Original Article

Association between Hyperhomocysteinemia and Diabetic Retinopathy

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Purpose: To study the association between hyperhomocysteinemia (Hcy) and retinopathy among diabetics and non diabetics.

Material and Methods: This Case control study was carried out at the department of Ophthalmology Liaquat National Hospital Karachi from March 2008 to November 2008. A total of 154 subjects were selected from Eye OPD, out of them 77 were diabetics with early retinopathy (cases) and 77 were non diabetics and had no history of ocular diseases (controls). Patients with advance proliferative DR were excluded. Sample size was calculated with the help of openepi software. Non probability purposive sampling was done.

Results: Serum Hcy levels measured higher than 12 µmol/L in 69 (85.2%) patients and lower than 12 µmol/L in 8 (10.9%) patients with diabetes. While serum Hcy levels were lower than 12 µmol/L in 65 (89.1%) patients and higher than 12 µmol/L in 12 (14.8%) patients of control groups. Serum Hcy levels were significantly higher in DR patients than non diabetics. According to the findings, serum Hcy levels more than 12 µmol/L were 47 times more frequent in diabetic patients with retinopathy than in non diabetics, with odds ratio of 46.71 (95% CI:17.95 to 121.6).

Conclusion: A significant association was observed between hyperhomocysteinemia and DR, with chi square value of 46.79 and P value 0.0005 at the end of the study.

Diabetes mellitus refers to the group of diseases that leads to high blood glucose levels due to defects in either insulin secretion or insulin action in the body[1]. Pakistan has a population of 154 million and more than 10% of its adult population has diabetes[2]. According to World Health Organization (WHO) estimates, there are 177 million diabetics in the world[3].

Diabetes mellitus is characterized by recurrent or persistent hyperglycemia as fasting plasma glucose level at or above 126 mg/dl, and plasma glucose at or above 200 mg/dl, two hours after a 75 gm oral glucose load as in a glucose tolerance test[4]. The current recommended goal for HbA1C in patients with diabetes is < 7.0 %, which is considered good glycemic control. People with diabetes who have HbA1c levels within this range have a significantly lower incidence of complications from diabetes including retinopathy and diabetic nephropathy[5,6]. Individuals with diabetes are 25 times more likely to become blind than individuals without this disease. In many developed countries diabetic retinopathy (DR) is a leading cause of new cases of visual impairment and blindness among adults aged 20 – 74 years.[8] Among people with type 1 diabetes, about 25% develop DR during the first five year and about 100% within two decades[8]. Among people who have type 2 diabetes, about 31% have retinopathy at diagnosis,[8] and more then 60% develop DR during the first two decades of the disease[9].

DR seems to be essentially a retinal vascular disorder probably beginning in the capillary bed. Epidemiological studies have shown that the risk and severity of DR are strongly related to the duration of diabetic mellitus, hyperglycemia and hypertension, and also but less consistently to hypercholesterolemia and smoking[10]. Another study showed an association

between the presence of DR and C677T Polymorphism of the methylenetetrahydrofolate reductase (MTHFR) gene among patients with type 2 DM[11]. DR involves both morphologic and functional changes of retinal capillaries[12,13]. Homocysteine (Hcy) is a sulfhydryl amino acid that is considered to play an important role in vascular injury resulting in the development of peripheral and coronary arterial disease[14].

Hyperhomocysteinemia may induce endothelial dysfunction and injury followed by platelet activation and thrombus formation, possibly by increasing oxidative stress;[15] therefore, it is conceivable that hyperhomocysteinemia is causally related to retinal vasculopathy through changes of the retinal vasculature and formation of microthrombi[16]. Hyperhomocysteinemia is a strong risk factor for overall mortality in diabetic patients than among diabetics and non-diabetics[17].

So, plasma Hcy should be assessed in all diabetic patients and any existing hyperhomocysteinemia should be treated with the aim of reducing the toxic effect of Hcy and preventing further capillary closure and hypoxia.

This research was an attempt to study the association between hyperhomocysteinemia and retinopathy in our population of diabetics and non diabetics, which may help in early diagnosis, treatment and prevention of new cases of visual impairment.

MATERIAL AND METHODS

This case control study was carried out at the department of Ophthalmology, Liaquat National Hospital Karachi from March 2008 to Nov 2008. A total of 154 subjects were selected. Sample size was calculated with the help of openepi software. Non probability purposive sampling was done.

Inclusion Criteria for case: patients between 20-60 years of either gender, suffering from DR of duration between 5-15 years, which was diagnosed on fundus examination using slit lamp. The fasting blood glucose should be >126 mg/dl or random blood glucose of >200 mg/dl or HbA1c should be between 6.0-9.0 mg%.

Inclusion Criteria for Controls: patients between 20-60 years of either gender who were non diabetic and had no history of ocular diseases.

Exclusion Criteria for cases: Diabetic patients without retinopathy and diabetic patient with retinopathy but duration of < 5 year. Diabeties with advance diabetic retonopathy with serum creatinine of >1.5 mg/dl.

Exclusion Criteria for controls: Patients who refused to get serum homocysteine levels checked or who did not have serum creatinine level of >1.5 mg/dl as increased serum creatinine level means there is spurious increase level of serum homocysteine, so it would not represent the true status of Hcy level.

Patients who fulfill exclusion and inclusion criteria were collected through ophthalmology department of Liaquat National Hospital. Controls were matched on age and gender, were selected from the same OPD, and were not suffering from diabetes as confirmed by investigations. From all patients serum Hcy levels was analyzed for determination of association in both groups which were matched according to the gender and age. Informed consent was taken from all patients and as there was no active intervention involved, ethical committee approval was not sought, the hospital approved to bear the cost of tests done for this study. History, ocular examination (via slit lamp biomicro-scopy through 90D) and Hcy levels were recorded in a performa. Patients with renal dysfunction associated with high Hcy levels were excluded from the study.

SPSS-10 was used to analyze data. Frequency and percentage were computed for categorical values like gender, DR and Hcy level (>12.0 μmol/L) {5.0 -12.0 μmol/L}. Mean and standard deviation were computed for quantitative variables like age and duration of diabetes. Odds ratio was computed to determine the relationship between DR and hyperhomocysteinemia using 2x2 table and significance was evaluated through the confidence interval (CI). P value <0.05 was considered as significant.

RESULTS

A total of 154 patients were included in this study, in which 77 patients with DR were taken as cases and 77 non diabetics with no history of the ocular disease were taken as control in the study. Controls were matched by age and gender and were selected from the same OPD.

The average age of the patients was 42.21±11.95 years (95% CI: 40.31 to 44.11). Similarly average Hcy level was 16.35 ± 9.83 μmol/l (95%CI: 14.79 to 17.92) and average duration of diabetes was 8.99 ± 3.44 years (95% CI: 8.21 to 9.77) as shown in table 1. Age and gender were similar in both groups because of matching.

Of the 77 diabetes patients, 34 (44.2%) patients were observed with duration of diabetes 8 to 10 years,

Table 1: Characteristics of study variables

Variables	n	Mean + SD	95% CI	Median (IQR)	Min - Ma
Age (years)	154	42.21 ± 11.95	14.31 to 44.11	42 (20)	20 - 60
Homocstiene level	154	16.35 ± 9.83	14.79 to 17.92	12 (17)	5 - 39
Duration of diabetes (years) Only case	77	8.99 ± 3.44	8.21 to 9.77	9 (5)	5 - 15

Table 3: Association between hyperhomocysteinemia and retinopathy among diabetic and non diabetic

Serum Homocysteine Level	Case n = 77	Control n = 77	Total	OR (95% CI)
> 12 umol/l	69 (85.2%)	12 (14.8%)	81	46.72 (17.95 to 121.6)
5 to 12 umol/l	8 (10.9%)	65 (89.1%)	73	

Table 2: Descriptive statistics according to type of diabetic retinopathy n = 77

Type of Diabetic Retinopathy	N	Mean ± SD
Background Diabetic Retinopathy	34	36.18 ± 9.82
Preproliferative Diabetic Retinopathy	30	40.47 ± 10.74
Proliferative Diabetic Retinopathy	13	57.77 ± 4.17

28 (36.7%) patients were with the duration of 5 to 7 years and 15 (19.5%) were observed with the duration of diabetes 11 to 15 years.

Out of 154 patients, 80 (51.9%) were male and 74 (48.1%) were female with 1.08:1 male to female ratio. There were 34 (44.2%) patients with background diabetic retinopathy (mean age 36.18 ± 9.82 years) and 13 (16.9%) patients with proliferative diabetic retinopathy (mean age 57.77 ± 4.17 years) and 30 (39%) patients with PPDR (mean age 40.47 ± 10.74 years) as shown in (Table 2).

Associations between hyperhomocysteinemia and DR in diabetics and non diabetics are presented in table 3. Serum Hcy levels measured higher than 12 µmol/L in 69 (85.2%) patients and lower than 12 µmol/L in 8 (10.9%) patients of cases. While serum homocysteine level lower than 12 µmol/L in 65 (89.1%) patients and higher than 12 µmol/L in 12 (14.8%) patients of control groups as presented in Table 3. Serum homocysteine level was significantly higher in diabetic retinopathy patients than no diabetics. According to the findings, serum homocysteine level more than 12 µmol/L. was 47 times more frequent in diabetic patients with retinopathy than non diabetics (Odds Ratio = 46.71, 95% CI: 17.95 to 121.6).

DISCUSSION

DR is a leading cause of blindness among patients with DM[18]. It involves both morphologic and functional changes of retinal capillaries[19,20]. PDR is augmented by retinal hypoxia[21]. Hyperhomocysteinemia may induce endothelial dysfunction and injury following platelet activation and thrombus formation, possibly by increasing oxidative stress[15]. So it is thought that hyperhomocysteinemia is casually related to retinal vasculopathy through changes of the retinal vasculature and formation of microthrombi[15,17]. Oxidative stress is thought to be increased in DM[22]; this may make them more susceptible to hyperhomocysteinemia induced oxidative damage.

Hoogeveen et al looked for an association between the Hcy level and retinopathy among subjects diabetics and non diabetics. There were 625 numbers of patients. They defined hyperhomocysteinemia as serum total Hcy level greater than 16 µmol/L. In their study the prevalence of retinopathy was 9.8% (28/285) in subjects with normal glucose tolerance, 11.8% (20/169) in those with impaired glucose tolerance, 9.4% (10/106) in those with newly diagnosed DM, and 32.3% (21/65) in those with known DM. It was 12.0% (64/534) in subjects with a serum total Hcy level of 16 µmol/L or less and 16.5% (15/91) in those with a serum total Hcy level of more than 16 µmol/L. After

stratification for DM and adjustment for age, sex, glycosylated hemoglobin, and hypertension, the odds ratio (95% confidence interval) for the relation between retinopathy and hyperhomocysteinemia was 0.97 (95% confidence interval, 0.42 - 2.82) in non-diabetic patients and 3.44 (95% confidence interval, 1.13 – 10.42) in diabetic patients with DM, P value was 0.08[23].

Ambrosch et al examined 65 patients with diabetes; 43 were found to have diabetic neuropathy and this subgroup had elevated levels of Hcy and a higher prevalence of hyperhomocysteinaemia[24].

Vaccaro et al studied 66 patients with diabetes and found patients with PDR; Hcy was significantly higher when compared to patients without retinopathy due to the genetic homozygote C677T mutation which was at least twice as frequent in the diabetic patients[25].

M Goldstein et al, evaluate the prevalence of hyperhomocysteinemia in diabetic patients with no DR with non proliferative diabetic retinopathy (NPDR) and with proliferative diabetic retinopathy (PDR) that study included 179 diabetic patients and 156 age matched controls with no diabetes and no history of the ocular disease, who were undergoing routine physical checkups. They were using high performance liquid chromatography (HPLC) technique for plasma Hcy level measurement. Hyperhomocysteinemia was defined when Hcy level were higher than 15 µmol/L. The mean plasma homocysteine level was 11.75 ± 0.24 in the control group, 13.46 ± 0.74 in the no DR group, 14.56 ± 0.64 in the N PDR group and 15.86 ± 1.34 in the PDR group. Mean Hcy levels were significantly elevated in the NPDR and PDR groups compared to the control group (P=0.001 and <0.0001, respectively). The prevalence of hyperhomocysteinemia was also higher in the NPDR and PDR groups compared to the control group (P=0.032 and 0.011, respectively). No statistically significant difference was found between the no DR and the control group[26].

A total of 154 patients were included in this study, 77 diabetic patients with DR including background, non proliferative and proliferative diabetic retinopathy and 77 age and gender matched controls with no diabetes and non history of ocular disease were selected from the same OPD. Plasma Hcy levels of all study participants were measured using Fluorescence Polarization Immunoassay Technique (FPIT)[27].

Serum homocysteine level measured higher than 12 µmol/L in 69 (85.2%) patients and lower than 12 µmol/L in 8 (10.9%) patients of cases. While serum homocysteine level lower than 12 µmol/L in 65 (89.1%) patients and higher than 12 µmol/L in 12 (14.8%) patients of control groups as presented in Table 3. Serum homocysteine levels were significantly higher in DR patients than non diabetics. According to the findings, serum homocysteine level more than 12 µmol/L was 47 times more frequent in diabetic patients with retinopathy than non diabetics, an odds ratio of 46.71 with 95% CI: 17.95 to 121.6. It was concluded that significant association was observed between hyperhomocysteinemia and DR, chi square 46.79 and P value 0.0005 at the end of the study.

It is considered that a higher plasma level of Hcy in diabetic patients may play a role in accelerating the micro vascular retinal changes, and may therefore contribute to the severity of DR.

The prevalence of hyperhomocysteinemia and mean plasma homocysteine level in DR patients were higher than in the control group, those patients who have PPDR and PDR have higher Hcy level than BDR. Therefore, a longer follow up period is needed to evaluate the long term effects of Hcy levels on the progression of DR. Hyperhomocysteinemia is one of the contributing factor to micro vascular angiopathy via thrombus formation in the capillaries and further impairment in blood supply to the affected tissue. It is necessary that plasma homocysteine should be assessed in all diabetic patients and that any existing hyperhomocysteinemia should be treated with the aim of reducing the toxic effect of Hcy and preventing further capillary closure and hypoxia.

CONCLUSION

Hyperhomocysteinemia may be a risk factor for retinopathy in patients of diabetes, but probably not in patients without diabetes and it partially explains the increased risk of micro vascular angiopathy in diabetic patients and can be used as a marker for the development of DR.

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