Haptoglobin Polymorphism and Disturbance of Lipid Parameters in Victims of Strokes

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Abstract: The objective of our study was to determine the frequency of the main genotypes of haptoglobin and their association with lipid parameters in subjects with stroke. This is a prospective, analytical and case-control study. 46 stroke patients and 46 matched controls were included by sex and age ± 2 years. Genotyping of Hp was performed using standard PCR with Proflex System PCR (Biosystems, Spain) and lipid parameters were assayed using enzymatic techniques with the Cobas c311 system (Roche, Germany). The mean age of our patients was 63 ± 14 years and the sex ratio was 0.70. Ischemic stroke was predominant with a frequency of 60.87% against 30.43% for hemorrhagic stroke. Allelic distribution in patients showed a high frequency of the Hp2 allele (51.09%) compared to control subjects (26.61%) (p <0,0001). The Hp2-2 genotype was more found in stroke subjects (36.96%) than in controls (17.39%) with a p = 0.002. A combination of the Hp2-2 genotype and the increase in mean LDL-c (p = 0.02), Triglycerides (p <0.005) and a decrease in mean HDL-c (p = 0.007) were found. Our study shows that the Hp2-2 genotype would be a predisposing factor to ischemic stroke. Its association with lipid parameters is found and could be considered as an additional risk factor.

Keywords: Stroke, Polymorphism of the Haptoglobin Gene, Lipid Balance

1. Introduction

Stroke is a real public health problem. The World Health Organization (WHO) defines a stroke as “the rapid development of localized or global clinical signs of cerebral dysfunction with symptoms lasting more than 24 hours and that can lead to death without any apparent cause other than vascular” [1]. There are two main types of stroke: ischemic stroke and hemorrhagic stroke.

They are one of the leading causes of morbidity and mortality after heart disease and cancer worldwide. They are the leading cause of acquired adult disability and the second leading cause of disability in the world. According to WHO, there are about 32 million cases, including 12.5 million deaths [2]. They represent 30% of hospitalizations and are responsible for nearly 2/3 of mortality in Senegal with an incidence of 1 to 2% of the general population [3].

The diagnosis is made by medical imaging mainly by CT and MRI. Biology is nowadays particularly absent from the stages of diagnosis and follow-up of the strokes. Thus, the search for biomarkers of prevention and/or follow-up is always more and more relevant.

The objective of our study was to determine the frequency of Hp genotypes and their association with lipid parameters
in stroke patients.

2. Methodology

2.1. Study Population

This is a prospective, analytical and case-control study. Included in our study were 46 stroke patients and 46 controls matched with cases by sex and age ± two years. Free and informed consent of all those involved in this study was obtained in advance in accordance with local ethical considerations. For all of our study population, genotyping of the Hp gene and assay of lipid parameters were performed.

2.2. Sampling

Blood samples were taken from fasting subjects, resting by venipuncture, and blood was collected in a dry tube for determination of lipid status parameters and in a tube with EDTA for genotyping the Hp gene.

2.3. Methods

2.3.1. Determination of Lipid Parameters

The determination of total cholesterol, HDL-c and triglycerides concentrations were carried out from Cobas C-311 (Roche, Germany) with enzymatic techniques. LDL-c was determined by the Friedewald formula.

2.3.2. Genotyping of the Haptoglobin Gene

The extraction of the DNA was carried out using the QIAmp® genomic DNA and RNA kits (QIAGEN, Paris) using the semi-automatic microcentrifuge technique. Genotyping of the Hp gene was performed using conventional PCR without enzymatic digestion with the ProFlex System PCR (Biosystems, Spain). The primers A (5’GAGGGGAGCTTGCCTTTCCATTG3’) and B (5’GAGATTTTTTGACCCCTGGCCTGT3’) were used for the amplification of a 1757pb sequence specific for the Hp1 allele and a 3481pb sequence specific for the Hp2 allele. Primer C (5’CCTGCCTCGTATTAACTGCACCAT3’) and D (5’CCGAGTGCTCCACATAGCCATGT3’) were used to amplify a 349pb sequence specific for the Hp2 allele. The PCR was carried out by adding to 2 µl of DNA, MgCl2 (1.5 mM), 2.5 µl of 10 mM dNTPs, 12.5 µl of Taq polymerase (Applied Biosystems, USA), each of the primers at a volume of 1 µl and sterile water in a final volume of 25µl. The PCR program used consisted of an initial denaturation at 95°C for 5 min, followed by 35 amplification cycles each comprising DNA denaturation at 95°C for 1 min, primer annealing, and 69° elongation. C for 2 minutes; final elongation was then carried out at 72°C for 10 minutes. The PCR product was visualized on 0.7% agarose gel in the presence of ethidium bromide (BET) and a molecular weight marker.

2.4. Statistical Evaluation

Our data was collected and used with Microsoft Excel 2013 software. The Student test was used to compare the mean values and the Chi-square test for frequencies. A value of p <0.05 was considered a statistically significant difference.

3. Results

The mean age of our subjects was 63 ± 14 years with extremes of 32 and 87 years. The analysis of the results of our study population showed a female predominance with a rate of 57% against 43% for men. The sex ratio was 0.70. Ischemic stroke was the most found form in our study population with a rate of 60.86% followed by hemorrhagic stroke at 30.43%. The typing of stroke was not done in 8.7% of our subjects (see Figure 1).
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Figure 2. Allelic and genotypic frequencies of Hp in stroke and control subjects.

The statistical analysis of the data showed that the average total cholesterol level in patients with stroke was 1.81 g/l against 1.60 g/l for controls (p = 0.057).

Figure 3. Valeurs moyennes des paramètres du bilan lipidique chez les AVC et les témoins.

In addition, no significant difference was found between stroke subjects and controls by dividing the subjects according to the three Hp genotypes. The mean HDL-c level was 0.36 g/l in stroke and 0.60 g/l in control subjects with p < 0.001. The comparison between these two groups showed significant differences for all three genotypes (see Figure 3). The subjects with stroke had an average LDL-c level of 1.34 g/l for a mean level in the controls of 0.80 g/l with a p < 0.001 showing a significant difference. The comparison between stroke and control subjects by genotype showed significant differences for Hp1-1 (p < 0.001) and Hp2-2 (p = 0.02) (see Table 1). The mean value of triglycerides in subjects with stroke (0.98 g/l) was increased more than in control subjects (0.85 g/l) with a p = 0.008 showing a significant difference. Mean TG levels for the different genotypes were 0.7 g/l, 1.1 g/l and 0.9 g/l for Hp1-1, Hp2-1 and Hp 2-2, respectively. Comparison with control subjects showed a significant difference only for the Hp2-2 genotype (p < 0.001) (See Table 1).
4. Discussion

Our study focused on a population of stroke patients in the search for biomarkers predisposing to this pathology. The average age of our subjects was 63 ± 14 years with a predominance of subjects aged 60 to 80 years. The distribution of subjects by sex showed a female predominance (59% vs. 41%) with a sex ratio of 0.70. In Senegal, the Diouf et al study reported a similar sex ratio (0.68) [5], however, other studies have shown a male predominance [6-7]. Nevertheless, according to the literature, there is variability in the prevalence of stroke in Africa according to sex with a predominance of studies in favor of the male gender [8].

The study of the type of stroke showed that the ischemic form was more frequent with a rate of 60.87% against 30.43% for the hemorrhagic form. This predominance of the ischemic form has been found in similar studies including those reported by Balogou et al [9], Diarra et al [7] and Bugnicourt et al [10].

The study of polymorphism of the Hp gene in subjects with stroke showed a higher frequency for the genotype Hp2-2 with a rate of 30.57% followed by the Hp1-1 genotype with a rate of 17.39%. Similar results were found by Harris et al who showed the same link between these two parameters in the context of the link between dyslipidemias and stroke [16-18]. Indeed, authors have noted that the efflux capacity of cholesterol was inversely related to the incidence of cardiovascular diseases [19]. However, this relationship between stroke and HDL-C is more complex and not universally established. The increase in LDL-C noted in our study is also found in the literature with several references [20-21]. Indeed, LDL-C is an important vector of cholesterol. When there is a decrease in specialized LDL-C receptors, its concentration increases and moreover its accumulation at the level of the artery wall, resulting in the formation of an atheromatous plaque whose rupture has many pathological consequences. It is in this sense that many studies have shown a strong and gradual correlation between LDL-C levels and the risk of atherosclerosis and cardiovascular diseases [22].

In our series, the comparison of mean TG rates between subjects with stroke and control subjects showed a significant difference (p <0.05) which may define a relationship between triglyceride levels and the risk of stroke, more specifically ischemic strokes. However, epidemiological studies of this relationship between ischemic stroke and TG had found controversial results [23]. According to a meta-analysis (64 studies), there would be a correlation between increased triglyceride levels and the risk of stroke for any 10 mg/dl increase in basal triglyceride. In addition, the study of the influence of the major genotypes of haptoglobin on the variations of the lipid parameters showed statistically significant differences. An association between the Hp2-2 genotype and the increase of the average level of LDL-C and Triglycerides and a decrease in the HDL-C level was found. These findings were also found in other studies including that of Sow A D et al., which showed the same link between these two parameters in the context of cardiovascular risk but in epileptic subjects [24]. In addition, the scarcity of studies associating these two parameters in the literature should be noted.

5. Conclusion

The polymorphism of Hp is always interesting to study because carriers of genotype Hp2-2 are more likely to
develop the disease. However, the impact of this polymorphism must be estimated in conjunction with the other genetic, metabolic and clinical risk factors associated with the pathogenesis of stroke. In this study, the Hp2-2 genotype comes out as a predisposing factor to stroke. Its association with the lipid parameters is found but must be confirmed in other studies with a larger cohort and in other cardiovascular pathologies.

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Conflict of Interest

The authors declare that they have no conflict of interest.

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