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Original Research Paper

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A STUDY TO ASSESS CORRELATION OF SEVERITY OF DIABETIC RETINOPATHY WITH SERUM HOMOCYSTEINE LEVELS IN TYPE2 DM

Dr Samiksha Agrawal

Dr Anjul Garg* MS*Corresponding Author

Dr U S Tiwari MS

ABSTRACT **Objective:** To evaluate the prevalence of high Plasma Homocysteine levels in diabetic patients and its correlation with nonproliferative diabetic retinopathy (NPDR) and with proliferative diabetic retinopathy (PDR) . Besides, this study also aims at determining association between glycemic control, serum creatinine levels and with serum homocysteine levels. Research design and methods: this hospital-based, case control study was conducted on 50 patients aging between 40 and 70 years attending the outpatient department of ophthalmology of J.A.Hospital, Gwalior from January 2018 to June 2019. Method used for Homocysteine estimation was Auto pure homocysteine assay.Results : There was statistically significant difference observed between the three groups viz. PDR, NPDR and NO DR, with respect to glycemic control (7.55±1.55, 7.60±2.88 and 5.97±.93 respectively, p = 0.04) and duration of diabetes (80±31.7, 68±44.3 and 39±54.8 respectively , p = 0.025) . Although the mean (+ SD) levels of serum homocysteine was higher among the PDR, NPDR as compared to NO DR, the difference was not statistically significant (15.56 ± 4.82µmol/L in PDR, 14.02±2.83 µmol/L in NPDR and 12.99±4.7µmol/L in NO DR ; p = 0.47). There was no statistically significant difference in the prevalence of hyperhomocysteinemia between cases and controls, when hyperhomocysteinemia was defined as serum homocysteine level > 15.00 µmol/L. **Conclusion:** Since diabetes is a microvascular occlusive disease, adjuvant risk factors contributing to a hypercoagulability state, such as increased levels of plasma homocysteine, may accelerate or aggravate the development or progression of diabetic retinopathy.

KEYWORDS : serum homocysteine level, diabetic retinopathy, type2 DM, vitamin B12 deficiency.

INTRODUCTION.

Diabetes is a microangiopathic atherosclerotic disease that affects the capillary bed in the kidneys, retina, and peripheral nervous system. Chronic hyperglycaemia is known to be the major determinant of diabetic retinopathy. Factors which have an effect on the development and progression of diabetic retinopathy are puberty, hypertension, and pregnancy. Modifiable risk factors for progression are blood glucose, blood pressure, serum lipids, and smoking. Nonmodifiable risk factors are duration, age, genetic predisposition, and ethnicity.

Diabetic retinopathy is predominantly a microangiopathy, in which the small blood vessels of the retina are damaged due to hyperglycemia. This results in capillary leakage and non-perfusion, leading to retinal edema and hypoxia. Retinal hypoxia and ischemia lead to neovascularization, which is diagnostic of proliferative diabetic retinopathy (PDR). This subsequently results in fibro vascular proliferation, vitreous hemorrhage and tractional retinal detachment, leading to severe, often irreversible visual impairment and blindness.

In recent years, hyperhomocysteinemia has been postulated as a potential risk factor for development and progression of retinopathy in patients with diabetes. Homocysteine is a sulfur containing amino acid derived from the methionine metabolism.

Elevated levels of plasma Homocysteine have been found in patients suffering from peripheral vascular occlusions, such as coronary artery disease, cerebral vascular accidents, and deep-vein thrombosis, as well as from ocular vascular occlusions, such as retinal vein and retinal artery and anterior ischemicopticneuropathy. 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. Since diabetes is a micro vascular occlusive disease, an adjuvant risk factor contributing to a hypercoagulability state, such as increased levels of plasma Homocysteine, may accelerate or aggravate the development or progression of diabetic retinopathy.

Homocysteine gets converted into an essential amino acid methionine after undergoing "methylation" in the presence of Vitamin B 12 . This is the step in its metabolism, where Vitamin B 12 and folic acid are intricately involved. Therefore Homocysteine levels are too elevated significantly in patients with Vitamin B 12 deficiency. 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. Hence, understanding and characterizing the role of hyperhomocysteinemia in the pathogenesis of DR may help in identifying a novel target to combat this potentially blinding disease.

MATERIALS AND METHODS

Design of study: This study was a hospital-based, case control study.

Patient selection: Patients with Type 2 DM, seen in the outpatient clinics of the Department of Ophthalmology, GRMC, Gwalior, from January 2018 to June 2019, were screened for eligibility for enrolment in the study. All patients underwent dilated fundus examination to diagnose and grade diabetic retinopathy, as part of their routine clinical evaluation.

Inclusion criteria

Patients with T2DM seen in the outpatient clinics of the Department of Ophthalmology

Cases: 15Patients with Non Proliferative Diabetic Retinopathy

(NPDR) and 15 Proliferative diabetic retinopathy (PDR)

Controls: 20patients without diabetic retinopathy (NO DR).

Exclusion criteria

Age less than 40 years and more than 70 year, History of liver disease, Pregnant or post-partum women , Hazy ocular media in one or both eyes, precluding adequate visualization of the fundus for diagnosis and grading of diabetic retinopathy . Ocular diseases that may result in ambiguity in the diagnosis and grading of diabetic retinopathy such as retinal vessel occlusion, retinal vasculitis and retinal changes/ vitreous hemorrhage associated with ocular trauma.

Methodology

A detailed questionnaire was administered to all the participants of the study. Measurement of blood pressure (BP) and estimation of Body mass index was done for all participants of the study.

Fasting (AC) and two-hour postprandial blood sugar levels (PC), glycosylated hemoglobin (Hba1c), lipid profile (LDL levels) and serum creatinine were done.

A fasting venous blood sample was collected from all participants for the estimation of serum homocysteine. Green tube containing heparinized lithium was used for sample collection, and serum homocysteine was analyzed by enzyme cycling method.

Method used for Homocysteine estimation

Principle: Auto pure homocysteine assay is based on the measurement of co-substrate measurement product. Oxidized homocysteine sample is reduced to free homocysteine which then react with SAM to form methionine and SAH. SAH is assessed by coupled enzyme reaction where adenosine is formed. The adenosine formed is hydrolysedintinosine and ammonia which react with glutamate dehydrogenase with concomitant conversion of NADH to NAD+. The concentration of homocysteine is proportional to the amount of NADH converted to NAD+ and is measured as change in absorbance at 340nm.

Component of reagent

AutoPure homocysteine is a ready to use, two liquid reagent system.

R1
- S – adenosylmethionine (SAM)
- NADH
- TCEP
- 2 – oxoglutarate

R2
- Glutamate dehydrogenase
- SAH hydrolase
- Adenosine deaminase
- Hcymethyltransferase

Patients with Type 2 DM in Ophthalmology OPD, who meet eligibility criteria of study

Dilated fundus examination

Patients with PDR and NPDR were cases, Age & gender matched patients without DR were controls.
Recruited into study after taking informed consent

Data collected using questionnaire

Estimation of serum homocysteine done with fasting blood samples from all cases &controls Data collated & analyzed

SUMMARY AND CONCLUSION

After obtaining approval from institutional ethical committee, this hospital-based, case control study was conducted on 50 patients aging between 40 and 70 years attending the outpatient department of ophthalmology of J.A.Hospital, Gwalior from January 2018 to June 2019.

The patients were divided into three group on the basis of severity of diabetic retinopathy using ETDRS classification.

1. No diabetic retinopathy (NO DR) = 20
2. Non proliferative diabetic retinopathy (NPDR).= 15
3. Proliferative diabetic retinopathy (PDR). = 15

Patients with history of liver disease, Pregnant or post-partum women, Hazy ocular media precluding adequate visualization of the fundus, vascular diseases of retina such as retinal vessel occlusion and ocular trauma were excluded from the study. Measurement of blood pressure, estimation of Body mass index, Fasting and two-hour postprandial blood sugar levels, glycosylated hemoglobin, lipid profileand serum creatinine were carried out.

A fasting venous blood sample was collected from all participants for the estimation of serum homocysteine.

The information so obtained was recorded in a predesigned proforma. The following observations were made:

1. Demographic profile were comparable between cases and control

2. Comparison of various risk factors in the three groups:

There was statistically significant difference observed between the three groups viz. PDR, NPDR and NO DR, with respect to glycemic control (7.55±1.55, 7.60±2.88 and 5.97±.93 respectively, p = 0.04) and duration of diabetes (80±31.7, 68±44.3 and 39±54.8 respectively , p = 0.025) . Among other variables like hypertension, hyperlipidemia, BMI and renal dysfunction, we were not able to demonstrate any statistically significant correlation with severity of diabetic retinopathy.

3. Comparison of serum homocysteine levels in the three groups:

Although the mean (+ SD) levels of serum homocysteine was higher among the PDR, NPDR as compared to NO DR, the difference was not statistically significant (15.56 ± 4.82µmol/L in PDR, 14.02±2.83 µmol/L in NPDR and 12.99±4.7µmol/L in NO DR ; p = 0.47). There was no statistically significant difference in the prevalence of hyperhomocysteinemia between cases and controls, when hyperhomocysteinemia was defined as serum homocysteine level > 15.00 µmol/L.

4. Correlation of serum homocysteine levels with other risk factors:

There was statistically significant correlation (r = 0.65; p = 0.008) between serum homocysteine and creatinine levels in the (PDR). Among other variables like HbA1c, LDL, BMI and age, we were not able to demonstrate any statistically significant correlation with serum homocysteine, in either cases or controls.

The following conclusion was drawn from our study:
- The prevalence and mean of hyperhomocysteinemia was higher in the PDR and NPDR, as compared to the controls with no retinopathy (15.56 ± 4.82µmol/L, 14.02±2.83µmol/L and 12.99±4.7µmol/L respectively; p = 0.47). However, the difference in the prevalence of hyperhomocysteinemia between the cases and controls was not statistically significant.
- Poor glycemic control which is known as risk factors for progression of diabetic retinopathy was found to be

significantly associated with DR.

- Duration of diabetes which is proven risk factor for progression of diabetic retinopathy was found to be significantly associated with DR.
- There was statistically significant positive correlation of serum creatinine with homocysteine levels in the PDR group ($r = 0.655$, $p = 0.008$).

LIMITATIONS OF THE STUDY

1. The smaller sample size as compared to previous studies.
2. We did not estimate 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.

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