ORIGINAL ARTICLE

The oxidative and antioxidative status of simple febrile seizure patients

Mahmut Abuhandan,[1] Mustafa Calik,[2] Abdullah Taskin,[3] Ilhan Yetkin,[4] Sahabettin Selek,[5] Akin Iscan[6]

Abstract

Objective: To evaluate the oxidative status following a seizure in children experiencing a simple febrile seizure.

Methods: The cross-sectional study was conducted at Harran University, Turkey, between January and September 2011. It comprised 32 paediatric patients who, within the preceding 8 hours, had experienced a seizure due to upper respiratory tract infection and had been diagnosed with simple febrile seizure, and 30 healthy children as the control group. Blood was taken from the patients 8 hours after the seizure. Total oxidant level and Total anti-oxidant level were measured according to the Erel technique and the oxidative stress index was calculated. Data was analysed using SPSS 11.5.

Results: The mean values of the total oxidant level and the oxidative stress index of the cases were found to be significantly high compared to the controls and the total anti-oxidant level was found to be significantly low ($p<0.01$, $p<0.01$, $p<0.03$ respectively).

Conclusion: The increased total oxidant level and decreased total anti-oxidant level resulting in increased oxidative stress associated with febrile seizure patients may increase the risk of experiencing febrile seizures.

Keywords: Febrile, Seizure, Oxidative status. (JPMA 63: 594; 2013)

Introduction

Febrile seizures are defined as a type of seizure accompanied by fever in children aged between six months and five years, without any central nervous system disease or electrolyte impairment.[1-3] It is seen at a frequency of 2.5% in childhood,[1,3-5] peaking in the 18-month-old age group.[1,6,7] Although there is no evident difference in race or gender, it is more often seen in Asians and males. The seizures are usually benign, but even if they are rare, as there is a risk of recurrence and of developing epileptic seizures, monitoring is important.[1,2,8]

Though the pathogenesis of febrile seizures is not completely known, the pathogenesis mechanisms of febrile seizures may be associated with increased excitatory amino acids, such as glutamate, and decreased inhibitory amino acids, such as g-aminobutyric acid. Oxide and carbon monoxide are also suggested to play roles in neurotoxicity caused by the excitatory amino acids during febrile seizures.[9]

Studies in recent years have shown that free oxygen radicals and lipid peroxidation have a role in the pathogenesis of several diseases. Oxidative stress has been shown to be related to several diseases such as asthma, diabetes mellitus, cardiological diseases such as myocardial infaction, rheumatological diseases such as rheumatoid arthritis, cancer, neurological diseases, stroke, epilepsy and inflammatory diseases.[10-13] The free radicals and lipid peroxidation which are formed increase cell destruction.[14] To prevent the harmful effects of oxidants, such as free radicals and lipid peroxidation, defensive mechanisms got developed in the body. These are glutathione reductase, glutathione peroxidase, superoxide dismutase, and catalase, which are known as the 'anti-oxidant defence systems.'[14,15]

This study aimed to evaluate the post-seizure oxidative status in patients with febrile seizures.

Patients and Method

The cross-sectional case-control study was conducted at Harran University Medical Faculty, Sanliurfa, Turkey. The study was approved by the Ethics Committee of the university, and written informed consent was obtained from all the participants or their parents. From January to September 2011, 32 consecutive patients with simple febrile seizure, aged between 9 and 60 months, who were admitted to the paediatric neurology and paediatric emergency polyclinics, and 30 age-gender matched control patients were included in the study.

Study patients were divided into two groups: group 1 consisted of patients with simple febrile seizure; and group 2 consisted of healthy subjects. Children in the control group were brought to the general paediatric

[1,4]Department of Paediatrics, Medical Faculty, [2,6]Department of Paediatric Neurology, Faculty of Medicine, [3,5]Department of Clinical Biochemistry, Medical Faculty, Harran University, Sanliurfa, Turkey.

Correspondence: Mahmut Abuhandan. Email: drabuhandan@mynet.com

polyclinic for vaccinations or health examination, and had no history of seizures, epilepsy or neurological impairment. The exclusion criteria comprised any cases of metabolic disease; chronic disease; history of asphyxia and afebrile seizure; neurological sequelae; neurological findings following a seizure with a diagnosis of degenerative and demyelinating disease of the central nervous system. As such, 10 cases were excluded.

A detailed anamnesis was taken of all the children and their status was determined by physical examination. Simple febrile seizure was defined as having a generalised tonic clonic seizure lasting less than 15 minutes and not repeated within 24 hours. Demographical and clinical characteristics were obtained from institutional records and clinical evaluation at admission. Blood samples were taken 8 hours after the febrile seizure. At the start of the study, total blood count, electrolytes, kidney and liver function tests were done by automatic blood count instrument (Celldyn 3500) for all the children in both the groups. For biochemical analysis, the blood samples taken from the cases were centrifuged at 3500rpm for 10 minutes. The formed elements were then discarded with the tube and the serum samples were stored at -80°C.

The Total Anti-oxidant Status (TAS) and Total Oxidant Status (TOS) were measured on the study day colormetrically, using the Erel method by auto-analyser (Abbott Aeroset, Abbott Diagnostics, Abbott Park, IL, USA). For the TAS results, mmol Trolox Eqiv/L units were used and for the TOS results, μmol H_2O_2 Eqiv/L units. The Oxidative Stress Index (OSI) was defined as percentage rate of TAS values to TOS values. Before the calculation the TAS test mmol unit value was translated to micromol units as in the TOS test. The results were expressed as Arbitrary units, calculated by the following formula: OSI (arbitrary unit) = TOS (μmol H_2O_2 equivalent/L) / TAS (mmol Trolox equivalent/L) x 10.

TAS of plasma was determined using a novel automated measurement method, developed by Erel.[16] In this method, the most potent biological radical, hydroxyl radical, is produced. In the assay, ferrous ion solution, which is present in reagent 1 [o-dianisidine (10 mM), ferrous ion (45 AM) in the Clark and Lubs solution (75 mM, pH 1.8] is mixed with hydrogen peroxide, which is present in reagent 2 [H_2O_2 (7.5 mM) in the Clark and Lubs solution]. The sequentially produced radicals such as brown coloured dianisidinyl radical cation, produced by the hydroxyl radical, are also potent radicals. Using this method, the anti-oxidative effect of the sample against the potent free radical reactions that is initiated by the produced hydroxyl radical, is measured. The assay has excellent precision values of lower than 3%. The results were expressed as mmol Trolox Equiv. L-1.

TOS of plasma was determined using a novel automated measurement method, developed by Erel.[17] Oxidants present in the sample oxidise the ferrous ion-o-dianisidine complex to ferric ion. The oxidation reaction is enhanced by glycerol molecules, which are abundantly present in the reaction medium. The ferric ion makes a coloured complex with xylenol orange in an acidic medium. The colour intensity, which can be measured spectrophotometrically, is related to the total amount of oxidant molecules present in the sample. The assay is calibrated with hydrogen peroxide and the results were expressed in terms of micromolar hydrogen peroxide equivalent per liter (Imol H_2O_2 Equiv. L-1.

The ratio percentage of TOS level to TAS level gave the OSI, an indicator of the degree of oxidative stres.[17]

Data was analysed using SPSS version 11.5. Distribution of parametric variables was assessed with one-sample Kolmogorov-Smirnov test. The results were presented as mean±standard deviation. Demographic data was analysed using chi-square test. Independent Samples T test was used to analyse data between the groups. A two-tailed p value of less than 0.05 was considered statistically significant.

Results

Among the 32 cases, there were 17 (53.1%) males and 15 (46.9%) females. Of the 30 controls, the corresponding numbers were 16 (53.3%) and 14 (46.7%).

The mean age was determined as 26.65±12.58 months for the cases and 30.46±11.53 months for the controls. No statistically significant difference was found between the two groups in terms of age or gender (p>0.05). In terms of

Table: Comparison of the TAS, TOS and OSI values of the febrile convulsions patient group and the control group.

	Patient group Mean ± SD	Control group Mean ± SD	P
TOS (μmol H2O2 Eqv./L	21,02 ± 4,68	18,17 ± 3,64	0.01
TAS (μmol H2O2 Eqv./L	0,90 ± 0,15	1,01 ± 0,17	0.01
OSI (AU)	2,38 ± 0,76	2,0 ± 0,55	0.03

Student t test for Independent Samples was used.
Significance was defined as $p < 0.05$.
TOS: Total oxidant stress.
TAS: Total anti-oxidant status.
OSI: Oxidative Stress Index.

upper respiratory tract infections in the patients, 22 (67.7%) had tonsilitis, and 10 (32.3%) had otitis.

The mean TOS and OSI values of the cases were found to be statistically significantly high compared to those of the controls, and the TAS values were significantly low ($p<0.01$, $p<0.05$, $p<0.01$ respectively) (Table).

Discussion

The balance status of the rate of formation of free radicals in an organism and the rate at which they are removed is known as oxidative balance. The process of achieving oxidative balance in the organism is not affected by free radicals. Impairment of this balance is caused by an increase in the rate of formation of these radicals or a decrease in the rate at which they are removed. The condition named 'oxidative stress' can be summarised as a serious imbalance between the formation of free radicals and the anti-oxidant defence mechanism, resulting in tissue damage.[18]

Free radicals affect all the important cell components such as lipids, proteins, deoxyribonucleic acid (DNA) and carbohydrates and cause defects in the structures. Examples of the causes of oxidants which are formed are free radicals such as reactive oxygen species (ROS) in the biological system, superoxide anion, hydroxyl radical, nitric oxide, peroxyl radical, and hydrogen peroxide.[19] Lipid peroxidation is one of the parameters of oxidant formation. Increased lipid peroxidation and changes in antioxidant enzyme levels in a febrile seizure cause cell damage and death by destroying neural cell membranes.[9]

Studies have shown that an increase in malondialdehyde (oxidant level) to be an indicator of lipid peroxidation and polyunsaturated fatty acid peroxidation which create oxidative stres.[20,21] In the current study, the TOS values of the febrile seizure group were found to be statistically significantly high in comparison with the TOS values of the control group. These results correlate with those of the aforementioned study. Thus it is thought that, as the brain is very sensitive to oxidative damage and the increased oxidative stress of epileptic seizures, stroke and neurological diseases such as neurodegenerative impairments and nerotrauma, are affected by the pathophysiology of the patient,[22,23] so the physiopathology of the febrile seizure patient may be influential.

The body has developed defence mechanisms to prevent the formation of ROS and the damage wrought by them. These are known as 'anti-oxidant defence systems'. As anti-oxidant molecules are structures which are sources of both endogenous and exogenous energy, they are able to render the damage caused by oxidant molecules ineffective through both internal and external protection of cells. External cell protection includes various molecules such as albumin, bilirubin, transferin, ceruloplasmin and uric acid. The main anti-oxidant defence intracellularly is provided by free radical scavenger enzymes. These are superoxide dismutase, glutathione-S-transferase, glutathione peroxidase, glutathione reductase, catalase and cytochrome oxidase. Trace elements such as copper, zinc and selenium are necessary for the functioning of these enzymes.[15]

A study of febrile seizure patients following a seizure, showed decreased superoxide dismutase, as an anti-oxidant with protective properties against free radicals, and an increase in glutathione peroxidase.[21] In another study of febrile seizure patients, an increase was shown in catalase, which has anti-oxidant properties.[20] A study of epileptic patients found glutathione reductase to be low in comparison with the control group.[24] In the current study, the TAS values of the febrile seizure group were found to be at a statistically significant low level compared to the TAS values of the control group. Studies have shown that in conditions of stress, anti-oxidants decrease and the oxidant levels increase.[25] This situation may arise from the increased stress during a seizure. The comparison of the OSI related to the decreased anti-oxidants and increased total oxidants in the patients, compared to that of the control group, was found to be statistically significant. The sample size was relatively small and the design of the study was cross-sectional which were the limitations of the study.

Conclusion

The study demonstrated that patients with simple febrile seizures had higher increased oxidative stress levels than healthy subjects. Increased oxidative stress may have played a role in leading to simple febrile seizures. Large-scale prospective cohort studies are needed to shed more light on the issue.

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