



Correlation between Plasma Homocysteine Level and Retinal Vein Occlusion: A Case Control Study

Authors

Dr Vinayak Ganesh Bhat MD¹, Dr Rajkumar Patra MS^{2*}

¹Assistant Professor, Department of Biochemistry, Maharajah's Institute of Medical Sciences, Nellimarla, Vizianagaram, Andhra Pradesh, India

²Assistant Professor, Department of Ophthalmology, Maharajah's Institute of Medical Sciences, Nellimarla, Vizianagaram, Andhra Pradesh, India

*Corresponding Author

Dr Rajkumar Patra MS

Assistant Professor, Department of Ophthalmology, Maharajah's Institute of Medical Sciences, Nellimarla, Vizianagaram, Andhra Pradesh, India

Abstract

Introduction: Retinal venous occlusion (RVO) is very common condition affecting retinal vasculature. It's caused by localized atherosclerosis and often associated with systemic disorders. Regular tests showed Hyperhomocysteinemia is seen to be highly associated in patients with RVO along with other systemic causes. Mild to moderate homocysteine levels thought to cause atherosclerosis in Retinal vasculature by damaging the blood vessel wall. This study assesses the correlation between plasma homocysteine and retinal vein occlusion.

Materials and Methods: this was a progressive case-control based study conducted in Maharajah's Institute of Medical Sciences, Vizianagaram, Andhra Pradesh over a period of 1 year. 58 patients with diagnosis of RVO were included in the study after exclusion criteria. Detailed ophthalmological examination along with plasma Homocysteine levels were checked along with coagulation and hematological tests. Patients were followed up for 1 year for progression of disease.

Results: there was a strong association between high levels of Homocysteine and RVO. A statistically significant association was found ($p < 0.001$) when compared to control. Analysis also revealed that prevalence of rise in plasma homocysteine levels in cases of Central Retinal vein occlusion (CRVO) (OR=13) compared to Branch Retinal Vein Occlusion (BRVO)(OR=5.03).

Conclusion: Hyperhomocysteinemia is more prevalent in CRVO than BRVO in our study subjects. So, treatment of Hyperhomocysteinemia by folic acid and Vitamin B12 supplementation in RVO patients should be considered.

Introduction

Retinal venous occlusion is very common condition affecting retinal vasculature. Its divided into two types. Central Retinal Venous Occlusion (CRVO) and Branch Retinal Vein Occlusion (BRVO). It's caused by localized atherosclerosis and often associated with systemic disorders. Both

local (Raised intra ocular Pressure) and systemic risk factors (Hyperlipidemia, Hypertension, Diabetes mellitus) have been associated with RVO¹. Among the types of RVO, CRVO has poorer prognosis than BRVO because of its sight of occlusion and type of consequent vascular damage.

Especially in young patients, it's thought to be caused by hypercoagulability of blood. Biochemical and other laboratory tests have not accounted for this cause². Regular tests done by Department of Biochemistry among all the parameters tested, Hyperhomocysteinemia is seen to be highly associated in patients with CRVO. Study conducted by Boyd et al³ reported that there is no significant increase in Von Willebrand factor (factor VIII) apart from homocysteine levels in cases of CRVO when compared to control subjects. Mild to moderate homocysteine levels have always been reported as risk factor for the development of atherosclerosis in Retinal, cerebral and coronary vasculature^{4,5,6,7}. Clinical features seen depends on the degree and site of atherosclerosis. Homocysteine is found to damage the blood vessel wall in multifactorial pathway^{8,9,10,11,12,13}.

Hyperhomocysteinemia is caused by various reasons. It can be caused by a rare genetic defect due to deficiency of enzymes cystathione β synthase (CBS) and methyltetrahydrofolate reductase. Even nutritional deficiencies like vitamin B12 and folate¹⁴ can cause mild Hyperhomocysteinemia as these vitamins play a key role in the Biochemical pathways.

All circulating Homocysteine is derived from Dietary methionine. Methionine acts as a methyl group donor as S adenosyl methionine (SAM). After receiving methyl group homocysteine becomes S adenosyl homocysteine (SAH) which is then converted back to Homocysteine. Homocysteine is a Sulphur containing non protein amino acid which is removed from blood in two ways.

- 1) Metabolized to cystathione by transsulfuration pathway which requires pyridoxine (Vitamin B6)^{15, 16}
- 2) Converted back to methionine with the help of Vitamin B12 and folate. It requires transmethylation reaction^{15, 16}.

There were also some contradictory reports suggesting the hypothesis that Hyperhomocysteinemia causes RVO^{17,18}. so, to

evaluate the correlation, current study was conducted for 1 year.

Materials and Methods

Study Design

A 1-year prospective case-control study was conducted in patients with diagnosis of CRVO. Study excluded those patients with other local or systemic diseases. This study was conducted in coordination of Department of Biochemistry and Department of Ophthalmology in Maharajah's Institute of medical sciences (MIMS), Nellimarla, Vizianagaram, Andhra Pradesh, India. Institutional ethical committee approval was taken for the study. All the patients who participated in the study have been explained the study in their own language and informed consent was taken. These were the patients who visited regular OPD in MIMS with history suggesting CRVO.

A detailed history was taken by the participants which included family history, Dietary habits, other habits like smoking, alcohol intake, history of systemic and other ocular diseases, drug history. Exclusion criteria included Diabetes mellitus, Hypertension, cardio vascular disease, high cholesterol, liver disease, renal disease, Coagulation and other hematological abnormalities as per Biochemical and Hematological tests conducted in the subjects.

Both eye ophthalmological examination was done. Visual acuity, relative afferent pupillary defect (RAPD), slit lamp bio microscopy and fundus examination was used to clinically diagnose RVO. 102 patients participated in the study. After thoroughly screening of inclusion and exclusion criteria, 58 patients were included in the study among which 21 had CRVO and 37 had BRVO. 50 subjects were taken as control who didn't have RVO. None of the study subjects had any history of major thromboembolic disease.

Biochemistry Testing

Patients were told to do overnight fasting. Venous blood sample was taken into EDTA containing

tube in Department of Biochemistry. Plasma was separated immediately by centrifugation at 1000 x g at 25°C for 3 minutes. Total plasma Homocysteine was estimated with reagent kit supplied by ERBA Mannheim clinical chemistry division.

Other biochemical tests were also done to subjects including Fasting plasma glucose, Lipid profile (LDL cholesterol, HDL cholesterol, VLDL Cholesterol, Total cholesterol, Triglycerides), Coagulation tests (CT, BT, PT), Hematological tests (TC, DC, ESR, Hb).

Results

In present study, Incidence of Hyperhomocysteinemia was also studied in current study. High homocysteine levels were found in 31 patients with RVO in which 14 were CRVO patients and 17 were BRVO patients. Normal homocysteine levels were found in 27 RVO patients in which 7 were CRVO and 20 were BRVO. In control subjects, 6 had high

Homocysteine levels and 36 had normal homocysteine levels.

mean ±SD Plasma total homocysteine levels of RVO was found to be 18.21 ± 5.48 µM/L as compared to control 12.51 ± 2.14 µM/L. When studied in detail, mean ±SD Plasma total homocysteine levels of CRVO was found to be 19.04±5.15 µM/L as compared to control 12.51 ± 2.14 µM/L. mean ±SD Plasma total homocysteine levels of BRVO was found to be 17.39±5.82 µM/L as compared to control 12.51 ± 2.14 µM/L. so it was found that, levels of Homocysteine were significantly high in RVO patients when compared to control subjects. (P < 0.001). analysis also showed that an elevated homocysteine level was a risk factor for RVO with an OR = 7.36.

It was seen that; Homocysteine levels were higher in CRVO than BRVO. Analysis also showed that Hyperhomocysteinemia is associated with increased incidence of CRVO (OR=13) compared to BRVO (OR=5.03)

Table 1 Mean total homocysteine levels in RVO and control subjects

Parameter	RVO (mean ±SD) (µM/L)	Control (mean ±SD) (µM/L)
Plasma total homocysteine	18.21 ± 5.48	12.51 ± 2.14

Value expressed as mean ±SD
P< 0.001 as compared to control.

Fig. 1 Comparison of plasma total homocysteine levels between RVO of cases and control

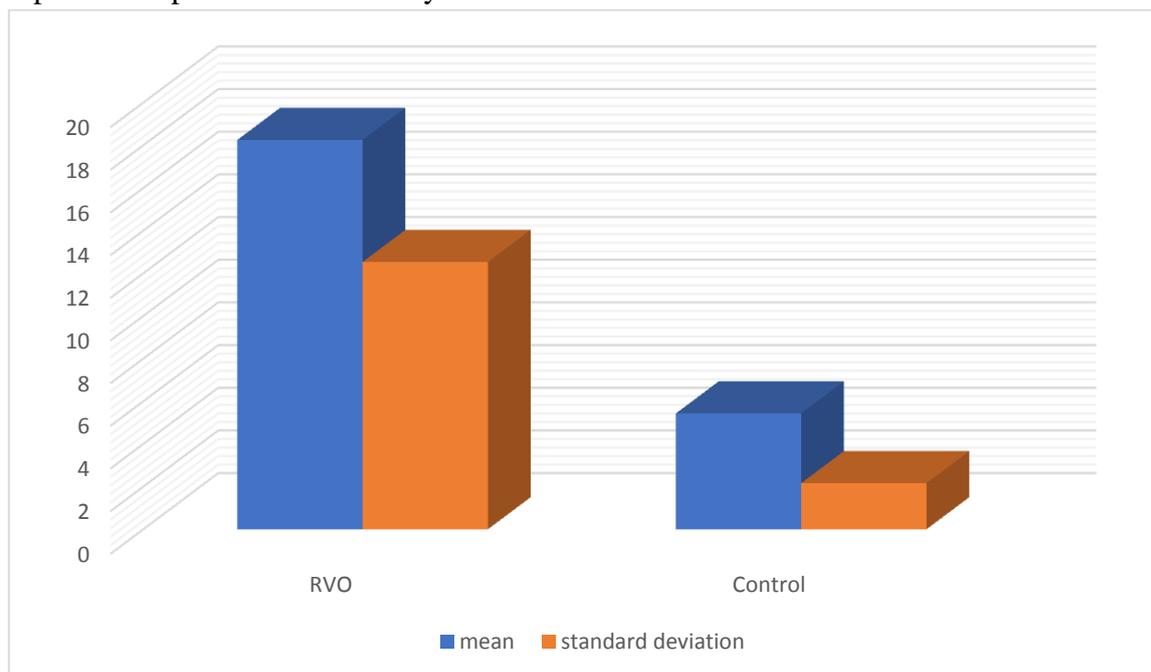


Table 2 Incidence of Hyperhomocysteinemia in RVO compared to control

Parameter	RVO	Control
High homocysteine	31	6
Normal homocysteine	27	36
total	58	42

OR = 7.36 for RVO

Table 3 Mean Total homocysteine levels in CRVO, BRVO and control subjects

Parameter	CRVO (mean ±SD) (µM/L)	BRVO (mean ±SD) (µM/L)	Control (mean ±SD) (µM/L)
Plasma homocysteine total	19.04±5.15	17.39±5.82	12.51±2.14

Value expressed as mean ±SD
 P< 0.001 as compared to control.

Fig 2 Mean Total homocysteine levels in CRVO, BRVO and control subjects

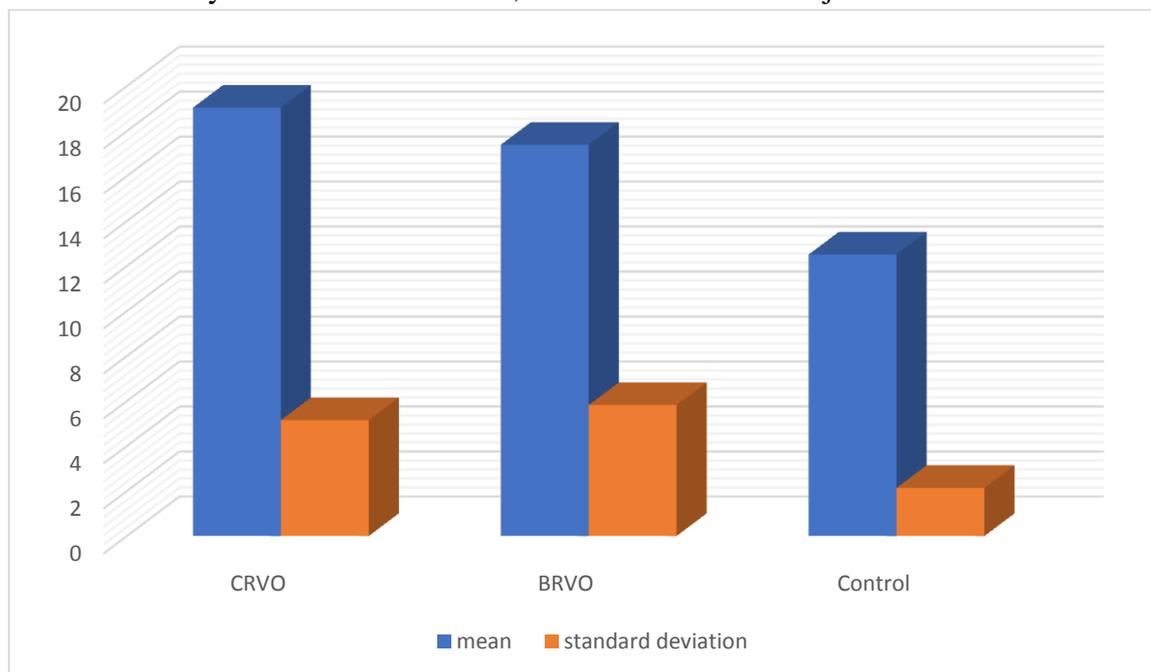


Table 4 Incidence of Hyperhomocysteinemia in CRVO and BRVO with respect to control

Parameter	CRVO	BRVO	Control
High homocysteine	14	17	6
Normal homocysteine	7	20	36
Total	21	37	42

OR = 13 for CRVO and OR = 5.03 for BRVO

Discussion and Conclusion

Some studies conducted in the world, strongly suggests Hyperhomocysteinemia as independent risk factor for CRVO^{18,19,20}. Study conducted by Gao W et al²¹ reported and Odds ratio (OR) of 1.3 for fasting homocysteine levels with CRVO done in Chinese population and study done by Lattanzio et al¹⁴ reported OR of 3.0. meta-analysis done by Janssen et al²² has also shown an overall

OR of 8.9 for homocysteine. Another meta analytical study conducted by Cahill et al²³ has shown that retinal vascular occlusion associated with increased plasma homocysteine and low serum folate levels. Study conducted by Poloshek et al²⁴ explained the possibility of direct cytotoxic effect of homocysteine on retinal vascular endothelial cells. He has also reported methionine deficiency in subjects.

There are various mechanisms, which explains endothelial dysfunction by homocysteine. It includes decreased bioavailability of nitric oxide⁹. Mitogenic effect on smooth muscle cells of artery¹⁰, Various thrombotic factors alter their expression, expression of acute stress related genes 11. homocysteine has sulfhydryl group, which has high pKa. So, homocysteine forms stable disulfide bonds with protein cysteine residues. Due to this reason, many proteins function is impaired. Molecular targets of homocysteine were found to be albumin, transthyretin, fibronectin, factor V and annexin II¹². Homocysteine is also converted to chemically reactive metabolite, *homocysteine– thiolactone*, which contributes for toxicity leading to endothelial dysfunction¹³.

In this study, the patients with RVO showed a significant elevation of homocysteine when compared to control. It can be due to direct cytotoxic effect of homocysteine on retinal endothelial cells along with its prothrombotic effects. Vascular smooth muscle cells when exposed to high homocysteine, there is inflammatory response upregulation which causes atherogenesis and other adverse vascular defects²⁵. There was complete exclusion of all major thromboembolic defects and coagulation disorders. Even hematological and coagulation parameters were normal in study patients. Hence, homocysteine induced thrombosis may be a crucial factor which is triggering vascular occlusion²⁶ which was also told by study done by Janssen et al²².

Hyperhomocysteinemia is more prevalent in CRVO than BRVO in our study subjects. So treatment of Hyperhomocysteinemia by folic acid and Vitamin B12 supplementation in RVO patients should be considered.

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