RESEARCH ARTICLE

Hyperhomocysteneia as a Potential Risk Factor for Diabetic Retinopathy : Experience from Outpatient Department of a Tertiary Care Hospital

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Abstract :

Background: Diabetic retinopathy is one of the most sights threatening microvascular complication with increasing prevalence of diabetes mellitus throughout the world. It can go undetected & not even noticed until irreversible damage. Hyperhomocysteinemia is related to retinal vasculopathy seen in diabetic retinopathy. The aim of this study is to explore the association of serum homocysteine level with the glycemic status of diabetes & diabetic retinopathy to limit the development & progression of retinopathy from diabetes mellitus. **Materials and method:** This cross-sectional study was conducted in the Department of Biochemistry and Molecular Biology, BIRDEM Academy over a period of six months. According to inclusion criteria, after taking informed written consent from 90 respondents and their caregivers, a structured questionnaire was filled up for each subject. The total study population were divided into three groups, as Group I- age & gender matched healthy subjects and Group II- DM without retinopathy and Group III- diagnosed case of diabetic retinopathy. Relevant biochemical parameters such as fasting and 2hours after breakfast blood sugar, HbA1c, serum homocysteine level were measured by appropriate method. Statistical analyses were done with the help of SPSS software. **Results:** In this study, comparison of glycemic parameters (FBS, 2hrs ABF and HbA1c) among three groups were significantly higher in group-III. A positive correlation of HbA1c was found with homocysteine. This study further carried out multiple logistic regression analysis & found significant positive association of diabetic retinopathy with HbA1C and serum homocysteine. **Conclusion:** This study concluded an idea about diabetic retinopathy and its correlation and association with glycemic status among T2DM patients in Bangladeshi population. This finding may help diabetic patients to achieve effective management strategy to prevent the progression of diabetic retinopathy.

Keywords: Diabetic retinopathy; Hyperhomocysteinemia; T2DM patients; HbA1c.

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Introduction:

Diabetes mellitus (DM) is a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both [1].

The prevalence of diabetes has reached now global epidemic proportions. Worldwide about 425 million people are living with diabetes, whereas two-thirds of people with diabetes (327 million) are working age

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(20 to 65 years) [2]. Diabetic complications range from acute life-threatening conditions such as hypoglycemia or ketoacidosis to chronic debilitating macrovascular and microvascular complications whereas cardiovascular disease and peripheral artery disease are macrovascular complications and microvascular complications are retinopathy, nephropathy and neuropathy [3]. Among them one of the major microvascular pathology is diabetic retinopathy [4]. Diabetic retinopathy (DR) is a disease of the retina which is leading cause of blindness in working adults [2]. The prevalence of diabetic retinopathy among diabetic patients is 34.6%[5]. Diabetic retinopathy is characterized by appearance of vascular lesions of increasing severity, culminating in the growth of new vessels. DR progresses from non-proliferative diabetic retinopathy (NPDR), then to proliferative diabetic retinopathy (PDR)[6]. Worldwide, approximately 93 million people have diabetic retinopathy, among them 17 million has PDR and 28 million has sight – threatening diabetic retinopathy. As the incidence of diabetes is increasing at an alarming rate people with vision-threatening retinopathy may cross 51 million by 2030 [7]. Early or NPDR is marked by retinal vascular microaneurysms, blot hemorrhages, cotton-wool spots, loss of retinal pericytes, increased vascular retinal ischemia. PDR, the more severe state , is marked by the formation of abnormal, fragile new blood vessels that are prone to hemorrhage [8]. There are several pathways such as polyol pathway, hexosamine pathway, activation of protein kinase C (PKC), advanced glycation end products (AGEs) and glyceraldeydeoxidation pathways cause of oxidative stress and pathogenesis of diabetes and DR [9]. Homocysteine (Hcys) is a sulfar-containing amino acid. Elevated plasma Hcys has been associated with ocular complications in secondary glaucoma optic atrophy, age-related macular degeneration (AMD) and DR [10, 11]. Hcys is a by- product of transmethylation reactions and detoxified by methionine synthetase [12].

So, the aim of this study is to evaluate homocysteine status in diabetes and DR patients. The result was help us to find out the association between homocysteine with the glycemic status of diabetes and diabetic retinopathy. The results of this study could be helpful to establish a biomarker for early screening of DR in Bangladesh.

Materials and method:

This cross-sectional study was conducted over a period of six months. A total of 90 subjects aged 20 to 65 years will be selected for this study from outpatient department of Ophthalmology of BIRDEM General Hospital for this study. 30 healthy subjects was be taken as Group I, 30 diabetic patients without retinopathy was taken as Group II and 30 diabetic retinopathy patients was taken as Group III. FBS, 2hours ABF, HbA1c and homocysteinewere measured from all study subjects. Patients having history of recent stroke and malignancy, assumption of psychotropic drugs, anti-thyroid medication, vitamin B6 and B12 within two months prior to the clinical assessment were excluded from this study.

Sample collection

After overnight fasting for 8-10 hours about 4ml of venous blood was drawn with aseptic precautions from antecubital vein from all the subjects and dispersed 2ml in Ethylenediamine Tetra Acetic Acid (EDTA) tube for HbA1c and 2ml was delivered in fluoride tube for estimation of fasting blood glucose. Two hours after breakfast 4ml blood sample was collected and 2ml was transferred in fluoride tube for 2hrs after blood glucose and 2ml in plane tube for measurement of homocysteine.

Statistical analyses

Statistical analyses were performed with the help of SPSS (23) version. Data were presented as mean±SD. Data were compared using appropriate statistical method like Pearson correlation test, independent sample Kruskal-Willis test and Multiple logistic regression.Tests were considered significant at the level of ≤ 5% and considered as test of significance when $P<0.05$.

Results :

Total patients were 90 and the age of the patients with DR was higher (64±11.27) (Table I). Comparison of glycemic parameters (FBS, 2hrs ABF and HbA1c) among three groups were significant and that was reflected in (Table II).In this study, a positive correlation of HbA1c was found with homocysteine (Table IV). This study further carried out multiple logistic regression analysis for glycemic status influencing diabetic retinopathy among type 2 diabetic subjects showed in (Table V), where found significant positive association of diabetic retinopathy with HbA1C and serum homocysteine.

Table I: Mean± SD of basic characteristic and biochemical variables of Group I, II,III

Variables	Group I Non-DM (30) Mean± SD	Group II DM without DR (30) Mean± SD	Group III DM with DR (30) Mean± SD
Age (years)	46.87±7.56	47.80±14.70	64±11.27
FBS (mmol/l)	5.06±.72	7.68±2.24	9.2±5.27
2 hrs ABF (mmol/l)	7.03±.58	12.87±3.34	15.10±6.33
HbA_1c (%)	5.61±.48	8.33±1.66	9.06±1.95
BMI (kg/m^2)	23.14±3.34	26.51±5.12	26.13±4.13
SBP (mm of Hg)	118±5.94	120.66±10.14	122.33±8.97
DBP (mm of Hg)	77.33±4.49	78.66±6.27	78 ±6.64
STC (mg/dl)	165.86±4.54	176±36.80	191.88±41.95
ST (mg/dl)	175.23±64.78	186.96±83.09	189.80±65.96
Serum LDL-C (mg/dl)	98.26±50.70	99.03±27.19	105.88±19.42
Serum HDL-C (mg/dl)	47.25±29.86	46.26±22.83	44.14±.72
Serum Creatinine (g/L)	0.92±.24	1.02±.31	1.15±.35
ACR (mg/g)	23.49±8.06	33.30±23.52	51.90±42.17
Serum Homocysteine (µmol/L)	8.94±3.45	9.81±2.87	16.23±2.95

All our results are presented in as mean±SD values. The demographic and laboratory data of the study population are shown in Table I.

Table II:Comparison of glycemic status between groups

Variables	Group I (n=30) Mean± SD	Group II (n=30) Mean± SD	Group III (n=30) Mean± SD	P value
FBS (mmol/l)	5.06± .72	7.687± 2.24	9.2± 5.27	<0.001
2 hours ABF (mmol/l)	7.03± .58	12.87±3.34	15.10± 6.33	<0.001
HbA_1C (%)	5.61± .48	8.33±1.66	9.06± 1.95	<0.001

Group I- Non DM Group II- DM Group III- DR
Statistical analysis was done by ANOVA test to compare among groups. Values are expressed as the mean± SD. *= significant. *p≤0.05, **p<0.01, ***p<0.001.

Table III: Comparison of biochemical variables of interest among the groups

Variables	Group I (n=30) Mean± SD	Group II (n=30) Mean± SD	Group III (n=30) Mean± SD	p value
Homocysteine (µmol/L)	8.94 ± 3.45	9.81± 2.87	16.23 ± 2.95	<0.001

Group I- Non-DM Group II- DM Group III- DR
Statistical analysis was done by ANOVA test to compare among groups. Values are expressed as the mean± SD. *= significant. *p≤0.05, **p<0.01, ***p<0.001.

Table IV: Correlation of overall study population/ DR with different variables of interest

Variables	r	p-value
HbA_1C	o.672	<0.001
Homocysteine (µmol/L)	0.570	<0.001

r, Pearson correlation coefficient *=significant.*p <0.05; ** p<0.01. *** p<0.001 Pearson correlation was used for statistical analysis.

Table V: Association of Diabetic retinopathy with different variables

Variable	Beta	p-value	95.0% Confidence Interval
HbA_1c	0.672	0.000***	0.200, 0.322
Homocysteine	0.220	0.037*	0.600, 0.196

Analysis was done by Multiple logistic regression. Beta for standardized regression coefficient. *=significant.*p <0.05; ** p<0.01. *** p<0.001.

Discussion :

In the last decade, hyperhomocysteinemia has emerged as another risk factor for the development and progression of diabetic retinopathy. However, there has been no definite evidence so far, to prove or disprove this association.Numerous factors have shown as having an effect on the development and progression of diabetic retinopathy. The results of our study revealed significantly higher concentration of serum homocysteine as well as a higher prevalence of hyperhomocysteinemia (serum homocysteine > 15 mol/L) in patients with T2DR compared to those without DR and hyperhomocysteinemia as an independent risk factor for DR. Table IV have showed serum Hcy level was increased in group III than group II and III (16.23 ± 2.95 vs 9.81± 2.87 vs 8.94 ± 3.45 respectively), that difference was statistically significant (p<0.001). The exact pathogenesis of DR is multifactorial and remains largely unclear but may involve [1] endothelial dysfunction and [2] low-grade chronic inflammation of the retinal capillaries. Hyperhomocysteinemia promotes these two pathophysiologic mechanisms [13]. It has long been recognized that oxidative stress is associated with the progression of diabetes and its complications.

The adverse effects of hyperhomocysteinemia on the endothelium maybe triggered by increased oxidative stress in the diabetic vasculature. Hyperhomocystainemia increases NADPH oxidase activity promotes uncoupling of endothelial nitric oxidesynthase [14] and inhibits the function of intracellular antioxidant enzymes, such as glutathione peroxidase and superoxide dismutase [15]. Moreover,autooxidation of excess hcy may directly lead to additional ROS production [16]. Accumulating ROS reacts with nitricoxide (NO) to form peroxynitrite radicals, leading to decrease NO bioavailability and activity and subsequent endothelial dysfunction. In our study we have found a positive correlation of HbA1C and homocysteine, table IV (r=o.672, r=0.570 respectively) with developing of DR. On the contarary, Gupta p et al (2020) did not find an association between homocysteine concentrations and increasing HbA1C in their study [17]. Our study also revealed that a negative association of folate and vitamin B12 (r= - 0.767,-0.426 respectively) with DR. they were statistically highly significant (p<0.001). This result also similar to the study of Gupta p et al (2020). So we also detected such an inverse association between folate and vitamin B_{12} with homocysteine and DR.Agreed by the other study done by Satynarayana A et al (2011) [18].This study further carried out multiple logistic regression analysis for factors influencing Diabetic retinopathy that showed in Table VII. Where found significant positive association of DR with HbA1C(Beta;0.672, p<.001) and serum homocysteine (Beta;0.220, p<.05). On the other hand significant negative association was found with folate (Beta;-0.499, p<.001) and vitamin B_{12} level (Beta;-0.345, p<.01) . With regard to the risk factors for DR, logistic regression analysis of our data confirmed that poor glycemic control& homocysteine are independent risk factors for the development and progression of DR. Patients with diabetes are usually on several drugs, such as oral hypoglycemic drugs like

metformin, statins as cholesterol reducing agents, diuretics for renal dysfunction. All these drugs may influence serum Hcy levels [19]. Metformin is a commonly used oral hypoglycemic drug in diabetes mellitus which may result in elevation of serum Hcy levels [20]. Although a number of studies have been conducted worldwide, in different populations, to elucidate the association of Dr with hyperhomocysteinemia, the results have not always been consistent. Some studies found a stong association of hyperhomocysteinemia with DR, [11,21] while others have failed to do so [20]. These studies have conducted that hyperhomocysteinemia may not be an independent risk factor for DR, and have suggested that other conditions associated with diabetes, such as decling renal function and the use of oral hypoglycemic agents, may cause elevation of serum Hcy levels in diabetic patients [22]. In our study the mean serum Hcy level was found to be higher among cases DR (group III) as Compared to disease control (group II) and healthy control (group I) (16.23±2.95µmol/L in cases vs 9.81±2.87µmol/L in case control vs 8.94±3.45µmol/L in healthy control), the difference was statistically highly significant ($p< 0.001$). Our study was dissimilar with study of Gupta P et al. They had found the mean serum Hcy was found to be higher among cases as compared to controls but the difference was not statistically significant ($p=0.87$) [18]. The serum Hcy levels depend upon the age profile of patients, as described by Moat et al. [23, 24]. There are many causes that have been suggested for increased Hcy levels, such as the deficiency of any of the enzymes involved the remethylationor transsulfurationpathways of the Hcy metabolism. Our studyshowed that thedifferencesintheserumHcy werestatisticallysignificant ($p<0.05$) between the control and diabetic groups. There were significantly association of Homocysteine with type 2 diabetes mellitus with and without diabetic retinopathology.

Conclusion :

Diabetic retinopathy is one of the microvascular complications of diabetes which may not have symptoms in the early stages. Control of these complications depends on proper management and monitoring of retinal status and blood glucose levels after the early detection of retinopathy, but may progress to a sight-threatening stage if left untreated. This study revealed that there is an associationof Homocysteineamong the patients of type 2 diabetes mellitus with and without diabetic retinopathy. Here is positive correlation of homocysteine with glycemic status of the study people. This evaluation may serve to identify diabetic patients predisposed to sight-threatening complications that may benefit from intensified screening and treatment strategies and aimed to limit or prevent the incidence and progression of diabetic retinopathy.

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