



Original Research Article

A study of serum homocysteine and serum vitamin B12 levels in patients with retinal vein occlusion and its correlation

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ABSTRACT

Purpose: To find out the association of serum homocysteine levels and serum vitamin B-12 levels in patients with retinal vein occlusion.

Materials and Methods: Patients of all age group and any sex who are coming in vitreoretinal OPD of department of ophthalmology at a tertiary care hospital of Western Maharashtra were studied. 50 cases of RVO and same number of age and sex matched controls were taken into consideration. All patients were underwent for assay of serum homocysteine by ECLIA method and serum Vitamin B12 by CMIA method.

Results: The highest number of cases were of BRVO (60%) followed by CRVO (30%) and only one case of TVO (2%) was noted. The average level of homocysteine in RVO and control group is found to be 23.80 and 12.43 whereas the standard deviation of homocysteine level in RVO group is 13.28 and control group is 10.58. Therefore levels of homocysteine are significantly higher in RVO group in comparison with control group. Vitamin B-12 level was 455.86 in control group and 325.8 in RVO group. The P value suggests that, Vitamin B12 was significantly lower in case group compared to controls. Vitamin B-12 levels were significantly lower in cases with a higher Homocysteine level.

Conclusion: Hyper-homocysteinemia and hypovitaminosis B12 is significantly present in cases of retinal vein occlusion irrespective of types and underlying systemic diseases.

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1. Introduction

Retinal vein occlusions (RVOs) have been defined as retinal vascular disorders characterized by engorgement and dilatation of the retinal veins due to increased retinal venous blood pressure, with secondary intraretinal hemorrhages intraretinal & (and partially subretinal edema including foveal region and which can lead to hard retinal exudates due to deposits of lipids; and a varying degree of retinal ischemia leading to cotton wool spots as signs of it.¹⁻⁹

RVOs are one of the most common causes of a retinal vascular abnormality and a frequent cause of visual loss.¹⁰ Being recognized at least as early as 1855,¹¹ RVOs have been the subject of more than 3,000 publications so far.

Current estimates of the prevalence of RVOs are derived from major population-based studies, such as the Blue Mountains Eye Study, the Beaver Dam Eye Study, and a combined analysis of the Atherosclerosis Risk in Communities and Cardiovascular Health Studies.¹²⁻¹⁴

Homocysteine is an intermediary metabolite of essential dietary amino acid methionine, metabolised by remethylation to methionine or by trans-sulfuration to cysteine. Remethylation requires vitamin B12 (cobalamin) essentially.¹⁵

Hyperhomocysteinemia results from absence, mutation of any of these enzymes in these reactions. As depicted below, this can lead to deficiencies in folic acid, vitamin B6, or vitamin B12 cofactors can lead to elevated total plasma homocysteine (tHcy) levels.¹⁶

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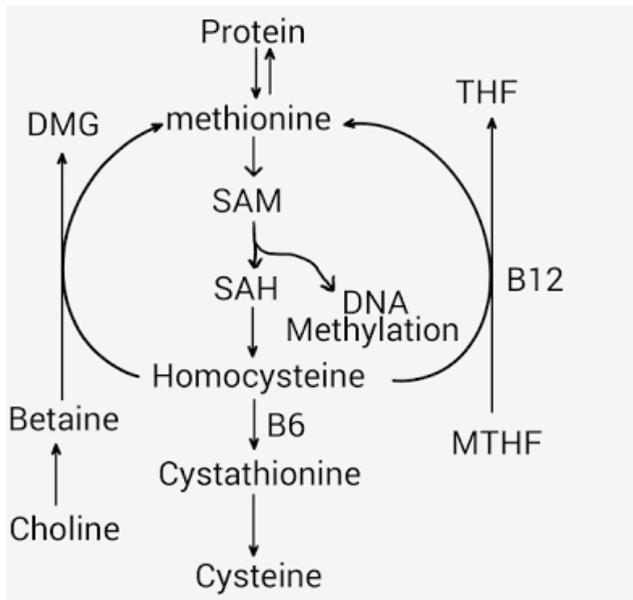


Fig. 1:

Hence, we will try to find correlation between Serum homocysteine and vitamin B-12 in patients with RVO. Additionally the study will elaborate serum homocysteine levels by means of ECLIA (Electro chemiluminescent immunoassay and vitamin B-12 levels by CMIA (chemiluminescent microparticle assay) and to find whether raised levels of homocysteine correlate with the types of RVO and also a correlation between low vitamin B12 levels and RVO and its types.

2. Materials and Methods

This study was conducted in out patient department (OPD) of Dr. D. Y. Patil medical college and hospital, Pimpri, Pune. A case control type of study was conducted from September 2017 to August 2019.

2.1. Sample size

100 (50 cases and 50 controls)

2.2. Inclusion criteria

1. Patients of all age groups coming in Ophthalmology OPD.
2. Patients coming with sudden loss of vision in Vitreoretinal OPD.
3. Patients giving history of previous known attacks of RVO.

2.3. Exclusion criteria

1. Patients who have been diagnosed for HTN retinopathy.

2. Patients having other retinal pathologies resembling RVO like Eale's disease, diabetic retinopathy, radiation retinopathy etc.
3. Patients having hazy/opaque media.

2.4. Methodology

Patients of all age group and any sex who are coming in vitreoretinal OPD of department of ophthalmology were studied.

Ethical clearance was obtained prior to study. Written informed consent was taken from all patients. 50 cases were selected as cases according to inclusion criteria. Age and sex matched controls were selected amongst non-diseased patients.

2.5. Data selection

Brief history was taken along with important demographic factors. Detailed history of other co-morbid conditions was taken.

Ocular examination including visual acuity (distant and near test types), BCVA (Best corrected visual acuity), intraocular pressure was measured by Goldmann applanation tonometer. Detailed slit lamp examination was done for anterior segment evaluation to find Neovascularization of iris (NVI), and Neovascularization of angle (NVA) etc were carried out. Dilated fundus of both eyes were evaluated. Types of RVO would be identified as central retinal vein occlusion (CRVO), branch retinal vein occlusion (BRVO), hemi-retinal vein occlusion (HCRVO) Retinal status was evaluated by direct ophthalmoscopy, slit lamp biomicroscopy by using 90D lens and indirect ophthalmoscopy after dilatation with Tropicamide/ Tropicamide plus eye drops. Serum Homocysteine was measured by Electro chemiluminescent immunoassay (ECLIA).

Hyperhomocysteinemia is defined as > 15 micromol/L

Serum Vitamin B-12 levels were assessed by Chemiluminescent Microparticle assay (CMIA) and levels would be assessed on following grades

- < 187 pg/ml – Low vitamin B12
- 187-883 pg/ml – Normal range
- > 883 pg/ml – High levels

2.6. Statistical analysis

Statistical analysis was done by using the soft ware Graphpad prism version 7. The results included mean values, standard deviations and p values calculated using unpaired students t test. Chi square was used where applicable.

3. Results

In our study group, Highest number of cases were of BRVO (60%) and only one case of TVO (2%) was noted.

Table 1: Type of Retinal vein occlusion

Type of RVO	No of cases	Percentage
CRVO	15	30%
BRVO	30	60%
HCRVO	4	8%
TVO	1	2%

Table 2: Homocysteine levels cases vs. controls

Homocysteine levels Micromol/L	RVO group	Control group
Mean	23.80	12.43
Std dev	13.28	10.58
P value	7.7E-06 (P<0.0001)	
Inference	Homocysteine levels were significantly higher in RVO group when compared to control group	

Table 1 depicts BRVO being in the highest and TVO type being the least RVO type of cases.

Table 2 shows us homocysteine levels in RVO and Control group. The average level of homocysteine in RVO and control group is found to be 23.80 and 12.43 whereas the standard deviation of homocysteine level in RVO group is 13.28 and control group is 10.58. Therefore we see that levels of homocysteine are significantly higher in RVO group in comparison with control group.

Table 3 shows the Vitamin B-12 level in RVO and control group. The average value was found be 455.86 in control group and 325.8 in RVO group. The P value suggests that, Vitamin B12 was significantly lower in case group compared to controls

Table 5 show we observe Vitamin B-12 level is exceptionally higher in normal homocysteine level cases of both the groups, that is the RVO group and control group. The average value is found to be 498.21 in normal HC level and 222.87 in high HC level cases of control and RVO groups.

Table 6 compares HC levels specifically in patients of CRVO vs. BRVO. Non significant difference was found between mean HC levels in patients with CRVO vs. BRVO.

The incidence of hyperhomocysteinemia was 60% in the cases with 28% incidence of low Vitamin B-12.

4. Discussion

It was found that the average age for males in RVO group was 56.98 and for the control group was 58.16 years. For RVO group the range of ages that were considered were 19-83 and for Control group 20-84 years. Martin SC et al.¹⁷ measured the homocysteine levels in 70 patients with retinal vessel occlusion and compared them with homocysteine levels in 85 controls without any evidence of retinal vessel occlusion. The mean age of subjects was 66.2 years whereas it was 51.5 years for the patients with retinal vein occlusion.

The study was age and sex matched to avoid any bias arising due to age and gender of the patients. This was done by taking equal number of male and female control group as per age distribution in cases by randomization method. This is depicted in table 3 and corresponding figure. Bharathi Devi SR et al.¹⁸ conducted a similar study where the patients were age and sex matched. Similarly many studies with age and sex matched of participants evaluating the homocysteine levels in patients of retinal vein occlusion were conducted.^{19–21} Like in our study incidence of RVO increases with advancing age.

Study shows that the type of retinal vein occlusion. It was noted that Highest number of cases were of BRVO(60%) followed by CRVO(30%) and only one case of TVO was found. The prevalence rates of retinal vein occlusion per 1,000 persons were 4.42 for BRVO and 0.80 for CRVO, which were age- and gender-standardized to the 2008 population of the world aged 30 years and older.¹⁶ Similarly in our study the prevalence of BRVO was higher when compared to other subtypes of RVO such as CRVO, HCRVO and TVO.

Study shows us homocysteine levels in RVO and Control group. The average level of homocysteine in RVO and control group is found to be 23.803 and 12.4274 whereas the standard deviation of homocysteine level in RVO group is 13.2805 and control group is 10.5811. Therefore we see that levels of homocysteine are significantly higher in RVO group in comparison with control group. Some authors hypothesized that elevated plasma homocysteine concentration could be a potential risk factor for RVO.^{22–24} This was evident in our study. Minniti G et al.¹⁹ correlated higher values of fasting homocysteine and low vitamin B-12 in patients with retinal vein occlusion. Mean plasma cysteine was significantly increased in patients with CRVO, suggesting that hyperhomocysteinemia may contribute to the pathogenesis of CRVO disorder was concluded in a study by Pinna A et al.²⁵ Martin SC et al.¹⁷ evaluated homocysteine levels in patients of retinal vein occlusion.

Table 3: Vitamin B-12 levels comparison in RVO group vs. Control group

Vitamin B-12 levels (pg/ml)	RVO group	Control group
Mean	325.8	455.86
Std dev.	181.53	289.5
P value	0.00862	
Inference	Vitamin B12 was significantly lower in case group compared to controls	

Table 4: Vitamin B-12 correlation with Homocysteine levels

RVO group	High HC level cases (>15micromol/L)	Normal HC level cases (4-15micromol/L)
Mean Vit-B 12 level (pg/ml)	240.27	454.1
Std dev	108.09	195.64
P value	0.00013	
Inference	Vitamin B-12 levels were significantly lower in cases with a higher Homocysteine level	

Table 5: Vitamin B 12 levels in context of HC level irrespective of Case/control group

Case+Controls	High HC level cases	Normal HC level cases
Mean Vit-B 12 level	222.87	498.21
Std dev	100.56	256.79
P value	5.3E-11 (P<0.0001)	
Inference	Vitamin B-12 levels are significantly lesser in patients with a higher level of homocysteine irrespective of presence of RVO.	

Table 6: Mean HC levels in CRVO vs. BRVO cases

HC levels	Mean	Std dev
CRVO	24.27	16.58
BRVO	23.88	11.41
P value	0.93429	
Inference	Non significant difference between mean HC levels in patients with CRVO vs. BRVO.	

Table 7: Incidence of increased HC and low Vit B-12 in cases vs. controls

	Incidence of raised HC	Percentage	Incidence of low Vit B-12	Percentage
Cases	30	60%	14	28%
Controls	9	18%	10	20%

Chua B et al.²⁶ that elevated serum homocysteine levels in the blood is associated with the presence of RVO, independent of other risk factors.

Study depicts the Vitamin B-12 level in RVO and control group. [Normal levels of vitamin B-12 is defined as 187-883 pg/ml and for homocysteine it is > 15 micromol/L.] The average value was found to be 455.86ng/ml in control group and 325.8ng/ml in RVO group. The standard deviation in control group is significantly higher than RVO group. Minniti G et al.¹⁹ concluded fasting homocysteine levels, low vitamin B12 levels, and hypertension are associated with a risk of RVO, especially for CRVO.

We can infer that average level of Vitamin B-12 in normal level of homocysteine participants was 454.1 ng/ ml and its standard deviation is 195.642 and in participants with high level of homocysteine it was 240.26 ng/ ml and its standard deviation is 108.08. These values depicts normal levels of

vitamin B-12 in normal level of homocysteine and lower level in higher level of homocysteine.

Study depicts Vitamin B 12 levels in context of Homocysteine levels irrespective of Case/control group. We observed that Vitamin B-12 level is normal in homocysteine level cases of both the groups, that is the RVO group and control group. The average value is found to be 498.213 in normal HC level and 222.872 in high HC level cases of control and RVO groups. We need to consider the patients with higher homocysteine and lower vitamin B-12 in context of a risk factor for development of RVO as this is an independent risk factor in control group patients.^{22,26}

HC levels specifically in patients of CRVO vs. BRVO. Non significant difference was found between mean HC levels in patients with CRVO vs. BRVO. The mean in CRVO group was 24.274 micromol/L and 23.8767 micromol/L in BRVO group. However, both are raised considering the cut

off for normal range of 4-15 micromol/L.²⁶

Study highlights the incidence of increased HC and low Vit B-12 in cases vs. controls. The incidence of hyperhomocysteinemia was 60% in the cases with 28% incidence of low Vitamin B-12 whereas in controls incidence of hyperhomocysteinemia was 18% with 20% incidence of low Vitamin B-12

5. Conclusion

1. Homocysteine levels were found to be significantly elevated in patients having retinal vein occlusion when compared to controls.
2. Vitamin B-12 levels were significantly lesser in patients of retinal vein occlusion when compared to controls.
3. All the patients having hyperhomocysteinemia have significantly lower levels of vitamin B12.
4. Patients having no underlying systemic conditions, Serum Homocysteine is the prime factor to study in patients with Retinal vein occlusion.
5. All patients with RVO should mandatorily be evaluated for presence of low B-12 levels and hyperhomocysteinemia.

6. Source of Funding

None.

7. Conflict of Interest

None.

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