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# Dynamic thiol/disulphide homeostasis in children with febrile seizure

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ABSTRACT

*Purpose:* Febrile seizure (FS) is the most common type of seizure in children and its etiopathogenesis is still not fully understood. We aimed to investigate the thiol/disulphide balance in children who had experienced FS in our study.
*Methods:* We included 40 FS and 40 control group subjects in the study. The total thiol, native thiol, and disulphide levels were measured and the disulphide/native thiol, disulphide/total thiol and native thiol/total thiol ratios were calculated in both groups.
*Results:* The mean age and gender distribution of the patients and control group subjects were similar. The total thiol level was lower in the FS group than the control group with no statistical significance ($p = 0.123$). Native thiol was significantly lower in the FS group than the control group ($p = 0.031$). The disulphide level and the disulphide/native thiol and disulphide/total thiol ratios were statistically significantly higher in the FS group than the control group while the native thiol/total thiol ratio was lower.
*Conclusion:* The fact that the disulphide level was higher and the native thiol level lower in the FS group than the control group suggests that the thiol/disulphide balance may have shifted in favor of oxidants and that oxidants may have a role in FS pathogenesis.


Febrile seizure is the most common reason of childhood seizures. The incidence is 2–5% and it is more common in Asian countries. The incidence peaks at 18 months. Although fever is a common symptom in children, it is difficult to explain why only some children with fever experience FS. FS is thought to occur due to various factors, mainly infections, and genetic susceptibility [1–3]. The role of the free oxygen radicals in the etiopathogenesis of FS as in many other diseases has attracted interest in recent years [2,3]. Oxidant and antioxidants are in a particular balance in the body. Oxidative stress develops if the increase in oxidants is not balanced by the antioxidants. The formed oxidants and free oxygen radicals create cellular damage with cell membrane lipid peroxidation and the brain is quite sensitive to such oxidative damage [4]. It has also been shown that antioxidants increase and the oxidants decrease in FS patients [2].

The dynamic thiol/disulphide homeostasis concept developed by Erel et al. [5] has been shown to play a role in the pathogenesis of many neurological disorders such as stroke and migraine [6,7]. However, there is no study on the relationship of FS with this newly developed method. The aim of this study was to find how this balance changes in children who have experienced FS and the factors playing a role in this change.

## 1. Material-methods

### 1.1. Patient design

A total of 40 pediatric patients diagnosed with FS at Yıldırım Beyazıt University Yenimahalle Training and Research Hospital's Pediatric Neurology Department and 40 healthy children were included in the study

Study inclusion criteria were as follows:
–Diagnosis of febrile seizure
–Seen within the initial 8 h after the seizure,
–No chronic disease
Study exclusion criteria were as follows:
–Parents did not accept participation in the study
–The post-seizure duration was over 8 h
–Patients with chronic disease

The FS patient group included patients aged 6 months to 5 years with a seizure in the febrile period, lack of central nervous system infection, lack of electrolyte disorder or metabolic disorders that

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could cause a seizure, and no history of recent afebrile seizure. The diagnosis of simple FS required a seizure lasting less than 15 min, a single seizure within 24 h, and lack of a generalized seizure or postictal pathological findings while the diagnosis of complicated FS required a seizure lasting longer than 15 min, more than one seizure within 24 h, focal onset/focal seizure, and lack of a postictal pathological finding [8].

Those who presented to the pediatric neurology outpatient department with non-infectious reasons, had no chronic disease and no history of medication use, and accepted to participate were included in the study as the healthy control group.

The age and gender data of the patient and control groups, the seizure number and the FS type (simple or complicated) were recorded.

Approval was obtained from Ankara Yıldırım Beyazıt University Yenimahalle Training and Research Hospital's Clinical Studies Ethics Committee.

### 1.2. Biochemical analysis

Venous blood samples were obtained from the patients and were centrifuged at 1500 cycles for 10 min. The serum was separated and kept at −80 °C. All bloods were then simultaneously thawed and analyzed.

A new and fully automated method developed by Erel and Neselioglu was used for the measurement of plasma native thiol, total thiol and disulfide levels, based on the reduction of dynamic disulfide bonds to functional thiol groups by sodium borohydrate (NaBH4) [5]. Formaldehyde was used to remove all the unused NaBH4 in order to prevent extra reduction of the 5,5′-dithiobis-2-nitrobenzoic acid (DTNB) and further reduction of the formed disulfide bond produced after the DTNB reaction. The total thiol content of the sample was measured using modified Ellman's reagent. Native thiol content was subtracted from the total thiol content and half of the obtained difference provided the disulfide bond quantity. In addition, the disulfide/thiol, disulfide/total thiol and thiol/total thiol ratios were calculated automatically and synchronously.

### 1.3. Statistical analysis

Measured values were evaluated and reported as means ± standard deviation (SD) using the SPSS 21.0 statistical program. The normality of the distribution of the groups was juxtaposed with the Kolmogorov-Smirnov test. The Independent T test was used in the analysis of normally distributed numerical variables. The chi-Square test was used in the evaluation of the gender distribution of the groups. A p value $< 0.05$ was considered statistically significant.

**Table 1**
General characteristics of the patients.

| Characteristics | FS group | Control group | P value |
|---|---|---|---|
| Age (months, Mean ± SD) | 24.95 ± 14.96 | 25.00 ± 16.02 | 0.534 |
| Gender (n) | | | |
| Female | 20 | 19 | 0.823 |
| Male | 20 | 21 | |

SD: Standard Deviation.

## 2. Results

We included 40 patients diagnosed with FS and 40 healthy children in the study. The mean age and gender distribution were similar in the two groups (Table 1)

There were 26 simple FS and 14 complicated FS patients. We had 17 patients younger and 23 older than 18 months. Eleven of the patients were included in the study after their first seizure and 19 after multiple seizures before presentation.

The total thiol level of the FS group was lower than the control group but with no statistical significance (p = 0.123). Native thiol was significantly lower in the FS group than the control group (p = 0.031). The Disulphide level, Disulphide/Native thiol and Disulphide/Total thiol ratios were statistically significantly higher (Fig. 1) and the Native thiol/Total thiol ratio lower in the FS group than the control group (Table 2)

When the patients in the FS group were divided as under and above 18 months, no statistically significant difference was found between the groups for the relevant parameters. When they were similarly divided into simple and complicated FS groups based on FS type or into groups of patients with single or multiple seizures, there was again no statistically significant difference (Table 3).

## 3. Discussion

Oxidative stress occurring as a result of lipid peroxidation and a decrease in antioxidant levels is known to play a role in seizure development [4,9,10]. An increase in free oxygen radicals or decrease in antioxidants has been found to be important in seizure recurrence. Besides, oxidative stress is thought to potentially play a role in seizure-related neuronal cell death. Lipid peroxidation was

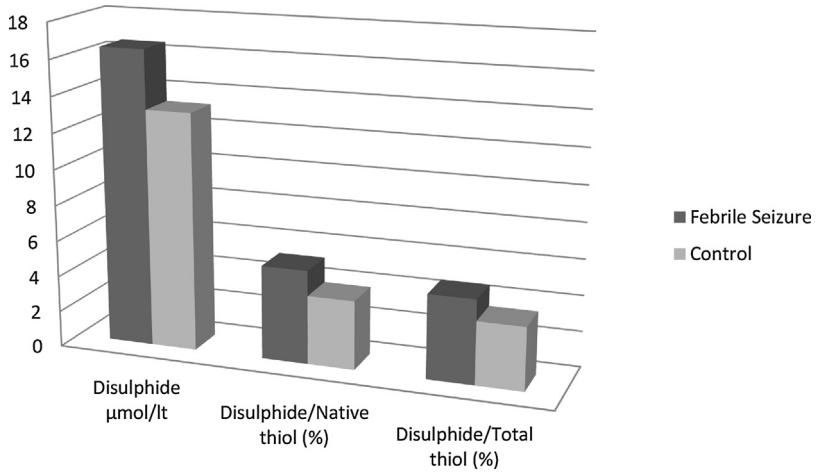


**Fig 1.** Disulphide levels and Disulphide/Native thiol, Disulphide/Total thiol.

**Table 2**
Thiol and disulphide levels of the FS and control groups.

| Variables | Febrile Seizure Mean ± SD | Control Mean ± SD | P value |
|---|---|---|---|
| Total thiol, μmol/lt | 360.34± 50.06 | 376.73 ± 43.82 | 0.123 |
| Native thiol, μmol/lt | 327.30 ± 50.61 | 350.22 ± 42.00 | **0.031** |
| Disulphide, μmol/lt | 16.51 ± 4.42 | 13.25 ± 5.67 | **0.005** |
| Disulphide/Native thiol (%) | 5.22 ± 1.92 | 3.83 ± 1.72 | **0.001** |
| Disulphide/Total thiol (%) | 4.68 ± 1.51 | 3.51 ± 1.48 | **0.001** |
| Native thiol/Total thiol (%) | 90.63 ± 3.02 | 92.96 ± 2.96 | **0.001** |

Bold values are statistically significant.

**Table 3**
Thiol and disulphide levels of the FS group.

| Features | | Total thiol, μmol/lt Mean ± SD | Native thiol, μmol/lt Mean ± SD | Disulphide, μmol/lt Mean ± SD | Disulphide/Native thiol (%) Mean ± SD | Disulphide/Total thiol (%) Mean ± SD | Native thiol/Total thiol (%) Mean ± SD |
|---|---|---|---|---|---|---|---|
| Age | 18 months >[a] | 351.55 ± 50.00 | 318.40 ± 51.80 | 16.57 ± 4.22 | 5.44 ± 2.12 | 4.84 ± 1.65 | 90.30 ± 3.31 |
| | 18 months <[b] | 366.84 ± 50.20 | 333.88 ± 49.83 | 16.47 ± 4.65 | 5.06 ± 1.78 | 4.55 ± 1.42 | 90.88 ± 2.84 |
| FS type | Simple[c] | 369.23 ± 46.53 | 336.48 ± 46.02 | 16.37 ± 4.45 | 4.97 ± 1.68 | 4.48 ± 1.34 | 91.02 ± 2.68 |
| | Complicated[d] | 343.83 ± 53.88 | 310.27 ± 55.95 | 16.78 ± 4.51 | 5.68 ± 2.29 | 5.03 ± 1.78 | 89.92 ± 3.5 |
| Number of FS | 1[e] | 353.85 ± 43.10 | 322.26 ± 45.67 | 15.79 ± 5.39 | 5.08 ± 2.15 | 4.55 ± 1.71 | 90.88 ± 3.43 |
| | >1[f] | 362.80 ± 52.95 | 329.22 ± 53.00 | 16.79 ± 4.06 | 5.27 ± 1.86 | 4.72 ± 1.45 | 90.54 ± 2.91 |
| P value | | a–b: 0.665 | a–b: 0.511 | a–b: 0.312 | a–b: 0.218 | a–b: 0.217 | a–b: 0 0.217 |
| | | c–d: 0.127 | c–d: 0.120 | c–d: 0.786 | c–d: 0.272 | c–d: 0.280 | c–d: 0.280 |
| | | e–f: 0.620 | e–f: 0.703 | e–f: 0.531 | e–f: 0.781 | e–f: 0.753 | e–f: 0.753 |

found to increase and antioxidant treatments to have a positive effect in rats with experimentally induced seizure [4,9,10,11]. The golden standard for studies investigating seizure and the antioxidant/oxidant relationship is to perform the measurements in the cerebrospinal fluid but it is not possible to do this in practice. Blood and cerebrospinal fluid oxidant/antioxidant levels have been found to show a correlation in various studies [12]. The plasma malondialdehyde level was found to be higher in patients with FS than in the control group and the cerebrospinal fluid malondialdehyde level was also higher in the FS patients [13].

The etiopathogenesis of FS is still a mystery. Although many mechanisms such as genetic factors, trace elements, and cytokines are thought to possibly play a role, considering a single factor seems simplistic. Another study found the blood antioxidant level to be higher and the antioxidant capacity to be lower in patients who had experienced FS than the control group [14].

Dynamic thiol disulphide homeostasis plays a role in many vital mechanisms such as antioxidant status, detoxification, signal transduction, apoptosis, regulation of enzymatic activity and transcription factors and cellular signalling mechanisms in the body. Studies have shown dynamic thiol disulphide homeostasis plays a role in the pathogenesis of many diseases [5,15,16], such as diabetes [17], coronary artery disease [18], hypertension [19], brain tumor [20], bipolar disorder [21], stroke [6], headache [7].

While we found the native thiol level of the FS group to be lower than in the control group, disulphide and disulphide/native thiol, disulphide/total thiol, native thiol/total thiol ratios were statistically quite significantly higher in the FS group. This shows the thiol/disulphide homeostasis in patients with FS to be significantly different than in the control group, but is it is difficult to comment on whether this change is a result of FS or whether FS is actually the result of this change.

Akarsu et al. [13] found the oxidant levels in children experiencing an afebrile seizure to be higher than after a febrile seizure and they thought this to be an indicator of the neuronal damage that developed at various degrees depending on seizure etiology in a study they conducted in children with febrile or afebrile seizures.

We found no difference between the parameters when FS patients were divided among themselves as simple FS and complicated FS in our study. Günes et al. [2] found the erythrocyte malondialdehyde and glutathione peroxidase level to be higher and superoxide dismutase to be lower than in the FS patients than in the control group. When they compared the patients with FS by subgroups as simple and complicated, they found no statistical difference between the oxidant/antioxidant levels, as in our study.

Patients who had single or multiple seizures were found to have similar parameters in our study. This may suggest that the main factor affecting the oxidant antioxidant balance is FS formation and that the seizure frequency and FS type factors are not effective.

In conclusion, the native thiol level was lower and the disulphide level much higher in the children with FS than the control group and this change in the thiol/disulphide homeostasis could play a role in FS pathogenesis.

## Declaration of conflicting of interests

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## Ethical approval

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