Hyperhomocysteinemia, as an Independent Risk Factor for Retinal Venous Occlusion in an Indian Population

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Abstract Total plasma homocysteine was analysed in 64 cases of retinal vein occlusion (RVO) of which 24 cases of central retinal vein occlusion (CRVO) and 40 cases of branch retinal vein occlusion (BRVO) and compared with 45 age and sex matched control. Homocysteine was significantly increased in RVO cases in respect to control ($P < 0.001$). Analysis also revealed that prevalence of rise of plasma homocysteine was more in cases of CRVO (OR = 13) than that of BRVO (OR = 5.03).

Keywords Homocysteine (Hcys) · Hyperhomocysteinemia (HHcys) · Retinal vein occlusion (RVO) · Central retinal vein occlusion (CRVO) · Branch retinal vein occlusion (BRVO) · Odds ratio (OR)

Introduction

Retinal venous occlusion (RVO) is one among the most common retinal vascular diseases. It is predominantly of two

types, central retinal venous occlusion (CRVO) and branch retinal venous occlusion (BRVO). The basic pathology of the disease is localized atherosclerosis. It is often associated with systemic diseases. In many cases, it also occurs in young adults with no other systemic disease. Both local (raised intra ocular tension) and systemic risk factors (diabetes mellitus, hypertension, hyperlipidaemia) have been associated with RVO [1]. Among the types of RVO, based on the site of occlusion and on the type of consequent vascular damage CRVO have poorer prognosis than the BRVO.

Though hypercoagulability has been reported in the pathogenesis of RVO in young patients, laboratory tests have not accounted for this cause in most of these patients [2].

Among the parameters tested, hyperhomocysteinemia (HHcys) and circulating antiphospholipid antibodies are reported to be significantly more common in the patients with CRVO [3]. Boyd et al. [4] have reported that there is no significant increase in factor VIII (von Wilbrand factor), apart from homocysteine (Hcys) levels in the CRVO cases compared with the control subjects.

Mild to moderate elevation of plasma Hcys is reported as a risk factor for atherosclerosis in the coronary, cerebral, and retinal vasculature [5–8]. Clinical feature depends on the site and degree of atherosclerotic changes. The mechanisms by which Hcys damages the blood vessel wall seems to be multifactorial [9–14].

The cause of HHcys is varied. Severe HHcys is due to rare genetic defects resulting in deficiencies of the enzymes cystathionine β -synthase (CBS) and methyltetrahydrofolate reductase. Mild HHcys is due to impairment in the enzymes in a transmethylation pathway associated with or without nutritional deficiencies such as B₁₂ and folate [15].

All circulating Hcys is primarily derived from dietary methionine, which acts as a methyl group donor in the form

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of *S*-adenosyl methionine. On donating the methyl group it forms *S*-adenosyl Hcys which is then converted to Hcys. Hcys is a sulfur-containing nonprotein amino acid that is either metabolized to cystathionine by the transsulfuration pathway, requiring B₆, or it is converted back to methionine by B₁₂ and folate, requiring transmethylation [16].

There were contradictory reports in support of the hypothesis that HHcys are associated with RVO cases [17–19]. Therefore, a 1 year prospective study in adult RVO cases was performed to evaluate the relationship between the plasma total Hcys (tHcys) with CRVO and BRVO.

Materials and Methods

Design

A 1 year prospective case–control study of consecutive, unrelated adult patients, with a diagnosis of CRVO and BRVO in the absence of any other local and systemic disease, was conducted at Regional Institute of Ophthalmology, Kolkata and Department of Biochemistry in R G Kar Medical College and Hospital, Kolkata. The institutional ethics committee approved the study and informed consent was obtained from all the study populations, in accordance with the Declaration of Helsinki. Age- and sex-matched control subjects were the patients attending the out patient department of Regional Institute of Ophthalmology, Kolkata for uncomplicated cataract surgery.

A detailed questionnaire on family history, social status, and dietary habits, including other habits such as smoking, alcohol intake, history of systemic diseases, other ocular diseases and drug history was completed by all the study subjects. Hypertension, diabetes mellitus, cardiovascular disease, high cholesterol, renal disease, liver disease, hematologic and coagulation abnormalities were ruled out in the present study based on the biochemical and hematologic tests apart from the questionnaire.

Ophthalmic examination of eyes, including visual acuity, relative afferent pupillary defect (RAPD), electroretinogram (ERG), and fundus examination, was used for the clinical diagnosis of RVO. A total of 109 subjects participated in the study. Based on the inclusion and exclusion criteria, 64 patients were included in the study. Among them 24 had CRVO and 40 had BRVO. All RVO cases were unilateral. A total of 45 subjects were taken as control subjects based on the questionnaire. None of the study subjects had any history of major thromboembolic episode.

Measurement of Plasma Hcys

Venous blood samples were drawn into EDTA-containing tubes after the participants fasted overnight. Plasma was

separated immediately from blood cells by centrifugation at 1,000×*g* at 25 °C for 3 min and brought to Biochemistry department of R G Kar Medical College and Hospital, Kolkata to be analysed. Total plasma Hcys was estimated with a Reagent kit, supplied by Lilac Clinical chemistry division (lot: CRB1010022A) [20].

Other biochemical tests—fasting plasma glucose, lipid profile (total cholesterol, triglyceride, HDL cholesterol, LDL cholesterol), liver function test (ALT, AST, Total Bilirubin, Direct Bilirubin, Total protein, Albumin), kidney function test (Urea, Creatinine), tests for hematologic (TC, DC, ESR, Hb) and coagulation defects (CT, BT, PT) were performed. RA factor, Anti Nuclear Antibody was also measured to exclude autoimmune diseases.

Results and Discussion

Arteriolosclerosis is an important causative factor for BRVO. Because a retinal arteriole and its corresponding vein share a common adventitial sheath, thickening of the arteriole appears to compress the vein. This causes secondary changes, including venous endothelial cell loss, thrombus formation, and potential occlusion. Similarly, the central retinal vein and artery share a common adventitial sheath at the arteriovenous crossings posterior to the lamina cribrosa so that atherosclerotic changes of the artery may compress the vein and precipitate the CRVO. It therefore appears that both arterial and venous disease contribute to RVO. Venous occlusion causes elevation of venous and capillary pressure with stagnation of the blood flow. This results in hypoxia of the retina drained by the obstructed vein, which in turn results in damage to the capillary endothelial cells and extravasations of blood constituents. Tissue pressure is increased, causing further stagnation of the circulation and hypoxia, so that a vicious cycle is established [1].

The present study indicates a significant increase in the Hcys levels in the patients with RVO (mean tHcys, 18.25 ± 5.43 μM/L) as opposed to the control subjects (mean tHcys, 12.53 ± 2.16 μM/L; $P < 0.001$) (Table 1; Fig. 1). Analysis also showed that an elevated Hcys level was a risk factor for RVO, with an OR = 7.36 (Table 2).

Of the 64 patients with RVO in the study, 24 had CRVO (37.5 %) and 40 had BRVO (62.5 %). A more increase in the Hcys levels was seen in the patients with CRVO (mean tHcys, 19.16 ± 4.96 μM/L) in comparison to the BRVO cases (mean tHcys, 17.71 ± 5.63 μM/L) (Table 3; Fig. 2). Analysis also showed that HHcys are associated with increased incidence of CRVO (OR = 13) than that for BRVO (OR = 5.03) (Table 4).

There are reports strongly indicating that HHcys is an independent risk factor for CRVO [18, 19, 21]. Lattanzio

Table 1 Mean plasma tHcys levels in RVO and control subjects

Parameter	RVO (mean $\pm$ SD) (μ M/L)	Control (mean $\pm$ SD) (μ M/L)
Plasma tHcys	18.25 $\pm$ 5.43*	12.53 $\pm$ 2.16

Value expressed as mean $\pm$ SD* $P < 0.001$ as compared with control **Fig. 1** Comparison of plasma total homocysteine level between RVO cases and control**Table 2** Incidence of hyperhomocysteinemia (HHcys) in RVO in respect to Control

Parameter	RVO	Control
High Hcys	34	6
Normal Hcys	30	39
Total	64	45

OR = 7.36 for RVO

Table 3 Mean plasma tHcys levels in CRVO, BRVO and control subjects

Parameter	CRVO (mean $\pm$ SD) (μ M/L)	BRVO (mean $\pm$ SD) (μ M/L)	Control (mean $\pm$ SD) (μ M/L)
Plasma tHcys	19.16 $\pm$ 4.96*	17.71 $\pm$ 5.63*	12.53 $\pm$ 2.16

Value expressed as mean $\pm$ SD* $P < 0.001$ as compared with control

et al. [15] have reported an OR of 3.0 for fasting HHcys in patients with CRVO and an OR of 1.3 was reported by another study in a Chinese population [22]. The meta-analysis by Janssen et al. [23] has also shown an overall OR of 8.9 (95 % CI, 5.7–13.7) for Hcys. The meta-analysis by Cahill et al. [24] has shown that the retinal vascular occlusion is associated with elevated plasma Hcys levels and low serum folate levels. One study has shown that defective Hcys remethylation caused by a deficiency of either methionine synthase or folate produces oxidative stress and endothelial dysfunction in the cerebral microcirculation of mice [25].

Fig. 2 Comparison of plasma total homocysteine level among CRVO cases, BRVO cases and control**Table 4** Incidence of hyperhomocysteinemia (HHcys) in CRVO and BRVO in respect to control

Parameter	CRVO	BRVO	Control
High Hcys	16	18	6
Normal Hcys	8	22	39
Total	24	40	45

OR = 13 for CRVO and OR = 5.03 for BRVO

The possibility of direct cytotoxic effect of Hcys and Hcys thiolactone in the retinal vascular endothelial cells has also been reported in a case report by Poloshek et al. [26]. He has reported methionine deficiency in this patient in association with microvascular damage to the retina.

There are various mechanisms reported regarding endothelial dysfunction by Hcys. These include decreased bioavailability of nitric oxide [10], altered expression of various thrombotic factors, mitogenic effect on arterial smooth muscle cells [11], and expression of acute stress-related genes [12]. Moreover, the high pK_a of the sulfhydryl group ($pK_a = 10.0$) of Hcys is responsible for the formation of stable disulfide bonds with protein cysteine residues and, in the process, alters or impairs the function of many proteins. Albumin, fibronectin, transthyretin, annexin II, and factor V have now been identified as molecular targets for Hcys [13]. Metabolic conversion of Hcys to a chemically reactive metabolite, Hcys-thiolactone is suggested to contribute to Hcys toxicity in humans (Hcys-thiolactone hypothesis) [14] leading to endothelial dysfunction.

In this study the patients with RVO show a significantly elevated Hcys in respect to control. There is a possibility of a direct cytotoxic effect of Hcys on the retinal endothelial cells, apart from its prothrombotic effects. Exposure of vascular smooth muscle cells to HHcys can lead to upregulation of the inflammatory response that characterizes early atherogenesis and may, in part, account for the adverse vascular effects of HHcys [27].

In the absence of any systemic disease or abnormal hematological and coagulation parameters, including hereditary thrombophilic defects, as ruled out by the exclusion criteria, no major thromboembolic activity or coagulation disorders were recorded in the adult patients with RVO recruited in the study. Hcys-induced thrombosis may be a crucial factor triggering vascular occlusion [28]. Regarding thrombophilic risk factors and retinal venous occlusion, as per the meta-analysis by Janssen et al. [23], there is evidence of association between HHcys and venous thrombosis.

HHcys is more prevalent in CRVO than that of BRVO in our study population. Therefore, B₁₂ and folate supplementation suggested to correct the HHcys, in patients with RVO would be worth considering.

Conflict of interest Nil.

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