The effect of catalase enzyme gene polymorphism A-21T (rs7943316) on epilepsy and its drug resistance after hypoxic ischemic brain injury

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Background
Epilepsy is one of the most common serious neurological disorders, affecting more than 4% of all children. One of the most common conditions leading to epilepsy is hypoxic ischemic encephalopathy (HIE), which is a condition that occurs when the entire brain is deprived of an adequate oxygen supply. Catalase (CAT) is a major cytoplasmic antioxidant enzyme. Considering that the A-21T and C-262T polymorphisms in the promoter region of CAT are associated with the activity of promoter of the CAT, subsequently it may alter the risk of oxidative stress-related disorders. Therefore, polymorphism of the CAT gene can be a candidate marker of the risk of epilepsy.

Objective
The aim was to assess if antioxidant CAT gene polymorphism A-21T (rs7943316) contributes to susceptibility to epilepsy, susceptibility to epilepsy after neonatal HIE, susceptibility to epilepsy owing to other causes than neonatal HIE, resistance to antiepileptic medications in epileptic patients after HIE, and/or resistance to antiepileptic medications in epileptic patients owing to causes other than HIE.

Patients and methods
This cross-sectional case–control descriptive analytical study included 105 participants: 70 patients with epilepsy (divided into two groups according to the etiology of epilepsy) were compared with 35 age-matched and sex-matched healthy controls. The patients were recruited from neuropediatrics clinic in Fayoum University Teaching Hospital during a period extending from September 2017 till February 2018. All samples were subjected to genomic DNA analysis of catalase enzyme polymorphism A-21T (rs7943316) using real-time polymerase chain reaction-based method.

Results
This study showed that there was a statistically significant difference ($P < 0.05$) between patients with epilepsy due to HIE and controls regarding genotyping, where AA (wild genotype) was higher among controls, whereas AT (heterozygous mutant genotype) was higher among cases. Moreover, AT (heterozygous mutant genotype) and T allele were statistically significantly higher among epilepsy with HIE cases when compared with epilepsy without HIE ($P < 0.001$ and < 0.01, respectively). However, there was no statistically significant difference in CAT rs7943316 genotype and allele frequency when patients with epilepsy were stratified by drug resistance, electroencephalography, or sex.

Conclusion
Our study revealed that there was a significant link between CAT A-21T (rs7943316) single nucleotide polymorphism and susceptibility to epilepsy after neonatal HIE. CAT polymorphism does not influence the overall risk of drug resistance among participants with epilepsy after neonatal HIE or owing to other causes than HIE.

Keywords:
catalase, epilepsy, hypoxic ischemic encephalopathy, single nucleotide polymorphism

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Introduction

Hypoxic ischemic encephalopathy (HIE) develops as a consequence of intrauterine or perinatal hypoxia associated with a variety of maternal, placental, and/or fetal conditions [1]. Perinatal asphyxia leads to a cascade of neurotoxic events involving energy failure, ultimately resulting in the accumulation of reactive oxygen species (ROS) [2]. The cell and tissue damage in the immature brain with its lower capacity for defending against ROS, in particular lower glutathione peroxidase activity, may eventually cause permanent neurological sequelae such as cerebral palsy and/or epilepsy [3].

Several antioxidant mechanisms protect cells against oxidative damage. The first-line defenses against ROS are the antioxidant enzymes manganese superoxide dismutase (SOD_2), glutathione peroxidase 1 (GPX_1), and catalase (CAT), which detoxify the superoxide anion and hydrogen peroxide [4]. Their activity and ability to protect cells and tissues from ROS and their damaging products are influenced by functional genetic polymorphisms in antioxidant genes [5].

Antioxidant enzymes represent an important defense mechanism against ROS. It has been suggested that generation of oxidative stress within the brain tissue might play an important role in epileptogenesis, and several studies have investigated whether antioxidant enzyme activity influences either susceptibility to epilepsy or its characteristics such as drug resistance. Catalase (EC 1.11.1.6, CAT, OMIM: 115500) is a heme-containing peroxisomal ubiquitous enzyme found in all organisms. It catalyzes the decomposition of hydrogen peroxide to oxygen and water; therefore, it is a major cytoplasmic antioxidant enzyme [6]. Several genetic polymorphisms in the CAT have been reported in human populations. Three genetic polymorphisms have been reported in the promoter region of the CAT, named A-21T (rs7943316), C-262T (rs1001179), and C-844T (rs769214) [7].

It has been suggested that these polymorphisms may alter the recognition sites of transcriptional factors; therefore, it might be concluded that these polymorphisms may alter the expression levels of the gene. Considering that the A-21T and C-262T polymorphisms in the promoter region of CAT are significantly associated with the activity of promoter of the CAT, subsequently it may alter the risk of oxidative stress-related disorders [8]. Previous studies have shown that CAT polymorphisms were associated with the risk of several multifactorial diseases [9].

Although a previous study by Esihet *et al.*[10] has showed that CAT enzyme polymorphism (rs1001179) does not influence risk of epilepsy or its drug resistance after neonatal hypoxic ischemic brain injury, no study investigated the effect of CAT enzyme polymorphism A-21T (rs7943316) on epilepsy after hypoxic ischemic brain injury, which is our study's aim.

Aim

This study aims to investigate if CAT enzyme polymorphism A-21T (rs7943316) contributes to susceptibility to epilepsy after neonatal HIE, susceptibility to epilepsy owing to other causes than neonatal HIE, resistance to antiepileptic medications in epileptic patients after HIE, and resistance to antiepileptic medications in patients owing to other causes than HIE.

Patients and methods

This cross-sectional case–control descriptive analytical study included 70 patients with epilepsy divided into two groups according to the etiology of epilepsy, who were compared with 35 age-matched and sex-matched healthy controls. Those patients were recruited from Neuropediatrics Clinic in Fayoum University Hospital during a period extended from September 2017 till February 2018. The inclusion criteria of the first group were infants and children with age between three months and twelve years, with neonatal HIE, with history of perinatal asphyxia and needed admission at neonatal ICU. The exclusion criteria of the first group is the presence of any other concurrent medical condition that may itself lead to the development of epilepsy (brain malformation, neonatal sepsis, genetic, metabolic or any other disorders that may be associated with neonatal encephalopathy). Patients with insufficient data regarding perinatal history were also excluded. The inclusion criteria for the second group were patients diagnosed as having idiopathic epilepsy or febrile seizures. The control group included 35 healthy individuals with no history of epilepsy or perinatal asphyxia. Patients who attended the clinic seeking medical advice such as preoperative assessment, nutritional follow-up, or routine care, were included as the control group. The study was carried out in accordance with the Code of Ethics of the World Medical Association (Declaration of Helsinki) and after the approval of the local Ethics Committee. Informed consent was obtained from all study participants after the nature of the study was explained.

All patients were subjected to the following: first, history taking: personal history including demographic data (age and sex), perinatal history, past history (previous neonatal ICU admissions), medication history (antiepileptic; types, duration of treatment, and response), developmental history including motor and mental development, family history including consanguinity, history of neurological problems, history of similar illness, disease history in family, etc., Second, thorough clinical examination included anthropometric measurements such as height, weight of the patients, and head circumference, regional examination of head and neck (dysmorphic features, eyes, pupils, ears, mouth, head shape, neck), extremities, skin, back and spine examination, local examination, cardiac, chest, and abdominal examination, neurological assessment to detect degree of spasticity and

extrapyramidal movements; motor system examination, muscle power assessment and reflexes, and cranial nerve examination. Third, investigations included imaging studies (computed tomography of the brain and brain MRI were done when indicated, as well as electrophysiological studies and EEG).

Detection of the presence of CAT enzyme gene polymorphism A-21T (rs7943316)

The test was done in two main steps:

(1) Extraction of genomic DNA from EDTA blood samples, which was done by GeneJET Whole Blood Genomic DNA Purification Mini Kit, provided by Thermo fisher Scientific (Biotechnology Company) - Kingfisher Dr, Swindon SN35BZ, United Kingdom.

(2) Detection of A-21T polymorphism in catalase gene using single nucleotide polymorphism (SNP) ready-made assay by real-time PCR. This was done using Taqman Genotyping Mastermix provided by Thermo Fisher Scientific and SNP ready-made assay (Thermo Fisher Scientific), which contains the following: first, forward and reverse primers Context Sequence [VIC/FAM] TTGGCTGAGCCTGAAGTCGCCACGG [A/T] CTCGGGGCAACAGCAGATT GCCT; second, probes VIC for A allele; and third, FAM for T allele. Viia 7 (Applied Biosystem) was used to perform real-time PCR with the following thermal profile: pre-read stage: 60°C for 30 s; hold stage: 95°C for 10 min; and PCR stage: 95°C for 15 s, and at 60°C for 1 min. The past two steps were done for 50 cycles at 60°C for 30 s. Interpretation was done automatically in almost all samples by Vii A 7 instrument software or manually using amplification plot or multicomponent plot. Amplification plot is a graphical representation of real-time PCR data representing CT vs Delta Rn. The threshold cycle (CT) is the PCR cycle number at which the fluorescence level meets the threshold. Rn is the fluorescence of the reporter dye divided by the fluorescence of a passive reference dye, that is, Rn is the reporter signal normalized to the fluorescence signal of Applied Biosystems ROX Dye.

Statistical analysis

Statistical analyses were carried out using the SPSS (Statistical Package for the Social Sciences, Chicago, Illinois, USA) version 17. The sample size gave a calculated power of 0.96 using the post-hoc power calculator. Data were subjected to the Kolmogorov–Smirnov test to determine the distribution and method of analysis. Skewed data (age) are expressed as median (range), and statistical significance was tested using the Mann–Whitney test as appropriate. Categorical variables are given as percentages. χ^2 test was used to compare the demographic data (sex), genotype and allele distributions, and clinical data (remission and drug resistance, normal and abnormal EEG) among the cases and the controls using odds ratios and 95% confidence interval. Allele frequencies were estimated by the gene counting method and expressed as a percentage of the total number of alleles. A P value (two tailed) less than 0.05 was considered as statistically significant [11].

Results

Of the 35 healthy controls, 23 (65.7%) were males and 12 (34.3%) were females, and the median age was 7 (0.75–12) years. Of the 35 patients with epilepsy with HIE, 22 (62.9%) were males and 13 (37.1%) were females, and the median age was 4 (0.30–12) years. Of the 35 patients with epilepsy without HIE, 20 (57.1%) were males and 15 (42.1%) were females, and the median age was 3.5 (0.50–11) years. There was no statistically significant difference regarding sex and age for patients with epilepsy compared with healthy controls, with *P* value more than 0.05.

Comparison between study groups regarding genotypes and alleles

AT genotype among all patients with epilepsy was 43 (61.4%), whereas in the control group, it was 17 (48.6%), but among cases, AA genotype was seen in 27 (38.6%) cases, whereas in the control group, it was present in 18 (51.4%). There was no statistically significant difference ($P > 0.05$) between cases and controls regarding genotype. A allele among epilepsy cases was 97 (69.3%) whereas in the control group it was 53 (75.7%). The T allele in cases was 43 (30.7%), whereas in the control group, it was 17 (24.3%). There was no statistically significant difference ($P > 0.05$) between cases and controls regarding genotypes and alleles (Table 1).

Comparison between healthy control and epilepsy due to HIE regarding genotype and alleles

AT genotype among cases of epilepsy due to HIE was 30 (85.7%), whereas in the control group, it was 17 (48.6%), as well as in cases of epilepsy due to HIE, AA genotype was 5 (14.3%), whereas in the control group, it was 18 (51.4%). The A allele in cases of epilepsy due to HIE was 40 (57.1%), whereas in the control group was 53 (75.7%). However, the T allele in cases of epilepsy due to HIE was 30 (42.9%), whereas in the control group was 17 (24.3%). The distribution of CAT (rs7943316) genotypes and alleles among patients with epilepsy after HIE was very highly statistically significant when compared with healthy controls (<0.001 and < 0.05,

respectively). AT genotype and T allele were higher among epilepsy after HIE (Table 2).

Comparison between healthy control and epilepsy owing to other causes than HIE regarding genotype and allele

AT genotype among cases with epilepsy without HIE was 13 (37.1%), whereas in the control group it was 17 (48.6%). Moreover, in cases with epilepsy without HIE AA genotype was 22 (62.9%), whereas in the control group, it was 18 (51.4%). There was no statistically significant difference ($P > 0.05$) between cases of this group and controls regarding genotype. A allele number among cases with epilepsy without was 57 (81.4%) whereas in the control group, it was 53 (75.7%). Among cases with epilepsy without HIE, T allele number was 13 (18.6%), whereas in controls, it was 17 (24.3%). The distribution of CAT rs7943316 genotypes and alleles among patients with epilepsy without HIE was not statistically significantly different when compared with healthy controls ($P > 0.05$). AA genotype and A allele were higher among epilepsy without HIE but did not reach statistical significance (Table 3).

Comparison between epilepsy due to HIE and epilepsy owing to other causes than HIE regarding genotype and alleles

Among cases of epilepsy with HIE, AT genotype was 30 (85.7%), whereas in cases of epilepsy without HIE, it was 13 (37.1%). AA genotype among cases of epilepsy with HIE was 5 (14.3%), whereas in cases of epilepsy without HIE, it was 22 (62.9%). There was very highly statistically significant difference ($P < 0.001$) between cases of both groups regarding genotypes. Among cases of epilepsy with HIE, A allele number was 40 (57.1%), whereas in cases of group epilepsy without HIE, it was 57 (81.4%). In cases of epilepsy with HIE, T allele number was 30 (42.8%), whereas in cases of epilepsy without HIE, it was 13 (18.5%). There was a highly statistically significant difference ($P < 0.01$) between both groups regarding genotypes and alleles (Table 4).

Comparison between study groups regarding antiepileptic drug response

Among all 70 patients with epilepsy, 45 (64.3%) achieved remission and 25 (35.7%) had drug-resistant epilepsy. There was no statistically significant difference in CAT rs7943316 genotype and allele frequency when patients with epilepsy were stratified by drug resistance ($P > 0.05$) (Table 5).

Comparison between patients in remission and drug-resistant epilepsy in those with HIE regarding genotypes and alleles

Among patients in remission, AT genotype was seen in 18 (85.7%), whereas among patients with drug-resistant epilepsy, it was 12 (85.7%). AA genotype among patients in remission was 3 (14.3%), whereas among those with drug-resistant epilepsy, it was 2 (14.3%). In patients in remission, A allele number was 24 (57.1%), whereas in cases of drug-resistant epilepsy, it was 16 (57.1%). In cases of

Table 1 Comparison between study groups regarding genotypes and alleles

Variables	Cases (*n*=70) [*n* (%)]	Control (*n*=35) [*n* (%)]	Odds ratio	95% CI	*P*	Significance
AT	43 (61.4)	17 (48.6)	0.593	0.261-1.345	>0.05	NS
AA	27 (38.6)	18 (51.4)				
A	97 (69.3)	53 (75.7)	1.382	0.719-2.657	>0.05	NS
T	43 (30.7)	17 (24.3)				

CI, confidence interval.

Table 2 Genotypes and alleles of patients with epilepsy with HIE compared with the control group

Variables	Epilepsy due to HIE (*n*=35) [*n* (%)]	Control (*n*=35) [*n* (%)]	Odds ratio	95% CI	*P*	Significance
AT	30 (85.7)	17 (48.6)	0.157	0.05-0.5	<0.01	S
AA	5 (14.3)	18 (51.4)				
A	40 (57.1)	53 (75.7)	2.338	1.135-4.818	<0.05	S
T	30 (42.9)	17 (24.3)				

95% CI, confidence interval; HIE, hypoxic ischemic encephalopathy; S, significant.

Table 3 Genotypes and alleles of patients with epilepsy without HIE compared with the control group

Variables	Epilepsy without HIE (*n*=35) [*n* (%)]	Control (*n*=35) [*n* (%)]	Odds ratio	95% CI	*P*	Significance
AT	13 (37.1)	17 (48.6)	1.598	0.616-4.148	>0.05	NS
AA	22 (62.9)	18 (51.4)				
A	57 (81.4)	53 (75.7)	0.711	0.315-1.604	>0.05	NS
T	13 (18.6)	17 (24.3)				

CI, confidence interval; HIE, hypoxic ischemic encephalopathy.

patients in remission, T allele number was 18 (42.9%), whereas in cases of drug-resistant epilepsy, it was 12 (42.9%). There was no statistically significant difference ($P > 0.05$) between both groups regarding genotypes and alleles (Table 6).

Comparison between patients in remission and drug-resistant epilepsy in those without HIE regarding genotypes and alleles

AT genotype among patients in remission was 8 (33.3%), whereas among patients of drug-resistant epilepsy it was 5 (45.5%). Among cases of patients in remission, AA genotype was 16 (66.7%), whereas among cases of drug-resistant epilepsy, it was 6 (54.5%). In patients in remission, A allele was 40 (83.3%), whereas in cases of drug-resistant epilepsy, it was 17 (77.3%). In cases of patients in remission, T allele was 8 (16.7%), whereas in cases of drug-resistant epilepsy, it was 5 (22.7%). There was no statistically significant difference ($P > 0.05$) between cases of both groups regarding genotypes and alleles (Table 7).

Discussion

To our knowledge, this is the first study investigating the contribution of antioxidant CAT A-21T (rs7943316) SNP in epileptic patients after neonatal HIE among Egyptians, as there are very limited data on antioxidant enzyme polymorphism in relation to the risk of epilepsy. Historical records show that the Egyptian population is one that has undergone genetic admixture and racial mixing, which created a heavily mixed population of modern Egyptians including several ethnic groups such as Bedouins, Peasants, Nubians, Berbers, and Urbanites [12]. The brain is an organ that consumes the highest amount of oxygen in the human body. The lack of antioxidant enzyme production and the rapidly developing brain tissue, which is thus overloaded with aerobic metabolism, renders newborns highly susceptible to oxidative damage [13].

It was hypothesized that the reduced capacity for defense against ROS may itself be responsible for epileptogenesis in the damaged brain tissue. To date, some studies have already suggested the possibility of the implication of antioxidant enzymes in the generation of epilepsy. Rumia *et al.* in 2013 analyzed

Table 4 Genotypes and alleles of patients with epilepsy with HIE compared with patients with epilepsy without HIE

Variables	Epilepsy with HIE (*n*=35) [*n* (%)]	Epilepsy without HIE (*n*=35) [*n* (%)]	Odds ratio	95% CI	*P*	Significance
AT	30 (85.7)	13 (37.1)	10.154	3.155-32.681	<0.001	S
AA	5 (14.3)	22 (62.9)				
A	40 (57.2)	57 (81.5)	0.304	0.141-0.654	<0.01	S
T	30 (42.8)	13 (18.5)				

CI, confidence interval; HIE, hypoxic ischemic encephalopathy; S, significant.

Table 5 Comparison between study groups regarding antiepileptic drug response

Variables	Remission (*n*=45) [*n* (%)]	Drug resistant (*n*=25) [*n* (%)]	Odds ratio	95% CI	*P*	Significance
AT	26 (57.8)	17 (68)	1.673	0.619-4.521	>0.05	NS
AA	19 (42.2)	8 (32)				
A	64 (71.1)	33 (66)	1.268	0.604-2.662	>0.05	NS
T	26 (28.9)	17 (34)				

CI, confidence interval.

Table 6 Comparison between patients in remission and drug-resistant epilepsy in those with hypoxic ischemic encephalopathy regarding genotypes and alleles

Variables	Remission (*n*=21) [*n* (%)]	Drug resistant (*n*=14) [*n* (%)]	Odds ratio	95% CI	*P*	Significance
AT	18 (85.7)	12 (85.7)	1	0.145-6.907	>0.05	NS
AA	3 (14.3)	2 (14.3)				
A	24 (57.1)	16 (57.1)	1	0.380-2.628	0.05	NS
T	18 (42.9)	12 (42.9)				

CI, confidence interval.

Table 7 Comparison between patients in remission and drug-resistant epilepsy in those without hypoxic ischemic encephalopathy regarding genotypes and alleles

Variables	Remission (*n*=24) [*n* (%)]	Drug resistant (*n*=11) [*n* (%)]	Odds ratio	95% CI	*P*	Significance
AT	8 (33.3)	5 (45.5)	0.6	0.139-2.581	>0.05	NS
AA	16 (66.7)	6 (54.5)				
A	40 (83.3)	17 (77.3)	1.471	0.42-5.149	>0.05	NS
T	8 (16.7)	5 (22.7)				

antioxidant enzyme levels in surgical brain specimens of patients with epilepsy and demonstrated an increase in CAT and a decrease in GPX levels, together with no change in SOD levels [14]. Their results thus suggest that CAT is the main enzymatic antioxidant in the human epileptic neocortex, whereas GPX and SOD did not appear to be major free radical scavenger systems in epilepsy. In addition, a previous study by Esih *et al.* [15], showed that CAT rs1001179 polymorphism has been statistically significantly associated with cerebral palsy in patients after HIE, even after taking prematurity into consideration in the multivariable analysis. There have been published papers mainly focusing on the fact that because of high concentrations of sensitive immature cells, metal-catalyzed free radicals, non-saturated fatty acids, and low concentrations of antioxidant enzymes, the brain requires high levels of oxygen supply and is, thus, extremely sensitive to hypoxia. Strong evidence indicates that oxidative stress plays an important role in pathogenesis and progression of HIE. Following hypoxia and ischemia, ROS production rapidly increases and overwhelms antioxidant defenses. A large excess of ROS will directly modify or degenerate cellular macromolecules, such as membranes, proteins, lipids, and DNA, and lead to a cascading inflammatory response, and protease secretion. These derivatives are involved in a complex interplay of multiple pathways (e.g. inflammation, apoptosis, autophagy, and necrosis), which finally lead to brain injury [16].

The study by Esih *et al.* [10], stated that CAT rs1001179 polymorphism could be used to identify children who have a higher susceptibility to cerebral palsy after perinatal HIE. Our study included 105 participants: 35 healthy controls and 70 patients with epilepsy, comprising 35 (50%) with HIE and 35 (50%) with other causes of epilepsy other than HIE. All were investigated for CAT SNP A-21T (rs7943316). Of the 35 healthy controls, 23 (65.7%) were males and 12 (34.3%) were females, with median age of 7 (0.75–12) years. Of the 35 patients with epilepsy with HIE, 22 (62.9%) were males and 13 (37.1%) were females, and the median age was 4 (0.30–12) years. In patients with HIE, 21 participants (60%) were in remission and 14 participants (40%) were resistant to antiepileptic drugs. The overall number of patients with epilepsy in remission was comparable to those with no remission. Regarding EEG results, 8 (42.1%) participants had normal EEG results and 11 (57.9%) had abnormal EEG results. Of the 35 patients with epilepsy without HIE, 20 (57.1%) were males and 15 (42.9%) were females. The median age was 3.5 (0.50–11) years. A total of 24 participants (68.5%) were in remission and 11 (31.4%) were females; moreover, 5 (26.3%) participants had normal EEG and 14 (73.7%) had abnormal EEG results.

In the study by Esih *et al.* [15], the participants comprised 214 patients with epilepsy (64 with and 150 without neonatal HIE) as well as 95 healthy controls, who were genotyped for SOD2 rs4880, GPX1 rs1050450, and CAT rs1001179 using real-time PCR-based methods. Of the 95 healthy controls, 46 (48.9%) were males and 49 (51.1%) were females, and the median age was 21 (20–23) years. Of the 64 patients with epilepsy with HIE, 32 (50%) were males and 32 (50%) were females, 22 participants (35.5%) were in remission, and 40 (64.5%) were resistant to antiepileptic drugs. Of the 150 patients with epilepsy without HIE participants, 67 (44.7%) were males and 83 (55.3%) were females. A total of 83 participants (55.3%) were in remission, and 67 (44.7%) were resistant to antiepileptic drugs.

The present study found no effect of CAT A-21T (rs7943316) on susceptibility to epilepsy as there was no statistically significant difference ($P > 0.05$) between cases of epilepsy and controls regarding genotypes and alleles.

This study revealed that there was a significant link between CAT A-21T (rs7943316) SNP and susceptibility to epilepsy after neonatal HIE, as results showed that the distribution of CAT (rs7943316) genotypes and alleles among patients with epilepsy after HIE was very highly statistically significant when compared with healthy controls (<0.001 and < 0.05, respectively), thus considering CAT (rs7943316) genotype and allele to be risk factors for developing epilepsy after HIE, and the distribution of CAT rs7943316 genotypes and alleles among patients with epilepsy without HIE was not statistically significantly different when compared with healthy controls ($P > 0.05$) and that specify the relation between the genotype and the risk of epilepsy after HIE. However, Esih *et al.* [15], stated that genotype of CAT rs1001179 does not influence the overall risk of epilepsy after HIE. Moreover, our results showed that among cases of epilepsy with HIE, AT genotype distribution was higher than among cases of epilepsy without HIE, and there was a very highly statistically significant difference ($P < 0.001$) between cases of both groups. Moreover, T allele number was higher among patients with HIE than those of epilepsy without HIE, and there was a highly statistically significant difference ($P < 0.01$) between both groups.

Meanwhile, our study revealed that genotype of CAT A-21T (rs7943316) does not influence the risk of drug-resistance epilepsy after HIE. This may be owing to the small number of patients with drug-resistant epilepsy within epilepsy due to HIE group, as it was only 14 (40%) patients of total patients with HIE. Moreover, there was no effect found on risk

of drug-resistance epilepsy in patients with epilepsy owing to other causes than HIE.

Limitations of the study

Our study had several limitations: the number of patients in the study group with HIE was rather small. Furthermore, the clinical data obtained were not sufficient to perform a more thorough statistical analysis as some of the clinical data were not available for all the patients. Owing to the cross-sectional nature of our study, it was not possible to collect relevant data of maternal factors, such as medication taken during pregnancy, as they are possible confounders which could be addressed if our study had been performed prospectively.

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Conflicts of interest

There are no conflicts of interest.

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