Original Article

Role of hyperhomocysteinemia in proliferative diabetic retinopathy: A case–control study

Prabha Gupta, Deepa John, Grace Rebekah[1], Sheeja S John

Purpose: Hyperhomocysteinemia has been postulated as a potential risk factor for the development and progression of diabetic retinopathy. The aim of this study was to determine the association of hyperhomocysteinemia with proliferative diabetic retinopathy (PDR). **Methods:** This was a hospital-based, case–control study, conducted at a tertiary care ophthalmic center in South India. Thirty-nine patients with proliferative diabetic retinopathy were enrolled as cases, and 39 age- and gender-matched patients with no diabetic retinopathy (No DR) were enrolled as controls. Fasting serum homocysteine estimation, as well as baseline investigations, were done in all participants. Data regarding demographic profile and risk factors were documented. Data were analyzed using Chi-square test and independent *t*-test, as appropriate. **Results:** The prevalence of hyperhomocysteinemia was higher in PDR (59%) compared to "No DR" (48.7%); however, this difference was not statistically significant ($P = 0.36$). Similarly, the mean serum homocysteine level in cases was higher than in controls, but this was not statistically significant (17.98 + 6.26 µmol/L vs. 17.71 + 8.17 µmol/L; $P = 0.87$). Longer duration of diabetes, hypertension, anemia, and renal dysfunction were found to be significantly associated with PDR. **Conclusion:** The prevalence of hyperhomocysteinemia as well as the mean serum levels of homocysteine were found to be higher in the cases with PDR, compared to the controls with No DR, although the difference was not statistically significant. Longer duration of diabetes, hypertension, anemia, and renal dysfunction were significantly associated with PDR.

Key words: Diabetic, hyperhomocysteinemia, proliferative, retinopathy

There is an estimated 451 million people with diabetes worldwide, as of 2017; these figures are expected to increase to 693 million by 2045.[1] Diabetic retinopathy is becoming an increasingly important cause of visual impairment due to increase in the diabetic population. At present, there are no known means to prevent the onset of diabetic retinopathy. However, there are various known risk factors that affect the disease progression.

Of the various risk factors that are hitherto known to cause progression of retinopathy, some are modifiable, while others are nonmodifiable. In recent years, hyperhomocysteinemia has been postulated as a potential risk factor for the development and progression of diabetic retinopathy. Homocysteine (Hcy), an intermediate molecule in the metabolism of methionine, has generated considerable interest as a risk factor for cardiovascular disease and other vaso-occlusive diseases, including retinal vessel occlusion.[2] High blood levels of homocysteine are toxic to the vascular endothelium through free radical formation. Free radicals cause disruption of endothelial integrity, leading to platelet activation, causing hypercoagulability and thrombus formation.[3]

Several studies have been done worldwide to investigate the role of hyperhomocysteinemia in diabetic retinopathy. Some of these studies have concluded that hyperhomocysteinemia is associated with increased risk for development and progression of diabetic retinopathy, as well as the development of diabetic macular edema,[2-6] while others have failed to find evidence to support such an association.[7,8]

Deficiency of vitamin B12 and folate has been associated with increased serum homocysteine levels. Hyperhomocysteinemia could, therefore, be a potentially modifiable risk factor for diabetic retinopathy. Dietary supplementation could be achieved at a very affordable cost, thereby saving the patient not only from the burden of morbidity caused by the disease but also from the economic impact of the medical expenses incurred. This is especially relevant in India, where there is a high prevalence of diabetes as well as vitamin B12 deficiency.[9-11] Hence, understanding and characterizing the role of hyperhomocysteinemia in the pathogenesis of diabetic retinopathy may help in identifying a novel target to combat this potentially blinding disease.

The aim of this study was to determine the association of hyperhomocysteinemia with proliferative diabetic retinopathy (PDR).

Methods

This was a hospital-based, case–control study conducted at a tertiary care ophthalmic center in South India, over a period

Departments of Ophthalmology, ¹Biostatistics, Christian Medical College, Vellore, Tamil Nadu, India

Correspondence to: Dr. Deepa John, Department of Ophthalmology, Christian Medical College, Vellore - 632 001, Tamil Nadu, India. E-mail: deeparebeccajohn@gmail.com

of 8 months, from January to August 2015, after receiving the approval of the Institutional Review Board (IRB Min No. 9150, dated 12/11/14). Patients with Type 2 diabetes, seen in the outpatient clinics, were screened for eligibility for enrolment in the study. The presence and level of diabetic retinopathy (DR) were assessed by dilated fundus examination, using slit lamp binocular indirect ophthalmoscopy. Diabetic retinopathy was classified according to the Modified Airlie House Classification (Early Treatment Diabetic Retinopathy Study).[11,12]

All eligible patients between 40 and 70 years of age were enrolled in the study after obtaining their informed consent. Patients with PDR were included in the study as cases, while age and gender-matched patients without diabetic retinopathy (No DR) were included as controls. Patients with history of liver disease, pregnant or postpartum women, patients with hazy ocular media in one or both eyes, precluding adequate visualization of the fundus for diagnosis and grading of diabetic retinopathy, and those with ocular diseases such as retinal vessel occlusion, retinal vasculitis, and retinal changes, or vitreous hemorrhage associated with ocular trauma, which may have resulted in ambiguity in the diagnosis and grading of diabetic retinopathy, were excluded from the study [Fig. 1].

A detailed questionnaire was administered to all the participants of the study. Measurement of blood pressure and estimation of body mass index (BMI), fasting (AC) and 2 h postprandial blood sugar levels (PC), glycosylated hemoglobin (HbA1c), lipid profile [low-density lipoprotein (LDL) levels], hemoglobin (Hb), and serum creatinine were done in all participants.

For the purpose of this study, good glycemic control was defined as HbA1c level <7%.[13] Hypertension was diagnosed if there was history of treatment for hypertension, or if systolic blood pressure was ≥140 mmHg, or diastolic blood pressure ≥90 mmHg.[14] Anemia was diagnosed if the patient was a previously diagnosed case of anemia on treatment, or if the hemoglobin was <13 and <12 g/dL in men and women, respectively.[15] Hyperlipidemia was diagnosed if the patient was a known case of hyperlipidemia on treatment, or if LDL cholesterol was ≥100 mg/dL.[13] Renal dysfunction was defined as estimated glomerular filtration rate [eGFR] <60 mL/min per 1.73 m^2), irrespective of the cause.[16] Body mass index (BMI) < 18.5 kg/m^2, BMI ≥ 18.5 to 24.9 kg/m^2, BMI ≥ 25.0 to 29.9 kg/m^2 and BMI ≥ 30 kg/m^2 was considered as Underweight, Normal weight, Overweight and Obesity respectively.[17]

With regard to smoking, all the study participants were divided into four groups: Group 1 (Nil smoking) – participants who did not smoke at all, Group 2 (Regular smoking) – participants who smoked more than once a week, Group 3 (Occasional smoking) – participants who smoked less than once a week, Group 4 (Quit smoking) – participants who stopped smoking at least six months earlier. Smoking was considered as positive in all those falling under Group 2. Alcoholism was assessed as per the CAGE criteria for screening of alcoholism.[18] The questionnaire included the following components, and two "yes" responses were taken as indicators to denote alcoholism.
1. Have you ever felt you needed to Cut down on your drinking?
2. Have people Annoyed you by criticizing your drinking?
3. Have you ever felt Guilty about drinking?
4. Have you ever felt you needed a drink first thing in the morning (Eye-opener) to steady your nerves or to get rid of a hangover?

Ophthalmological investigations such as fundus fluorescein angiography and optical coherence tomography, which were required for the routine management of diabetic retinopathy, were performed as per standard clinical indications.

A fasting venous blood sample was collected from all participants for the estimation of serum homocysteine, using direct chemiluminescent technology by ADIVA Centaur HYC competitive immunoassay. As hyperhomocysteinemia is still an emerging risk factor for diabetic retinopathy, different studies have taken different cut off values for hyperhomocysteinemia, and consequently, the prevalence of hyperhomocysteinemia reported in various studies also shows wide variation.[2,3,5] As this study was conducted to evaluate the role of hyperhomocysteinemia in PDR, and to determine if there was a significant difference in the prevalence of hyperhomocysteinemia between the cases (with PDR) and the controls (no DR), we took the median serum homocysteine value of controls as the cut-off for hyperhomocysteinemia.

Data analysis was done using PASW Statistics 18 – SPSS software. Descriptive statistics were reported using mean ± SD for continuous variables, and n (%) for categorical variables. Chi-square test was used to assess the association of the categorical variables with the cases and controls. Independent two-sample t-test was used to compare the means between cases and controls, for continuous variables. Data regarding confounding factors or suspected effect modifiers were obtained by history, clinical examination, and laboratory investigations. Age and gender were matched between cases and controls. Other factors such as duration of diabetes, glycemic control, hypertension, anemia, hyperlipidemia, nephropathy, smoking, alcoholism, obesity, malabsorption syndromes, and use of medication were documented and analyzed. The laboratory personnel who processed and
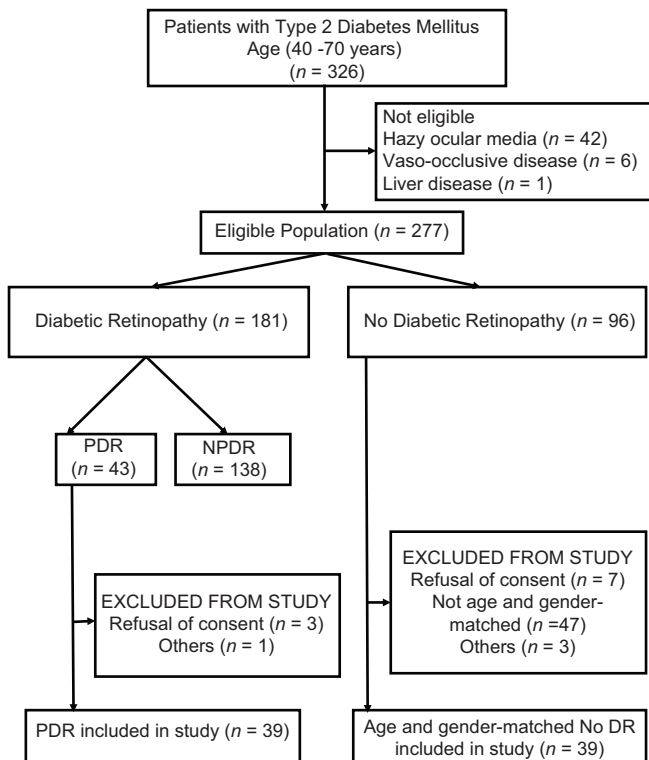

Figure 1: Flow chart of the study patient selection

analyzed the blood samples were masked toward the group to which the patient belonged (whether case or control) [Fig. 2].

Results

Seventy-eight patients, who fulfilled the eligibility criteria, were recruited into the study – 39 patients with PDR (PDR – cases), and 39 age- and gender-matched patients without diabetic retinopathy (No DR – controls).

All baseline demographic variables such as age, gender, place of residence, and dietary pattern were comparable between the two groups [Table 1]. There was no significant difference between the two groups with respect to glycemic control, hyperlipidemia, BMI, smoking, alcohol intake, and malabsorption syndromes. However, significantly longer duration of diabetes and higher prevalence of hypertension, anemia and renal dysfunction were found in cases with PDR as compared to controls with No DR [Table 2].

Although the use of drugs such as fibrates, multivitamins, vitamin B12, vitamin B6, nicotinamide, and proton pump inhibitors was more in the cases as compared to the controls, the difference was not statistically significant. There was also no significant difference between the two groups with respect to the intake of drugs such as antiepileptics and H2 blockers. However, there was a statistically significant difference between the two groups with respect to the use of drugs such as metformin, diuretics, and folate supplementation, with the use of metformin being more common in controls, and the use of diuretics and folate supplementation being more common in cases. There was no history of intake of other drugs such as levodopa, sulfalsalazine, trimethoprim, pyrimethamine, methotrexate, and oral contraceptive pills, in either of the two groups. Table 3 summarizes the details of drug intake in the two groups.
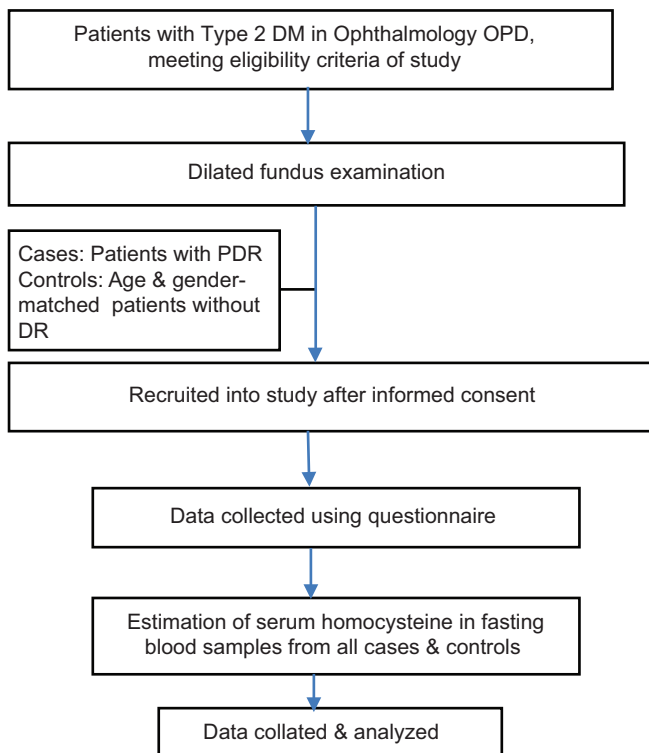

Figure 2: Diagrammatic algorithm of the study

We found that the prevalence of hyperhomocysteinemia was higher (59%) in cases with PDR, as compared to controls with No DR (48.7%), with hyperhomocysteinemia defined as serum homocysteine level >16.19 μmol/L, which was the median value of serum homocysteine in controls. However, this difference was not statistically significant ($P = 0.36$). There was also no significant difference between cases and controls when hyperhomocysteinemia was defined as serum homocysteine levels >10, >12, or >15 μmol/L [Table 4].

Similarly, the mean serum homocysteine level was found to be higher among cases as compared to controls (17.98 + 6.26 μmol/L in cases vs. 17.71 + 8.17 μmol/L in controls), but the difference was not statistically significant ($P = 0.87$).

Discussion

In the last decade, hyperhomocysteinemia has emerged as a novel risk factor for the development and progression of diabetic retinopathy.[19] However, there has been no definite evidence so far, to prove or disprove this association.

Table 1: Demographic profile of the two groups

Variable	Mean±SD, *n*%		P
	PDR (*n*=39)	No DR (*n*=39)	
Age (yrs)	55.3±5.4	54.8±6.1	0.70
Male	33 (84.6)	33 (84.6)	1.0
Female	6 (15.4)	6 (15.4)	
Residence			
In Tamil Nadu	27 (69.2)	26 (66.7)	0.80
Outside Tamil Nadu	12 (30.8)	13 (33.3)	
Vegetarian diet	5 (12.8)	7 (17.9)	0.53
Non-vegetarian diet	34 (87.2)	32 (82.1)	

Table 2: Comparison of risk factors between the two groups

Risk factors	Mean±SD, *n*%		P
	PDR *n*=39	No DR *n*=39	
Duration of diabetes			
<5 yrs	6 (15.4)	18 (46.2)	
5-10 yrs	12 (30.8)	10 (25.6)	<0.001
>10-15 yrs	7 (17.9)	10 (25.6)	
>15 yrs	14 (35.9)	1 (2.6)	
Poor glycemic control	25 (64.1)	28 (71.8)	0.4
Good glycemic control	14 (35.9)	11 (28.9)	
Hypertension	33 (84.6)	20 (51.3)	<0.01
No hypertension	6 (15.4)	19 (48.7)	
Anemia	29 (74.4)	11 (28.2)	<0.001
No anemia	10 (25.6)	28 (71.8)	
Hyperlipidemia	26 (66.7)	32 (82.1)	0.12
No hyperlipidemia	13 (33.3)	7 (17.9)	
Renal dysfunction	17 (43.6)	3 (7.7)	<0.001
No renal dysfunction	22 (56.4)	36 (92.3)	
Smoking	3 (7.7)	1 (2.6)	0.6
No smoking	36 (92.3)	38 (97.4)	
Alcoholism	0 (0)	1 (2.6)	1
No alcoholism	39 (100)	38 (97.4)	
Malabsorption	0 (0)	0 (0)	1
BMI	26.3±4.1	27.0±4.2	0.47

Table 3: Comparison of the two groups with respect to drug intake

Drug	PDR *n*=39	Percentage	No DR *n*=39	Percentage	*P*
Fibrates	2	5.1	-	-	0.49
Multivitamins	7	17.9	3	7.7	0.18
Cyanocobalamin	9	23.1	4	10.3	0.13
Vitamin B6	6	15.4	3	7.7	0.48
Nicotinamide	7	17.9	3	7.7	0.18
Proton pump inhibitors	7	17.9	2	5.1	0.15
Antiepileptics	-	-	1	2.6	1
H2 Blockers	-	-	1	2.6%	1
Metformin	18	46.2	36	92.3	<0.001
Diuretics	12	30.8	3	7.7	0.01
Folate	10	25.6	-	-	0.001
Levodopa	-	-	-	-	
Sulfasalazine	-	-	-	-	
Trimithoprim	-	-	-	-	
Pyrimethamine	-	-	-	-	
Methotrexate	-	-	-	-	
Oral Contraceptive pills	-	-	-	-	

Although a number of studies have been conducted worldwide, in different populations, to elucidate the association of diabetic retinopathy with hyperhomocysteinemia, the results have not always been consistent. Some studies have found a strong association of hyperhomocysteinemia with diabetic retinopathy,[2-6,11,19] while others have failed to do so.[7,8] These studies have concluded that hyperhomocysteinemia may not be an independent risk factor for diabetic retinopathy, and have suggested that other conditions associated with diabetes, such as declining renal function and the use of oral hypoglycemic agents, may cause elevation of serum homocysteine levels in diabetic patients.[7,8] In a meta-analysis done by Xu *et al.*, including 31 studies and 6394 patients, it was found that the homocysteine levels in the blood of patients with diabetic retinopathy were higher than that of patients in the control group, although there was statistical heterogeneity among the studies.[20] Xu *et al.* also observed that the role of hyperhomocysteinemia was probably more significant in Type 1 diabetes mellitus or in mixed (Type 1 + 2) diabetes,[20] rather than in patients with Type 2 diabetes mellitus, who constituted our study population.

The cut-off for defining hyperhomocysteinemia has been arbitrary and has differed substantially among different studies. The global disparity in defining hyperhomocysteinemia may be due to different genetic constitutions, lifestyles, environmental, nutritional, and dietary factors, as well as the fact that hyperhomocysteinemia is still an emerging risk factor for diabetic retinopathy. The cut-off values defined in various studies range from 12 μmol/L to 16 μmol/L.[2,3,5] Consequently, the prevalence of hyperhomocysteinemia reported in various studies also shows wide variation.[2,3,5] As our study was conducted to evaluate the role of hyperhomocysteinemia in PDR, and to determine if there was a significant difference in the prevalence of hyperhomocysteinemia between the cases (with PDR) and the controls (no DR), we took the median serum homocysteine value of controls as the cut-off for hyperhomocysteinemia (>16.19 μmol/L). In our study, the prevalence of hyperhomocysteinemia was higher (59%) in cases with PDR, as compared to controls with no retinopathy (48.7%). However, this difference was not statistically

significant (*P* = 0.36). In view of the heterogeneity in the definition of hyperhomocysteinemia, we tried analyzing our results, taking different cut-off values for defining hyperhomocysteinemia. However, we could not find a statistically significant difference between the two groups in our study, with cut-off levels at >10 μmol/L (92.3% vs. 89.7%; *P* = 1.0), >12 μmol/L (84.6% vs. 76.9%; *P* = 0.39), and >15 μmol/L; (64.1% vs. 53%; *P* = 0.36) [Table 4].

In our study, the mean serum homocysteine level was found to be higher among cases as compared to controls (17.98 + 6.26 μmol/L in cases vs. 17.71 + 8.17 μmol/L in controls), but the difference was not statistically significant (*P* = 0.87). The serum homocysteine levels depend upon the age profile of patients, as described by Moat *et al.*[21-23] We, therefore, chose age-matched controls for each of our cases with PDR. Levels of homocysteine are usually higher in men compared to women.[24] Hence, we selected gender-matched controls for each of our cases. Other baseline demographic variables such as place of residence and dietary pattern were also comparable between the two groups.

Duration of diabetes, hypertension, anemia, and renal dysfunction are proven risk factors for the development and progression of diabetic retinopathy. We found a significantly longer duration of diabetes and higher prevalence of hypertension, anemia, and renal dysfunction in cases with PDR as compared to controls with no retinopathy. However, there was no difference between the two groups with respect to glycemic control, hyperlipidemia, BMI, smoking, alcohol intake, and malabsorption syndromes.

Patients with diabetes are usually on several drugs, such as oral hypoglycemic drugs like metformin, statins as cholesterol reducing agents, diuretics for renal dysfunction, and several multivitamin preparations, which contain vitamin B12 and folic acid. All these drugs may influence serum homocysteine levels.[25,26] Moreover, in India, the prevalence of multinutrient deficiency, including vitamin B12 deficiency, is quite high, which may have a bearing on serum homocysteine levels.[5] There was a higher number of cases with PDR in our study, who were on multivitamin, vitamin B12, B6, nicotinamide, and folate

Table 4: Prevalence of hyperhomocysteinemia (*n*, %) with different cut-off levels of serum homocysteine

Serum homocysteine level (micromol/L)	PDR (*n*=39) *n* (%)	No DR (*n*=39) *n* (%)	*P*
≥ 16.19 micromol/L	23 (59.0)	19 (48.7)	0.36
≥ 10micromol/L	36 (92.3)	35 (89.7)	1.0
≥ 12micromol/L	33 (84.6)	30 (76.9)	0.39
≥ 15micromol/L	25 (64.1)	21 (53)	0.36

supplementation, compared to the controls. This difference was statistically significant in the case of folate supplementation, with 25.6% of the cases being on folate supplementation, while none of the controls were taking folate supplements ($P = 0.001$). This could have resulted in lower serum homocysteine levels in the cases in our study. We did not assess the serum folate and vitamin B12 levels in our study subjects due to financial constraints.

Metformin is a commonly used oral hypoglycemic drug in diabetes mellitus, but it is known to cause vitamin B12 and folate deficiency, which may result in elevation of serum homocysteine levels.[25,26] This could also have affected our results, as there was a significantly higher number of controls taking metformin (36 controls, 92.3%), compared to the cases (18 cases, 46.2%) ($P < 0.001$).

Limitations of the study

The study included only patients with Type 2 diabetes. Therefore, we may not be able to extrapolate the results of the study to all diabetic patients. There was a difference between the two groups with respect to the use of many drugs that are known to alter serum homocysteine levels. This difference was statistically significant in the case of folate and metformin use. This may have undermined the significance of the estimated difference between the two groups. We also did not assess the serum levels of vitamin B12 and folate in our study subjects due to financial constraints, and therefore, we could not find out if their serum homocysteine levels were influenced by the vitamin B12 and folate levels.

Conclusion

In this study, the prevalence of hyperhomocysteinemia as well as the mean serum levels of homocysteine were found to be higher in the cases with PDR, as compared to the controls with no retinopathy, although the difference was not statistically significant. Longer duration of diabetes, hypertension, anemia, and renal dysfunction, which are known risk factors for progression of DR, were found to be significantly associated with proliferative diabetic retinopathy.

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Conflicts of interest

There are no conflicts of interest.

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