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TO STUDY HOMOCYSTEINE LEVELS IN DIABETIC WITH AND WITHOUT RETINOPATHY

Sreeramulu A[1], Viswa S Reddy T[2]*

[1]Professor, Department of Ophthalmology, Kamineni Institute of Medical Sciences, and Research foundation, Amalapuram, Andhra Pradesh, India.
[2]Assistant Professor, Department of Ophthalmology, Kamineni Institute Of Medical Sciences, and Research foundation, Amalapuram, Andhra Pradesh, India.

ABSTRACT
Homocysteine is an emerging risk factor for cardiovascular and nondiabetic ocular vaso-occlusive diseases. However, studies of the relationship between homocysteine and diabetic retinopathy have reported inconsistent results. The aim of our study was to evaluate plasma tHcy levels in diabetic patients with and without retinopathy in order to investigate the role of tHcy in the progression of the diabetic retinopathy. Plasma total homocysteine concentration may be a useful biomarker and/or a novel risk factor for increased risk of diabetic retinopathy in people with type 2 diabetes.

Keywords:- Serum homocysteine level, Diabetic retinopathy, Type 2 DM, Vitamin B12 deficiency.

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INTRODUCTION

Homocysteine has generated considerable interest in recent years as both a sensitive biomarker of folate deficiency and an emerging risk factor for cardiovascular disease (even within the normal range of homocysteine concentrations) [1] and has been linked to vasoocclusive diseases in the eye [2]. The known determinants of higher fasting plasma homocysteine levels are older age, male sex, and certain genetic abnormalities, while the major risk factors for hyperhomocysteinemia (elevated plasma total homocysteine concentration) are impaired renal function and poor vitamin B status (particularly folate status but also vitamin B6 and B12 status). In the elderly (age 75 years), hyperhomocysteinemia is generally associated with low folate status or renal impairment [3].

Studies over the last two decades have shown that hyper homocysteinemia is associated with several macrovascular diseases, such as coronary artery disease, cerebrovascular disease, peripheral arterial disease, and deep-vein thrombosis [4–6]. There is also evidence supporting that Hcy abundance is closely related to renal status in the elderly. These results all suggest that Hcy is a marker of impaired renal function in diabetic patients. However, it remains unclear whether Hcy accumulation is playing a causative role that precedes early renal injury, or is only a secondary effect caused by impaired renal function in diabetic patients. There are several possible mechanisms that may lead to plasma Hcy accumulation during DN development in diabetic patients. Several studies indicate that mild elevations of homocysteine in plasma are associated with an increased risk for occlusive vascular disease, thrombosis, and stroke [7-11]

There are few reports on the relationship between plasma homocysteine and diabetic retinopathy. The aim of our study was to evaluate plasma tHcy levels in diabetic patients with and without retinopathy in order to investigate the role of tHcy in the progression of the diabetic retinopathy.

Corresponding Author : Dr. T.Viswa S Reddy **Email:** drpebyreddy@yahoo.com

MATERIAL AND METHOD:

This study was conducted at the department of Ophthalmology, Kamineni Institute Of Medical Sciences and Research Foundation, Amalapuram, Andhra Pradesh, India. After approval by Institutional Ethical Commitee, informed written consent was taken from the patients prior to inclusion in the study. Patients from both genders, aged between 35-65years, with recent or earlier diagnosis of type 2 DM were included through non-probability convenience sampling. Patients with hypertension, heart disease, renal disease, use of anti-hyperlipidaemia drugs, and history of ophthalmic diseases or surgery were excluded. The exclusion criteria included (1) patients who were already on lipid lowering drugs or glitazones; (2) females taking oral contraceptive pills or hormone replacement therapy; (3) familial hypercholesterolemia; (4) hypothyroidism; (5) patients with chronic liver disease; (6) patients with kidney disease. Assessment of DR was performed by ophthalmoscopy and or biomicroscopy through dilated pupils by a retinal specialist, and fluorescein angiography was obtained when indicated. Examination of the retina was done through dilated pupils to determine the level of nonproliferative DR or proliferative DR or diabetes without retinopathy. The DR is characterized by retinal microvascular signs that indicate the progression of the disease, from nonproliferative diabetic retinopathy (NPDR) to proliferative diabetic retinopathy (PDR), leading to macular oedema (DMO) and the commonest cause of blindness in diabetic patients. Sample size was 140 in each group keeping level of significance as p< 0.5. The data was analysed using SPSS software 11.5 version.

Table 1: Demographic Characteristic of the Study Population.

Patients with Type II Diabetes Mellitus (n=88)	
Female/Male	48/40
Age (Years)	64.3 ± 11.9
BMI (Kg/m^2)	26.0 ± 3.3
Waist Circumference (cm)	94.0 ± 8.72
HIP Circumference (cm)	96.3 ± 8.28
Waist to hip ratio	0.96 ± 0.09
Systolic Blood Pressure (mmHg)	138.1 ± 13.1
Diastolic Blood Pressure (mmHg)	81.6 ± 8.8

Table 2: (a) Laboratory Parameters of Subjects Included in the Study

Parameter	PDR 32	NPDR 30	Without DR 23
Cholesterol total (mmol/L)	6.09 ± 1.01	5.69 ± 1.02**	5.81 ± 1.05*
HDL (mmol/L)	1.36 ± 0.30	1.30 ± 0.34*	1.36 ± 0.036*
LDL (mmol/L)	4.31 ± 0.35	4.06 ± 0.30***	4.16 ± 0.31**
Triglycerides (mmol/L)	1.82 ± 0.60	1.53 ± 0.60**	1.56 ± 0.48**
Fasting Plasma Glucose (mg/dl)	1.70 ± 25	1.31 ± 29***	144 ± 23***
HbA1C (%)	8.6 ± 0.5	7.1 ± 0.7***	6.3 ± 0.5***
Comparisons between PDR and Other Groups: p=NS*; P<0.05**; P<0.001***			

Table 2: (b) Laboratory Parameters of Subjects Included in the Study

Parameter	Randomly Selected Patients (n=36)	PDR (n=32)	NPDR (n=30)	Controls (Healthy Subjects - n = 80)	Diabetic without retinopathy (n=23)
Creatinine (mg/dL)	83 ± 18.2$^{CE\$\$\$@}$	93 ± 16.4$^{***D\$\$\$@@}$	91.6 ± 15.8$^{**A\$\$\$@}$	63 ± 12.6$^{***CF@@@}$	83 ± 13.8$^{*BD\$\$\$}$
HCY (µmol/L)	1.01 ± 4.8$^{CF\$\$@}$	18.1 ± 5.8$^{***F\$\$\$@@@}$	14.1 ± 6.8$^{***C\$\$\$@}$	7.6 ± 6.6$^{**CF@@@}$	12.0 ± 6.9$^{*CD\$\$\$}$
Comparison between random selected patients and other groups: p=NS*; P<0.05**: P<0.001***					
Comparison between PDR and other groups: P=NS^A; P<0.05^B; P <0.001^C					
Comparison between NPDR and other groups: P=NS^D; P<0.05^E; P<0.001^F					
Comparison between controls and other groups: P=NS$^\$$; P<0.05$^{\$\$}$; P<0.001$^{\$\$\$}$					
Comparison between diabetic without retinopathy and other groups: P=NS$^@$; P<0.05$^{@@}$; P<0.001$^{@@@}$					

Discussion:

Many factors, some of them are yet unidentified, that affect the development and progression of DR, hyperhomocysteinemia seems to be associated with DR, at least as a biomarker. Longer diabetes duration and lower folic acid and vitamin B12 status appear to be important determinants for hyperhomocysteinemia in DR patients. Monitoring serum homocysteine concentration, as well as folate and vitamin B12 status in T2DM patients, could be used as an indicator for assessing microvascular risk in DM. Treatment of existing hyperhomocysteinemia with folic acid and vitamin B12 may be useful in reducing the risk of microvascular complications in T2DM.

Numerous factors may have an effect on progression of diabetic retinopathy. In our study higher plasma levels of homocysteine have been found in diabetic with proliferative diabetic retinopathy compared to both nonproliferative DR and diabetics without retinopathy. A previous study found moderate hyperhomocysteinemia to be a stronger cardiovascular risk factor in patients with type 2 diabetes than in nondiabetic subjects, suggesting that synergistic effects of diabetes and excessive circulating homocysteine accelerate the development of atherosclerosis [7].

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 [8]. 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 may be triggered by increased oxidative stress in the diabetic vasculature. Hyperhomocysteinemia increases NADPH oxidase activity [9], promotes uncoupling of endothelial nitric oxide synthase [10], and inhibits the function of intracellular antioxidant enzymes, such as glutathione peroxidase and superoxide dismutase [11]. Moreover, autooxidation of excess homocysteine may directly lead to additional ROS production [12]. Accumulating ROS reacts with nitric oxide (NO) to form peroxynitrite radicals, leading to decreased NO bioavailability and activity and subsequent endothelial dysfunction.

Significant differences were observed when comparing subjects with PDR versus subjects with NPDR in total cholesterol ($P < 0.05$), LDL ($P < 0.001$), and triglycerides ($P < 0.05$).The comparison between PDR and subjects without DR showed differences in LDL ($P < 0.05$) and triglycerides ($P < 0.05$). Significantly higher concentration of serum homocysteine as well as a higher prevalence of hyperhomocysteinemia (serum homocysteine > 15 μmol/L) in patients with T2DR compared to those without DR and hyperhomocysteinemia as an independent risk factor for DR. For homocysteine levels, randomly selected patients showed significant differences compared to PDR ($P < 0.001$), NPDR ($P < 0.001$), and controls ($P < 0.05$); PDR showed differences compared to randomly selected patients ($P < 0.001$), NPDR ($P < 0.001$), healthy controls ($P < 0.001$), and diabetics without retinopathy ($P < 0.001$).

Methionine and homocysteine metabolism depends upon adequate stores of folic acid and vitamins B2, B6, B12, which act as cofactors or substrates in the metabolism and are important nutritional determinants of serum homocysteine. A number of studies have shown an inverse association between blood folic acid and homocysteine concentration [13-14].

In patients with diabetes mellitus the odds ratio for hyperhomocysteinemia was 4.24 and 1.16 in PDR and NPDR, respectively. Increasing evidence suggested that the proliferation rate of cells would cause an elevation of circulating tHcy or an increase in the concentration of cells would deplete folate and inactivate the methionine synthase catalyzed remethylation reaction. This potential link between the microvascular changes that occur in diabetic retinopathy and hyperhomocysteinemia may be useful as a predictor for retinopathy. 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. Homocysteine and diabetes increase oxidative stress and reduce nitric oxide formation and may cause endothelial dysfunction [15-16]. Homocysteine enhances smooth muscle proliferation and affects the extracellular matrix. Thus elevated homocysteine level may act as a pathogenetic link or an instrument through which various risk factors may exert their deleterious effect on the promotion of diabetic retinopathy.

CONCLUSION:

Understanding and characterizing the tHcy role in the pathogenesis of diabetic retinopathy could help in identifying novel target to combat this blinding disease which is the major cause of blindness in adults. Homocysteine is a marker for microvascular disease risk or actually has a causal role for people with diabetes, homocysteine could become a useful measurement for assessing both macro vascular and microvascular risk in diabetes. Among the many factors, some of them are yet unidentified, that affect the development and progression of DR, hyperhomocysteinemia seems to be associated with DR, at least as a biomarker. Longer diabetes duration and lower folic acid and vitamin B12 status appear to be important determinants for hyperhomocysteinemia in DR patients. Monitoring serum homocysteine concentration, as well as folate and vitamin B12 status in T2DM patients, could be used as an indicator for assessing microvascular risk in DM.

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