Haptoglobin gene polymorphism and ischemic stroke: A case control study

Sir,

Human haptoglobin (Hp), an acute-phase protein that prevents oxidative tissue damage, exists in two allelic forms, Hp-1 and Hp-2. Three major genotypes include Hp 1-1, Hp 1-2, and Hp 2-2. Hp 2-2 seems to have inferior antioxidant properties compared with other Hp proteins leading to hemoglobin-related oxidative damage, high-density lipoproteins (HDL), macrophage-induced inflammation, and apoptosis, promoting increased atherogenesis.[1] Studies on the association of haptoglobin polymorphisms and stroke are limited. A meta-analysis showed that the incidence of cardiovascular events in diabetic patients with the Hp 2-2 genotype was significantly increased as compared with non-Hp 2-2 patients.[2] HP-1 alleles have also been reported to be more frequent in lacunar stroke.[3] It has also been described as a risk factor for refractory hypertension in patients with essential hypertension.[4] A study in Italian subjects has reported increased risk of non-cardioembolic ischemic stroke in patients carrying the haptoglobin 2-2 phenotype.[5] HP-2 variant was also shown to promote premature cardiovascular death in stroke survivors.[6]

We performed a case-control study at a tertiary care center in northern India to investigate the association of haptoglobin polymorphisms with acute ischemic stroke (AIS). The study was approved by the institutional ethics committee and informed consent was obtained from all participants. The study population involved 113 participants including 54 patients of AIS and 59 (±2 years) age- and sex-matched controls. Blood samples were analyzed within 2 h using Randox commercial kit available for analysis. Haptoglobin analysis was done using polyacrylamide in disc electrophoresis in which samples were run for 1.30 h at 150 V. Allele frequency was calculated, and genotypes were counted manually. Hardy Weinberg equilibrium assumption was tested among cases and controls for genotypic distributions. Measure of relative risk was calculated using odds ratio along with 95% confidence interval. To understand the effect of various parameters on different genotype of Hp among cases, the KruskalWallis test was performed and the statistical significance was considered at $P < 0.05$.

The Hp genotype frequencies among cases were 4% (Hp 1-1), 31% (Hp 2-1), and 65% (Hp 2-2) resulting in HP 1 allele frequency of 20%. The distribution of Hp genotype among cases and controls respectively was found to be in Hardy Weinberg equilibrium. The Hp-2 allele frequency was found to be higher among cases than among controls. Both groups differed significantly with respect to Hp distribution, where Hp 2-2 genotype was found to be higher among the cases [Table 1]. Thus, the low frequency of HP1 allele among cases was due to an over-representation of HP 2-2 genotype among cases. The genic effect of the Hp-2 allele was found to be significant ($\chi^2 = 7.31$, df = 1, P value = 0.0068) hence individuals having Hp-2 allele, be it in a homozygous or heterozygous form, were considered to be at risk. Odds ratio between cases and controls vis-à-vis Hp genotypes was found to be 2.40 ($P = 1.000$) indicating non-significant association of HP genotype with the occurrence of stroke [Table 2]. Four parameters, i.e., cholesterol level (P value = 0.001), triglyceride level (P value = 0.001), alcohol use (P value = 0.02) and hypertension (P value = 0.001) were found to be significantly high among cases compared to controls. However, when KruskalWallis test was applied, no significant difference

Table 1: Relative haptoglobin genotype count and allele frequencies among cases and control participants

Population	Observed genotypic count			Allele Frequency		*P*
	1-1	2-1	2-2	1	2	
Cases	2(4%)	17(31%)	35(65%)	0.20	0.80	0.013
Control	5(8%)	32(54%)	22(37%)	0.36	0.64	

Table 2: Odds ratio and its probability among cases and control participants

Population	No. of Individuals		Odds Ratio (95% CI)	*P*
	At risk individual	Not at risk		
Cases	52	2	2.40(0.447-12.962)	1.00
Control	54	5		

in the biochemical parameters among Hp subtypes was observed. Therefore, high cholesterol and triglyceride levels associated with atherosclerosis in the present study are not due to Hp polymorphism. However, considering that there was a difference in the distribution between cases and controls vis-à-vis Hp genotype, an alternative pathway, one that differs from the conventional pathway of atherosclerosis, may be associated with the pathogenesis of stroke. As stroke is a multi-factorial disease, establishing a direct causal association between a particular genotype and phenotype is a difficult task, as a multitude of environmental factors will influence the final outcome. The present study gives some evidence that it would be unfair to equate haptoglobin subtype with pathogenesis of stroke without considering other factors (such as cholesterol level, triglyceride level, hypertension, and alcohol use).

Patients with the Hp2 allele seem to have higher risk for neurological and cardiovascular disorders (CVD) with the Hp 2-2 genotype having specific implications in increasing the risk of stroke and myocardial disease in the diabetic population.[7] Further, when we consider the distribution of Hp genotype globally, the genotype 2-2 is the highest among Asians and lowest among Africans. In the American and European population, the values are intermediate. On the contrary, the prevalence of CVDs is high among Americans and European population.[5] Hence, it would be unfair to postulate a one-to-one correlation between prevalence of stroke and Hp globally on the basis of statistical association of HP 2-2 type with CVD reported in a few papers. However, one cannot exclude the possibility of involvement of extra-genic factors of modern lifestyle (eating habits, hypertension) together with Hp type (and/or with other genetic risk factors for stroke) being responsible for such preponderance of CVD in developed countries like Europe and America. Keeping in mind that HP 2-2 is found in a high frequency in India, it may indicate a higher risk for stroke given the right environmental factors. The present study showing a higher odds ratio and a longer confidence interval does not reveal a positive association of Hp-2 allele with stroke. However, our study shows a trend toward positive association which could be confirmed with a larger sample size and an improvised study design with a homogeneous ethnic background of both cases and controls.

Specifically in the Indian context, there are numerous breeding units (caste groups, tribal, and religious groups) who maintain their respective sympatrically allocated gene pools (residing side by side without admixture) by virtue of long-practiced endogamy. This has made their respective gene pools, to some extent, exclusive from each other and hence, one can expect variation in the results of epidemiological (genetic) studies conducted thereof. Thus, a study with a more homogenous, possibly endogamous group of a larger population size, may be required to completely assess the relationship between Hp types and stroke along with other genetic and environmental risk factors.

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Conflicts of interest

There are no conflicts of interest.

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