hcy elevation in DR. Defects in the genes of remethylation and transsulfuration enzymes could partly explain the association between Hcy and DR. Vaccaro et al. [17] reported higher frequency of homozygosity for the C677T mutation in the MTHFR gene, a common genetic defect associated with moderate HHC, in patients with PDR and/or microalbuminuria than in healthy controls (50% vs 13%, $p < 0.004$). Likewise, the MTHFR C677T allele frequency was noticeably higher in T1DM patients with PDR (75%) than in those with NPDR (38%) and DR-free ones (30%) [17]. A meta-analysis of 18 studies, including 1747 DR and 3146 DR-free type 1 and 2 diabetic patients, linked the MTHFR 677C/T variant to the risk of developing DR [30]. Another meta-analysis of 23 studies, including 2707 DR patients and 4264 controls, concluded a strong association between the MTHFR C677T polymorphism and DR [31]. Furthermore, Kowluru et al. [12] found that the enzymes responsible for Hcy breakdown, including CBS, CSE, and MTHFR, were 40–60% less active in the retinal microvasculature of patients with DR. These data support a Hcy-related genetic susceptibility to DR. Other genes controlling inflammation, angiogenesis, and neurogenesis are likely involved in DR etiopathogenesis [32, 33].

The study established a plasma Hcy cutoff of 10.7 μmol/L for detecting DR in T1DM. This suggests that DR risk initiates at concentrations below the conventional threshold of 15 μmol/L. In agreement, studies conducted in diabetic populations have often highlighted an increased risk of DR starting at concentrations of 13.7 μmol/L [34], 10 μmol/L [35], and 7.7 μmol/L [36]. In the current study, the threshold of 10.7 μmol/L was associated with modest sensitivity and specificity, making plasma Hcy a fairly diagnostic biomarker of DR. However, it could be useful for screening PDR in T1DM patients.

The study ascertained eGFR, microalbuminuria, and HbA1c as independent predictors for DR, confirming the role of altered renal function, associated nephropathy, and poor diabetes control as major determinants of DR [37]. Specifically, a 1% increase in HbA1c was associated with more than a twofold higher risk of DR. However, sex, smoking status, BMI, diabetes duration, blood pressure, and plasma lipids, which are recognized as risk factors for DR [10, 11, 19, 37–39], did not arise as independent predictors for DR in this series. The lack of independent association for these variables might be due to a tight matching of the groups for age and sex, as well as the small variation in body size and diabetes duration in our cohort. Given their role as cofactors for Hcy remethylation, folate and vitamin B12 were evaluated. Paradoxically, plasma folate was significantly higher in the DR group, while vitamin B12 concentrations tended to be higher without reaching statistical significance. This contrasts with studies reporting reduced B-vitamin levels in patients with DR [7, 40]. Importantly, mean concentrations of both

vitamins were within the normal range in the two groups, and no patient presented with biochemical deficiency. These findings therefore reflect the absence of clinical deficiency in our cohort rather than a genuine protective effect, and indicate that the elevation of plasma homocysteine in the DR group is not attributable to impaired B-vitamin status. The well-established metabolic link between B-vitamin deficiency and HHC [15] offers a therapeutic avenue for DR. A recent review identified B-vitamin supplementation alongside betaine and other homocysteine-lowering strategies as promising targets, while emphasizing that definitive evidence from randomized controlled trials remains lacking [15].

The study has limitations that warrant consideration. The single-center design may limit the generalizability of the findings. The relatively modest sample size may have reduced the statistical power to detect associations of smaller magnitude. Nevertheless, recruiting 120 patients with T1DM, with and without DR, while matching the two groups for age and sex, is challenging. On the other hand, our sample size is comparable to or larger than previous studies addressing the topic in type 1 diabetes. In particular, the comparison between the NPDR and PDR subgroups (n = 30 each) was likely underpowered to detect a modest graded effect, and the absence of a difference in mean plasma Hcy between these subgroups, despite a higher prevalence of hyperhomocysteinemia in PDR, should be interpreted with caution. The case–control design, in which exposure and outcome were assessed concurrently, precludes any causal inference. Thus, it remains unclear whether the elevation in plasma Hcy contributes to DR or results from it. Although substantial evidence supports that Hcy might contribute to DR, high Hcy levels may result from microvascular damage or shared pathogenic pathways. Therefore, reverse or bidirectional relationships cannot be excluded. Long-term prospective studies or clinical trials are needed to clarify the association. Finally, our study did not include a genetic evaluation of Hcy-related pathways, including relevant polymorphisms (e.g., MTHFR C677T) and the expression of key metabolic enzymes. Further research is needed to explore these pathways, which may help elucidate Hcy involvement in DR. Despite limitations, the study demonstrates that Hcy is associated with DR in patients with T1DM. These findings may be extrapolated to populations sharing environmental and genetic characteristics with the Tunisian population but require confirmation in diverse ethnic and geographic settings.

Conclusions

This study provides evidence for a significant and independent association between elevated plasma homocysteine and diabetic retinopathy in patients with type 1 diabetes mellitus, which persisted after adjustment for traditional risk factors, including glycemic control and renal function. HHC was more prevalent among patients with DR and tended to be associated with more advanced, proliferative stages. A plasma Hcy cutoff value of 10.7 µmol/L discriminates DR with moderate performance, suggesting that Hcy may serve as a useful complementary biomarker for risk stratification rather than as a stand-alone screening test. Larger prospective cohorts are needed to validate plasma Hcy as a reliable DR biomarker, and well-designed randomized controlled trials, informed by lessons from cardiovascular research, are required to establish HHC as a modifiable risk factor and to determine whether homocysteine-lowering interventions, including B-vitamin supplementation, can improve retinal outcomes in T1DM.

Abbreviations

ADA
American Diabetes Association
AUC
area under the curve
BMI
body mass index
CBS
cystathionine β-synthase
CI
confidence interval
CKD-EPI
Chronic Kidney Disease Epidemiology Collaboration
CSE
cystathionine-γ-lyase
CV
coefficient of variation
DR
diabetic retinopathy
eGFR
estimated glomerular filtration rate
HbA1c
glycated haemoglobin
Hcy
homocysteine
HDL-C
high-density lipoprotein cholesterol
HHC
hyperhomocysteinaemia
IL-6
interleukin-6
LDL-C
low-density lipoprotein cholesterol
MTHFR
methylenetetrahydrofolate reductase
NF-κB
nuclear factor kappa B
NPDR
non-proliferative diabetic retinopathy

OR
odds ratio
PDR
proliferative diabetic retinopathy
ROC
receiver operating characteristic
SD
standard deviation
SE
standard error
T1DM
type 1 diabetes mellitus
T2DM
type 2 diabetes mellitus
TC
total cholesterol
TNF-α
tumour necrosis factor alpha
VEGF
vascular endothelial growth factor.

Declarations

Ethics approval and consent to participate

The study protocol was approved by the Ethics Committee of the National Institute of Nutrition and Food Technology, Tunis, Tunisia (approval number: 22/2023, date: 25/05/2023). The study was conducted in accordance with the ethical principles of the Declaration of Helsinki. Written informed consent was obtained from all participants prior to inclusion.

Consent for publication

Not applicable. The manuscript does not contain any individual person's identifiable data, images or videos.

Availability of data and materials

The datasets generated and analysed during the current study are not publicly available in order to protect participant privacy, but are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

Funding

This study was supported by the Research Committee of the National Institute of Nutrition and Food Technology, Tunis, Tunisia. The funding body had no role in the design of the study, in the collection, analysis and interpretation of the data, or in the writing of the manuscript.

Authors' contributions

MC and IK conceived and designed the study. MC, ABH and MA recruited the patients and collected the clinical data. ET, HB performed the biochemical analyses. KD and CA performed the ophthalmological examinations and graded diabetic retinopathy. OF and MJ performed the statistical analysis and interpreted the data. MC drafted the manuscript. IK, CA and MJ critically revised the manuscript for important intellectual content. All authors read and approved the final manuscript.

Acknowledgements

Not applicable.

Authors' information (optional)

Not applicable.

References

1. Duncan, B. B., Magliano, D. J. & Boyko, E. J. IDF Diabetes Atlas 11th edition 2025: global prevalence and projections for 2050. *Nephrol. Dial Transpl.* **41** (1), 7–9. 10.1093/ndt/gfaf177 (2025).
2. American Diabetes Association Professional Practice Committee. 2. Diagnosis and Classification of Diabetes: Standards of Care in Diabetes-2025. Diabetes Care. ;48(1 Suppl 1):S27-S49. (2025). 10.2337/dc25-S002
3. American Diabetes Association Professional Practice Committee. 12. Retinopathy, Neuropathy, and Foot Care: Standards of Care in Diabetes-2025. *Diabetes Care*. **48** (1 Suppl 1), S252–S265. 10.2337/dc25-S012 (2025).
4. Diabetes Control and Complications Trial (DCCT)/Epidemiology of Diabetes Interventions and Complications (EDIC) Research Group et al. Effect of intensive diabetes therapy on the progression of diabetic retinopathy in patients with type 1 diabetes: 18 years of follow-up in the DCCT/EDIC. *Diabetes* **64** (2), 631–642. 10.2337/db14-0930 (2015).
5. Teo, Z. L. et al. Global prevalence of diabetic retinopathy and projection of burden through 2045: Systematic review and meta-analysis. *Ophthalmology* **128** (11), 1580–1591. 10.1016/j.ophtha.2021.04.027 (2021).
6. Schreur, V. et al. Risk factors for development and progression of diabetic retinopathy in Dutch patients with type 1 diabetes mellitus. *Acta Ophthalmol.* **96** (5), 459–464. 10.1111/aos.13815 (2018).

7. Kowluru, R. A. Cross talks between oxidative stress, inflammation, and epigenetics in diabetic retinopathy. *Cells* **12** (2), 300. 10.3390/cells12020300 (2023).
8. De Bree, A. et al. Effect of the methylenetetrahydrofolate reductase 677C > T mutation on the relations among folate intake and plasma folate and homocysteine concentrations in a general population sample. *Am. J. Clin. Nutr.* **77** (3), 687–693. 10.1093/ajcn/77.3.687 (2003).
9. Beeri, M. S. et al. Normal homocysteine levels in well-functioning oldest-old individuals despite low kidney function. *J. Am. Geriatr. Soc.* **54** (10), 1623–1625. 10.1111/j.1532-5415.2006.00903.x (2006).
10. Homocysteine Studies Collaboration. Homocysteine and risk of ischemic heart disease and stroke: a meta-analysis. *JAMA* **288** (16), 2015–2022. 10.1001/jama.288.16.2015 (2002).
11. Clarke, R. et al. Folate, vitamin B12, and serum total homocysteine levels in confirmed Alzheimer's disease. *Arch. Neurol.* **55** (11), 1449–1455. 10.1001/archneur.55.11.1449 (1998).
12. Kowluru, R. A., Mohammad, G. & Sahajpal, N. Faulty homocysteine recycling in diabetic retinopathy. Eye Vis (Lond). ;7:4. (2020). 10.1186/s40662-019-0167-9. Erratum in: Eye Vis (Lond). 2020;7:11. doi:10.1186/s40662-020-00178-3.
13. Xu, C. et al. Relationship between homocysteine level and diabetic retinopathy: a systematic review and meta-analysis. *Diagn. Pathol.* **9**, 167. 10.1186/s13000-014-0167-y (2014).
14. Lei, X. et al. Association between homocysteine level and the risk of diabetic retinopathy: a systematic review and meta-analysis. *Diabetol. Metab. Syndr.* **10**, 61. 10.1186/s13098-018-0362-1 (2018).
15. Lei, C., Lv, Z., Ran, Q., Jiang, F. & Zhang, M. Homocysteine and diabetic retinopathy. *Exp. Eye Res.* **262**, 110729. 10.1016/j.exer.2025.110729 (2026).
16. Looker, H. C. et al. Homocysteine as a risk factor for nephropathy and retinopathy in type 2 diabetes. *Diabetologia* **46** (6), 766–772. 10.1007/s00125-003-1104-x (2003).
17. Vaccaro, O. et al. Plasma homocysteine and its determinants in diabetic retinopathy. *Diabetes Care*. **23** (7), 1026–1027. 10.2337/diacare.23.7.1026 (2000).
18. Bulum, T., Blaslov, K. & Duvnjak, L. Plasma homocysteine is associated with retinopathy in type 1 diabetic patients in the absence of nephropathy. *Semin Ophthalmol.* **31** (3), 198–202. 10.3109/08820538.2014.912338 (2016).
19. Agardh, E., Hultberg, B. & Agardh, C. D. Severe retinopathy in type 1 diabetic patients is not related to the level of plasma homocysteine. *Scand. J. Clin. Lab. Invest.* **60** (3), 169–174. 10.1080/003655100750044811 (2000).
20. Soedamah-Muthu, S. S. et al. Plasma homocysteine and microvascular and macrovascular complications in type 1 diabetes: A cross-sectional nested case-control study. *J. Intern. Med.* **258** (5), 450–459. 10.1111/j.1365-2796.2005.01560.x (2005).
21. Friedewald, W. T., Levy, R. I. & Fredrickson, D. S. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. *Clin. Chem.* **18** (6), 499–502 (1972).

22. Inker, L. A. et al. New creatinine- and cystatin C-based equations to estimate GFR without race. *N Engl. J. Med.* **385** (19), 1737–1749. 10.1056/NEJMoa2102953 (2021).
23. Li, H. et al. Research progress on the pathogenesis of diabetic retinopathy. *BMC Ophthalmol.* **23** (1), 372. 10.1186/s12886-023-03118-6 (2023).
24. Feng, Y., Shan, M. Q., Bo, L., Zhang, X. Y. & Hu, J. Association of homocysteine with type 1 diabetes mellitus: a meta-analysis. *Int. J. Clin. Exp. Med.* **8** (8), 12529–12538 (2015).
25. Mohamed, R. et al. Hyperhomocysteinemia alters retinal endothelial cells barrier function and angiogenic potential via activation of oxidative stress. *Sci. Rep.* **7** (1), 11952. 10.1038/s41598-017-09731-y (2017).
26. Cahill, M. T., Stinnett, S. S. & Fekrat, S. Meta-analysis of plasma homocysteine, serum folate, serum vitamin B(12), and thermolabile MTHFR genotype as risk factors for retinal vascular occlusive disease. *Am. J. Ophthalmol.* **136** (6), 1136–1150. 10.1016/s0002-9394(03)00571-3 (2003).
27. Kowluru, R. A. & Mohammad, G. Mitochondrial fragmentation in a high homocysteine environment in diabetic retinopathy. *Antioxid. (Basel)*. **11** (2), 365. 10.3390/antiox11020365 (2022).
28. Malaviya, P. & Kowluru, R. A. Homocysteine and mitochondrial quality control in diabetic retinopathy. *Eye Vis. (Lond)*. **11** (1), 5. 10.1186/s40662-023-00362-1 (2024).
29. Brazionis, L., Rowley, K., Sr, Itsiopoulos, C., Harper, C. A. & O'Dea, K. Homocysteine and diabetic retinopathy. *Diabetes Care*. **31** (1), 50–56. 10.2337/dc07-0632 (2008).
30. Luo, S., Shi, C., Wang, F. & Wu, Z. Association between the angiotensin-converting enzyme (ACE) genetic polymorphism and diabetic retinopathy-A meta-analysis comprising 10,168 subjects. *Int. J. Environ. Res. Public. Health*. **13** (11), 1142. 10.3390/ijerph13111142 (2016).
31. Shen, C., Zhao, M., Li, Y. Y. & Liu, N. P. Methylenetrahydrofolate reductase gene C677T polymorphism and diabetic retinopathy: A meta-analysis. *Chin. Med. Sci. J.* **35** (1), 71–84. 10.24920/003535 (2020).
32. Sharma, A., Valle, M. L., Beveridge, C., Liu, Y. & Sharma, S. Unraveling the role of genetics in the pathogenesis of diabetic retinopathy. *Eye (Lond)*. **33** (4), 534–541. 10.1038/s41433-019-0337-y (2019).
33. Pei, X., Huang, D. & Li, Z. Genetic insights and emerging therapeutics in diabetic retinopathy: from molecular pathways to personalized medicine. *Front. Genet.* **15**, 1416924. 10.3389/fgene.2024.1416924 (2024).
34. Fotiou, P. et al. Vitamin status as a determinant of serum homocysteine concentration in type 2 diabetic retinopathy. *J. Diabetes Res.* **2014**, 807209. 10.1155/2014/807209 (2014).
35. Chen, X. et al. Circulating level of homocysteine contributes to diabetic retinopathy associated with dysregulated lipid profile and impaired kidney function in patients with type 2 diabetes mellitus. *Eye (Lond)*. **37** (7), 1383–1389. 10.1038/s41433-022-02144-w (2023).
36. Luo, W. M. et al. The association of homocysteine and diabetic retinopathy in homocysteine cycle in Chinese patients with type 2 diabetes. *Front. Endocrinol. (Lausanne)*. **13**, 883845. 10.3389/fendo.2022.883845 (2022).

37. Braffett, B. H., Rice, M. M., Young, H. A. & Lachin, J. M. Mediation of the association of smoking and microvascular complications by glycemic control in type 1 diabetes. *PLoS One*. **14** (1), e0210367. 10.1371/journal.pone.0210367 (2019).
38. Berrabeh, S. et al. Prevalence and risk factors of retinopathy in type 1 diabetes: A cross-sectional study. *Cureus* **15** (10), e47993. 10.7759/cureus.47993 (2023).
39. Chen, Y. et al. Sex differences in the development of experimental diabetic retinopathy. *Sci. Rep.* **14** (1), 22812. 10.1038/s41598-024-73279-x (2024).
40. González-Lamuño, D. et al. Vitoria-Miñana I. Hyperhomocysteinemia in adult patients: A treatable metabolic condition. *Nutrients* **16** (1), 135. 10.3390/nu16010135 (2023).

Figures

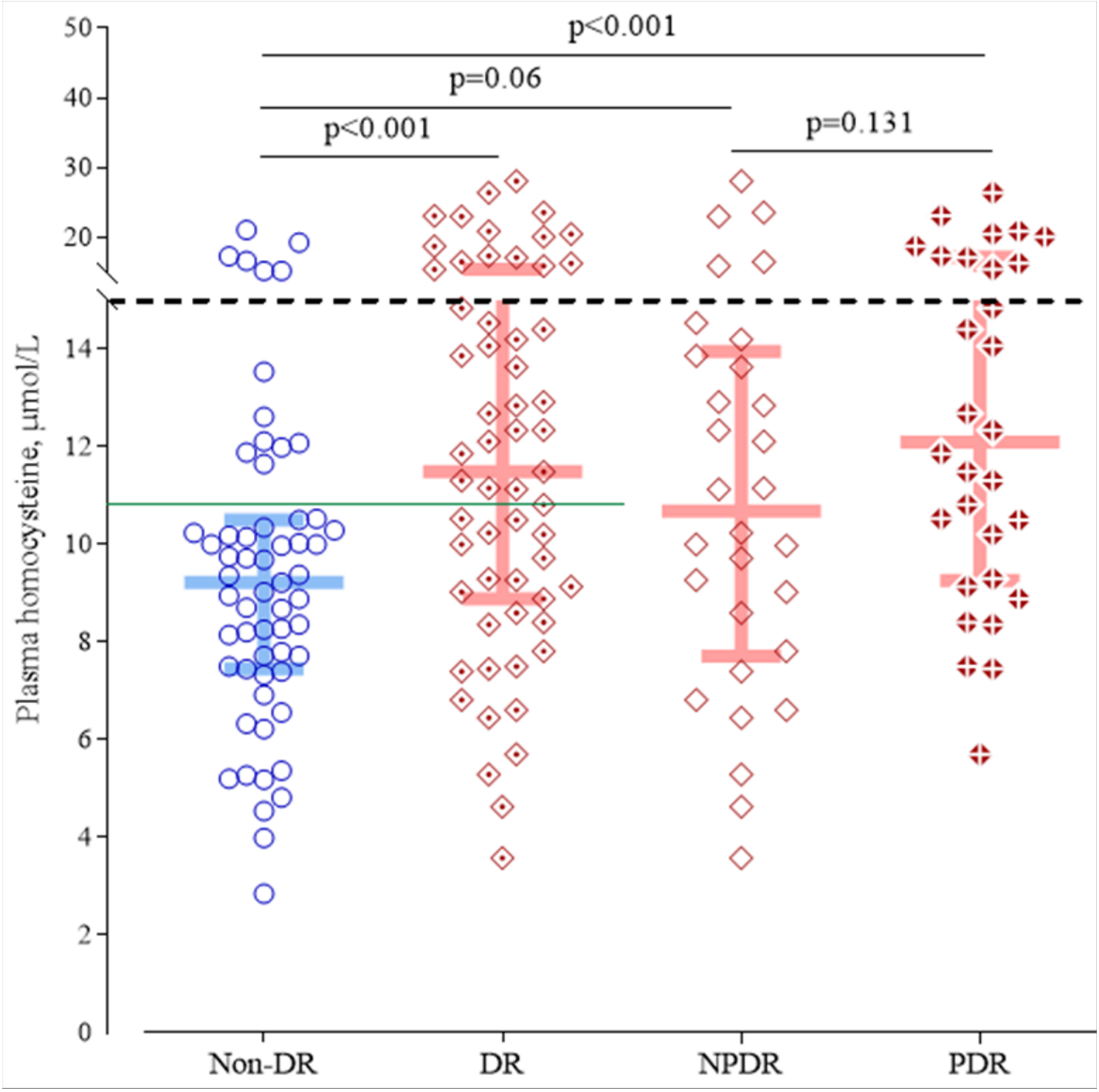


Figure 1

Plasma homocysteine concentrations according to diabetic retinopathy presence and severity.

Distribution of plasma homocysteine concentrations in patients with type 1 diabetes, according to the presence and severity of diabetic retinopathy. The dashed bold horizontal line indicates the conventional normal limit of plasma homocysteine values. Marks above the line represent elevated homocysteine in each group. The continuous thin horizontal line indicates the optimal cutoff value differentiating DR from non-DR groups.

DR, diabetic retinopathy; NPDR, non-proliferative diabetic retinopathy; non-DR, non-diabetic retinopathy; PDR, proliferative diabetic retinopathy.

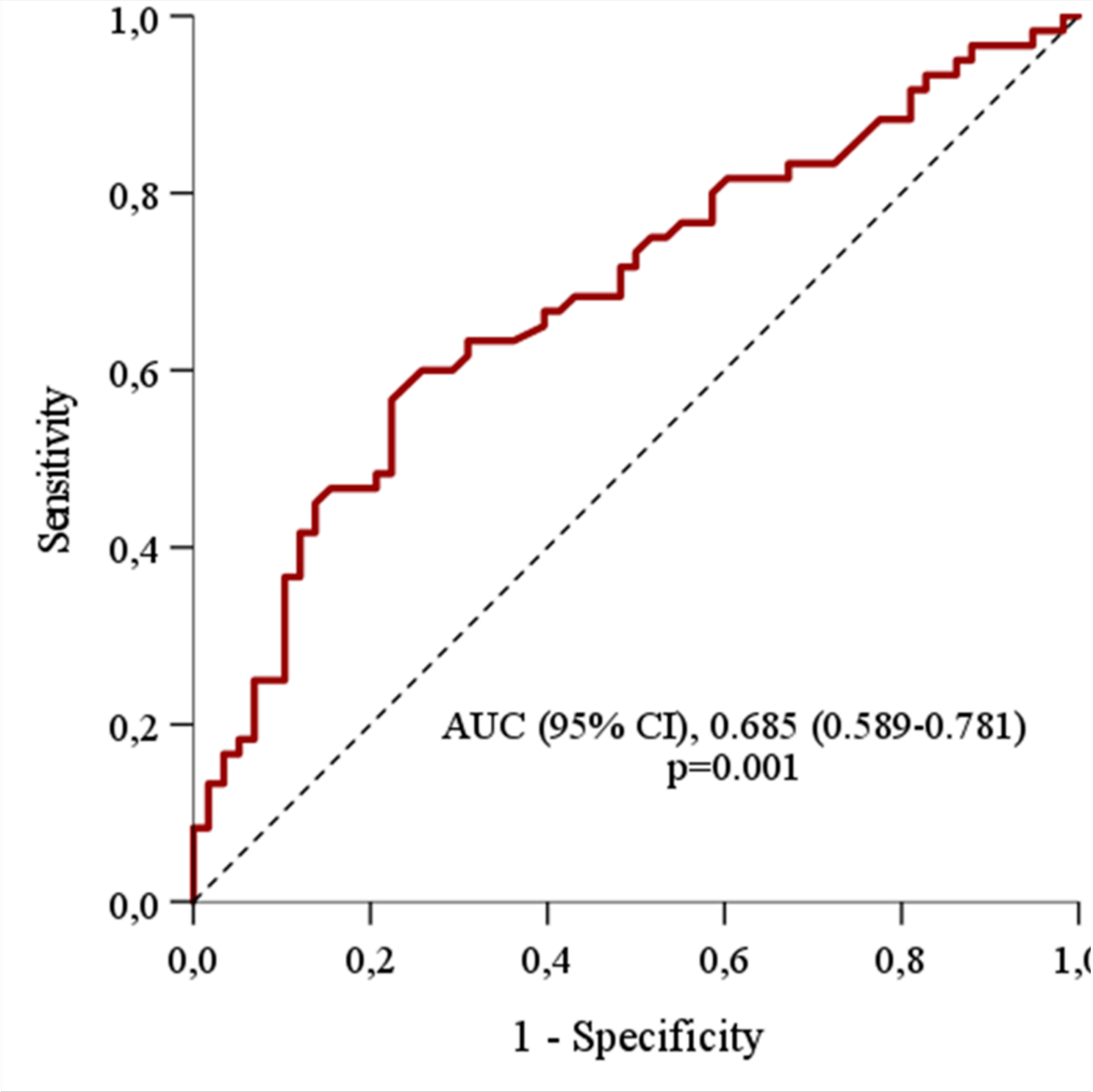


Figure 2

ROC curve of plasma homocysteine for diabetic retinopathy in type 1 diabetes.

Receiver operating characteristic (ROC) curve assessing the discriminative ability of plasma homocysteine for diabetic retinopathy in type 1 diabetes mellitus. AUC, area under the curve; CI,

confidence interval.