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Haptoglobin polymorphism and cardiovascular risk factors in followed epileptic patients at Fann National University Hospital

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The aim of our research was to evaluate the cardiovascular risk factors on epileptic patients treated at the Fann University Hospital and to study the influence of haptoglobin (Hp) polymorphism on disease progression. In order to do that, eighty-six (86) patients followed in neurology for at least 2 years were recruited. Each patient was matched to a control according to age and sex. Hp phenotyping was performed by electrophoresis on polyacrylamide gel, and lipid peroxidation was quantified by the dosage of the thiobarbituric acid reacting substances (TBARS). The determination of a number of biochemical parameters was performed in both patients and controls. The evaluation of lipid parameters showed significant differences in total cholesterol levels, triglycerides, low-density lipoprotein (LDL) cholesterol and atherogenic index between patients and controls. For C-Reactive Protein-ultra sensible (CRP-us) values greater than 3 mg / L, a statistically significant difference was found (p = 0.009). The frequencies of the three major phenotypes of patients compared to controls has shown significant difference only for Hp2-2 phenotype (p = 0.042). The significant increase of TBARS for patients compared to controls suggested an oxidative mechanism. Results have shown a risk of developing cardiovascular diseases during the progression of epilepsy. The influence of Hp polymorphism in modulating oxidative stress suggests that taking antioxidants may have a beneficial effect, especially in patients of phenotype Hp2-2.

Key words: Epilepsy, haptoglobin polymorphism, cardiovascular risks.

INTRODUCTION

Epilepsy is considered as one of the most widespread diseases in the world with a prevalence of 0.5 to 1% in the population (Hauser et al., 1991). It is a chronic pathology that often requires long-term antiepileptic drug

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therapy (McCorry et al., 2004). This long-term therapy poses a dilemma for practitioner since it was reported that it is associated to an endothelial dysfunction with the appearance of adverse reactions that can accelerate the occurrence of atherosclerosis (Hamed et al., 2007; Tan et al., 2009).

Oxidative stress was recognized as an important factor in the onset of atherosclerosis (Stocker and Keaney, 2004). Indeed, the role of low-density lipoprotein (LDL) oxidation in the transformation of macrophages into foam cells was one of the key points of the genesis of the atheromatous plaque (Moatti, 2003). This oxidative stress was potentially involved in many diseases as a trigger, or associated to complications in their evolution. If it is the case, it is logical to think that taking antioxidants or the intervention of an antioxidant mechanism may delay or prevent the appearance of such diseases.

The severity of oxidative stress and the ability of the body to reduce it may explain differences in susceptibility at the occurrence of atherosclerosis (Melamed-Frank et al., 2001). Therefore, the functional polymorphism of genes encoding certain enzymes and / or proteins may play a particularly important role in explaining the differences in susceptibility among patients with different phenotypes of epilepsy. Haptoglobin is a plasma protein encoded by two alleles rated 1 and 2; it has an antioxidant power which is a dependent phenotype (Melamed-Frank et al., 2001; Mackellar and Vigerust, 2016). The study of the Hp polymorphism effects on the progression of various diseases has been the subject of numerous researches in cardiovascular diseases, diabetes, infectious diseases, hematological and neurological disorders (Gogishvili et al., 1985; Guetta et al., 2007).

Apart from all the conditions mentioned above, the influence of Hp polymorphism on the development of many diseases, including neurological ones epilepsy (Panter et al., 1984), and lung or ovary cancers (Langlois and Delanghe, 1996; Anderson et al., 2009) was studied. Hp 2-2 phenotype cases have an increased susceptibility to adverse developments, compared with other phenotypes in these pathologies. Clinically, the 2-2 phenotype is associated with the risk of cardiovascular diseases and diabetes mellitus in patients (Tseng et al., 2004). The diabetic patients with the Hp 1-1 phenotype are markedly resistant to the development of diabetic retinopathy, diabetic nephropathy, and cardiovascular disease (Levy et al., 2000; Nakhoul et al., 2001). In a prospective study, participants homozygous for the Hp 2-2 allele had a 5-fold increased risk for the development of cardiovascular diseases as compared to participants homozygous for the Hp 1-1, allele (Asleh et al., 2003). The risk in heterozygous Hp 2-1 participates is intermediate (Asleh et al., 2003).

The aim of this research was to study the frequency of major Hp phenotypes in epileptic patients treated at the University Hospital of Fann and evaluate the number of cardiovascular risk factors.

METHODS

This is an analytical prospective study. Eighty-six (86) patients followed at the Neurological Clinic of the Hospital University-Fann for at least two years and with good adherence were recruited. Each patient was matched to a control according to age and sex. Controls were recruited at the National Blood Transfusion Center. During the recruitment, patients were informed about the objectives of the study and were recruited after consent. This work received the approval of the local ethical committee.

The blood sample was taken by venipuncture on subjects at rest, at the elbow crease after fasting for at least 12 h. The blood was collected into a tube containing an anticoagulant mixture (ethylenediaminetetraacetate, EDTA) and antiglycolytic (sodium fluoride, NaF), and another tube without anticoagulant or antiglycolytic. All tubes were centrifuged at 3000 revs/min for 5 min. Serum and plasma were then packaged in NUNK® tubes and stored at -20° C until dosage.

In patients and controls, cardiovascular risk factors, conventional lipid parameters and C-Reactive Protein-ultra sensible (CRP-us) were determined as. Besides these parameters, other parameters such as blood glucose, creatinine, urea and CRP were determined.

Hp phenotyping was performed by polyacrylamide gel electrophoresis followed by peroxidase staining according to Raymond method (Raymond, 1962). Samples were prepared by mixing 75 µL of plasma with 75 µL of a freshly prepared human Hb solution (1.6 µmol/L) and 100 µL of 0.29 mol/L sucrose. The samples were incubated for 10 min at room temperature and 10 µL of 0.001% (w/v) bromophenol blue were added to each sample prior the running on the gel. The electrophoresis of Hp-Hb complex was performed on a 5% polyacrylamide gel with running buffer containing 0.1 mol/L Tris and 0.09 mol/L boric acid, pH 8.4. Electrophoresis was run at a constant voltage of 200 V for 2 h. Afterwards, the Hp-Hb complexes were stained using their peroxidase activity by soaking the gel in a staining solution prepared by adding 0.2 g of dimethoxybenzidine to 100 mL of acetic acid solution (0.9 mol/L). After 2 h incubation in the dark at 37°C, 5 mL of 3% hydrogen peroxide (w/v), freshly prepared, were mixed with the solution covering the gel (100 mL); the bands corresponding to the Hp-Hb complexes, characteristic for each phenotype, were readily visible within 15 min.

The determination of total cholesterol, HDL-C, LDL cholesterol and triglycerides were made from a type of controller on Cobas C111 (Roche, Basel, Switzerland) with the manufacturer's reagents. The assay for TBARS was performed according to Yagi's method (Yagi, 1976), but modified (Conti, 1991). Briefly, 50 ul of each sample were mixed with 100 ul of TBA and trichloroacetic acid (TCA) in HCl to precipitate protein. The reaction was performed at pH 2 to 3 at 95°C for 20 min.

TBARS were extracted by the addition of 500 ul of n-butanol, then centrifuged at 1000 *g* for 5 min. Fluorescence of the butanolic phase was measured at 553 with a 532-nm excitation wavelength using an SFM 25 spectrofluorimeter (Kontron Inst., Montigny Le Bretonneux, France). Results were expressed in nmol of TBARS. A standard curve was produced using 1,19,3,39- tetraethoxypropane.

Statistical analysis

Statistical analyzes were performed thanks to Statview software using the Mann Whitney test to compare variables between patients and controls. The Kruskal Wallis test helped to determine significant differences among varying types of haptoglobin phenotypes. A p-value less than 0.05 was considered significant.
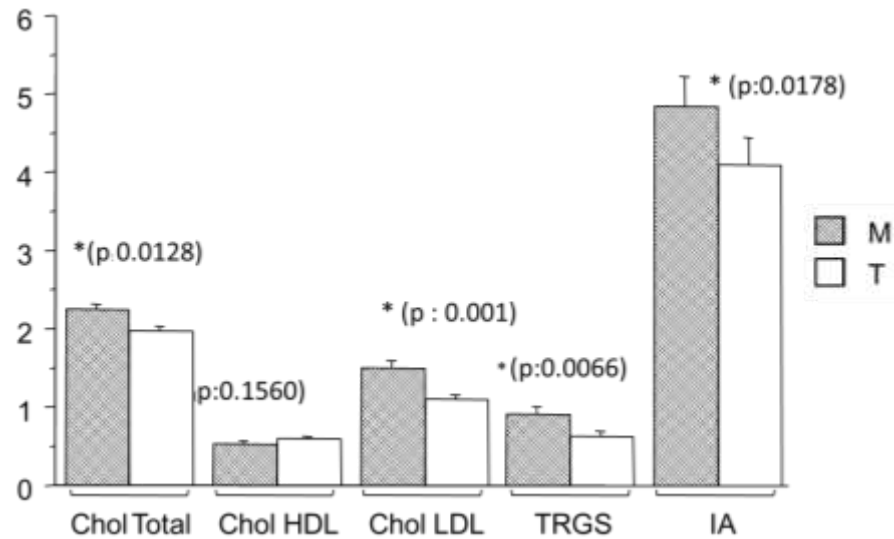
Figure 1. Comparison of average lipid parameters between epileptic patients and controls.
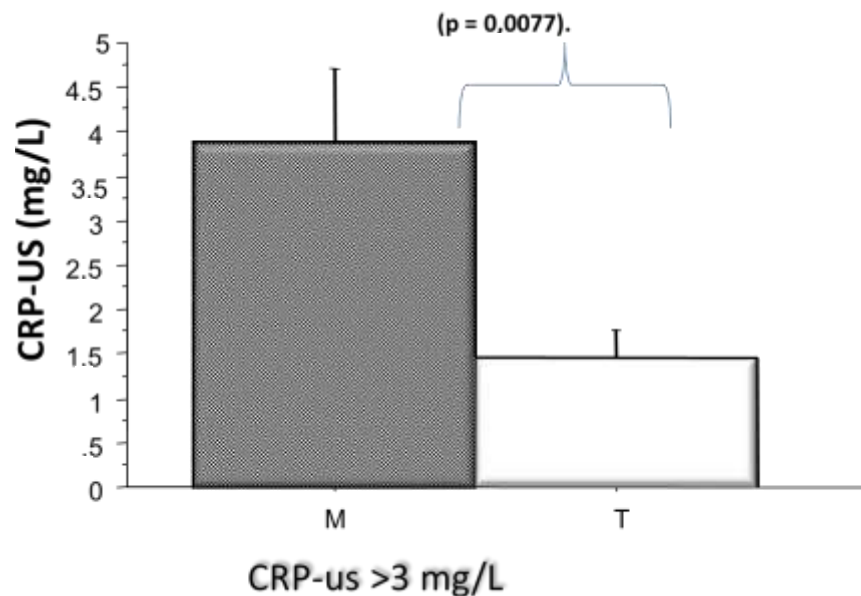

Figure 2. Comparison of average of CRP-us between epileptic patients and controls.

RESULTS

The study included 86 patients with 86 matched controls with a predominance of women (53%). The average age of the patients was 30.1y/o. A prevalence of generalized seizures (63%) versus partial seizures (37%) was found.

Evaluation of lipid parameters showed statistically significant differences between patients and controls (Figure 1) with regard to triglycerides (p = 0.0066), total cholesterol (p = 0.012) and LDL-cholesterol (p = 0.001), with the exception of HDL cholesterol (p = 0.1560).

Analysis of CRP and CRP-us results did not show statistically significant differences between patients and controls. However, the CRP-us values above 3 mg (Figure 2) was considered corresponding to a high-risk factor for cardiovascular diseases, a statistically significant difference was found (p = 0.0077).

The study of the Hp polymorphism revealed frequencies of 41.48% for Hp2-2 phenotypes, 32.36% for Hp1-1 and 23.16 for Hp2-1. Concerning controls, frequencies of 39.62, 45.07 and 13.31% were respectively found for Hp1-1 phenotype, Hp2-1 and Hp2-2. The frequencies
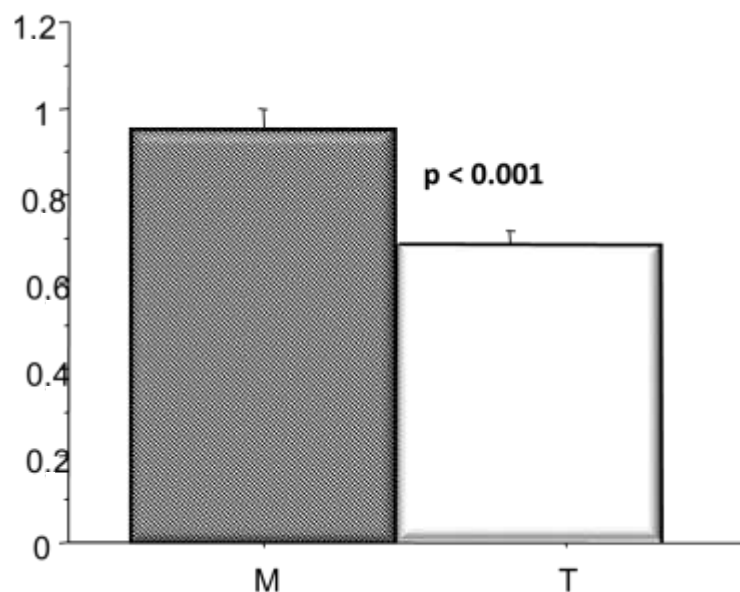
Figure 3. Comparison of TBARS level between epileptic patients and controls.

of the three major phenotypes of patients compared to controls showed significant difference only for Hp2-2 phenotype (p = 0.042); the other two phenotypes differences did not appear significant.

Analysis of the results of urea and creatinine showed a statistically significant difference between patients and controls (p <0.0001). However, no pathological value was noticed in both groups. On the other side, the Hp2-2 phenotype of patients had a significant increase in serum creatinine compared to Hp1-1 and Hp2-1 phenotypes. This difference was not found in controls.

The comparison of results between patients and controls showed no statistically significant differences (p = 0.267). The evaluation of oxidative stress through the TBARS dosage showed a statistically significant difference between patients and controls p<0.001 (Figure 3).

When the distribution was made according to the Hp phenotype, the Hp2-2 phenotype subjects showed elevated TBARS rates compared to other types of Hp but the differences were not found to be significant.

DISCUSSION

The aim of this study was to evaluate the cardiovascular risk factors, oxidative stress and the influence of haptoglobin polymorphism in epileptic patients at the National University Hospital-Fann.

In order to do that, 86 patients with 86 matched controls were recruited. In this study cohort, a predominance of men (53%) compared to women (47%) was observed. This predominance of men has been reported by some authors (Ngoungou et al., 2006). This finding could be explained by an under-reporting of the disease among young women of marrying age. It has been reported by Ngoungou et al. (2006) that among six studies, four have taken place in Nigeria with a predominance of female. The hypothesis of a rural exodus from men (Osuntokun et al., 1982, Osuntokun et al., 1987) or a higher male mortality (Rwiza et al., 1992) may explain this finding.

In the study cohort, the average age is 30.1 years. This value is similar to the one found by N'diaye (2010) which is 30 years. However, several authors have reported an early age of epilepsy, which occurs before the age of 20 in more than 60% of the cases. The bimodal distribution found in industrialized countries does not appear to exist in sub-Saharan Africa (Ngoungou et al., 2006). In few studies, the proportion of elderly patients is still low; this could be explained by a lower life expectancy, which limits the study of incidence rates in the higher age groups (Tekle-Haimanot et al., 1997).

A prevalence of generalized seizures (63%) was found compared to partial seizures (37%). These results are similar to those reported by Fall et al. (2015) who noted frequencies of around 67 and 33%, respectively for generalized seizures and partial seizures (Fall et al., 2015; Isnard et al., 2000; Aaberg et al., 2016).

In sub-Saharan Africa, many studies have reported a predominence of generalized seizure. Sub-medicalization and more specifically the absence of neurologist and Electroencephalography apparatus could be the cause of a poor classification of crises (Ngoungou et al., 2006).

This study was interested in cardiovascular risk factors such as the type of lipid abnormalities and the dosage of

the CRP-us.

The evaluation of lipid parameters showed statistically significant differences between patients and controls with regard to triglycerides (p = 0.0066), total cholesterol (p = 0.012) and LDL-cholesterol (p = 0.001), with the exception of HDL cholesterol (p = 0.1560).

These results would suggest an exposure of patients with cardiovascular disease during the treatment. This was reported by Tan et al. (Tan et al., 2009) who reported an exposure to the occurrence of cardiovascular diseases via changes in lipid parameters.

Indeed, dyslipidemia has been known as one of the major risk factors in the development of atherosclerosis (Kullo and Ballantyne, 2005), and LDL cholesterol plays an important role in the atherosclerotic process by increasing the endothelial permeability, lipoprotein retention in the intima of blood vessels, and the recruitment of foam cells (Kullo and Ballantyne, 2005; Stocker and Keaney, 2004).

In addition to these results, some authors have reported a negative influence of long-term treatment on lipid profile of epileptic patients (Isojärvi et al., 1993; Eiris et al., 1995; Nikolaos et al., 2004).

Analysis of CRP and CRP-us results did not show statistically significant differences between patients and controls.

However, when CRP-us values above 3 mg (corresponding to a high-risk factor for cardiovascular disease) was considered, a statistically significant difference was found (p = 0.0077). This is similar to the results found by Tan et al. (2009).

The association of the results on the evaluation of lipid parameters and CRP-us, an independent risk factor predictive of the rate of increase of atherosclerosis, would suggest that a long-term therapy with antiepileptic exposes to the occurrence of cardiovascular disease in epileptic patients.
Indeed, it has been reported that antiepileptic medication such as phenobarbital, carbamazepine and valproate contribute to an acceleration of atherosclerosis by changing the metabolism of homocysteine and folic acid (Schwaninger et al., 1999; Attilakos et al., 2006; Hamed et al., 2007; Stocker and Keaney, 2004). Furthermore, high plasma homocysteine is an independent risk factor for the progression of atherosclerosis (Hassan et al., 2004; Temple et al., 2000).

The study of the seizure's frequency in the study population shows a predominance of Hp2-2 (60.52%) compared to Hp2-1 (26.31%) and Hp1-1 (18.41%). These results are similar to those found by other authors (Sadrzadeh et al., 2004; Ilzecka, 1996; Al-Balaghee et al., 2015). Indeed, they found a significant relation between the Hp2-2 phenotype and the frequency of seizures. In their studies, 67% of epileptic patients with one or more seizures had the Hp2-2 phenotype. Saccucci et al. (2004) found an under-representation of phenotypes Hp2-1 and Hp1-1 in two populations of children with generalized epilepsy, compared to controls.

These results, compared to those found in this study, suggest that the Hp2-2 phenotype cases would be more subject to seizures than those with other phenotypes. The evaluation of oxidative stress through the TBARS dosage showed a statistically significant difference between patients and controls (p <0.001). Indeed, the auto-oxidation of the homocysteine promotes an overproduction of reactive oxygen species and hinders the cellular mechanisms of antioxidant defenses (Tyagi et al., 2006). This indicates that the oxidative mechanisms play an important role in the pathogenesis of cardio-vascular diseases.

The diversity in antioxidant activity of the Hp phenotypes may explain, in part, the clinical outcome by which Hp phenotype is associated with differential susceptibility to free-radical related atherosclerosis and autoimmune disorders [Levy et al., 2002; Bernard et al., 1997]. A correlation between phenotype-dependent modulation of oxidative stress and prostaglandin synthesis has been reported (Bernard et al., 1997).

Urea and creatinine results showed a significant difference between patients and controls (p <0.0001). However, no pathological value was noticed in both groups. In the other side, patients with Hp2-2 phenotype have shown a significant increase in serum creatinine compared to those with Hp1-1 and Hp2-1 phenotypes. This difference have not found in controls.

The comparison of results between patients and controls showed no statistically significant differences (p = 0.267).

Conclusion

The overall results suggest that there is a risk of occurrence of cardiovascular diseases in the evolution of epilepsy, and therefore the monitoring of renal function and lipid parameters is appropriate in this pathology. In addition, the results highlight the influence of Hp poly-morphism in modulating oxidative stress and suggest that taking antioxidants could have a particularly beneficial effect on patients with Hp2-2 phenotype.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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