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Negative Association of MTHFR C667t Polymorphism with Intracerebral Hemorrhage in Kashmiri Population- An Unexpected Result

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ABSTRACT

Introduction: Intra Cerebral Hemorrhage carries a substantial morbidity and mortality and is particularly prevalent in our Kashmir Valley. Genetic risk plays a significant part in ICH prevalence. MethylTetraHydroFolateReductase (MTHFR) C 677 -T Polymorphism is a widely studied genetic mutation all over world not only because of its association with the prevalence of stroke, but also because of the associated hyperhomocysteinemia and potentially preventive nutritional interventions.

Aims and Methodology: We conducted a hospital based case control study with 75 confirmed cases of ICH against 100 matched controls, and using a pretested, semi-structured questionnaire, collected information on the clinical parameters including Gender, Age, presence of Hypertension, Location of ICH and ICH severity Scoring. We employed the PCR-RFLP technique to study MTHFR C677T Polymorphism association with ICH the various clinical parameters.

Results: The allelic and genotypic frequencies of MTHFR C677T in ICH cases and controls was found to be significantly different (p=0.01; OR=0.40; CI=0.19-0.84). The association between MTHFR C677T polymorphism with that of the clinical parameters of ICH cases was not found to be significant.

Conclusion: MTHFR C667T Polymorphism appears to be protective in our ICH population, an unexpected result given the literature published so far.

Keywords: MetylTetraHydroFolateReductase (MTHFR), C677T Polymorphism, IntraCerebral Hemorrhage and Kashmiri population

INTRODUCTION

Intra-Cerebral Haemorrhage (ICH) victims have a grim outcome with ensuing death or severe disability for more than 50%.[1] Of the thousands of stroke survivors each year, approximately 30% require assistance with activities of daily living, 20% require assistance with ambulation, and 16% require institutional care.[2] Intra-Cerebral Haemorrhage (ICH) victims have a grim outcome with ensuing death or severe disability for more than 50%.[1] Of the thousands of stroke survivors each year, approximately 30% require assistance with activities of daily living, 20% require assistance with ambulation, and 16% require institutional care.[2]

Overall, ICH accounts for 10–35% of stroke cases depending on the population studied [3] Like some series from Asian countries such as that from Shibata, Japan, with reported several-fold higher incidence rates of ICH[4], ICH in our valley was reported to be the commonest stroke-type observed in Kashmir accounting for close to two third of strokes with male preponderance.[5] Consequently it becomes more relevant to study ICH in Kashmir.

The conventional risk factors of ICH (Hypertension, low cholesterol levels, heavy alcohol intake, advanced age, and male gender)[6] could not explain all cases of ICH. . This infers that other influences, including genetic ones, are involved in stroke risk.[7] Genetic predisposition to stroke can be categorized as either a single gene disorder (with minor contribution) or as a polygenic disorder (major genetic contributor for predisposition to stroke)[8]. Among polygenic disorders one important genetic polymorphism studied is MTHFR (MethyleneTetraHydroFolateReductase) gene polymorphism.[9]

Many studies have shown that the plasma homocysteine level is associated with the risk for atherosclerosis [10,11,12] and interestingly it has been also reported that elevated plasma homocysteine levels are associated with hemorrhagic strokes also.[13] Several mechanisms have been proposed for this association. Homocysteine is reported to be actively involved in the oxidative stress event [14], endothelial dysfunction including induction of NADPH oxidase and decreasing bioavailability of nitric oxide (NO).[15], enhanced platelet adhesion to endothelial cells[16] and also promotion of growth of vascular smooth muscle cells.[17] This in turn may be accomplished by Protein N-homocysteinylation contributing finally to vascular inflammation, atherogenesis, hypercoagulation status, and vulnerability to establishment of atherosclerotic plaques.[18]. Regarding predisposition to ICH, increased promotion to plaque rupture has been proposed as a possible mechanism.[19]

MTHFR is a key enzyme in homocysteine metabolism which catalyzes irreversibly conversion of 5,10-MTHF to 5-MTHF, which is the main circulatory form of folate. Human MTHFR gene is located on chromosome 1p36.3.[20] C667T polymorphism of MTHFR gene is a C-to-T (Cytosine to Thymine) transition at nucleotide 677 (C677T) in exon 4, which results in an alanine (Ala) to valine (Val) substitution in the MTHFR enzyme[21] and makes the enzyme thermo-labile and less active which leads to hyperhomocysteinemia and hypomethylation in homozygous mutant state and this makes it an important marker for thrombotic events.[21,22] Functionally, MTHFR enzyme activity is reduced 35% with the heterozygous CT genotype and 70% with the variant TT genotype.[23] Polymorphisms in the MTHFR gene, including mainly C677T and to some extent A1298C A/V, have been shown to be associated with increased homocysteine levels and have shown evidence of causality of both stroke subtypes.[24,25] Similarly, MTHFR C677T polymorphism has been studied and speculated in ICH.[26,27] However, results from published genetic association studies were contradictory rather than conclusive. Some reported a significant impact of C677T polymorphism on ICH risk[28,29] while some failed to replicate these findings.[19,30]

Identification and management of possible risk factors to prevent stroke is an important strategy to reduce human and economic burden of stroke.[31] A potential advantage of delineating the at-risk population with these mutations could be primary preventive interventions including nutritional supplementation. E.g, a meta-analysis suggested that folic acid supplementation could significantly reduce the risk of stroke by 18%.[32] It has been also reported that vitamin B12 deficiency is positively correlated with hyperhomocysteinemia, which is partly attributed to low levels of folate.[33] B vitamin supplementation has been shown to decrease plasma homocysteine levels, although the effect on cardiovascular end points has been mostly negative.[34] Despite the inherent contradictions, the fact of the matter is that vitamin treatment remains potentially a cheap and safe mode of primary prevention of stroke.

METHODOLOGY:

We followed a candidate gene case-control approach, which is generally taken to study genetic risk factors and associations for stroke [35], to evaluate the association of C677T polymorphism (in Exon 4) of MTHFR gene in patients of Haemorrhagic stroke and in the control population and to assess the clinical implications, if any, of the gene polymorphism in stroke patients including association with the subtype of ICH, severity and other risk factors of ICH.

Inclusion/ Exclusion Criteria:

All consecutive ICH admitted to the Neurology Department of SKIMS, Soura were considered for the study excluding ICH patients with history of trauma and suspected or documented aneurysm or intra cranial space occupying lesion (ICSOL).

A pretested, semi-structured questionnaire was used to collect the information on clinical parameters. A total of 75 ICH cases with prior consent were

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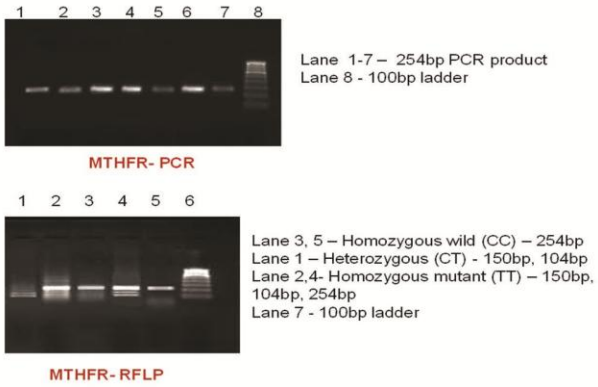
included in our study with 100 age and gender matched controls. The data collected included information about Gender, Age, Hypertension, Location of ICH and ICH severity Scoring.[36]The study was approved by the ethical committee of the institute (No. SIMS 1 131/IEC-SKIMS/2017-239; Dated: 03-01-2017).

The collected blood samples were subjected to DNA polymorphism analyses using PCR - RFLP technique[37]

(Figure 1)



Statistical analysis was performed by using SPSS software (V.11.5). Chi Square test for homogeneity of proportions was used to determine significance of mutation pattern and Odds ratio was used to determine association of presence of polymorphism with various Clinico-epidemiological characteristics such as age, gender and hypertension. Statistical significance was considered with p-value ≤ 0.05.

RESULTS:

A total of 75 ICH cases and 100 healthy controls were included in this study.

No significant gender- or age-related differences were observed between the groups (*p>0.05*) (Table 1).

Variable		Cases ICH (n=75)	Controls (n=100)	p-values
Age (years)	≤55	31	49	0.35
	>55	44	51	
Gender	Male	50	56	0.16
	Female	25	44	

Table 1: Frequency distribution analysis of selected demographic factors in ICH cases and controls

The distribution of MTHFR C677T genotypic and allelic frequency in ICH cases and controls is given in Table 2.

Genotype	**ICH Cases n=75(%)**	**Controls n=100(%)**	***OR (95% CI); P¥; Fψ***
MTHFR C677T			
CC	62(82.66%)	66(66%)	1.0 (Reference)
CT +TT	13+0=13(17.33%)	29+5=34(34%)	**0.40(0.19 – 0.84);0.01**
Allele(2N)	***(2N)*=150**	***(2N)*=200**	***OR (95% CI); P¥; Fψ***

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C *T*	137 13	161 39	 **0.39(0.20 – 0.76);0.005**
¥ = Pearson's P Value, ψ = Fisher Exact P Value. Significant P values are shown in bold.			

Table 2: Allelic and Genotypic frequencies of *MTHFR C677T* polymorphisms in ICH cases and controls

The allelic and genotypic frequencies of MTHFR C677T in ICH cases and controls was found to be significantly different (*p=0.01; OR=0.40; CI=0.19-0.84*).

Furthermore the association between MTHFR C677T polymorphism with that of the clinical parameters of ICH cases was also carefully analysed (Table 3), and none of these clinical parameters among ICH cases were found to be significantly associated with MTHFR C677T polymorphism.

Parameter	**No of ISC cases (n=75)**		
	CC(62)	***CT(13)***	***p-value***
Age ≤55years(31) >55years(44)	 27 35	 4 9	 0.57(0.16-2.07);0.53
Gender Females(25) Males(50)	 21 41	 4 9	 0.86(0.23-3.15);1
Hypertension Hypertensive(62) Non- Hypertensive (13)	 50 12	 12 1	 2.88(0.34-24.35);0.44
Location of ICH Lobar(18) Non-Lobar(57)	 17 45	 1 12	 0.22(0.02-1.82);0.16
ICH Score 0(14) 1(18) 2(28) 3(13) 4(2)	 10 16 24 11 1	 4 2 4 2 1	P=0.489 1.0 (Reference) 3.2(0.49-20.81);0.36 2.4(0.49-11.53);0.40 2.2(0.32-14.72);0.64 0.4(0.01-8.07);1

Table 3: Association between *MTHFR C677T* polymorphism and various clinical variables in ICH patients

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DISCUSSION:

In the present study, we examined whether the MTHFR C677T polymorphism is associated with patients with hemorrhagic stroke. Also because, our population, akin to certain other regions of the world[38,39], has an unusually higher incidence of haemorrhagic stroke as well.[40] In Kashmiri population, previously, no study evaluating association of haemorrhagic stroke with MTHFR C677T gene polymorphism has been conducted.

In our study the frequency of MTHFR C677T polymorphism was found to be significantly different in the ICH cases than healthy controls with *p=0.01; OR=0.40; CI=0.19-0.84*. This finding suggests that the TT genotype is not a predisposition to ICH. Our findings correlate with many a studies and contrast with some. In India a recent study done in 2015 by Das S et al, concludes that CT genotype of MTHFR (C677T) polymorphism is a risk factor for ICH among the South Indians from Andhra Pradesh.[41] However Somarajan BI et al, had conducted a case–control study in a Northern Indian population, in 2011, and proved the otherwise.[42] Outside India, studies proving a casual association include, Ali Sazki et al in 2006 (Turkey),[43] and, Hultdin J et al in 2011 (Sweden)[44]. In contrast , and akin to our study, Dikmen et al (2006) did not detect any significant role of C677T polymorphism in hemorrhagic stroke risk in a Turkish population[45], nor did Nakata Yet al., in people of Chinese Han ethnicity.[46] Various Metaanalysis done in this respect have mostly favoured the association, including Shan Gao et al in 2012 in Asian and Caucasian populations[47], Shan Kang et al in 2013[48], Zhao X et al in Asian and Caucasian populations in 2013[49] and Xin Hu et al in 2015 in Chinese Han population.[50]

The reasons for these disparities are multifactorial, including firstly the racial-ethnic differences, for example, the association was found by some to be stronger in Asian than European population.[47,51] Secondly, the alleles have been shown to at best only modestly increase risk of ICH. Consequently the no. of cases studied need to be more. With the result, there is lack of adequate power for association in many studies, including our study. Also, various haplotypes, unevaluated in most studies, including ours, may confer a protective effect or a risk, irrespective of the results of the association study.[44]

Among the controls we found the frequency of MTHFR CC, CT and TT genotypes to be 66 (66%), 29 (29%) and 5(5%), while in the ICH cases it was 62(82.66%) and 13(17.33%) and 0% respectively. This means that TT genotype/T allele appeared protective for ICH in our population. This finding is in contrast to almost all the studies till date those have shown an association of ICH with MTHFR polymorphism, with TT genotype contributing to increased risk of ICH.[41,47,48] The reason for this unusual finding is not clear and at best hypothetical. The protective effects of 677T allele of MTHFR has been long known in various diseases, especially in Cervical[52] and Colonic carcinogenesis.[53,54] Similarly, It was concluded in a study that subjects with MTHFR 677C>T polymorphism have lower likelihood of renal insufficiency.[55] While as, in a metaanalysis, It was concluded that lower eGFR was a risk factor for both ischaemic and haemorrhagic stroke.[56] So theoretically, at least, in our subpopulation and our genetic pool this apparent protective effect of MTHFR 677C>T on eGFR and consequently on ICH may be phenotypically overt. Also it has been observed that several alleles and various mutation predisposing to ISC stroke may be protective to ICH and vice versa.[57] For example Factor V Lieden mutation has been found to be protective to ICH in a metaanalysis.[57] Similarly MTHFR mutation, a risk factor for ISC, may be protective in ICH.

Our aim was also to investigate the association of MTHFRC677T genetic polymorphism with the risk factors of ICH in our population. Some investigators like Markan S et al. found that MTHFR 677 CT/MTHFR 1298 CC genotypes are associated with increased risk of hypertension in Indians[58] and Heux S et al. found that MTHFR gene variant C677T is a risk factor for essential hypertension in Caucasian[59]. However , our study did not confer to the association. Also, our study did not reveal any association of the location of ICH (Lobar vs Non-Lobar) with the polymorphism which is in agreement with a study by Somarajan B.I. et al.[42] Lastly, we did not find the said association with the severity of ICH as has been proposed by some researchers.[60]

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