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CASE SERIES OF METHYLENE TETRAHYDROFOLATE REDUCTASE GENE POLYMORHISM IN CEREBRAL VENOUS SINUS THROMBOSIS PATIENTS

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ABSTRACT

Background: Cerebral venous sinus thrombosis (CVST) is a rare form of stroke with diverse etiologies, including genetic and metabolic factors. Hyperhomocysteinemia is a known prothrombotic risk factor, and polymorphisms in the methylene tetrahydrofolate reductase (MTHFR) gene particularly C677T and A1298C may contribute to elevated homocysteine levels. This study aimed to investigate the frequency of MTHFR gene polymorphisms in CVST patients with hyperhomocysteinemia.

Materials and Methods: A case series was conducted involving nine patients diagnosed with CVST and hyperhomocysteinemia. Clinical data including age, gender, lifestyle habits, and family history were recorded. Blood samples were analysed for plasma total homocysteine, serum folate, and vitamin B12 levels. Genotyping for MTHFR C677T and A1298C polymorphisms was performed using real-time PCR (RT-PCR). **Results:** The mean age of participants was 44.55 ± 10.32 years, with a female predominance (66.67%). Mean plasma homocysteine level was 23.76 ± 21.33 mmol/L, and 44.44% had homocysteine levels above the 90th percentile. One patient (11.1%) was homozygous and one (11.1%) heterozygous for the MTHFR C677T mutation, while two patients (22.2%) were heterozygous for the A1298C variant. Folate and vitamin B12 levels were below optimal in several cases, possibly contributing to elevated homocysteine levels.

Conclusion: This study suggests a potential association between MTHFR gene polymorphisms and hyperhomocysteinemia in CVST patients. MTHFR genotyping, alongside homocysteine assessment, may aid in identifying individuals at increased thrombotic risk and support personalized management approaches.

KEYWORDS: *Cerebral venous thrombosis, MTHFR, Hyperhomocysteinemia*

INTRODUCTION

Cerebral venous sinus thrombosis (CVST) is a rare but serious neurological condition, accounting for less than 1% of all strokes [1]. It predominantly affects younger individuals and can lead to significant morbidity and mortality. CVST is now recognized as a multifactorial disorder, with thrombophilia playing a central role in its pathogenesis [2]. Among the prothrombotic factors, hyperhomocysteinemia (HHcy) has emerged as a strong and independent risk factor, present in 27–43% of CVT patients compared to 8–10% of controls, with an odds ratio of 4 to 7 [3,4].

Even mild elevations in plasma homocysteine are associated with both arterial and venous thrombosis [5]. HHcy may result from genetic mutations in enzymes such as MTHFR, methionine synthase, and cystathionine β-synthase, or from deficiencies in vitamins B6, B9 (folate), and B12, all essential for homocysteine metabolism [5]. The common MTHFR C677T mutation, present in homozygous form in 10–13% of the white population, is one of the most studied [6].

Nutritional deficiencies particularly in folate and B vitamins are important contributors, especially in resource-limited settings. The interaction between genetic predisposition and poor nutritional status may further elevate thrombotic risk [6, 7].

Although the association between HHcy and CVST is well-established globally, with a 4- to 5-fold increased risk [8], data from the Indian population remain limited. Given the high prevalence of vitamin deficiencies and possible genetic factors in India, this case series aims to highlight the clinical relevance of HHcy as a modifiable risk factor and to emphasize the importance of targeted screening and early intervention in at-risk populations.

MATERIALS ANS METHODOLOGY

The present case series was conducted involving eight patients diagnosed with cerebral venous sinus thrombosis (CVST) and hyperhomocysteinemia.

Patients were recruited from Medicine department MGM Medical College and Hospital. Clinical data, including baseline homocysteine levels and relevant demographic and medical history, were recorded.

Sample Collection and RNA Isolation

Peripheral venous blood samples were collected in EDTA-coated tubes from each participant. Samples were processed within two hours of collection to preserve RNA integrity. Total RNA was extracted using a commercial RNA isolation kit (MTHFR gene polymorphism kit). The RNA was quantified using a spectrophotometer and assessed for purity via A260/A280 ratio before proceeding to reverse transcription.

Reverse Transcription and RT-PCR Amplification

Reverse transcription was performed to synthesize complementary DNA (cDNA) from the extracted RNA using reverse transcriptase enzymes. Target-specific primers were designed to detect common mutations associated with hyperhomocysteinemia, specifically the C677T and A1298C mutations in the *MTHFR* gene. RT-PCR amplification was carried out using a thermal cycler with optimized conditions to ensure specificity and efficiency of mutation detection.

Data Analysis

The presence or absence of the targeted mutations was assessed qualitatively. Homocysteine levels were documented for each patient, and descriptive statistics were used to explore associations between mutation status and homocysteine concentration. Due to the small sample size, formal inferential statistical analysis was not performed; results were presented as individual case profiles with mutation status and clinical correlation.

RESULTS

Table-1: Demographic characteristics of the study participants

Variables	No of cases	Percentage
Age (mean + SD)	44.55 ± 10.32	
Gender		
Male	3	33.33%
Female	6	66.67%
Total	9	100.00%
Alcohol	1	11.11%
smoking	2	22.22%
Family history of vascular disease	3	33.33%
Plasma total homocysteine, mmol/L (mean + SD)	23.76 ± 21.33	
Hyperhomocysteinemia, n (%; >90th percentile)	4	44.44%
Folates, nmol/L (mean + SD)	5.78 ± 2.13	
Vitamin B12, pmol/L (mean + SD)	343.56 ± 429.00	

In present case series involving 9 patients with hyperhomocysteinemia, the mean age was 44.55 ± 10.32 years. The majority were female (66.67%), with males comprising 33.33%. Among the participants, 11.11% reported alcohol consumption, 22.22% were smokers, and 33.33% had a family history of vascular disease. A vegetarian diet was followed by 44.44% of the group. The mean plasma total homocysteine level was 23.76 ± 21.33 mmol/L, with 44.44% of participants showing hyperhomocysteinemia (>90th percentile). Mean folate and vitamin B12 levels were 5.78 ± 2.13 nmol/L and 343.56 ± 429.00 pmol/L, respectively.

Table-2: MTHFR Detection by RT-PCR

MTHFR Detection by RT-PCR	Detected (Positive)	Not detected (Negative)
Homozygous Mutant for MTHFR C677T mutation	1	8
Homozygous Wild type for MTHFR C677T mutation	0	9
Heterozygous Mutant for MTHFR C677T mutation	1	8
Homozygous Mutant for MTHFR A1298C mutation	0	9
Homozygous Wild type for MTHFR A1298C mutation	0	9
Heterozygous Mutant for MTHFR A1298C mutation	2	7

MTHFR gene mutation analysis by RT-PCR showed that 1 patient (11.1%) was homozygous mutant and 1 (11.1%) was heterozygous mutant for the C677T mutation. For the A1298C mutation, 2 patients (22.2%) were heterozygous mutants. These findings suggest that MTHFR mutations may contribute to hyperhomocysteinemia in a subset of patients. The presence of C677T and A1298C variants, known to affect folate metabolism and homocysteine levels, highlights a potential genetic influence. Although based on a small sample, the results support the role of MTHFR genotyping in evaluating patients with elevated homocysteine, especially in the absence of other risk factors.

DISCUSSION

Cerebral venous sinus thrombosis (CVST) is a rare form of cerebrovascular disease, accounting for less than 1% of all strokes [9]. It is more frequently observed in younger individuals, particularly women during pregnancy, the puerperium, or those using oral contraceptives, as well as in patients with inherited or acquired thrombophilic disorders [10]. Due to its highly variable clinical

presentation, ranging from isolated headache to seizures or focal neurological deficits, a high index of suspicion is essential for prompt diagnosis and management [11].

In the present case series involving nine patients with CVST and hyperhomocysteinemia, a female predominance (66.67%) was noted, with a mean age of 44.55 years. This demographic distribution is comparable to that reported by Gogu AE et al. [10] who observed a similar female preponderance (65.79%) in a younger cohort with a mean age of 37.55 years. Such findings underscore the continued relevance of gender and age-related risk profiles in CVST.

The current case series identified elevated mean plasma total homocysteine levels (23.76 ± 21.33 mmol/L), with 44.44% of patients meeting the criteria for hyperhomocysteinemia. Several factors may contribute to this discrepancy, including the high prevalence of nutritional deficiencies in developing countries. Dietary patterns, particularly vegetarianism a lifestyle observed by 44.44% of our cohort can lead to folate and vitamin B12 deficiencies, which in turn elevate homocysteine levels. These nutritional deficiencies may arise from socioeconomic, cultural, or religious practices, as previously described by **Bharatkumar et al.** [12]

The role of genetic predisposition in hyperhomocysteinemia was explored through MTHFR gene polymorphism analysis. Among our participants, one patient (11.1%) was homozygous and another (11.1%) was heterozygous for the MTHFR C677T mutation, while two patients (22.2%) were heterozygous for the A1298C variant. The presence of both C677T and A1298C variants known to impair folate metabolism suggests a potential genetic contribution to hyperhomocysteinemia. Our findings are consistent with those of **Cantu C et al.,** [8] who reported a higher frequency of the thermolabile TT genotype in CVT patients (22%) compared to controls (10%), and with **Bharatkumar et al.,** [12] who found the 677T allele in 19.5% of cases. These results support the hypothesis that MTHFR polymorphisms may play a contributory role in CVST, particularly in patients lacking conventional risk factors.

A review of the literature revealed two case reports: a 45-year-old male with heterozygous C677T mutation and hyperhomocysteinemia, and a 21-year-old with seizures and heterozygous A1298C mutation. The prevalence of the MTHFR 677T allele varies widely by ethnicity, ranging from 20–55% in European populations to 4–38% in Asian populations [13]. In India, **Devi et al.** [13] and **Rai et al.** [14] reported prevalence rates of 10.12% and 12%, respectively, in South and Eastern Indian cohorts. The occurrence of CVST in patients with MTHFR C677T polymorphism remains relatively rare; however, our identification of two such cases further reinforces its clinical relevance.

Interestingly, **Maji et al.** [15] also described a case of heterozygous C677T polymorphism with normal homocysteine in 9 year old boy with folate, and vitamin B12 levels, highlighting the role of gene-nutrient and gene-environment interactions in modulating the phenotypic expression of MTHFR variants. This underscores the multifactorial nature of homocysteine metabolism, influenced by genetic, nutritional, and lifestyle factors. Overall, despite the small sample size, the current case series supports the utility of MTHFR genotyping in patients with CVST and hyperhomocysteinemia, particularly when traditional risk factors are absent. Early identification of such genetic polymorphisms could guide more targeted management strategies, including vitamin supplementation and lifestyle modification.

CONCLUSION

This case series underscores the association between hyperhomocysteinemia and MTHFR gene polymorphisms in patients with cerebral venous sinus thrombosis. A notable proportion of patients exhibited elevated homocysteine levels, with several carrying heterozygous or homozygous mutations in the MTHFR C677T or A1298C loci. These findings underscore the importance of evaluating genetic factors in the pathogenesis of CVST, particularly in patients without conventional risk factors. Despite the small sample size, our findings indicate that MTHFR genotyping, alongside homocysteine level assessment, may aid in risk stratification and inform targeted therapeutic strategies in CVST patients.

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