A Study Of Correlation Of Plasma Homocysteine With Serum Lipid Profile In Retinal Vein Occlusion

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Abstract

Purpose: Both hyperhomocysteinemia and dyslipidaemia are considered as an independent risk factor in retinal vein occlusion. This study was done to find out the correlation of plasma homocysteine with serum lipids in patients with retinal vein occlusion.

Material & Methods: A total of 84 retinal vein occlusion cases and 65 age and sex matched controls were assayed to explore the relationship of plasma homocysteine with serum lipid profiles in this observational, cross sectional, open, comparative, eight month study. Results: Plasma homocysteine, total cholesterol, triglyceride, LDL cholesterol and VLDL cholesterol levels were elevated significantly (P <0.001) and HDL cholesterol was decreased significantly (P <0.001) in the patients with RVO as opposed to the control subjects. Significant negative correlation was found between homocysteine and HDL cholesterol in RVO patients (r = -0.273, P < 0.029).

Conclusions: Patients with low HDL cholesterol should be screened for HHcys as association of low HDL cholesterol and HHcys might have a synergistic effect on the retinal circulation. Future study is needed to see whether treatment of HHcys will increase the HDL cholesterol level and that can be an important preventive measure against development as well as treatment of retinal vein occlusion.

Keywords: Homocysteine (Hcys), Hyperhomocysteinemia (HHcys), High density lipoprotein (HDL), Low density lipoprotein (LDL), Retinal vein occlusion (RVO).

Retinal vein occlusion (RVO) is the 2nd most common retinal vascular disorder after diabetic retinopathy with prevalence ranging from 0.7% to 1.6%1. It is of three types depending on the site of occlusion and the consequent vascular damage - central retinal vein occlusion (CRVO), branch retinal vein occlusion (BRVO) and hemi central retinal vein occlusion (HCRVO)2. Among them, CRVO have poorer prognosis than the BRVO. The basic pathology of the disease is localized atherosclerosis. Both local (Raised intra ocular tension) and systemic risk factors (Diabetes mellitus, Hypertension, Hyperlipidaemia) have been associated with RVO3.

In 1969, McCully suggested that moderate levels of hyperhomocysteinemia (HHcys) might be associated with atherosclerosis4,5. Mild hyperhomocysteinemia (HHcys) is also reported as a risk factor for atherosclerosis in the coronary, cerebral, and retinal vascularuteture6-9. There were also reports in support of the hypothesis that HHcys were associated with RVO cases10-12.

Hcys is an amino acid containing sulfur. It is derived from dietary methionine which is initially converted into S-adenosyl methionine that donates the methyl group to a methyl accepter and itself forms S-adenosyl Hcys which is eventually converted back to Hcys. It is either reconverted to methionine requiring B12 and folate or metabolized to cystathionine requiring B613. Enzymes deficiency like cystathionine â-synthase (CBS) and methylytetrahydrofolate reductase or nutritional deficiencies such as B12, B6 and folate are the major causes of HHcys14. Dyslipidaemia3, 15 and hyperhomocysteinemia10 were considered as an independent risk factor in retinal vein occlusion but no study was done to find out the correlation between them in RVO. In this context, this study was performed to find out the correlation of plasma homocysteine with serum lipids in patients of RVO.

Materials and methods

This observational, cross sectional, open, comparative, eight month study was conducted in ESIPGIMSR & ESIC Medical College, Joka with a total of consecutive 84 (47 males and 37 females) unilateral RVO cases attending the outpatient department of Ophthalmology. Sixty five (35

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Received on : 27/11/2014, Accepted on : 30/12/2014
Conflict of Interest : None, Financial Disclosure : None
males and 30 females) age and sex matched controls were included in the study. Presence of any of the following conditions like pregnancy, lactation, malignancy, sepsis, liver and renal failure, recent vascular accidents (< 6 months), previous thromboembolic events, inflammatory disorders, thyroid disorder, diabetes mellitus, vitamin intake (B6, B12, and folate), alcohol, drugs (methotrexate, fibrates) and smoking were excluded from the study population by detailed history, clinical and biochemical examination. The study was approved by the institutional ethics committee and informed consent was obtained from all the study populations, in accordance with the Declaration of Helsinki.

Fasting blood samples were collected from the patients and the controls. The blood samples were collected by vein puncture in EDTA vial and plain vial using disposable syringe. The blood collected in EDTA vial was immediately centrifuged at 1000g at 25°C for 3 minutes and plasma was separated and analyzed for Hcys. Samples were stored tightly capped at 2-8 °C for up to 48 hours if testing was delayed. Plasma Hcys was estimated by enzymatic method in autoanalyser (Toshiba TBA40FR Biochemistry analyser) with a Reagent kit, supplied by Lilac Clinical chemistry division16. (Linearity extends to 50 ìmol/L)

The blood collected in plain vial was kept in tilted position for 30 minutes at room temperature and then centrifuged to separate serum for the estimation of lipid profile. Serum total cholesterol was measured after enzymatic hydrolysis and oxidation17. The High density lipoprotein (HDL) cholesterol level was determined after precipitating the chylomicrons, Very low density lipoprotein (VLDL) and low density lipoprotein (LDL) fragments, using phosphotungstic acid and magnesium chloride18. Serum triglyceride level was determined after enzymatic hydrolysis with lipases19. The serum LDL and VLDL cholesterol levels were determined using the formula of Friedwald T (1972)20. Serum lipid profiles were estimated in autoanalyser (Toshiba TBA40FR Biochemistry analyser) with a Reagent kit, supplied by coral.

Statistical analysis was performed using the Student’s t-test and Pearson’s correlation coefficient by SPSS software (Versions 16.0).

Results

The mean age of RVO patients and control participants were 44.1 ± 15.2 years and 50.2 ± 10.6 years respectively. Of the 84 RVO cases 59 patients were BRVO, 22 were CRVO and 3 were HCRVO.

Hcys levels were increased significantly in the patients with RVO (mean total Hcys, 17.86 ± 5.13 ìmol/L) as opposed to the control subjects (mean total Hcys, 12.05 ± 2.11 ìmol/L; P < 0.001). (Figure- 1)

Total cholesterol (209.2 ± 53 mg/dl) levels were elevated significantly as opposed to the control (159.2 ± 30 mg/dl) (P <0.001). Triglyceride (175.3 ± 45.6 mg/dl) levels were
Table-1: Correlations of Homocysteine with Total cholesterol, Triglycerides, LDL cholesterol, HDL cholesterol and VLDL cholesterol in RVO patients (N=84).

<table>
<thead>
<tr>
<th>Homocysteine</th>
<th>Pearson Correlation</th>
<th>Total cholesterol</th>
<th>Triglycerides</th>
<th>LDL cholesterol</th>
<th>HDL cholesterol</th>
<th>VLDL cholesterol</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0.102</td>
<td>-0.061</td>
<td>0.036</td>
<td>-0.273*</td>
<td>-0.189</td>
</tr>
<tr>
<td>Sig. (2-tailed)</td>
<td></td>
<td>0.424</td>
<td>0.626</td>
<td>0.776</td>
<td>0.029</td>
<td>0.135</td>
</tr>
</tbody>
</table>

* Correlation is significant at the 0.05 level (2-tailed).

Discussion

A retinal arteriol and its corresponding vein share a common adventitial sheath. Thickening of the arteriol 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 extravasation of blood constituents. Tissue pressure is increased, causing further stagnation of the circulation and hypoxia, so that a vicious cycle is established.3

HHcys was reported as an independent risk factor for CRVO11-12, 21. An Odds Ratio (OR) of 3.0 for fasting HHcys in patients with CRVO was reported by Lattanzio et al14 and an OR of 1.3 was reported by another study in a Chinese population22. Janssen et al23 have observed an overall OR of 8.9 (95% CI, 5.7–13.7) for Hcys. The meta-analysis by Cahill et al24 have shown that raised plasma Hcys levels and low serum folate levels were associated with retinal vascular occlusion. Dayal S et al25 have observed that deficiency of either methionine synthase or folate produces oxidative stress leading to the endothelial dysfunction in the cerebral microcirculation of mice. The direct cytotoxic effect on retinal vascular endothelial cells by Hcys and Hcys thiolactone has also been reported in a case report by Poloshek et al26.

The results of the present study indicated that hcys levels were increased significantly in the patients with RVO (mean tHcys, 17.86 ± 5.13 ìmol/L) as opposed to the control subjects (mean tHcys, 12.05 ± 2.11 ìmol/L ; P < 0.001). (Figure-1)

There are various mechanisms reported regarding endothelial dysfunction by Hcys. These include diminished bioavailability of nitric oxide27, abnormal expression of various thrombotic factors28. Hcys can form stable disulfide bonds with protein cysteine residues and, in the process, alters or impairs the function of many proteins like albumin, fibronectin, transthyretin, annexin II, and factor V29. Hcys may be metabolised into Hcys-thiolactone which is a highly reactive compound that contributes to Hcys toxicity in humans30 (Hcys-thiolactone hypothesis) leading to endothelial dysfunction. HHcys can lead to upregulation of the inflammatory response in the vascular smooth muscle cells that characterizes early atherogenesis31.

This study also observed that total cholesterol, triglyceride, LDL cholesterol and VLDL cholesterol levels were elevated significantly (P <0.001) as well as HDL cholesterol was decreased significantly (P <0.001) in the patients with RVO as opposed to the control subjects. There are various mechanisms reported regarding endothelial dysfunction
by LDL Cholesterol. It is initially converted into oxidized LDL cholesterol by free radicals. The oxidized LDL is taken up by scavenger receptor on monocyte macrophages leading to foam cell. It decreases the expression of endothelial nitric oxide synthase and in turn inhibiting nitric oxide mediated vasorelaxation. It also stimulates Interleukin-1, tumor necrosis factor-alpha and interferon-gamma which cause leukocyte recruitment and adhesion to the endothelial cell.

Although Jadav et al did not find any correlation between Hcy with lipid profile in ischemic heart disease patients but our study observed a significantly negative correlation with HDL Cholesterol in RVO patients (Table-1). This can be explained by possible action of Hcys in reducing the expression of peroxisome proliferator-activated receptor (PPARα) and decreased the ApolipoproteinA-I promoter activity and its protein levels. In addition to its influence on ApolipoproteinA-I, hyperhomocysteinemia inhibits reverse cholesterol transport by reducing circulating HDL via inhibiting ApolipoproteinA-I protein synthesis and enhancing HDL cholesterol clearance in mice.

It was well established that both HHcys and Dyslipidemia can induce atherosclerotic changes. Our study showed that low HDL cholesterol was aggravated by HHcys in RVO cases as evidenced by strong negative correlation between them. So the Patients with low HDL cholesterol should be screened for HHcys as association of low HDL cholesterol and HHcys might have a synergistic effect on the retinal circulation. Future study is needed to see whether treatment of HHcys will increase the HDL cholesterol level and that can be an important preventive measure against development as well as treatment of retinal vein occlusion.

References:


34. Witztum JL. Immunological response to oxidized LDL. Atherosclerosis 1997;131:S9-S11.

