Presence of Haptoglobin-2 Allele Is Associated with Worse Functional Outcomes After Spontaneous Intracerebral Hemorrhage

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Key words
- Clinical outcomes
- Haptoglobin phenotype
- Intracerebral hemorrhage

Abbreviations and Acronyms
CI: Confidence interval
Hp: Haptoglobin
ICH: Intracerebral hemorrhage
IVH: Intraventricular hemorrhage
mRS: modified Rankin Scale
OR: Odds ratio
SAH: Subarachnoid hemorrhage

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OBJECTIVE: **To determine if the haptoglobin (Hp) phenotype, which has been shown to be a predictor of clinical outcomes in cerebrovascular disorders, particularly subarachnoid hemorrhage, was predictive of functional outcomes after spontaneous intracerebral hemorrhage (ICH).**

METHODS: **Patients admitted with a diagnosis of ICH were prospectively included and divided into 3 groups based on their genetically determined Hp phenotype: 1-1, 2-1, and 2-2. Outcome measures included mortality and 30-day modified Rankin Scale scores. Demographics and outcomes were compared for each phenotype using multivariate linear regression analysis.**

RESULTS: **The study included 94 patients. The distribution of Hp phenotype was Hp 1-1, 12 (13%); Hp 2-1, 46 (49%); and Hp 2-2, 36 (38%). The 3 Hp subgroups did not differ in terms of demographic variables, comorbidities, or ICH characteristics. There was a nonsignificant trend toward increased mortality in Hp 2-1 and Hp 2-2 compared with Hp 1-1, with mortality of 8% in Hp 1-1, 17% in Hp 2-1, and 25% in Hp 2-2 ($P = 0.408$). In the regression model adjusted for confounders, Hp 2-1 (odds ratio = 0.05, 95% confidence interval = 0.01–0.47, $P < 0.001$) and Hp 2-2 phenotypes (odds ratio = 0.14, 95% confidence interval = 0.02–0.86, $P = 0.045$) had significantly lower odds of modified Rankin Scale scores 0–2 compared with Hp 1-1.**

CONCLUSIONS: **After ICH, individuals with the Hp-2 allele (2-1 and 2-2) had worse functional outcomes than individuals with the Hp-1 allele (Hp 1-1). There was a nonsignificant association between Hp phenotype and mortality. Larger prospective studies with better surrogates of ICH outcomes are warranted.**

INTRODUCTION

The role of serum inflammatory markers in predicting outcomes after spontaneous intracerebral hemorrhage (ICH) has been extensively studied (19). Genetic markers have emerged as a potential source of predicting outcomes, and one such marker is the haptoglobin (Hp) gene, with the Hp 2 allele defined by the presence of a 1.7-kb intragenic duplication of exons 3 and 4 of the Hp 1 allele (4). An individual has 1 of 3 genetically determined Hp phenotypes: 1-1, 2-1, or 2-2 (4). Population studies have reported an increased risk of cardiovascular disease such as stroke and myocardial infarction with the Hp 2 allele (8). Hp 2-1 and 2-2 phenotypes have been associated with higher incidences of diabetic nephropathy and restenosis after coronary angioplasty (11). Hp 2-2 also correlates with a higher rate of deep venous thrombosis compared with the other phenotypes (16).

Phenotypic studies in patients with acute brain injury have yielded similar results. For instance, patients with aneurysmal subarachnoid hemorrhage (SAH) have worse outcomes, in particular, a higher incidence of vasospasm with Hp 2-2 (12). These results were confirmed in animal models of SAH, in which we showed that the Hp 2-2 phenotype is correlated with increased arterial constriction and worse functional outcomes (4). We hypothesized that Hp phenotype may play a similar role in determining clinical outcomes in patients with ICH. This is the first study exploring an association between the Hp phenotype and ICH outcomes in the clinical setting.

METHODS

Patient Selection

We prospectively studied consecutive patients admitted with a diagnosis of ICH to the neurosciences critical care unit at Johns Hopkins Medical Institutions in Baltimore, Maryland, between January 2008 and July 2013. The study was approved by the institutional review board. The inclusion criteria were: 1) age >18 years, 2) nontraumatic ICH, and 3) presentation within 24 hours after ICH. Exclusion criteria were death on arrival and surgical evacuation of the ICH. The hematoma volume was measured using computed tomography scans at admission. The severity of ICH was assessed using the ICH score, which is a validated composite score that takes into account age, Glasgow

Coma Scale score, intraventricular hemorrhage, hematoma volume, and infratentorial location (6). Systolic blood pressure was maintained at <160 mm Hg. Anticoagulant and antiplatelet use before ICH was identified on admission.

Hp Phenotyping

Blood samples were collected from patients before transfusion of blood products and stored at −20°C until they were used for phenotyping. Hp phenotyping was performed at the Technion Institute using polyacrylamide gel electrophoresis, which provides a signature-banding pattern for each of the 3 possible Hp phenotypes (7).

Outcomes

The main outcomes of the study were modified Rankin Scale (mRS) score of 0–2 at 30 days (favorable outcome) and in-hospital mortality. The functional outcome score was determined at the 3-month outpatient follow-up, and the clinician was blinded to the results of the Hp phenotype.

Statistical Methods

Pearson χ^2 and Fisher exact tests were used to examine categorical variables. For continuous variables, one-way analysis of variance was used when data were normally distributed, and Kruskal-Wallis test was used when data were not normally distributed. Univariate logistic regression calculated the odds of each outcome for each independent variable. Multivariable logistic regression models were constructed to study the effect of Hp genotype on outcomes, after adjusting for confounders. The Hosmer-Lemeshow goodness-of-fit test was used to assess the fit of the multivariable regression models. Statistical analyses were performed using IBM SPSS Statistics version 21.0 (IBM Corp., Armonk, New York, USA). All analyses were 2-tailed, and significance level was determined by $P < 0.05$.

RESULTS

Demographics

The distribution of Hp phenotype in 94 patients with ICH was as follows: Hp 1-1, 12 (13%); Hp 2-1, 46 (49%); Hp 2-2, 36 (38%). The mean age in the cohort was 59.5 years (SD, ±17.38; median, 57.5 years). Age was significantly higher in the combined Hp 2-1/2-2 cohort compared with the Hp 1-1 cohort (60.89 years vs. 50.0 years, $P = 0.042$). The mean hematoma volume was 31.4 mL (SD, ±12.9; median, 21.3 mL), whereas the mean ICH score was 2.14 (SD, ±0.8; median, 2). Analysis of the demographics and comorbidities otherwise showed no significant difference in the 3 groups or in the combined Hp 2-1/2-2 groups (Table 1). ICH characteristics such as hematoma volume, ICH score, and Glasgow Coma Scale score were similar in the groups.

Outcomes

The overall mean mRS score was 3.87 (SD, ±1.6; median, 4.0). A favorable outcome was achieved in 10 patients with the distribution of mRS score 0–2 being 4 (33.4%) in Hp 1-1, 2 (4.3%) in Hp 2-1, and 4 (11.1%) in Hp 2-2 ($P = 0.015$) as shown in Figure 1. There were 18 deaths in the ICH cohort, which included 1 (8.3%) in Hp 1-1, 8 (17.4%) in Hp 2-1, and 9 (25.0%) in Hp 2-2 ($P = 0.408$). We observed a nonsignificant trend toward increased mortality with the number of Hp 2 alleles or the "allele dose effect," with mortality rates of 8% in Hp 1-1, 17% in Hp 2-1, and 25% in Hp 2-2. In the unadjusted regression analysis (Table 2), Hp 2-1/2-2 phenotypes were less likely to have favorable outcomes at 30 days compared with Hp 1-1 (odds ratio [OR] = 0.16, confidence interval [CI] = 0.04–0.68, $P = 0.013$), but there was no significant difference in mortality (OR = 2.88, CI = 0.37–23.8, $P = 0.408$). In the multivariable regression model examining Hp 2-1 and Hp 2-2 separately, Hp 2-1 and Hp 2-2 were each less likely to have favorable outcomes compared with Hp 1-1 (Table 3). Hp phenotype was not associated with mortality. The covariates considered in the regression model included age, sex, hypertension, ICH score, and Hp phenotype. In a second regression model comparing Hp 2-1 and Hp 2-2 combined (Hp 2-1/2-2) with Hp 1-1 as reference, Hp phenotype was an independent predictor of functional outcomes with Hp 2-1/2-2 having lower odds of achieving mRS score 0–2 compared with Hp 1-1 (OR = 0.13, CI = 0.03–0.71, $P = 0.018$); however, there was no significant effect on mortality (OR = 1.70, CI = 0.15–19.1, $P = 0.667$).

DISCUSSION

The present study shows that patients with Hp 2-1/2-2 phenotypes had worse functional outcomes compared with patients with Hp 1-1. Although a trend was apparent, we found no significant association between Hp phenotype and mortality. The pathophysiologic mechanism of injury entails free radical–mediated damage of the vascular endothelium from the free hemoglobin released after ICH (14). In addition, there is inhibition of vasodilation by blocking nitric oxide (1). This secondary injury leads to disruption of the blood-brain barrier, resulting in cerebral edema and death of brain parenchymal cells (1). Hp indirectly exerts a broad range of antiinflammatory activities and plays an antioxidant role most notably by virtue of its ability to bind free hemoglobin, accelerating the rapid clearance of hemoglobin via CD163 scavenger receptors on macrophages (3). Animal studies on the spatial distribution of Hp using murine ICH models colocalized the signals in the oligodendrocytes within the gray matter and the myelinated tracts in the corpus callosum and striatum (18). The oligodendrocytes overexpressing Hp had significantly less injury after ICH compared with Hp-deficient neurons (18). However, there are intrinsic differences among the Hp phenotypes in the ability to bind hemoglobin, with the hemoglobin-binding ability strong in Hp 1-1, intermediate in Hp 2-1, and weak in Hp 2-2 (4). In the Western population, Hp 2-1 is the most predominant form and is present in 47%–55% of patients, whereas Hp 1-1 is the least common form and is seen in 10%–20% of patients (10). The distribution of Hp phenotype in our study was similar to previous population-based studies.

The effect of Hp phenotypes on clinical outcomes has been studied extensively in SAH. Studies in murine SAH models have shown a higher rate of vasospasm with Hp 2-2 (4). This finding has been replicated in clinical studies. Borsody et al. (2) noted higher rates of vasospasm on transcranial Doppler imaging in patients with SAH with Hp 2-1/2-2 versus Hp 1-1 (87% vs. 29%, $P = 0.001$). Another study involving 95 patients with SAH also observed higher angiographic vasospasm with Hp 2-2 (12). More recently, Kantor et al. (9) reported