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A novel screening and diagnostic indicator for assessing the risk of diabetic retinopathy

Hany K. Mansour [1], Raef M. Botros [1], Ahmed M. Bahaa Eldin [2], Mona E. EL-deep[1], Nesma H. Ahmed [1]*

[1]*Faculty of Medicine, Ain Shams University, Egypt.*

[2]*Assiut University, Egypt.*

Abstract

Diabetes mellitus (DM) is a collection of metabolic disorders characterised by chronic hyperglycemia brought on by insufficient insulin production, action, or both. It is the leading cause of visual loss in those of working age, can have catastrophic consequences, one of which is diabetic retinopathy (DR). To investigate the use of plasma homocysteine as a screening and diagnostic marker for determining the risk of diabetic retinopathy in individuals with DM. An observational study involving ninety diabetic patients at the diabetes outpatient clinic at Ain Shams University Hospital looked at plasma homocysteine as a screening and diagnostic indicator for figuring out the risk of diabetic retinopathy in patients with diabetes mellitus over an eight-month period, from April 2022 to December 2022. Our research indicates that there was a substantial statistical difference between the groups under investigation with respect to the patient's age and the length of their diabetes mellitus, as well as clinical and laboratory data (FBS, HBA1C, eGFR, and albumin/creatinine ratio). The study also demonstrates that there was a substantial statistical difference in homocysteine levels between the groups under investigation. Homocysteine level and (age, duration of diabetes, HbA1C and FBG, eGFR and ACR) were observed to positively correlate in our study. Furthermore, there was no statistically significant variation in BMI, smoking status, or gender among the groups under study. DR is one of the primary side effects of DM. A significant relationship was seen between the level of homocysteine in plasma and DR. A useful and trustworthy test for figuring out whether diabetics have DR is plasma homocysteine levels when utilised as an indicator of diabetic retinopathy.

Keywords: Homocysteine, Assessing Diabetic Retinopathy, Diabetes Mellitus.

Full length article **Corresponding Author*, e-mail: mona.eldepp@gmail.com

1. Introduction

DM is a collection of metabolic diseases characterised by consistently elevated blood sugar levels brought on by inadequate insulin action, synthesis, or both. which result in long-term damage and impairment to a number of organs, including the heart, blood vessels, kidneys, nerves, and eyes [1]. One of the most dangerous microvascular effects of diabetes is DR, which affects 95% of patients [2]. Considering that it is estimated that 3–8% of people with type 1 DM will become blind [3]. Proliferative and nonproliferative DR are the two main subtypes. The growth of abnormal blood vessels, or neovascularization, in the retina is referred to as "proliferative" depending on whether it occurs or not. Unfortunately, until blindness and irreversible damage have happened, DR may go untreated or unrecognised [4]. Homocysteine (Hcy), an amino acid containing sulphur, is created when methionine is broken down. Elevated amounts of plasma homocysteine damage the vascular endothelium by producing free radicals. These free radicals cause direct damage to the endothelium by rupturing its integrity and exposing the smooth muscle and vascular matrix underneath. By triggering platelets and thrombus formation, this encourages a state of hypercoagulability [5]. It is claimed that it contributes to the oxidation-reduction process and generates oxidative stress [6]. Furthermore, due to its pro-proliferative and endotoxic properties, which may interfere with methylation and glutathione synthesis, it has been connected to vascular diseases [7]. Since diabetes is a microvascular occlusive disease, an adjuvant risk factor that raises plasma homocysteine levels and induces a hypercoagulability condition may account for the higher homocysteine levels seen in proliferative diabetic retinopathy compared to nonproliferative diabetic retinopathy [8]. Although the mean blood levels of Hcy in the PDR patients were found to be greater than in the NDR control group, this difference was not statistically significant [9]. A different

study, according to Xu et al. (2014), showed that DM patients with PDR had higher plasma levels of Hcy than did patients without either DR or NDR. The blood-retina barrier most likely broke down in PDR patients with elevated Hcy in their vitreous after vitreoretinal surgery [9]. On the other hand, there is no appreciable difference in Hcy levels between people with proliferative retinopathy (PR) and people without it, according to multiple studies. However, patients with PR have significantly higher homocysteine levels than people without PR [10]. In people with diabetes mellitus, the study evaluated plasma homocysteine as a screening and diagnostic marker for DR development.

2. Subjects and methods

Between April 2022 and December 2022, this observational study was conducted at the Ain Shams University Hospital's diabetes outpatient clinic. Based on the study's inclusion and exclusion criteria, participants were selected at random. The study included ninety patients in total, divided into two groups: group B included sixty diabetic individuals with diabetic retinopathy, and group A included thirty diabetic patients without the condition. Group B was divided into two subgroups, Group B1 and Group B2, which included thirty diabetic patients with nonproliferative DR and thirty diabetic patients with proliferative DR, respectively.

2.1. Inclusion criteria

Patients with a minimum of ten years' history of diabetes mellitus, encompassing both type 1 and type 2 diabetes, regardless of gender.

2.2. Exclusion criteria

Individuals with vascular or inflammatory disorders known to elevate homocysteine levels, such as a recent stroke or myocardial infarction, as well as those with chronic liver disease and eGFRs less than 60 mL/min/1.73 m2. Every patient was requested to give their full medical history, which included information about their diabetes mellitus (age of start, duration, dosages, and compliance with treatment). Overall assessment: Anthropometric measurements [weight, height, BMI (Weight/(Height in meters)2)], vital signs (blood pressure, pulse).

2.3. Laboratory investigations

FBS, PPBS (mg/dl), HBA1C (%), Estimated GFR (mL/min/1.73m2), Serum plasma homocysteine (Umol/L) and Albumin/creatinine ratio (mg/g).

2.4. Fundus examination

At the Ophthalmology outpatient clinic at Ain Shams University Hospital, a specialised ophthalmologist conducted a fundus examination and rated diabetic retinopathy. Following pupillary dilatation with 1% tropicamide and 10% phenylephrine eye drops, the ophthalmoscope was employed. Based on what was discovered in the worse eye, retinopathy was categorised. A fundus lens-equipped slit lamp biomicroscope (Magnon SL-450, Japan) and a binocular indirect ophthalmoscope (Keeler Instruments Inc. PA, USA) were used to investigate the fundus.

2.5. Statistical analysis

The collected data were entered into IBM SPSS version 23, altered, and labelled. Whereas nonparametric data were shown as median with interquartile range (IQR), parametric data were shown as mean, standard deviations, and ranges. Furthermore, numerical values and percentages were provided for the qualitative attributes. The significance level of the p-value was determined as follows: P-value > 0.05 denoted non-significant (NS), P-value < 0.05 marked significant (S), and P-value < 0.01 indicated highly significant (HS).

3. Results

Age differences were substantial, but there was no statistically significant difference in smoking or BMI among the three study groups whose sexes were matched (Table 1). Between the study groups, there was a significant statistical difference in the duration of diabetes and hypertension (Table 2). There was no statistically significant difference found between any of the study groups regarding 2-hour PPBG, although there was a significant statistical difference found regarding FBG, HbA1c, eGFR, and ACR (Table 3). There was a significant statistical difference in homocysteine levels across the study groups (p-value 0.001). The homocysteine levels between group A and group B1 (p-value 0.001) and between group A and group B2 (p-value 0.001) differed significantly from one another, however there was no statistically significant difference between groups B1 and B2 (Table 4). When homocysteine levels in hypertension and non-hypertensive people were compared, there was a significant statistical difference (P value <0.001), with higher homocysteine levels in hypertensive patients (Table 5). Additionally, a substantial statistically significant difference was observed in the homocysteine levels between individuals receiving different treatment modalities, with the oral antidiabetic P value (0.001) showing the highest difference (Table 6). After doing a correlation analysis, the homocysteine level was favourably connected with age, the length of diabetes, HbA1C and FBG, eGFR, and ACR. However, there was no relationship found between BMI and 2hpp and homocysteine levels (Table 7).When multivariable binary logistic regression was utilised to determine predictors of diabetic retinopathy, it was found that ACR and homocysteine level were risk factors (Table 8).

4. Discussion

Diabetes's most common microangiopathic side effect is diabetes retinopathy (DR). An increasing body of research indicates that systemic inflammation contributes significantly to the early and late stages of DR, promoting the growth of new blood vessels and macular edoema, upsetting the glial cross, and resulting in neuronal loss [11]. Because homocysteine generates free radicals, it is detrimental to the vascular endothelium. By upsetting the integrity of the endothelium and exposing the underlying vascular matrix and smooth muscle, these free radicals directly damage the endothelium. This promotes hypercoagulability by activating platelets and causing thrombus formation [5].

Table 1: Comparison between 3 groups as regards demographic data.

		Group A Normal fundus **(N=30)**	**Group B1** Non PDR **(N=30)**	**Group B2** PDR **(N=30)**	**P-value**
Age (years)		39.967 ± 12.121	44.767 ± 11.144	50.367 ± 7.247	•0.001*
Gender	**Male**	8(26.67%)	9(30.00%)	12(40.00%)	#0.516
	Female	22(73.33%)	21(70.00%)	18(60.00%)	
Smoking	**No**	25(83.33%)	26(86.67%)	24(80.00%)	#0.787
	Yes	5(16.67%)	4(13.33%)	6(20.00%)	
BMI (kg/m²)		27.520 ± 5.123	28.680 ± 5.472	29.050 ± 4.633	•0.481

*#Chi-Square test, • ANOVA test, *significant, N: number of patients.*

Table 2: Comparison between 3 groups as regards clinical data.

		Group A Normal fundus **(N=30)**	**Group B1** Non PDR **(N=30)**	**Group B2** PDR **(N=30)**	**P-value**
Duration (years)		12.133±2.825	12.367±3.316	15.133±5.494	•0.008*
HTN		5(16%)	13(43%)	17(56%)	•0.001*
DM Treatment	**Oral antidiabetics**	3(10.00%)	4(13.33%)	6(20.00%)	#0.075
	Insulin	14(46.67%)	12(40.00%)	4(13.33%)	
	Both	13(43.33%)	14(46.67%)	20(66.67%)	

*#Chi-Square test, • ANOVA test, *significant, N: number of patients.*

Table 3: Comparison between 3 groups as regards laboratory investigations.

	Group A Normal fundus **(N=30)**	**Group B1** Non PDR **(N=30)**	**Group B2** PDR **(N=30)**	**P-value**
FBG(mg/dl)	165.967±69.689	225.033 ± 83.468	239.267±92.152	• 0.002*
2HPP(mg/dl)	239.333±108.599	254.867±81.332	259.867±81.386	0.663
HBA1C(%)	8.540±2.482	9.137 ± 2.314	10.443±3.391	0.029*
eGFR(mL/min/1.73m2)	98.367 ± 9.353	91.833 ± 6.701	81.233±6.484	• <0.001*
ACR (mg/g)	24.333±7.203	40.700±18.925	213.833±55.209	• <0.001*

*• ANOVA test, *significant.*

Table 4: Comparison between three groups as regards Homocysteine level.

	Group A Normal fundus	**Group B1** Non PDR	**Group B2** PDR	**•P-value**	**A& B1**	**A &B2**	**B1& B2**
Homocysteine (Umol/L)	11.653 ± 3.745	19.473 ± 5.853	22.573 ± 9.353	<0.001*	“ <0.001*	“ <0.001*	“ 0.181

*• ANOVA test, *significant, ‘‘TUKEY’S Test*

Table 5: Homocysteine level in subclasses according to hypertension state in studied patients.

Hypertensive state	**N**	**Mean ±SD**	**P-value**
No	55	14.969±5.485	^ <0.001*
Yes	35	22.506±9.390	

*^ independent T test, *significant, N=number of patients.*

Table 6: Homocysteine level in subclasses according to diabetes treatment (oral antidiabetics, insulin orboth) in studied patients.

Diabetic treatments	N	Mean ±SD	P-value
Oral antidiabetics	13	24.454±12.148	^0.001*
Insulin	30	14.947±6.807	
Both	47	17.972±6.430	

*^ independent T test, *significant, N=number of patients.*

Table 7: Correlation study of homocysteine level and other parameters

Correlations								
	Homocysteine							
	Group A Normal fundus (N=30)		Group B1 Non PDR (N=30)		Group B2 PDR (N=30)		All patients (N=90)	
	r	P-value	r	P-value	R	P-value	r	P-value
Age (Years)	0.320	0.085	0.087	0.647	-0.209	0.268	0.232	0.028*
Duration (Years)	0.150	0.428	0.153	0.418	0.404	0.027*	0.382	<0.001*
BMI (kg/m²)	0.227	0.228	-0.151	0.426	-0.106	0.577	0.033	0.755
HBA1C (%)	0.047	0.805	-0.168	0.376	0.436	0.016*	0.313	0.003*
FBG (mg/dl)	0.182	0.336	-0.196	0.299	0.427	0.019*	0.355	0.001*
2HPP (mg/dl)	0.000	0.998	-0.211	0.264	0.033	0.864	0.022	0.839
GFR (mL/min/1.73m2)	-0.127	0.502	-0.075	0.695	0.116	0.540	-0.364	<0.001*
ACR (mg/g)	0.025	0.894	-0.248	0.187	-0.169	0.373	0.360	<0.001*

**significant, N=number of patients.*

Table 8: Multivariable binary logistic regression.

Linear regression	Unstandardized Coefficients		Standardized Coefficients	t	P-value
	B	S.E	Beta		
Age (years)	0.000	0.005	-0.004	-0.059	0.953
Duration (years)	-0.002	0.011	-0.010	-0.186	0.853
HBA1C (%)	-0.032	0.022	-0.112	-1.469	0.146
FBG (mg/dl)	0.001	0.001	0.146	1.882	0.063
Homocysteine (Umol/L)	0.024	0.006	0.241	3.848	<0.001*
GFR (mL/min/1.73m2)	-0.011	0.007	-0.144	-1.703	0.092
ACR (mg/g)	0.006	0.001	0.622	9.215	<0.001*
HTN	0.064	0.093	0.038	0.696	0.489
Dependent Variable: Fundus					

*S.E: standard error, t: indipendant t test, * significant.*

Elevated homocysteine compromises the integrity of both the inner and outer BRB. Homocysteine increases BRB permeability and compromises the structural and functional integrity of the retina. According to another study, altered retinal vasculature associated to homocysteine is manifested by retinal ischemia, neovascularization, vascular leakage, and a poor blood–retinal barrier [12]. Our study's goal was to investigate the utility of plasma homocysteine as a diagnostic marker and screening tool for diabetes mellitus patients to assess their risk of developing diabetic retinopathy (DR). Our findings indicate that there was a significant statistical difference (p value <0.001) between the age groups that were studied. This could be explained by the fact that arteriosclerotic changes in the retinal arteries become apparent with ageing. These changes become considerably more pronounced in the presence of diabetes or hypertension. Arteriosclerosis, in turn, contributes to the onset and advancement of diabetic retinopathy. It was found that the incidence of diabetic retinopathy increased with patient age, are consistent with this outcome [6, 13]. In contrast to our findings, it was found that a patient's age is not a risk factor for diabetic retinopathy. Variations in participant characteristics, race or ethnicity, and research methods could account for this disparity [6]. Our study revealed a statistically significant difference (p-value of 0.008) in the duration of DM among the study groups. This could be explained by the effects of chronic hyperglycemia on retinal arteries. Diabetes mellitus-related systemic inflammation destroys the glial cross and causes neuronal loss. These elements have a critical role in the development of diabetic retinopathy. They also cause macular edoema and the formation of new blood vessels. This result is in line with other researches which found a strong link between the duration of diabetes mellitus and a high incidence of retinopathy [14,15]. Our findings indicated that hypertension was more common among diabetics with PDR. This may be explained by the fact that hypertension-related changes to small arteries increase peripheral blood flow resistance overall, thus hastening the onset of diabetic retinopathy. These results align with those, who found a significant association between untreated or poorly controlled hypertension and diabetic retinopathy [16]. Our findings indicate that the glycaemic profile (FBS and HbA1c) of the groups under study differed statistically significantly, with diabetics with PDR having higher values (P value: 0.002, 0.029), respectively. The effects of chronic hyperglycemia, such as oxidative stress, thickening of the retinal capillary basement membrane, increased permeability of the retinal vascular system, tissue ischemia, and the release of multiple vasoactive chemicals, are responsible for the neovascularization phenomenon. This result is in line with other researches which discovered a direct correlation between insufficient glycemic control as measured by glycated haemoglobin and FBG and the initiation and progression of diabetic retinopathy [13, 17,18].

Our findings indicate that there was a significant statistical difference in eGFR and ACR between the study groups, with the latter being higher in PDR-diabetic people (p value 0.001, 0.001). The comparable pathophysiology of diabetic kidney disease (DKD) and diabetic retinopathy may help to explain this. This involves inappropriate activation of the renin-angiotensin system, aberrant activation of protein kinase C, accumulation of advanced glycation end products, increased formation of reactive oxygen species, and oxidative stress brought on by hyperglycemia, among other things. Specifically, this may provide light on the connection between DR and DKD, which appears in ACR as one DR-predictive factor. The association between the phases of DR and eGFR and ACR was found in prospective observational studies [19-21]. This finding is in line with other research. Our findings showed that there was no statistically significant difference in gender between the groups that were being studied (p value 0.516). The fact that our experiment was randomised and there was no sex selection may help to explain this. This result is consistent with two researches which similarly did not discover any proof that gender and diabetic retinopathy were significantly correlated [22-23]. In contrast to our results, it was found that men were more likely than women to acquire diabetic retinopathy. Variations in participant characteristics, race or ethnicity, and research methods could account for this disparity [13]. Our findings indicate that there was no statistically significant variation in smoking between the study groups (p value 0.787). This may be explained by the connection between our Egyptian culture and the study's prevalence of female participants. This result is in line with the findings of Magliah et al. (2018), who found no correlation between smoking and the risk of developing diabetic retinopathy [22]. Our findings showed that smokers were far more likely to acquire diabetic retinopathy [24]. Variations in participant characteristics, race or ethnicity, and research methods could account for this disparity. Our findings showed that the patients' BMI did not differ statistically significantly between the study groups (p value 0.481). Consistent with our findings, it was reported that a high BMI is not a risk factor for diabetic retinopathy [22,25]. On the other hand, studies demonstrated that the primary risk factor for retinopathy was obesity [26-27]. Variations in participant characteristics, race or ethnicity, and research methods could account for this disparity. Our study found that there was a statistically significant difference in the homocysteine level between the study groups, with a higher amount in PDR patients (p value <0.001). This can be explained by the possibility that elevated levels of plasma Hcy, an adjuvant risk factor that contributes to a state of hypercoagulability and may precipitate the onset or progression of diabetic retinopathy, because diabetes mellitus is a microvascular occlusive condition. Thrombosis is brought on by hcy, which is harmful to the vascular endothelium and can exacerbate hypoxic conditions such as DR. This result is in line with studies that found mean plasma total homocysteine concentrations in diabetic patients with retinopathy were higher than in those without retinal. Furthermore, they found that mean plasma total homocysteine concentrations were higher in PDR patients than in non-PDR patients [9, 28, 29].

Our study revealed a statistically significant difference (p value<0.001) in homocysteine levels between people with and without hypertension. Because both homocysteine and hypertension result in endothelial dysfunction and increased arterial stiffness, which lowers nitric oxide availability, it is possible to explain the association between the two disorders. This result is in line with researches that found that hypertension is a risk factor for higher plasma homocysteine levels [30,31]. According to our analysis, there was a significant statistical difference in the homocysteine levels across the treatment modalities, with

the homocysteine levels being higher in those who received oral antidiabetics (p value). This result is in line with a study which found that people on oral anti-diabetics had higher mean homocysteine concentrations than people receiving insulin [32]. The results of this investigation showed a positive correlation between the homocysteine level and the duration of DM, FBG, and HA1C. This result is in line with a study that found a significant positive correlation between homocysteine and the duration of diabetes mellitus [33]. This result is in line with a study which found that the levels of FBG and HbA1c were correlated with a rise in homocysteine [34]. As per our findings, there was no discernible variation in homocysteine levels across genders that was statistically significant. This result is in line with the findings of Zhao et al. (2021), who found that gender is not a risk factor for high plasma homocysteine (Hcy) levels [35]. The results of Xu et al. (2020), who found that men had significantly higher Hcy levels than females, are in conflict with this finding. Variations in participant characteristics, race or ethnicity, and research methods could account for this disparity [36]. Our investigation revealed that there was no statistically significant difference in the levels of homocysteine between nonsmokers and smokers. This could be explained by the small proportion of smokers in our sample, which comes from the relationship between our Egyptian culture and the study's preponderance of female participants. According to a 2004 study by Sobczak et al., smoking was linked to higher plasma homocysteine (Hcy) levels. This conclusion contradicts their findings. Variations in participant characteristics, race or ethnicity, and research methods could account for this disparity [37]. When compared to HbA1c and FPG (diabetes metabolic management), our results show that serum homocysteine levels rise sharply and are highly substantially related with FPG and HbA1c in diabetic individuals. The results of this study suggest that elevated homocysteine is a valuable and practical marker for predicting the onset of DR in diabetic individuals. It is higher in PDR patients than in NPDR patients and normal fundus patients.

5. Conclusions

Increased levels of plasma homocysteine cause thrombosis, which aggravates hypoxic diseases such diabetic retinopathy by directly harming the vascular endothelium. Plasma homocysteine is a practical and helpful metric for predicting the presence of DR in diabetic patients since it is higher in PDR patients than NPDR patients and can be used as a diagnostic of diabetic retinopathy.

Conflict of Interest Statement

The authors declare no potential conflict of interest on time of submission.

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