Title: Determining Pro-Oxidant Antioxidant Balance in Febrile Children with and without Seizure: A comparative Study

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To appear in: **Basic and Clinical Neuroscience**

Received date: 2024/05/27

Revised date: 2024/06/01

Accepted date: 2024/06/5

Please cite this article as:

Salari Zare, M., Mir, M., Shobeiri, S.S., Tehrani, H., Amiri, P., Azra Aldaghi, M., et al. (In Press). Determining Pro-oxidant Antioxidant Balance in Febrile Children with and without Seizure: A comparative Study. Basic and Clinical Neuroscience. Just Accepted publication Jul. 10, 2024. Doi: http://dx.doi.org/10.32598/bcn.2024.6601.1

DOI: http://dx.doi.org/10.32598/bcn.2024.6601.1

Abstract

Background: A benign condition known as a febrile seizure (FS) occurs between the ages of six and sixty months when the patient has a fever of at least 38 ^{o}C, is not suffering from a CNS infection or metabolic disorder, and has no prior history of febrile seizures. It is thought that oxidative stress contributes to the emergence of a number of neurodegenerative disorders. In light of this, the current study examined the pro-oxidant antioxidant balance (PAB) in febrile children with and without seizures.

Methods: The current cross-sectional study was done in the Heshmatieh hospital of Sabzevar University of Medical Sciences, between March 2020 and March 2021. Forty febrile children with a temperature of 38 degrees and above (F) and forty febrile children who experienced their first seizure (FS) were included in the study, and blood samples were taken from all patients. The Pro-oxidant-antioxidant balance was evaluated using a special PAB assay. Pro-oxidant-antioxidant ratio values were expressed in HK.

Results: The HK variable in the FS group was 180.43 ± 9.28 and in the F group was 131.83 ± 17.73. In fact, the FS group had a higher mean serum level of PAB than F group (Pvalue <0.05), which indicated that the level of oxidative stress in patients with febrile seizure was higher than the febrile patients without seizure.

Conclusion: It seems that the serum antioxidant level is related to the possibility of seizures in febrile children.

Keywords: Antioxidant, Febrile seizure, Fever, Oxidative stress, Pro-oxidant

1. Introduction

Febrile Seizure (FS) is a benignant circumstance that occurs in six and sixty months of age with a temperature of 38 ºC or more, which is not a result of CNS infection or metabolic imbalance and has no history of previous seizures (Bakhtiari, Heydarian, Azmoudeh, Kaffashbashi, & Heidarian, 2023). FS is the most common type of seizure in children with a prevalence of 2–5% (Delpisheh, Veisani, Sayehmiri, & Fayyazi, 2014; Rivas-García et al., 2022). risk factors of FS include premature birth, brain disorder, and family history (Bakhtiari et al., 2023; Sharawat, Singh, Dawman, & Singh, 2016). There are two types of febrile seizures: simple and complex. A simple febrile seizure is an initial generalized seizure, usually tonic-clonic, accompanied by fever, which lasts at most 15 minutes and does not recur within 24 hours. Complex febrile seizures are those that last at least 15 minutes, are accompanied by focal neurologic symptoms, or reoccur within 24 hours (Smith, Sadler, & Benedum, 2019). Various factors contribute to recurrence of attacks, which include age, sex, family and personal history of seizures, body temperature at the time of seizure, and the interval between the onset of fever and the seizure (TALEBIAN MD & MOHAMMADI MD, 2009).

A family of unstable molecules known as reactive oxygen species (ROS) is made up of oxygen and one or more unpaired electrons in the outermost shell. ROS are compounds that are easily reactive due to the presence of these unpaired electrons (León Navarro, Crespo, & Martín, 2020). The most prevalent ROS are hydroxyl radical, superoxide anion, and hydrogen peroxide. In a healthy body, these radicals are crucial for cellular communication, development, apoptosis, and systemic functions like blood pressure regulation and immunological response (Brieger, Schiavone, Miller Jr, & Krause, 2012; León Navarro et al., 2020). On the other hand, oxidative stress happens when the body produces more ROS than the antioxidant defense system can eliminate. Basic cell macromolecules including DNA, lipids, and proteins can oxidize in these circumstances (León Navarro et al., 2020). Important enzymes may alter in both structure and function as a result of protein oxidation (Stadtman, 2001). Lipid peroxidation can change the fluidity, permeability, and protein-membrane functions, which can result in hyper-excitability (Wong-Ekkabut et al., 2007). It is believed that an imbalance between the production of ROS and the antioxidants that scavenge them causes oxidative stress (León Navarro et al., 2020).

Recent research has demonstrated the involvement of lipid peroxidation and free oxygen radicals in the pathophysiology of several diseases. Numerous diseases, including asthma, diabetes mellitus, rheumatologic conditions like rheumatoid arthritis, cancer, neurological conditions including stroke and epilepsy, and inflammatory disorders have all been linked to oxidative stress (Kumar & Kumari, 2017; Leung, Hon, & Leung, 2018). The production of lipid peroxidation and free radicals accelerates the death of cells (Bakhtiari et al., 2023). The body developed defense systems to stop oxidants, such free radicals and lipid peroxidation, from doing damage. These are the "antioxidant defense systems," which include glutathione reductase, glutathione peroxidase, superoxide dismutase, and catalase (Bakhtiari et al., 2023; Fetveit, 2008; Vidailhet et al., 2017) A disturbance in the equilibrium of pro-oxidant antioxidants is commonly referred to oxidative stress, and it plays a role in the development of multiple neurodegenerative diseases, autoimmune disorders, and cancer (Avval et al., 2018). In this regards, the present

research aimed to evaluate the pro-oxidant antioxidant balance (PAB) in febrile children with and without seizure.

2. Methods

The current cross-sectional study was done in the Heshmatieh hospital of Sabzevar University of Medical Sciences, Sabzevar, Iran, between March 2020 and March 2021. This research followed the Helsinki ethical principles. Informed consent was obtained from the parents or legal guardians of patients and the ethics committee of Sabzevar University of Medical Sciences approved this research [IR.MEDSAB.REC.1398.118].

Eighty children aged six months to five years were selected among the patients admitted to Heshmatieh hospital in Sabzevar using non-random sampling. 40 febrile children with a temperature of 38 degrees and above (F) and 40 febrile children who had experienced their first seizure (FS) were included. Blood samples were taken from each patient. In the case of patients with fever and seizures, the serum sample was taken in the second hour after the seizure, that is, between one and two hours after the seizure, and in the case of patients with fever and without seizures, the blood sample was taken when the fever was 38 degrees or higher. Patients with the presence of seizures without fever and also a history of seizures without fever in the past, suffering from developmental disorders and cerebral palsy, suffering from metabolic and genetic diseases and all syndromes that cause or accompany seizure were excluded. The patient's serum samples were prepared and all samples were kept at -20 $^{\circ C}$ until performing the PAB (pro-oxidant-antioxidant balance) test.

2.1.The pro-oxidant-antioxidant balance (PAB) test

The PAB test or pro-oxidant-antioxidant balance is a test to determine the oxidants and antioxidants of the target sample simultaneously in a single test (Alamdari et al., 2007). The PAB measurement method in our study was such that the activity of the oxidant and antioxidants were investigated together and tetra-methyl-benzoyl (TMB) was used as an oxidation-reduction index due to its electrochemical and optical properties to evaluate the pro-oxidant-antioxidant balance (Boskabadi et al., 2022). Pro-oxidant-antioxidant ratio values are expressed in HK (HK unit, H: Hamidi Alamdari, K: George Koliakos) (Boskabadi et al., 2022). HK is actually an optional unit that is calculated based on the percentage of hydrogen peroxide absorbed in the standard solution (Samani et al., 2022). An improved PAB was used according to the previously described method (Avval et al., 2018; Boskabadi et al., 2022; Tavana et al., 2016). Briefly, a standard curve was drawn using 0- 100% ratios of 250 μM hydrogen peroxide with 3 μM uric acid (in 10 mM sodium hydroxide). Based on the concentration of hydrogen peroxide in the reaction, the peroxidase enzyme oxidizes the TMB substrate, which is observed as a blue color. At the end of the reaction, hydrochloric acid produces a yellow color at a wavelength of 450 nm. The values were measured with the help of an ELISA reader at 450 nm with a reference wavelength of 620 nm. Finally, pro-oxidant-antioxidant ratio values were expressed in HK units.

2.2. Statistical analysis

In the description of the data, appropriate statistical tables and indices such as mean, standard deviation, etc. were used, and in the analysis of the data, the normality of the data was first investigated using the Shapiro-Wilk test, which was confirmed for normality, appropriate parametric methods such as Student's t test were used, and in case of non-normality, Mann-Whitney and Wilcoxon tests were used. Pearson Chi-Square test was used in data analysis with nominal scale, and in cases where more than 20% of the expected frequencies of the tables were less than 5 (Cochran), Fisher's Exact Test was used. The software used in this research was IBM-SPSS v.20.

3. Results

In this study, 80 patients including 32 girls (40%) and 48 boys (60%) with an average age of 36.67 ± 18.12 months and an age range of 6 to 60 months. HK and fever variables were evaluated in FS (fever and seizures) and F (fever) groups and the variables of duration of fever, duration of seizures, type of seizures, frequency of seizures, history of seizures, history of epilepsy in first-degree relatives of the patient, history of epilepsy in other relatives of the patient, history of fever and seizures in first-degree relatives of the patient, and history of fever and seizures in other relatives were examined only in FS group patients.

The age range (maximum and minimum age) in both groups was 6 to 60 months. The mean and standard deviation of age in FS group was 35.63 ± 18.20 months and in F group was 37.73 ± 18.21 months, and there was no significant difference between the two groups in terms of age ($p=0.567$). The number of girls in each of FS and F groups was exactly 16 (40 percent). The number of boys in each of FS and F groups was exactly 24 (60 percent). Gender distribution in the studied groups did not differ significantly from each other ($p=1.00$). Therefore, the two groups were similar in terms of age and gender variables.

3.1. Comparison of HK between FS and F groups

The range of observed HK (the difference between the highest and the lowest HK) was 43.54 in FS group and 66.93 in F group. The mean and standard deviation of HK in FS group was 180.43 ± 9.28 and in F group was 131.83 ± 17.73. In terms of the average of HK, the two groups were significantly different from each other ($p<0.001$) and F group had lower HK as compared to FS group (Fig. 1).

3.2. Comparison of fever between groups

According to patients' results, the number of patients with a fever of 38 to 39 degrees was 28 persons (70 percent) in FS group and 29 persons (72.5 percent) in F group. The number of patients with a fever of more than 39 degrees in FS and F groups was 12 persons (30%) and 11 persons (27.5%), respectively. The distribution of patients with different degrees of fever in the studied groups did not significantly differ from each other ($p=0.805$).

3.3. Comparison of HK between groups of fever degrees according to FS and F groups

In FS group, the average HK in the group with fever of 38 to 39 degrees was lower than the group with fever over 39 degrees, but the difference was not significant ($p=0.095$). In F group, the average HK was higher in the group with fever of 38 to 39 degrees compared to the group with fever over 39 degrees, but the difference was not significant ($p=0.083$).

3.4. Relationship between age and HK variables

In FS group, age had an inverse relationship with HK, but the correlation value was not significant (rsp=-0.032, p=0.845) and in F group, age had a direct relationship with HK, but the correlation value was not significant (rsp=0.102, p=0.529).

3.5. Comparison of variables between girls and boys in FS group

The frequency distribution of the history of fever and seizures in other relatives of male patients was significantly higher than that of female patients (p=0.027). There was no significant difference between girls and boys in the frequency distribution of other variables (Table 1).

3.6. Comparison of variables between different groups of fever in FS group

The frequency distribution of the time from the onset of fever to the onset of seizures was significantly different between patients in the group of more than 39 degrees and the group of 38 to 39 degrees (p=0.013). The frequency distribution of fever and complex seizures was significantly higher in the group of more than 39 degrees than in the group of 38 to 39 degrees (p=0.047). The distribution of the frequency of fever and seizures in 24 hours was significantly higher in the group of more than 39 degrees than in the group of 38 to 39 degrees (p<0.001). There was no significant difference in the frequency distribution of other variables between patients in the group of more than 39 degrees and the group of 38 to 39 degrees (Table 2).

3.7. HK comparison between different variables in FS group:

In FS group, the average HK between the levels of any of the variables of time from the onset of fever to the onset of seizures, duration of seizures, type of fever and seizures, frequency of seizures in the last 24 hours, previous history of fever and seizures in the patient, history of epilepsy in first degree relatives of the patient, history of epilepsy in other relatives of the patient, history of fever and seizures in first degree relatives, history of fever and seizures in other relatives of the patient had no significant difference (p<0.05) (Table 3).

4. Discussion

In this study, 80 patients including 32 girls (40%) and 48 boys (60%) with an average age of 36.67 ± 18.12 months and an age range of 6 to 60 months in the groups of febrile patients with and without seizures were evaluate in terms of HK variable and degree of fever.

In our study, the average age was 36.67 ± 18.12 months and the age range was 6 to 60 months. Also, the age range (maximum and minimum age) in both groups was 6 to 60 months. The mean and standard deviation of age in the FS group was 35.63 ± 18.20 months and in the F group, it was 37.73 ± 18.21 months, and there was no significant difference between the two groups in terms of age. In the study of Iyshwarya et al., the average age of children with fever and seizures was 3.05 ± 2.19 years, and in children with only fever it was 3.11 ± 1.24 years and in the control group it was 3.86 ± 1.18 (Iyshwarya, Kalyan, Suma, & Aruna, 2013). In the study of Abu-Handan et al., the average age was determined as 26.65 ± 12.58 months for the group with fever and seizures and 11.53 ± 30.46 months for the control group. In this study, there was no statistically significant difference between the two groups in terms of age (Abuhandan et al., 2013). In other study, the average age in the group of patients with fever and seizures was 2.13 ± 0.82 years, and in the group of febrile children without seizures as a control, the average age was 2.47 ± 1.16 years, which had no significant difference in terms of age (El-Masry, Sadek, Hassan, Ameen, & Ahmed, 2018).

Our results showed that the number of girls in each of the FS and F groups was exactly 16 (40%). The number of boys in each of the FS and F groups was exactly 24 (60 percent). Gender in the studied groups did not differ significantly from each other. In the study of Iyshwarya et al., it was observed that there was no significant gender difference in children with fever and seizures (Iyshwarya et al., 2013). In a cross-sectional study conducted in Iran, no significant gender difference was observed regarding febrile seizures in children aged 6 months to 5 years (Ehsanipour, TALEBI, Vahid, & Kani, 2009). However, the Mollah et al research revealed that male children were more prone to fever and seizures than female children (Mollah et al., 2008).

In current study, out of 40 patients with fever and seizures (FS group), 34 had simple fever and seizures (85%) and 6 had complex fever and seizures (15%). Similar to our findings, in the study of Gönes et al., out of 31 patients in the fever and seizure group, 25 patients (80%) had simple fever and seizures, and 6 patients (20%) had complex febrile seizures (Güneş et al., 2009).

Febrile seizures are those between six and sixty months of age with a temperature of 38 or more, which is not a result of CNS infection or metabolic imbalance and has no history of previous febrile seizures. Many studies have considered factors such as fever background, seizures in first degree relatives, lack of micronutrients, immunological reactions and oxidative stress as its aggravating factors (Hartfield, 2010; Pacitti et al., 2013). Seizures are the most common cause of Emergency department calls and hospitalizations in children (Salmi et al., 2021). Children with early-onset fever and seizures, especially those with recurrent fever and seizures, may be at greater risk for poorer verbal and processing performance, and are therefore at risk of cognitive and functional impairments (Billstedt et al., 2020). A complex interaction between brain, genetics, epigenetics and early environment is involved in fever and seizures (Mewasingh, Chin, & Scott, 2020). Fever and convulsions are considered to be the response of the developing brain to fever, but its exact physiopathology remains unknown. In general, a set of environmental and genetic factors are involved in its creation. Specific mutations in ion channels make a person susceptible to fever and seizures. Disturbance of the balance between antioxidants and pro-oxidants in the body is called oxidative stress (Momen Beitollahi et al., 2010). Antioxidants are compounds that are able to eliminate free radicals in the body. On the other hand, compounds that are able to produce oxygen free radicals in the body are called pro-oxidants (Kunz, 2002). Some types of free radicals contain nitrogen and some that are produced during harmful processes contain oxygen, which after formation damages proteins, lipids and DNA (Momen Beitollahi et al., 2010). On the other hand, seizures lead to the production of free radicals. Therefore, oxidative stress and the production of free radicals are currently known effects of seizures (Valko et al., 2007). Recent data have shown that the disorder in the antioxidant system makes the nervous system vulnerable and makes the person susceptible to convulsive attacks (Martinc, Grabnar, & Vovk, 2012). In other words, the production of free radicals provokes the occurrence of convulsive attacks (Laus et al., 2017).

In this research, pro-oxidant-antioxidant ratio values were expressed in HK units. In fact, it is an optional unit that is calculated based on the percentage of hydrogen peroxide absorbed in the standard solution. The mean and standard deviation of HK in the FS group was 180.43 ± 9.28 and in the F group, it was 131.83 ± 17.73. In terms of the mean of HK, the two groups were significantly different from each other ($p<0.001$). Therefore, the amount of HK in the patients of FS group was significantly higher than the patients of F group, and this proved that the amount of oxidative stress in patients with fever and seizures is higher than in the fever without seizures group, and in fact, the pro-oxidant-antioxidant balance in the FS group was disturbed compared to the F group.

Compared to previous studies, no similar study had been found that had measured and compared the pro-oxidant-antioxidant balance of serum samples in fever and seizures and fever without seizures patients

with the PAB test, but in other studies, the levels of antioxidants and the amount of oxidative stress had been measured and compared in patients with fever and seizures and fever patients with other methods. In the study of Akarsu et al., the levels of erythrocyte arginase, plasma malondialdehyde, erythrocyte catalase, cerebrospinal fluid malondialdehyde, cerebrospinal fluid nitric oxide, and plasma nitric oxide were measured. Their results indicated that the level of free radicals was significantly higher in the febrile seizures group than in the control group. Also, the authors showed that the risk of free radicals in seizures without fever was much higher than seizures with fever (Akarsu et al., 2007). In the study of Günes et al., erythrocyte malondialdehyde and glutathione peroxidase were significantly higher and superoxide dismutase was significantly lower in the group of patients with fever and seizures. This result, in accordance with the result of our study, showed a direct relationship between the occurrence of seizures caused by fever and the increased level of free radicals (Güneş et al., 2009). Increased levels of malondialdehyde indicate loss of fatty acids, which leads to cell membrane damage and cell death. Akarsu et al, by investigating the effects of fever and fever with seizures, on the oxidant status in children, found that the plasma malondialdehyde level was increased in children who had febrile seizures (Akarsu et al., 2007). Moreover, superoxide dismutase and glutathione peroxidase enzymes play a protective role against free radicals. Günes et al.'s research findings showed that superoxide dismutase levels decreased and glutathione peroxidase levels increased after febrile seizures. Overproduction of superoxide may be present in fever and seizures, and decreased levels of superoxide dismutase may result in reduced catalyzing of superoxide to oxygen and hydrogen peroxide. The glutathione peroxidase activity may enhance to convert hydrogen peroxide to water as a compensatory mechanism (Güneş et al., 2009). Although it is well known that seizures cause oxidative stress, the effects of fever and seizures on oxidative balance remain unclear (Akarsu et al., 2007). In the Iyshwarya et al. research, the increase of the stress marker, malondialdehyde, among children with fever and seizures, and the decrease of the serum level of zinc and magnesium as antioxidants in this group indicated that oxidative stress had increased among those with Fever and seizures (Iyshwarya et al., 2013).

Along with our study, Abu-Handan et al., showed that in patients with fever and simple seizures, oxidative stress was higher than in healthy individuals, and oxidative stress may play an important role in the occurrence of febrile seizures (Abuhandan et al., 2013).

5. In conclusion

According to our findings, average serum level of pro-oxidant-antioxidant balance in the febrile children with seizures was significantly higher compared to those without seizures, and this can prove that the amount of oxidative stress in patients with fever and seizures was higher than in the control group. In fact the pro-oxidant-antioxidant balance in the case group (febrile children with seizures) had been disturbed compared to the control group (febrile patients without seizures). An increase in oxidative stress and a decrease in the level of antioxidants in patients with fever and seizures compared to patients with fever is a problem that had been continuously revealed in similar studies. But this time we used another laboratory method to prove this issue, which is very accurate and reliable. The results of our study may indicate the correctness of this hypothesis that if the serum level of antioxidants is low in a febrile child, the probability of fever and seizures in this child is higher than a similar case with a higher antioxidant serum level. To prove this hypothesis, there is a need for more extensive studies with a larger statistical population with a long follow-up.

Acknowledgment

This paper is based on a student thesis approved by Sabzevar University of Medical Sciences. The authors express their gratitude to the Vice-Chancellor of Research and Technology of this university.

Authors' Contributions

M.SZ: Methodology, Investigation, review & editing, M.M: Methodology, Investigation, review & editing, S.Sh: Investigation, Writing original draft, review & editing, H.T: Conceptualization, Methodology, Investigation, review & editing, P.A: Methodology, Investigation, review & editing, M.A: Investigation, review & editing, K.H: Conceptualization, Methodology, Investigation, review & editing. All authors read and approved the final manuscript.

Ethical Approval

The Research Ethics Committee of Sabzevar University of Medical Sciences approved the study protocol (Approval ID: IR.MEDSAB.REC.1398.118).

Funding

This work was supported by the Sabzevar University of Medical Sciences, Sabzevar, Iran (Grant number: 98036).

Conflict of Interest

The authors declare that they have no competing interests.

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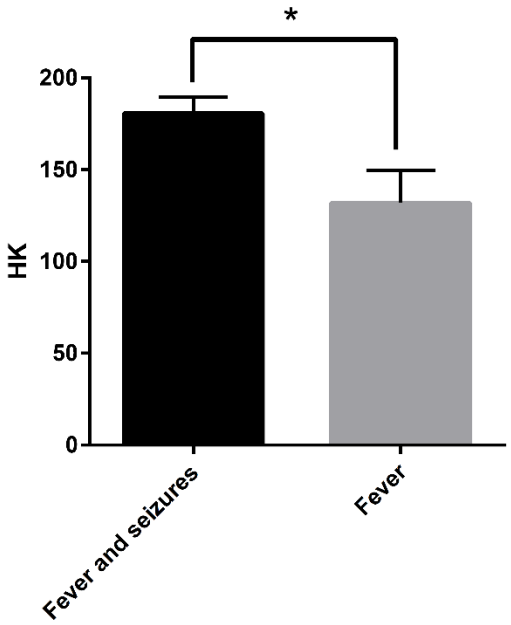

Figure 1 Comparison of HK between Fever and seizure (FS) and Fever (F) groups

Table 1 Comparison of variables between girls and boys in FS group

Variable		Female	Male	Total	Fisher's exact test
The time from the onset of fever to the onset of seizures	less than 1 hour	2(12.5)	5(20.8)	7(17.5)	P=0.463¥
	1 to 24 hours	13(81.3)	18(75)	31(77.5)	
	More than 24 hours	1(6.3)	1(4.2)	2(5)	
Seizure duration	less than 5 minutes	10(62.5)	21(87.5)	31(77.5)	P=0.084¥
	5 to 15 minutes	5(31.3)	2(8.3)	7(17.5)	
	More than 15 minutes	1(6.3)	1(4.2)	2(5)	
type of fever and seizure	Simple	14(87.5)	19(82.6)	33(84.6)	P=1.00
	Complex	2(12.5)	4(17.4)	6(15.4)	
Number of seizures in the last 24 hours	One time	15(93.8)	19(79.2)	34(85)	P=0.373
	More than one	1(6.3)	5(20.8)	6(15)	
Previous history of fever and seizures	No	10(62.5)	20(83.3)	30(75)	P=0.159
	Yes	6(37.5)	4(16.7)	10(25)	
History of epilepsy in first degree relatives of the patient	No	14(87.5)	20(83.3)	34(85)	P=1.00
	Yes	2(12.5)	4(16.7)	6(15)	
History of epilepsy in other relatives of the patient	No	14(87.5)	20(83.3)	34(85)	P=1.00
	Yes	2(12.5)	4(16.7)	6(15)	
History of fever and seizures in first degree relatives	No	15(93.8)	18(75)	33(82.5)	P=0.210
	Yes	1(6.3)	6(25)	7(17.5)	
History of fever and seizures in other relatives of the patient	No	15(93.8)	14(58.3)	29(72.5)	P=0.027
	Yes	1(6.3)	10(41.7)	11(27.5)	

¥: the result of Tau Kendall B test

Table 2 Comparison of variables between different groups of fever in FS group

Variable		38-39 degrees	More than 39 degrees	Total	Fisher's exact test
The time from the onset of fever to the onset of seizures	less than 1 hour	7(25)	0(0)	7(17.5)	P=0.013¥
	1 to 24 hours	20(71.4)	11(91.7)	31(77.5)	
	More than 24 hours	1(3.6)	1(8.3)	2(5)	
Seizure duration	less than 5 minutes	23(82.1)	8(66.7)	31(77.5)	P=0.257¥
	5 to 15 minutes	5(17.9)	2(16.7)	7(17.5)	
	More than 15 minutes	0(0)	2(16.7)	2(5)	
type of fever and seizure	Simple	25(92.6)	8(66.7)	33(84.6)	P=0.047
	Complex	2(7.4)	4(33.3)	6(15.4)	
Number of seizures in the last 24 hours	One time	28(100)	6(50)	34(85)	P<0.001
	More than one	0(0)	6(50)	6(15)	
Previous history of fever and seizures	No	21(75)	9(75)	30(75)	P=1.00
	Yes	7(25)	3(25)	10(25)	
History of epilepsy in first degree relatives of the patient	No	26(92.9)	8(66.7)	34(85)	P=0.055
	Yes	2(7.1)	4(33.3)	6(15)	
History of epilepsy in other relatives of the patient	No	23(82.1)	11(91.7)	34(85)	P=0.418
	Yes	5(17.9)	1(8.3)	6(15)	
History of fever and seizures in first degree relatives	No	23(82.1)	10(83.3)	33(82.5)	P=1.00
	Yes	5(17.9)	2(16.7)	7(17.5)	
History of fever and seizures in other relatives of the patient	No	19(67.9)	10(83.3)	29(72.5)	P=0.451
	Yes	9(32.1)	2(16.7)	11(27.5)	

¥: the result of Tau Kendall B test

Table 3 Comparison of HK between different variables in FS group

Variable	No.	Mean	SD	Minimum	Maximum	Median	Results

Time from the onset of fever to the onset of seizures	Less than 1 hour	7	182.17	7.06	168.90	192.23	181.50	X^2=1.40 P=0.496
	1 to 24 hours	31	180.23	10.01	150.95	194.49	183.08	
	More than 24 hours	2	177.56	4.23	174.57	180.56	177.56	
Duration of seizures	Less than 5 minutes	31	181.08	7.40	163.55	194.49	181.50	X^2=1.49 P=0.475
	5 to 15 minutes	7	175.79	15.57	150.95	186.54	183.71	
	More than 15 minutes	2	186.73	4.28	183.71	189.76	186.73	
Type of fever and seizures	Simple	33	179.59	9.97	150.95	194.49	181.50	Z=1.03 P=0.311
	Complex	6	184.24	2.90	181.50	189.76	183.39	
Frequency of seizures in the last 24 hours	Once	34	180.06	9.98	150.95	194.49	182.76	Z=0.09 P=0.926
	More than once	6	182.55	2.82	178.67	186.54	182.29	
Previous history of fever and seizures in the patient	No	30	181.94	7.05	163.55	194.49	182.76	T=1.35 P=0.206
	Yes	10	175.92	13.53	150.95	190.01	182.29	
History of epilepsy in first degree relatives of the patient	No	34	179.84	9.85	150.95	194.49	182.45	Z=0.68 P=0.517
	Yes	6	183.77	4.02	178.67	189.76	183.23	
History of epilepsy in other relatives of the patient	No	34	181.14	6.90	163.55	194.49	182.45	T=0.62 P=0.562
	Yes	6	176.45	18.32	150.95	192.23	185.08	
History of fever and seizures in first degree relatives	No	33	180.24	9.65	150.95	194.49	183.08	Z=0.23 P=0.835
	Yes	7	181.36	7.87	170.16	194.37	182.76	
History of fever and seizures in other relatives of the patient	No	29	181.00	9.11	150.95	194.49	183.71	Z=0.88 P=0.385
	Yes	11	178.95	10.01	155.36	194.37	180.87	

Z: the result of the Mann-Whitney test, T: the result of the independent t-test, X^2: the result of the Kruskal-Wallis test