Introduction

Febrile seizures or febrile convulsions (FC) are the most common neurologic disorder in children 6-60 months of age. Zinc (Zn) and copper (Cu) play role as cofactors in more than 300 enzymatic activities significantly. The aim of this study was to evaluate the relationship serum levels of Zn and Cu with seizure occurrence in febrile children. In this case-control study, 270 children with 6 month to 6 years were evaluated. The patients were enrolled in three groups: a) children with febrile convulsion, b) febrile children without convulsion and c) healthy ones. After recording of all patients’ characteristics, 5 mL blood was taken from peripheral vessels at the first 12 hours of hospitalization. Absorption of all samples was read by BRAIC (Rayleigh instrument) company, WFX-130 model with calibration diagram, considering samples dilution levels. The mean of serum Zn levels in children with FC were significantly lower than other two groups. Mean serum Cu levels in children with FC and non-FC patients were significantly higher than healthy children. No meaningful differences were observed in serum levels of Zn and Cu among the girl or boy cases. This study showed significant lower serum zinc level in children with febrile seizure and meaningful higher serum copper level than control group cases. There was no significant difference in level of serum zinc and copper in term of sex.

Keywords: Children, seizure, fever, zinc, copper
Hypothalamus controls the central body temperature in normal conditions, and set within the normal range (36.5-37.5 °C). An exogenous pyrogen (external fever-inducing substance such as gram-negative bacteria lipopolysaccharide) or endogenous ones (such as interleukin-1) caused fever by acting directly on the hypothalamic thermoregulatory center and then rise body temperature by releasing epinephrine, vessels contraction (particularly peripheral vessels), finally reach a new regulation point and fever occurs (9,10).

Considering of febrile seizures’ incidence and probably their complications, high hospitalization costs, and ability to fear parents, identification of causes for the prevention are very essential. This study was evaluated the relationship serum levels of Zn and Cu with seizure occurrence and fever intensity in febrile children.

Materials and methods
In this case–control study, serum Zn and Cu levels of 270 children with febrile seizure, referring to a teaching hospital (Bu-Ali Sina, Sari, Iran), during 2 years evaluated. The study was approved by the Ethical and Research Committee of Mazandaran University of Medical Sciences (No: 88-142). Patients were 6 month to 6 years age bracket (samples number has been calculated based on previous studies sample volume & sample volume formula) (3-5). After explaining to parents and getting their consent, cases entered to study and examined by a pediatric neurology specialist to place in one of 3 groups: a) children with febrile convulsion, b) febrile children without convulsion and c) healthy ones (without fever and convulsion).

The exclusion criteria for patients in this study were including age younger than 6 months and older than 6 years, mental or cerebral retardation or sings of genetic syndrome, complex convulsion (atypical), chronic disease (heart, liver, kidney), malnutrition and situations that lead to decrease study metals levels in serum including hemolysis, dehydration, vomiting, dysentery and pneumonia.

After physical exams and measuring the body temperature to confirm the fever of case and controls, 5 mL blood was taken from peripheral vessels at the first 12 hours of hospitalization. All patients’ characteristics were recorded and under sterile conditions, samples transferred to hospital laboratory for centrifuge and isolation of serum from globule and then serum was kept at -20 °C. At the end of sampling, all samples were defreeze and diluted using 10% triton in 0.1 normal nitric acid solutions (Merck, Germany) to 1:10 level. Four standard concentrations were made for Zn and Cu.

Standard powders of Zn and Cu prepared from Merck (Germany) and atomic absorption level of standard solutions was measured by using atomic absorption method and then concentration–absorption calibration diagram was drawn. Absorption of all samples was read by the same instrument with calibration diagram, considering samples dilution levels with atomic absorption spectrophotometer made by Beijing Rayleigh Analytical Instrument Corporation (BRAIC) company, WFX-130 model (China).

Statistical analysis
Data were analyzed by SPSS16 software (Chicago, USA), independent samples t-test and ANOVA were used to compare serum levels between study groups and P value ≤0.05 was considered statistically significant.

Results
Patients demographic characteristics presented in Table 1. It was insignificantly differences between three groups in age, weight and gender.

No meaningful differences were observed in serum levels of Zn and Cu among the girl or boy cases (Table 2 and 3).

The mean of serum Zn levels in children with FC (0.43 ± 0.38 mg/l) were significantly lower than other two groups (Table 4). Also, serum Zn levels in convulsion free febrile children (0.66 ± 0.37 mg/l, respectively) had meaningful difference with healthy group patients (0.97 ± 0.15 mg/l).

Serum Cu concentrations in three study groups were reported in table 5. Mean serum Cu levels in children with FC and non-FC patients (1.6 ± 0.38 and 1.53 ± 0.76 mg/l, respectively) were significantly higher than healthy children 0.53 ± 0.24 mg/l (p value < 0.05).

Discussion
The results of the present study demonstrated children with fever convulsion had significantly lower serum Zn levels than two other groups (febrile children without
**Table 1** Demographic characteristics of the study patients

|               | FC group  | Non-FC group | Healthy group |
|---------------|-----------|--------------|---------------|
| **Gender**    |           |              |               |
| **Boys**      | 31        | 50           | 41            |
| **Girls**     | 61        | 43           | 52            |
| **Age (month)** | 26.15 ± 16.60 | 29.22 ± 20.25 | 26.57 ± 14.03 |
| **Weight (kg)**  | 11.98 ± 3.20   | 12.52 ± 3.78   | 13.64 ± 2.31   |

FC: febrile convulsion

**Table 2** Relation of serum Zn concentration by gender

|               | Zn level (mg/L) | FC group Mean ± SD | Non-FC group Mean ± SD | Healthy group Mean ± SD | p value |
|---------------|-----------------|--------------------|------------------------|-------------------------|---------|
| **Boys**      |                 | 0.58 ± 0.27        | 0.614 ± 0.29           | 0.75 ± 0.32             | 0.78    |
| **Girls**     |                 | 0.51 ± 0.29        | 0.7 ± 0.33             | 0.76 ± 0.46             | 0.37    |
| **p value**   |                 | 0.78               | 0.37                   | 0.94                    |         |

**Table 3** Relation of serum Cu concentration by gender

|               | Cu level (mg/L) | FC group Mean ± SD | Non-FC group Mean ± SD | Healthy group Mean ± SD | p value |
|---------------|-----------------|--------------------|------------------------|-------------------------|---------|
| **Boys**      |                 | 1.18 ± 0.37        | 1.24 ± 0.62            | 0.76 ± 0.36             | 0.58    |
| **Girls**     |                 | 1.11 ± 0.26        | 1.56 ± 0.4             | 0.48 ± 0.16             | 0.06    |
| **p value**   |                 | 0.58               | 0.06                   | 0.16                    |         |
convulsion and healthy children, without fever and convulsion). There was a statistically significant difference in mean serum zinc level in between febrile children without convulsion and healthy group patients. Mean serum Cu levels in children with FC and non-FC patients were significantly higher than healthy children. There were no statistically meaningful differences in the mean serum Zn and Cu levels in boys and girls.

Yılmaz and Balcı; Talebian et al. (12) Ganesh et al. (13), Amiri et al. (14) reported no significant difference in serum Z level in relation to sex, this is in agreement with result of our study. Gheini et al. (15) Vidyasagar et al. (16) and Burhangnoglu et al.; (17) found the lower mean serum Zn level in children with febrile seizure than the other children without fever. Also, Mahyar et al. (18), Saghazadeh (19) and Amiri et al. (14) reported the lower serum Zn level in children with febrile seizure compared to control group. There was a correlation between serum Zn level and febrile seizure in Margaretha et al.; study (20). They reported that lower serum Zn level related to longer the duration of seizure. Results of our study was similar to Modarresi et al. (21) and Ehsanipour et al. (22) studies who found that serum Zn level was lower in children with febrile seizure than the other two control groups (febrile with non-convulsion and healthy children).

Children with febrile seizure in our study had meaningful higher serum Cu level than control group cases. This is in agreement with Prasad et al. (23) that found serum Cu levels in children with seizures were significantly increased and opposed to Amiri et al. (14) Yilmaz and Balcı; (11) who reported no significant change in serum Cu level in cases of febrile convulsion or Gheini et al.

Table 4 Comparing of serum Zn concentration in 3 study groups

|            | FC group Mean ± SD | Non-FC group Mean ± SD | Healthy group Mean ± SD |
|------------|--------------------|------------------------|------------------------|
| Zn level (mg/l) | 0.43 ± 0.38        | 0.66 ± 0.37            | 0.97 ± 0.15            |
| p value     | 0.008 (1 and 2 groups) | 0.002 (2 and 3 groups) | 0.001 (1 and 3 groups) |

Table 5 Comparing of serum Cu concentration in 3 study groups

|            | FC group Mean ± SD | Non-FC group Mean ± SD | Healthy group Mean ± SD |
|------------|--------------------|------------------------|------------------------|
| Cu level (mg/l) | 1.16 ± 0.38        | 1.53 ± 0.76            | 0.53 ± 0.24            |
| p value     | 0.005 (1 and 2 groups) | 0.001 (2 and 3 groups) | 0.001 (1 and 3 groups) |
who found mean serum Cu level in the control group was significantly lower than that of the case group.

**Conclusion**
We observed significant lower serum Zn level in children with febrile seizure and meaningful higher serum Cu level than control group cases. There was no significant difference in level of serum Zn and Cu in term of sex.

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**Conflict of interest**
The authors declare that they have no competing interests.

**References**
1. American Academy of Pediatrics. Clinical Practice Guideline Febrile Seizures: Guideline for the Neurodiagnostic Evaluation of the Child with a Simple Febrile Seizure. Pediatrics 2011; 127: 389-94.
2. Menkes J, Sarnat H. Paroxysmal disorders. In: Menkes J, Sankar R. Child Neurology. 6th edition. Philadelphia, USA: Lippincott Williams & Wilkins, A wolters Kluwer Company 2000; 987-90.
3. Tütüncüoğlu S, Kütükçüler N, Kepe L, Coker C, Berdeli A, Tekgül H. Proinflammatory cytokines, prostaglandins and zinc in febrile convulsions. Pediatr Int 2001; 43:235-39.
4. Pisacane A, Sansone R, Impagliazzo N, Coppola A, Rolando P, D’Apuzzo A, Tregrossi, C. Iron deficiency anaemia and febrile convulsions: case-control study in children under 2 years. BMJ 1996; 313; 343.
5. Prakash O, Deepak M, Ram SU. Cerebrospinal fluid zinc, magnesium, copper and gamma- amino butyric acid levels in febrile seizures. J Pediatr Neurol 2007; 5: 39-44.
6. Anonymous, Minerals and trace elements. Available at: http://mefanet-motol.cuni.cz/articles.php?aid 610. 14 Sep, 2009.
7. Tuula E. The adverse effects of Zinc deficiency. J Orthomolecular Med 1995; 10:146-64.
8. Izumi Y, Ishii K, Akiba K, Hayashi T. Hypozincemia during fever may trigger febrile convulsion. Med Hypotheses 1990; 32:77-80.
9. Dinarello CA. Infection, fever, and exogenous and endogenous pyrogens: some concepts have changed. J Endotoxin Res 2004; 10:201-22.
10. Gatti S, Vezzani A, Bartfai T. Mechanisms of fever and febrile seizures: putative role of the interleukin-1 system. Academic Press 2002; 169-88.
11. Yilmaz D, Balci O. Serum selenium and copper levels in children with simple febrile seizure and viral infections. Pediatr Practice Res 2014; 2:41-5.
12. Talebian A, Vakili Z, Talar SA, Kazemi M, Mousavi GA. Assessment of the relation between serum zinc and magnesium levels in children with febrile convulsion. Iran J Pathol 2009; 4:157-60.
13. Ganesh R, Janakinamam L. Serum Zinc levels in children with simple febrile seizure. Clin Pediatr (Phila) 2008; 47:164-6.
14. Amiri M, Farzin L, Moassesi ME, Sajadi F. Serum Trace Element Levels in Febrile Convulsion. Biol Tr Elems 2010; 135: 38-44.
15. Gheini S, Kiani A, Sedighehi M, Hojabri K. Assessment of serum zinc, selenium and copper in simple febrile convulsions in children aged 6 to 60 months in Mohammad Kermanshahi Hospital in 2012. J Kermanshah Univ Med Sci 2015;19:16-23 (Persian).
16. Vidyasagar V, Venugopal BL, Darshan MS. Comparison of serum zinc level in patients with simple febrile seizure versus acute febrile illness. Sch J App Med Sci 2015; 3:2210-19.
17. Burhanoglu M, Tutuncuglu S, Coker C, Tekgüll H, Ozgür T. Hypozincæmia in febrile convulsion. Eur J Pediatr 1996; 155:498-501.
18. Mahyar A, Pahlavan A, Varasteh-Nijad A. Serum zinc level in children with febrile seizure. Acta Medica Iranica 2008; 46:477-80.
19. Saghazadeh A, Mahmoudi M, Meysamie A, Gharedaghi M, Zamponi GW, Rezaei N. Possible role of trace elements in epilepsy and febrile seizures: a meta-analysis. Nutr Rev 2015;73:760-79.
20. Gattoo I, Harish R, Quyoom Hussain S. correlation of serum zinc level with simple febrile seizures: A Hospital based Prospective Case Control Study. Int J Pediatr 2015;3:509-15.
21. Rabbani MW, Ali I, Latif HZ, Basit A, Rabbani MA. Serum zinc level in children presenting with febrile seizures. Pak J Med Sci 2013; 29:1008-11.
22. Ehsanipour F, Talebi-Taher M, Harandi NV, Kani K. Serum zinc level with febrile convulsion and its comparison with that of control group. Iran J Pediatr 2009; 19: 65-8.
23. Prasad R, Singh A, Das B, Upadhyay R, Singh T, Mishra O. Cerebrospinal fluid and serum zinc, copper, magnesium and calcium levels in children with Idiopathic seizure. J Clin Diagn Res 2009; 3:1841-6.