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Original Research Article

Role of Plasma Homocysteine Levels in Diabetic Retinopathy: A Case-Control Study

Sangam[1], Jaishree Singh[2], Pooja Kapadia[3]

[1]Junior Resident, Department of Ophthalmology, Government Medical College, Kota, Rajasthan,

[2]Sr. Professor and Head of Department of Ophthalmology, Government Medical College, Kota, Rajasthan

[3]Junior Resident, Department of Ophthalmology, Government Medical College, Kota, Rajasthan

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Corresponding author: Dr. Sangam

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Abstract

Background: Higher blood levels of homocysteine are thought to be harmful to the vascular endothelium through the production of free radicals, so hyper homocysteinemia may be a risk factor for diabetic retinopathy that is potentially modifiable.

Aim: To identify hyper homocysteinemia's contribution to diabetic retinopathy.

Material and Methods: A total of 80 patients with Type 2 DM who were seen in the outpatient clinics of the Department of Ophthalmology were screened for eligibility to participate in the study. All patients underwent a dilated fundus examination to diagnose diabetic retinopathy as part of their routine clinical evaluation. Patients were equally divided into two groups: one with retinopathy and one without retinopathy. Samples were sent for measuring levels of serum homocysteine along with blood was sent to determine levels.

Results: On statistical analysis using a t test, the difference in mean homocysteine levels in both groups was statistically significant. The mean value of homocysteine in cases is higher than the mean of controls.

Conclusion: As a result of our study's findings, which showed that diabetic patients with DR had significantly higher prevalence of hyperhomocysteinaemia and mean plasma homocysteine levels than a matched control group without DR, homocysteine may be a useful biomarker or a novel risk factor for elevated risk of diabetic retinopathy in type 2 diabetic patients.

Keywords: Homocysteine, Diabetic Retinopathy.

Introduction

The hallmark of the spectrum of metabolic illnesses known as diabetes mellitus (DM) is hyperglycemia. Diabetic retinopathy (DR) is one of the most specific vascular complications of diabetes mellitus [1]. Microangiopathy, often known as diabetic retinopathy, is a disorder where hyperglycemia harms the retina's microscopic blood vessels. Retinal edoema and hypoxia are as a result of capillary leakage and non-perfusion. Neovascularization is a defining feature of

proliferative diabetic retinopathy and is caused by retinal hypoxia and ischemia. (DR). This causes tractional retinal detachment, vitreous hemorrhage, and fibrovascular development, which result in severe, frequently irreversible vision impairment and blindness [2]. Due to the exponential development in the number of diabetics, all diabetes patients will experience some type of DR within 20 years after the onset of the disease, making DR a major contributor to visual impairment in India [3]. As a result, it is thought that a number of factors contribute to the increased risk of retinopathy in people with diabetes. If the risk factors for its development are recognized, it might be possible to postpone the onset and development of retinopathy. Modifiable risk factors include some of the several ones that have been previously recognised as accelerating retinopathy. Recently, the possibility of hyperhomocysteinemia as a risk factor for the development and progression of diabetic retinopathy has been raised. Homocysteine (Hcy), an intermediary molecule in the metabolism of methonine, has attracted more attention recently as a potential risk factor for cardiovascular disease and other vaso-occlusive conditions such retinal artery occlusion [4]. Higher blood homocysteine levels are thought to damage the vascular endothelium by generating free radicals. Free radicals compromise the integrity of the endothelium, which causes platelets to become activated, coagulability to increase, and thrombus formation [5]. So, hyperhomocysteinemia may be a potentially modifiable risk factor for diabetic retinopathy. Dietary supplementation could be carried out for a very low cost, saving the patient from the financial burden of incurred medical costs as well as the burden of morbidity brought on by the disease. This is particularly true in India, where a lack of vitamin B12 and a high prevalence of diabetes [6-8].

Therefore, understanding how hyper homocysteinemia contributes to the development of the DR may help identify a new target for treating this potentially blinding illness.

Aim and Objectives

To ascertain whether hyperhomocysteinemia is related to diabetic retinopathy.

Material and Methods

This study looked into the relationship between plasma homocysteine levels and diabetic retinopathy in diabetic patients who were seen in the outpatient ophthalmology department of the government medical college and affiliated hospitals. The research was carried out between May 2021 and November 2022. In total, 80 participants were included in the study, and they were equally split into two groups:

Section I (Cases): During the study period, patients with diabetic retinopathy were seen in the Department of Ophthalmology's outpatient clinics.

The controls in Section II:

- Patients with similar ages and genders but without diabetic retinopathy.
- Patients with Type 2 diabetes who also have diabetic retinopathy meet the inclusion criteria.

Exclusion standards

Patients with known cardiovascular illness, known cerebrovascular disease, liver disease, end-stage renal disease, disease of the ovary and pancreas, and a genetic abnormality linked to high homocysteine levels were excluded. Pregnancy, cloudy ocular media, and cancer were further disqualifying criteria.

The study's information leaflet was given to every patient. Hindi and English information

leaflets were available. The information page was read aloud to participants who were illiterate in a language they could understand. After gaining their informed consent, patients were subsequently recruited in the trial.

Each study participant was given a thorough questionnaire to complete. All study participants had their blood pressure (BP) measured and their body mass index (BMI) estimated. The normal clinical indications were followed for doing ophthalmological studies including fundus fluorescein angiography and optical coherence tomography, which were necessary for the routine therapy of diabetic retinopathy.

If performed within the previous three months, fasting (AC), two-hour postprandial blood sugar levels (PC), glycosylated haemoglobin (Hba1c), lipid profile (LDL levels), haemoglobin (Hb), and serum creatinine levels were also noted. If these tests were performed within the last three months, it was because it was common practise for the treatment of diabetes patients.

Each participant gave a fasting venous blood sample for the fasting blood sugar test. To determine postprandial blood sugar levels and the HbA1c value, a two-hour postprandial venous blood sample will also be taken. The World Health Organization's (WHO) criteria for type 2 diabetes mellitus diagnosis were used (fasting glucose 7 mmol/L or post glucose 11.1 mmol/L and/or HbA1c 6.5%). The aforementioned biochemical tests were carried out at Central Laboratory with the assistance of the Department of Biochemistry, MBS Hospital, Kota, Rajasthan.

Direct chemiluminescent technology was used in a competitive immunoassay to measure the amounts of homocysteine in the blood. > 15 micromol/L is the threshold for hyperhomocysteinemia.

The categorical variables' associations with the cases and controls were evaluated using the chi square test. For continuous variables, an independent t test was performed to compare the means between cases and controls. A statistically significant result was one with a p value less than 0.05.

Observations and Result

Both cases and controls had similar average ages. While controls were on average 51.2750 years old (SD: 15.7350), patients were on average 50.77 years old (SD: 15.4513). In instances, there were 62.5% females and 37.5% males. Comparably, of the controls, 52.50 percent of the population was female. To eliminate confounding, cases and controls were matched for gender.

In comparison to the mean haemoglobin of controls, which is 11.28 g/dl (9SD=0.217), the mean haemoglobin of cases is 11.38 g/dl (SD=0.217). 47.50% of patients had excess weight, compared to 17.80% of controls. The statistical significance of this difference is 0.0086 (p value). This suggests a connection between BMI and diabetic retinopathy. 17.5% of cases were taking vitamin B12, and 47.5% of cases had previously taken metformin. Metformin and vitamin B12 were not administered to the controls. In both the cases and the controls, there was no drug using history.

Table 1: Homocysteine levels in Cases and Controls

	No of Observation	Mean	SD
Cases	40	21.8650	14.0551
Controls	40	15.3175	14.3122

T test value is 2.0644 and p value is.0423.

The average homocysteine level in cases and controls is shown in Table 1 at 21.8650 (SD 14.0551), with controls at 15.3175. (SD 14.3122). The T test was used to do a statistical analysis on this difference, and it revealed that it was statistically significant. (p = 0.0423). Therefore, we can conclude that there is a sizable difference between the homocysteine mean values in cases and controls.

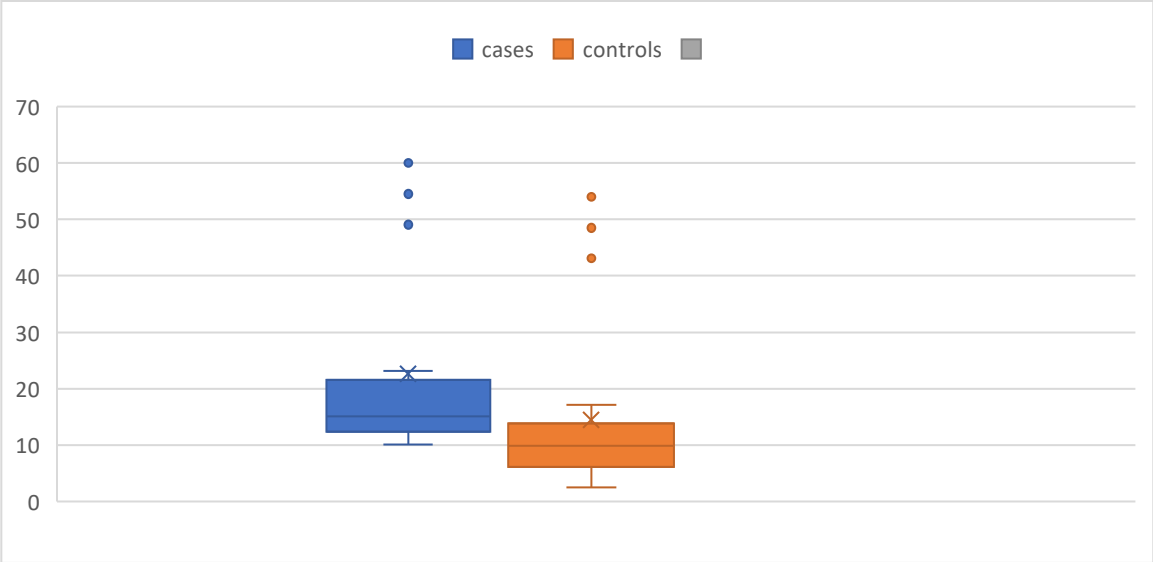

Figure 1: Homocysteine Levels of Cases and Controls

Table 2: Duration of Diabetes in Cases

	Number	Percentage
<5 years	7	17.5%
5-10 years	13	32.5%
10-15 years	13	32.5%
>15 years	7	17.5%

The length of diabetes in cases is shown in Table 2. 32.5% of cases had diabetes from 5 to 10 years and 10-15 years, whereas 17.5% had it for less than 5 years and more than 15 years. Most individuals had diabetes for five to fifteen years prior to developing diabetic retinopathy.

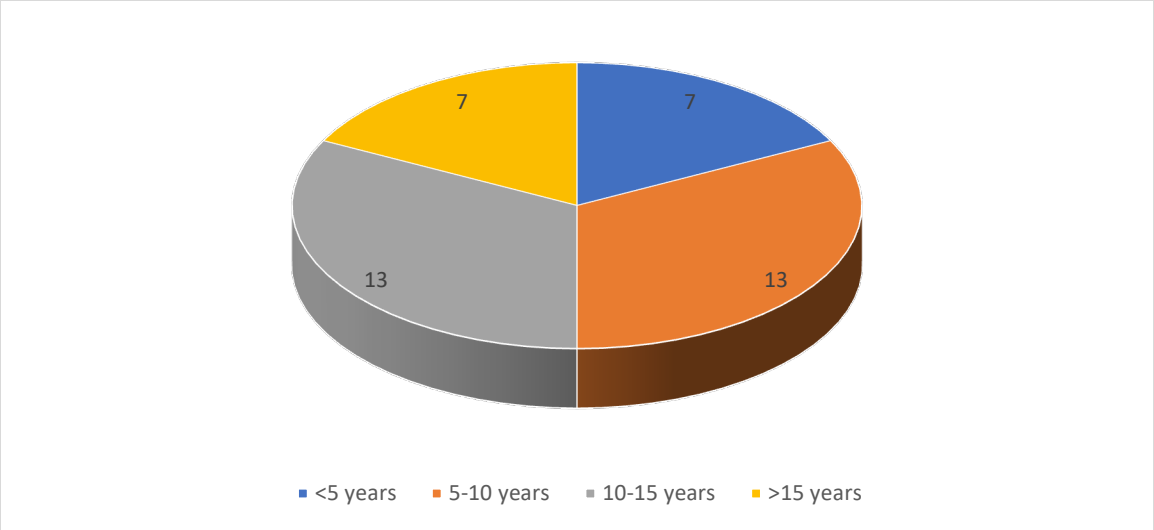

Figure 2: Duration of Diabetes in cases

Table 3: Comparison of Hb1Ac in Cases and Controls

	No of Observation	Mean	SD
Cases	40	6.4950	0.8227
Controls	40	5.3675	0.4160

T test result: 7.7350, 0.000 as the p-value

The Hb1Ac levels in patients and controls are contrasted in the table. Mean Hb1Ac was 6.495 (SD=0.8227) for patients and 5.3675 (SD=0.4160) for controls. The T test revealed that the mean difference was statistically significant (p value = 0.00). This demonstrates that the Hb1Ac between patients and controls differs significantly.

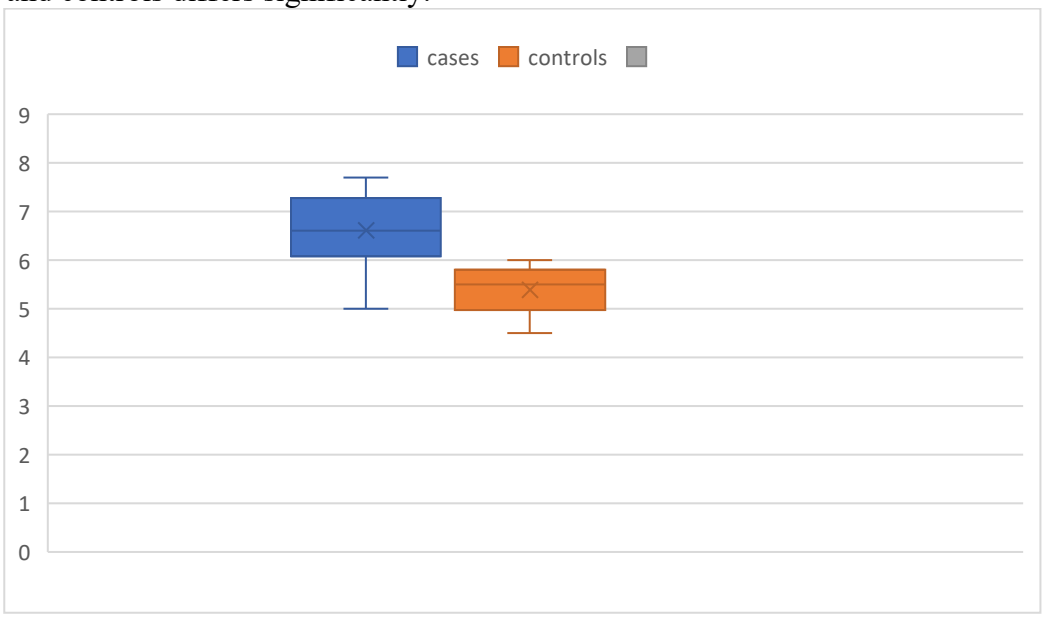

Figure 3: Mean Hb1Ac of Cases and Controls

Discusssion

The mean age of the diabetes and non-diabetic groups in our study sample is determined to be (50.77 years and 51.27 years in the DR and no DR groups, respectively). The study populations of Brazionis et al and Fotiou et al were significantly older than our research population, with median ages of 66.5 years in the DR group and 65 years in the No DR group, respectively [4,8]. The age profile of the patients does affect the serum homocysteine levels, according to Moat et al Therefore, we chose age-matched controls for each of our DR patients. Typically, men's homocysteine levels are higher than women's [9]. To eliminate confounding, patients and controls were matched for gender. In instances, there were 62.5% females and 37.5% males. Comparably, of the controls, 52.50 percent of the population was female. (p value=0.497) The difference was not statistically significant. In the studies of Brazionis et al and Fotiou et al, gender has not been looked at as a risk factor [4,8]. Retinopathy and other macro and micro vascular issues have been connected to longer diabetes duration [10]. According to our data, 17.5% of cases had diabetes for between five and fifteen years, while 32.5% had the disease for between ten and fifteen years. Most individuals had diabetes for five to fifteen years prior to developing diabetic retinopathy. The duration of diabetes in the retinopathy group in the trials by Brazionis et al and Fotiou et al was significantly longer than in the group without retinopathy [4,8]. Although the mean duration of diabetes in the study by Satyanarayana et al was 11.03 + 6.9 years compared to 10.16 + 6.9 years in the DR and no DR groups, the difference was not statistically significant (p = 0.09) [11]. In our investigation, the mean Hb1Ac was 6.495 (SD = 0.8227) for patients and 5.3675 (SD = 0.4160) for controls.

The T test revealed that the mean difference was statistically significant (p value = 0.00). According to studies conducted by Satyanarayana et al, the mean/median (with SD/IQR) HbA1c levels were significantly higher in patients with DR compared to patients without DR (10.3 + 2.9% vs. 9 + 2.5%; p 0.01), showing that there is a significant difference in HbA1c between cases and controls [11]. Fotiou et al (7.4%(6.6 -8.9%) vs. 6.7% (6.0 - 7.6%); p 0.001) and Brazionis et al (8.6% (7.1 - 10.2%) vs. 7.6% (6.6 - 8.7%); p = 0.003)(4)(8). 47.50% of patients had excess weight, compared to 17.80% of controls. The statistical significance of this difference is 0.0086 (p value). This suggests a connection between BMI and diabetic retinopathy. which was essentially identical to the study conducted by Satyanarayana et al [11].

In our study, the mean homocysteine level in Cases and Controls was 21.8650 (SD 14.0551), while in Controls, it was 15.3175. (SD 14.3122). The T test was used to statistically test for this difference, and it was discovered to be statistically different. (p = 0.0423). Therefore, we can conclude that the mean values of homocysteine in the cases and controls differ significantly. Satyanarayana et al found that the group without retinopathy had significantly higher homocysteine levels than the control group without diabetes. Additionally, they showed that patients with diabetic retinopathy had significantly higher serum homocysteine levels than those in the group without retinal damage [11]. Malagaurnera et al compared the serum homocysteine levels of patients with PDR, NPDR, no DR, and healthy controls. The mean homocysteine level in the PDR group was significantly higher than in the no DR group (18.2 + 5.6 vs. 12.1 + 6.8 mol/L; p 0.01). They also found a direct link between increasing homocysteine levels and retinopathy severity. This study found that

the odds ratios for hyperhomocysteinemia were 4.2 and 1.2, respectively, for the PDR and NPDR groups [12]. Hultberg et al's study revealed that PDR patients with T1DM had significantly higher mean + SD homocysteine levels (15.0 + 6.3 mol/L; p 0.001) than patients with little or minimal DR (10.7 + 4.3 mol/L) or controls (11.0 + 3.4 mol/L). [13].

Conclusion

Our study's findings showed that diabetic patients with DR had a significantly higher prevalence of hyperhomocysteinaemia and higher mean plasma homocysteine levels than a matched control group without DR. These results may help to partially explain why diabetic retinopathy may worsen over time in diabetic patients with greater amounts of homocysteine than in diabetics with no DR having reduced homocysteine level, but the shorter duration of the dialysis was not considered.

Significant correlations between DR and established risk factors for the evolution of diabetic retinopathy, such as longer duration of diabetes, hypertension, anemia, and renal failure, were discovered.

In order to determine the long-term impact of homocysteine levels on the emergence of diabetic eye disease, a longer time of observation is necessary.

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