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Investigation of methylenetetrahydrofolate reductase C677T and factor V Leiden mutation as a genetic marker for retinal vein occlusion

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Abstract:

PURPOSE: Thromboembolic phenomenon is one of the causes of retinal vein occlusion (RVO) which is in fact a multifactorial disease. Therefore, we aimed to study methylenetetrahydrofolate reductase gene polymorphism (MTHFR C677T) and factor V Leiden as genetic risk factors of RVO.

MATERIALS AND METHODS: A total of 50 (19 males and 31 females) cases of RVO were compared with 50 age- and sex-matched (21 males and 29 females) controls. Complete ocular examination was done for all samples. The diagnosis of RVO was made clinically; however, fundus fluorescein angiography and optical coherence tomography were performed when needed. Serum homocysteine levels were estimated by automatic chemiluminescence analyzer, whereas MTHFR C677T and factor V Leiden mutations were detected by polymerase chain reaction–restriction fragment length polymorphism method.

RESULTS: The mean age of RVO cases and controls was 54.62 ± 13.92 years and 58.72 ± 11.20 years, respectively. 48.3% of cases and 51.7% of controls were diabetic. 65.3% of cases were hypertensive proving hypertension as a strong risk factor ($P = 0.003$) of RVO. Serum homocysteine was also found significantly high ($P = 0.025$) with mean values of 19.98 ± 9.03 μmol/L and 16.98 ± 8.29 μmol/L in cases and controls, respectively. The MTHFR genotype (CT) was found in 83.3% patients of central RVO group and 78.6% cases of branch RVO group that was significantly associated with high serum homocysteine levels. Factor V Leiden mutation was absent in all individuals.

CONCLUSION: Hyperhomocysteinemia is an important risk factor for RVO, especially in patients with MTHFR C677T gene polymorphism.

Keywords:

Factor V Leiden, hyperhomocysteinemia, methylenetetrahydrofolate reductase, retinal vein occlusion

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Introduction

Retinal vein occlusion (RVO) is the second most common visually disabling disease affecting the retina after diabetic retinopathy.[1] Obstruction of retinal venous flow leads to damage to the vasculature, hemorrhage, and tissue ischemia.[2] It may occur in the central, branch, or tributary (or macular branch vein) retinal vein affecting the entire retina, a quadrant or macular region, respectively. Sometimes, a hemisphere gets involved as a consequence of hemi RVO.

Systemic inflammation leads to RVO through induction of systemic hypercoagulability. In the coagulation cascade, factor V along with factor X on activation converts prothrombin to thrombin for thrombus formation. Normally after adequate thrombosis, activated factor V is cleaved by activated

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protein C. Resistance to this process causes venous thrombosis. A single-point mutation was discovered in the year 1994 in factor V gene (FVR 506Q) at position 1691 where guanine was replaced by adenosine. This is called factor V Leiden mutation. From time to time, this mutation has been associated with sporadic RVOs.[3,4]

Methylenetetrahydrofolate reductase (MTHFR) normally catalyzes the conversion of 5,10-methyltetrahydrofolate to 5-methyltetrahydrofolate. However, in the presence of MTHFR gene C677T polymorphism, MTHFR activity slows down even with normal folic acid levels in body. The resulting hyperhomocysteinemia causes severe vascular endothelial cell injury. The damaged lumen of blood vessels acts as a precursor for thrombotic events, one of which being RVO.[5]

The currently available treatment options for RVO often leave the patients with unsatisfactory visual improvement. Therefore, there are ongoing attempts to explore the role of genetic factors in thromboembolic events to find better treatment options. However, in Indian population, such correlation has not been scrutinized so far. Hence, the authors found it necessary to study the genetic markers of RVO so that they may be targeted specifically to treat the condition with favorable visual outcome.

Materials and Methods

Study design

This prospective, cross-sectional case–control study was conducted between the years 2014 and 2016.

Subjects

Fifty patients diagnosed with RVO were included in the study irrespective of their age and sex. They were compared with age- and sex-matched normal population (control group) whose fundus examination neither revealed any feature of RVO nor had history of stroke or cardiovascular disease. The patients suffering from chronic kidney disease or who were on long-term anticoagulants and corticosteroids were excluded from the study.

Clinical examination

All patients underwent a meticulous ophthalmic examination including recording of best-corrected visual acuity, slit-lamp biomicroscopy, measurement of intraocular pressure (IOP), dilated fundus examination by indirect ophthalmoscopy, and gonioscopy. Fundus fluorescein angiography and optical coherence tomography were done when felt necessary.

The patients were examined by a physician for recording pulse, blood pressure, electrocardiogram, and complete systemic evaluation to rule out atherosclerosis, peripheral artery disease, and cardiovascular, renal, and cerebrovascular comorbidities. Laboratory and other investigations were done for confirming the clinical diagnosis.

Laboratory examination

5 ml of blood was drawn from peripheral vein under aseptic conditions in ethylenediaminetetraacetic acid and plain vacutainer. Hemoglobin, prothrombin time, blood urea, serum creatinine, and serum homocysteine were measured using automatic analyzer.

Molecular analysis

DNA was extracted from blood using spin column-based DNA extraction kit (HiPurA, Himedia, India). After isolation of DNA, the polymerase chain reaction (PCR)–restriction fragment length polymorphism method was used for identification of G1691A mutation. A 267 bp fragment was amplified for factor V Leiden. Amplified PCR product was digested with 1.5 U of *MnlI* restriction enzyme and electrophoresed on a 2% agarose gel. In normal controls, the 267 bp fragment was digested by the *MnlI* restriction enzyme to three subfragments. The mutation, however, abolishes a restriction site, thus producing only two subfragments.[6]

For the detection of MTHFR C677T, a 198-bp region of exon 4 was amplified. The C to T substitution creates a *HinfI* recognition sequence, analyzed by 3% agarose gel electrophoresis, which digests into two subfragments.[7]

All the data were entered into Microsoft Excel sheet for Windows. Chi-square test was applied to see the association of various risk factors such as gender, IOP, diabetes, hypertension, smoking, alcohol consumption, MTHFR, and factor V Leiden genotypes with RVO and also in between its types (central RVO [CRVO], branch RVO [BRVO], and macular tributary vein occlusion). Student's *t*-test was applied to see the difference in mean age, serum homocysteine, and other continuous variables in different RVO. $P < 0.05$ was considered statistically significant. While performing statistical analysis, Minitab statistical software (Minitab Inc, Pennsylvania USA) (free trial) was used.

Results

The age of patients ranged between 18 and 86 years. The mean age of cases and controls was 54.62 ± 13.92 years and 58.72 ± 11.20 years, respectively. There was no significant gender predisposition. The logMAR value of best-corrected visual acuity was better among the patients of BRVO in comparison to CRVO cases. Of the cases who had macular edema, 82.8% were cases of BRVO.

On comparing all cases with controls, gender, IOP, diabetic status, personal habits of smoking and alcohol consumption, dietary habits, serum creatinine, and prothrombin time did not reveal any significant association with RVO [Table 1]. Significantly a high number of hypertensive patients were observed in RVO cases as compared to controls.

Mean values of serum homocysteine in cases and controls were 19.98 ± 9.03 μmol/L and 16.98 ± 8.29 μmol/L, respectively (P = 0.025, making hyperhomocysteinemia significantly associated with RVO) [Figure 1].

The frequency of MTHFR CT (heterozygous mutant) genotype was statistically similar in cases and controls. MTHFR TT (homozygous mutant) genotype was not observed in any case or control. There was no significant association of MTHFR genotype with RVO (P = 0.663). We also did not observe any significant difference in MTHFR genotype in BRVO as compared to CRVO [Table 2].

Table 1: Demographic and clinical characteristics of cases and controls

Parameters	Case	Control	*P*
Age (in years)	54.62±13.92	58.72±11.20	
Female	31	29	0.838
IOP >5 mm Hg	2	0	-
Macular edema	29	0	-
Diabetes mellitus	14	15	0.826
Hypertension	32	17	0.003
Smokers	6	7	0.766
Alcohol consumption	8	6	0.564
Dietary habits			
Vegetarian	29	36	0.245
Eggetarian	1	0	
Nonvegetarian	20	14	
Serum homocysteine (μmol/L)	19.98±9.03	16.98±8.29	0.025

IOP=Intraocular pressure

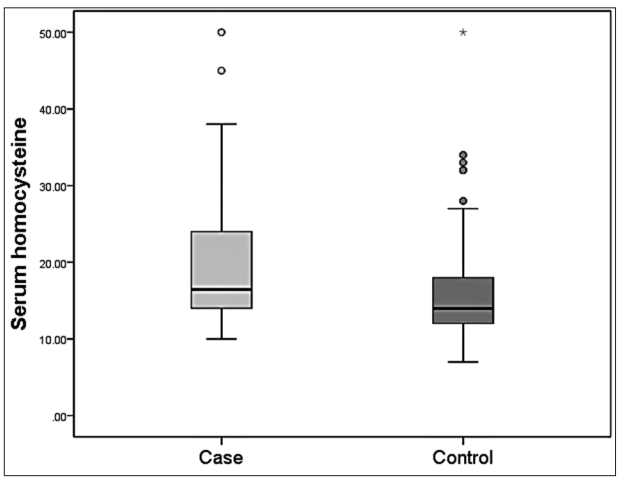

Figure 1: Whisker Box plot showing serum homocysteine levels with standard deviation in cases and controls. Horizontal line in box represents median. Box represents the standard deviation. Vertical line represents the range

Finally, on comparing serum homocysteine with CC (homozygous normal) and CT variants of MTHFR genotype, it revealed a significant (P value = 0.022) association of raised serum homocysteine with CT variant of MTHFR genetic polymorphism in patients of RVO [Figure 2]. Hyperhomocysteinemia (homocysteine >15 μmol/L) was observed in 31 RVO cases, of which 20 had CC genotype and 11 had CT genotype. Nineteen RVO cases had normal homocysteine levels, of which only 3 had CT genotype.

Factor V Leiden mutation was not observed in any of the studied individuals.

Discussion

RVO presents as sudden painless diminution of vision.[8] The building blocks of etiopathogenesis of this disease entity are hypercoagulable state of blood and thromboembolic phenomena due to vascular structural alterations.[9] It can be due to environmental factors or predisposing genetic mutation in an individual, disturbing the milieu interior for a normal blood circulation.[8]

Hyperhomocysteinemia is independently associated with an increased risk of thrombosis.[10] However, elevation of plasma homocysteine is also found in 5%–7% of normal population.[11] In the study carried out by Salaun *et al*.,[12] the risk factors associated with CRVO were hyperhomocysteinemia, arterial hypertension, and intraocular hypertension. The only notable risk factor for BRVO was arterial hypertension. Hyperhomocysteinemia was discovered to be significantly associated with RVO in the present study. Li *et al*.[5] and McGimpsey *et al*.[13] in their meta-analyses found an increased risk of RVO

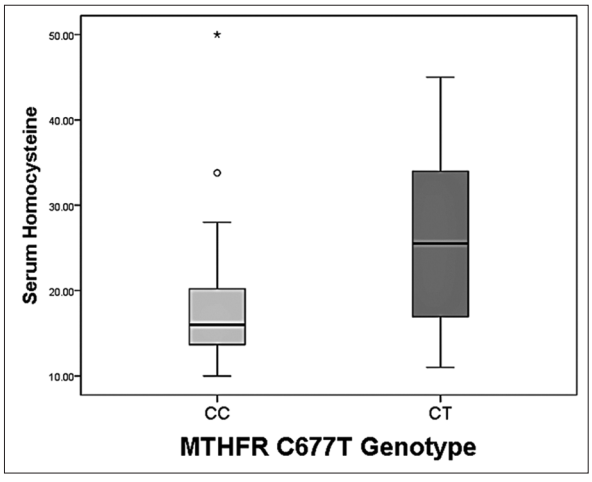

Figure 2: Whisker Box plot showing serum homocysteine levels with standard deviation in different genotypes of MTHFR C677T polymorphism. Horizontal line in box represents median. Box represents the standard deviation. Vertical line represents the range

Table 2: Frequency of methylenetetrahydrofolate reductase C677T gene polymorphism in different groups

	CC	CT	*P*
Cases	36	14	0.663
Controls	34	16	
BRVO	6	3	0.694
CRVO	30	11	

BRVO=Branch retinal vein occlusion, CRVO=Central retinal vein occlusion

with raised plasma homocysteine. Al Wadani *et al*. also reported hyperhomocysteinemia as a risk factor for RVO.[14] However, there was no evidence to suggest an association between homozygosity for the MTHFR C677T genotype and RVO.[5,14] Similarly, in our study, we did not observe any association of MTHFR gene mutation and RVO. Therefore, we hypothesize that MRHFR C677T mutation is associated with higher serum homocysteine level but is not at the same time associated with RVO occurrence which can be due to still unknown factor which subsequently leads to a protective effect on RVO. Therefore, the integrated effects of MTHFR C677T mutation on serum homocysteine level and still unknown factor lead to a neutralizing effect on RVO occurrence by MTHFR C677T mutation. Glueck *et al*. hypothesized hyperestrogenemic state to be a risk factor for vascular occlusion in patients with thrombophilic mutation.[15]

Seventeen RVO patients and 21 controls were heterozygous for the MTHFR C677T mutation in a study executed by Cruciani *et al*.[16] They studied 29 patients under 50 years of age and compared with 62 age-matched controls. Three RVO patients and 23 controls were homozygous for the MTHFR C677T mutation. Three RVO patients and two controls were heterozygous for factor II G20210A mutation. One control was heterozygous for the factor V Leiden. This study failed to demonstrate that genetic mutations are risk factors for RVO in patients >50 years of age.

Paovic *et al*.[17] showed that patients with CT genotype of MTHFR C677T polymorphism had raised values of homocysteine that correlated with causation of RVO. The authors further concluded that in the absence of retinal vasculitis, the existence of a single-mutated allele for MTHFR C677T gene did not correlate with RVO. On the contrary, many patients with MTHFR genotype have normal homocysteine levels (phenotypic expression is critically dependent on folate levels and folic acid intake).[18] There can be many causes for hyperhomocysteinemia, besides genetic mutations, such as vitamin deficiencies (pyridoxine, folic acid, and Vitamin B12), chronic illnesses (chronic renal failure, diabetes, hypertension, and cancer), drugs (methotrexate, antiepileptic agents), and enzyme deficiencies.[17]

Finally, in our study, on comparing serum homocysteine with CC and CT variants of MTHFR, serum homocysteine levels were higher in patients with CT variant of MTHFR polymorphism in patients of RVO. It suggests that C677T MTHFR polymorphism may indirectly but occasionally cause RVO. The need for a larger population-based study cannot be ruled out. It is necessary to carry it out on randomized samples in larger numbers in Indian subcontinent, especially for validating this study.

Meta-analyses have not identified a statistically significant relationship between RVO and heritable thrombophilia, but suggested that factor V Leiden mutation might be a weak risk factor.[19] A more recent analysis confirmed only a moderately high prevalence of factor V Leiden mutation in patients with RVO as compared to controls and the authors recommended to not to screen for factor V mutation in all cases of RVO.[20] However, in our study, none of the individuals had factor V Leiden mutation which is in concordance with most of the previous studies that found factor V Leiden mutation to not to be associated with RVO.[10,16,21]

Conclusion

We found a strong correlation of serum homocysteine with RVO. However, none of the studied individuals had factor V Leiden mutation. MTHFR C677T gene polymorphism was linked significantly with elevated serum homocysteine levels.

In light of all the above facts, a similar study on a larger population would not only provide a greater significance but possibly open doors to newer approaches to combat RVO at molecular level and find ways for preventing the disease in the next generation.

Ethical approval

The study was conducted in accordance with the Declaration of Helsinki and was approved by the local ethics committee of the institute. Informed written consent was obtained from all patients prior to their enrollment in this study.

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Nil.

Conflicts of interest

The authors declare that there are no conflicts of interests of this paper.

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