Acta Scientific Ophthalmology (ISSN: 2582-3191)

Volume 7 Issue 5 May 2024

Research Article

Association of Serum Homocysteine Level with Retinal Vein Occlusion: A Comparative Cross-Sectional Study

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DOI: 10.31080/ASOP.2024.07.0755

Received: February 13, 2024

Published: April 02, 2024

Abstract

Purpose: To evaluate the association of serum homocysteine level with retinal vein occlusion.

Methods: This cross-sectional study was conducted in the Department of Ophthalmology, BSMMU, Dhaka from March 2017 to August 2019. After informed consent, a total of 46 subjects were selected with age ranging from 35-75 years. Among them, 23 subjects with RVO were considered as the study group and 23 aged and sex matched subjects without RVO was considered as control group for comparison. The study parameters were serum homocysteine level, folate and vitamin B_{12} were estimated in the Department of Biochemistry, BSMMU, Dhaka. Unpaired Students "t" test, Chi Square test, Pearson's correlation coefficient (r) test and Logistic regression analysis were done as applicable. p value < 0.05 was accepted as level of significance.

Results: In this study, mean plasma homocysteine level was 23.75 ± 6.21 µmol/L and 9.29 ± 3.19 µmol/L, serum folate level was 3.67 ± 0.96ng/ml and 10.49 ± 3.67 ng/ml and serum vitamin B12 level was 376.09 ± 176.07 pg/ml and 540.26 ± 300.57 pg/ml respectively in study and control groups. Logistic regression model demonstrated that hyperhomocysteinemia was about 6 times (OR = 5.655; 95% CI = 2.279 to 14.032) more chance to develop RVO than the subjects of normal plasma homocysteine level. Serum homocysteine level showed negative correlation with serum folate and vitamin B_{12} levels in RVO patients which was statistically significant (< 0.001).

Conclusion: It can be concluded that serum Homocysteine level was significantly increased in RVO patients.

Keywords: RVO; Homocysteine; Folate; Vitamin B_{12}

Introduction

Retinal vein occlusion (RVO) is the second most common retinal vascular disease after diabetic retinopathy. There are three types of RVO: branch retinal vein occlusion (BRVO), central retinal vein occlusion (CRVO) and hemi-CRVO with involvement of only one half of the retina surface. Among them BRVO is more common [1-3].

Various systemic and ocular risk factors have been found to be associated with retinal vein occlusions (RVO). Among them hyperhomocysteinemia appears to be one of the risk factors for RVO. Homocysteine is a sulfur amino acid. Its metabolism through two different pathways is affected by genetic enzyme defects or deficiency of vitamins that are needed as precursors of these enzymes [4]. The former can cause severe hyperhomocysteinemia, while folic acid and vitamin B12 deficiency could cause mild hyperhomocysteinemia. A diet rich in vegetables, fruits and dairy products usually prevents vitamin deficiency related hyperhomocysteinemia [5-7]. Although the exact mechanisms by which hyperhomocysteinemia

causes thrombosis are unknown. It has been postulated that elevated homocysteine levels in blood shown to be directly toxic to endothelial cells, impair thrombomodulin expression, directly activate factor V and inhibit protein C activation [8-10].

Various researchers of different countries suggested an association of serum homocysteine level with retinal vein occlusion [11-14]. But no published data are available in our country. So, present study has been designed to evaluate the association of serum homocysteine level with retinal vein occlusion. The findings of this study may also be helpful to provide evidence-based information to the physician as well as patient groups about the association of serum homocysteine level with retinal vein occlusion. Therefore, early diagnosis can reduce the incidence and severity of visual loss.

Subjects/Materials and Methods

This cross-sectional study was conducted in the Department of Ophthalmology, BSMMU, Dhaka from March 2017 to August 2019. After taking informed consent, a total of 46 subjects that has the inclusion criteria were taken as sample after informed consent. Among them, 23 subjects with RVO were considered as the study group and 23 aged and sex matched subjects without RVO was considered as control group for comparison. Ethical clearance was obtained from the Institutional Review Board (IRB) of BSMMU. Detail history and physical examination of each patient were performed and recorded. Detail history and physical examination of each patient was performed and recorded. Visual acuity for distance was noted using Snellen's literate chart held at a 6-m distance from the patient. The best visual acuity of the involved eye was noted using a pinhole and with refractive correction. If the person could not correctly recognize the top letter of the chart, visual acuity was noted using the finger counting method at a 3-m distance. If both the eyes had RVO, the findings of the eye with the worse visual acuity were taken for the analysis. The ocular pressure was measured using a non-contact tonometer (Medtronic, Minneapolis, USA). The details of the anterior segment were evaluated using a slit-lamp bio-microscope (Topcon, Oakland, USA). The posterior segment was evaluated after dilation of the pupil using 10% phenylephrine eye drops. A binocular indirect ophthalmoscope (Keeler, Windsor, UK) and + 20 D Volk lens was used for this purpose.

With all aseptic precautions, 10 ml of venous blood was collected from antecubital vein by a disposable plastic syringe from each subject after an overnight fast (at least 12 hours) for biochemical tests. Blood was transfused in a de-ionized glass test tube and kept in slanted position. At last, the blood sample was centrifuged at a rate of 3000 rpm for 15 minutes. After that, supernatant serum was collected in labeled Eppendorf tube and sent for analytical measurement of serum homocysteine, folic acid and vitamin B12 levels in the Department of Biochemistry, BSMMU, Dhaka.

An aneroid sphygmomanometer was used to measure systolic and diastolic blood pressures. Those known to have hypertension and already on medication was labeled as hypertensive even though their pressure readings were within the normal range. In patients ≥50 years of age, a systolic pressure of more than 140 mmHg and diastolic pressure more than 90 mmHg was considered as high. In patients < 50 years of age, systolic and diastolic pressure of more than 120 and 80 mmHg, respectively, was considered as high. All the information's was recorded in a prefixed questionnaire.

Ethical approval

Ethical clearance was obtained from the Institutional Review Board (IRB) of BSMMU. All principles outlined in the Declaration of Helsinki (2008) must be followed while conducting the study.

Statistical analysis

All the data were compiled and sorted properly and the quantitative data were analyzed statistically by using Statistical Package for Social Science (SPSS-25). Comparisons of continuous variables between the two groups were made with Unpaired Student's t-tests. Comparisons of proportions between the two groups were made with Chi-square tests. Pearson's correlation coefficient (r) test was performed to assess correlation between serum homocysteine with serum folate and vitamin B12 levels. Logistic regression analysis was performed to calculate Odds ratio and 95% confident interval (CI). $p < 0.05$ were considered as the level of significant.

Results

The results are expressed as frequency, percentage and mean ± SD and presented in figure and table as below.

Data were expressed as frequency, percentage and Mean ± SD. Chi-Square test were performed. $p < 0.05$ was accepted as level of significant. ns = not significant. N = Total number of study subjects, n_1 = no. of subjects in Study group, n_2 = no. of subjects in control group.

Age	Study group (n_1 = 23)	Control group (n_2 = 23)	p value
35-44	2 (8.7%)	4 (17.4%)	0.693^{ns}
45-54	8 (34.8%)	9 (39.1%)	
55-64	9 (39.1%)	8 (34.8%)	
65-75	4 (17.4%)	2 (8.7%)	
Mean ± SD	56.09 ± 9.69	55.55 ± 9.80	

Table 1: Distribution of study population according to Age (N = 46).

In this study, majority of the study subjects were in age group of 45-54 years (8; 34.8% and 9; 39.1%) and 55-64 years (9; 39.1% and 8; 34.8%) in both study and control groups. Mean age was 56.09 ± 9.69 and 55.55 ± 9.80 years respectively. No significant (p = 0.693) difference was observed in age of both groups.

Sex	Study group (n_1 = 23)	Control group (n_2 = 23)	p value
Female	9 (39.1%)	13 (56.5%)	0.765^{ns}
Male	14 (60.9%)	10 (43.5%)	

Table 2: Distribution of study population according to Sex (N = 46).

Data were expressed as frequency and percentage. Chi-Square test was performed. p < 0.05 was accepted as level of significant. ns = not significant. N = Total number of study subjects, n_1 = no. of subjects in study group, n_2 = no. of subjects in control group.

Among 46 subjects, majority were male (60.9%) in study group and female (56.5%) in control group. This difference was not statistically significant (p = 0.765).

Habit	Study group (n_1 = 23)	Control group (n_2 = 23)	p value
Smoker	12(52.2%)	7(30.4%)	0.134^{ns}
Nonsmoker	11(47.8%)	16(69.6%)	

Table 3: Distribution of study population according to personal habit (N = 46).

Data were expressed as frequency and percentage. Chi-Square test was performed. p < 0.05 was accepted as level of significant. ns = not significant. N = Total number of study subjects, n_1 = no. of subjects in Study group, n_2 = no. of subjects in control group.

In this study, majority were smokers (12; 52.2%) in study group and nonsmokers (16; 69.6%) in control group. But this difference was not statistically significant (p = 0.134).

Comorbidity		Study group (n_1 = 23)	Control group (n_2 = 23)	p value
Dyslipidemia	Yes	15 (65.2%)	5 (21.7%)	0.002**
	No	8 (34.8%)	18 (78.3%)	
DM	Yes	8 (34.8%)	7 (30.4%)	0.753^{ns}
	No	15 (65.2)	16 (69.6%)	
HTN	Yes	12 (52.2%)	7 (30.4%)	0.134^{ns}
	No	11 (47.8%)	16 (69.6%)	
IHD	Yes	7 (30.4%)	7 (30.4%)	1.000^{ns}
	No	16 (69.6%)	16 (69.6%)	

Table 4: Distribution of study population according to comorbidity (N = 46).

Data were expressed as frequency and percentage. Chi-Square test was performed. P < 0.05 was accepted as level of significant. *** = significant, ns = not significant. N = Total number of study subjects, n_1 = no. of subjects in Study group, n_2 = no. of subjects in control group.

In the study, 15 (65.2%) and 5 (21.7%) of the subjects had Dyslipidemia, 12 (52.2%) and 7 (30.4%) had DM, 12 (52.2%) and 7 (30.4%) were hypertensive and 7 (30.4%) and 7 (30.4%) subjects had history of IHD in both groups respectively. This difference was statistically significant (p = 0.002) for Dyslipidemia and not significant (p > 0.05) for DM, HTN and IHD.

Data were expressed as Mean ± SD. Unpaired Student t test was performed. p < 0.05 was accepted as level of significant.*** = significant. N = Total number of study subjects, n_1 = no. of subjects in study group, n_2 = no. of subjects in control group.

Parameters	Study group (n_1 = 23)	Control group (n_2 = 23)	p value
Plasma Homocysteine (µmol/L)	23.75 ± 6.21	9.29 ± 3.19	<0.001***
Serum Folate (ng/ml)	3.67 ± 0.96	10.49 ± 3.67	<0.001***
Serum Vitamin B_{12} (pg/ml)	376.09 ± 176.07	540.26 ± 300.57	<0.001***

Table 5: Study parameters of the study subjects in both groups (N = 46).

In this study, mean plasma homocysteine level was 23.75 ± 6.21 µmol/l and 9.29 ± 3.19 µmol/l, serum folate level was 3.67 ± 0.96ng/ml and 10.49 ± 3.67 ng/ml and serum vitamin B_{12} level was 376.09 ± 176.07 pg/ml and 540.26 ± 300.57 pg/ml respectively in both groups. Plasma homocysteine level was significantly (p = < 0.001) increased and serum folate level and vitamin B_{12} level was significantly (p = < 0.001) decreased in study group than control group.

Plasma Homocysteine (µmol/L)	Study group (n_1 = 23)	Control group (n_2 = 23)	OR (95% CI)	p value
<15	2 (8.7%)	19(82.6%)	5.655 (2.279 to 14.032)	<0.001***
>15	21(91.3%)	4 (17.4%)		

Table 6: Logistic regression analysis of predictors of RVO (N = 46).

Data were expressed as Mean ± SD. Logistic regression analysis was performed. p < 0.05 was accepted as level of significant. *** = significant. N = Total number of study subjects, n_1 = no. of subjects in study group, n_2 = no. of subjects in control group. Majority (21; 91.3%) of the RVO subjects had serum homocysteine level > 15 µmol/L. Logistic regression model demonstrated that hyperhomocysteinemia was about 6 times (OR = 5.655; 95% CI = 2.279 to 14.032) more chance to develop RVO than the subjects of normal plasma homocysteine level.

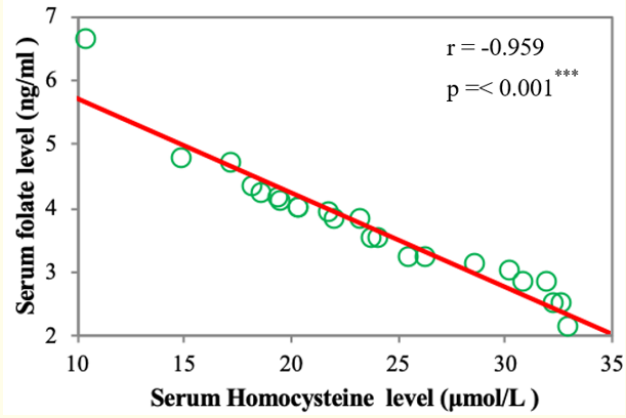

Figure 1: Showing correlation of serum homocysteine level with serum folate in study group (n1 = 23).

In this study, serum homocysteine level showed negative correlation (r = -0.959) with serum folate level in RVO patients which was statistically significant (<0.001).

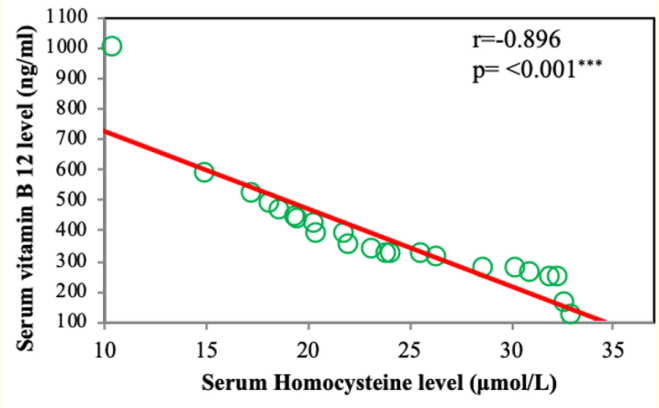

Figure 2: Showing correlation of serum homocysteine level with serum vitamin B12 level in study group (n1 = 23).

In this study, serum homocysteine level showed negative correlation (r = - 0.896) with serum vitamin B12 level in RVO patients which was statistically significant (<0.001).

Discussion

Hyperhomocysteinemia may contribute to the pathogenesis of retinal vascular disorder by oxidative injury to the vascular bed. It is associated with proliferation of vascular smooth muscle, decreased bioavailability of nitric oxide, altered endothelial function, enhanced thrombogenicity and expression of acute stress-related genes [16].

In present study, plasma Homocystine level was significantly ($p < 0.001$) higher and serum folate and vitamin B12 levels were significantly ($p < 0.001$) lower in study group than that of control group. Similar types of observations were found by some researchers of different countries [12,15,16]. However, some researchers found no significant changes in folate and vitamin B12 levels. This dissimilarity is may be due different in methodology and nutritional variation [17-20].

Logistic regression model demonstrated that hyperhomocysteinemia was about 6 times (OR = 5.655; 95% CI = 2.279 to 14.032) more chance to develop RVO than the subjects of normal plasma homocysteine level. Similar observation was found by Moghimi., *et al*. [20]. Their results showed that plasma homocysteine level above 15 µmol/l was 4.71 times (95% CI = 1.46-15.19) more risk of development of RVO.

Correlation analysis of plasma homocysteine level of RVO patients showed negative correlation with serum folate and vitamin B12 levels which was statistically significant ($p < 0.001$). Similar types of observations were found by some researchers of different countries [14-16]. However, some researchers [17-20] found no significant relation with plasma homocysteine level and serum folate and vitamin B12 levels. As their result showed increased homocysteine level but normal folate and vitamin B12 levels. This dissimilarity is may be due different in geographical and nutritional variation.

There are some postulated mechanisms suggested by various researchers of different countries which may imply the possible mechanism regarding the changes in the present study. Homocysteine is an intermediate product of methionine metabolism [19]. vitamin B12 as cofactor and methyl tetrahydrofolate as a co-substrate are required in remethylation pathway where homocysteine is remethylated to methionine [16,23]. So, increased homocysteine may lead to decreased in serum folate and vitamin B12.

Again, methylation of RNA, DNA, and various proteins causes repair and reorganization of damaged tissue [24,25]. An increase in methylation reactions after tissue injury, such as RVO, results in the conversion of Sadenosyl methionine (SAM) to S-adenosylhomocysteine (SAH), which leads to the generation of homocysteine [16,21,26]. Thus, leads to increased Homocysteine levels from in RVO patients [16].

In conclusion after analyzing the results of present study, it can be concluded that serum homocysteine is associated with RVO. It is evidenced by serum Homocysteine was significantly increased and serum folate and vitamin B12 levels were significantly decreased in RVO patients. Thus, suggesting that hypercysteinemia may contribute to the pathogenesis of retinal vascular disorder. The limitation of our study was having small sample size and short study period in comparison to other studies. Further study with a large sample size and longer study period is recommended.

Acknowledgements

- **Conflicts of Interest:** None of the authors declare any conflicts of interests.

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