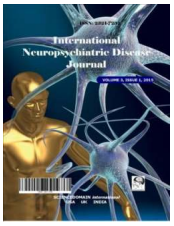

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Can Serum Levels of C-Reactive Protein (CRP), Interleukin-6 and Copeptin Discriminate between Simple and Complex Febrile Seizures?

Osama Abdel Salam[1*], Sanaa El Sadek[2], Mohamed Abdel-Moneim[3] and Zakaria Mohamed Ahmed[4]

[1]Department of Neurology, Faculty of Medicine, Mansoura University, Mansoura 35516, Egypt.
[2]Department of Pediatric, Faculty of Medicine for Girls, Al-Azhar University, Egypt.
[3]Department of Neurology, Faculty of Medicine, New Domiatta, Al-Azhar University, Egypt.
[4]Department of Neurology, Faculty of Medicine, Al-Azhar University, Egypt.

Authors' contributions

This work was carried out in collaboration between all authors. Author OAS designed the study and wrote the protocol. Author SES preformed the statistical analysis, collecting patients and examined them. Author MAM managed the literature search and wrote the first draft of the manuscript with assistance from author ZMA. All authors read and approved the final manuscript.

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ABSTRACT

Objectives: To evaluate serum levels of C-reactive protein (CRP), Interleukin-6 (IL-6) and copeptin in children with febrile seizure (FS) and their ability to discriminate between simple (SFS) and complex FS (XFS).
Patients and Methods: The study included 80 febrile children; 40 did not develop febrile seizure (FC), 29 developed SFS and 11 developed XFS. The study also included 10 healthy children as negative control (NC). Clinical evaluation included full history taking, general examination and neurological examination to evaluate patients' general conditions and to confirm inclusion criteria. On admission; body temperature was measured and a venous blood sample was obtained for

**Corresponding author: E-mail: osama.neuro2003@gmail.com;*

determination of complete blood count (CBC.) and ELISA estimation of serum CRP, IL-6 and copeptin.
Results: Male-to-female ratio was 2.64:1 and frequency of family history of FS was 17.5%. At admission; body temperature was significantly higher in febrile patients with significantly higher temperature in FS patients than in FC patients. Haemoglobin (Hb.) concentration of febrile patients was significantly lower than NC children with significantly lower Hb. Conc. in FS patients compared to FC patients. Serum CRP, IL-6 and copeptin levels and TLC were significantly higher in febrile patients compared to NC children and in FS patients compared to FC patients. Receiver operating characteristic (ROC) curve analysis defined high serum copeptin, IL-6, CRP, at admission body temperature, low Hb. Conc. and high total leucocytic count (TLC) as predictors for FS, in decreasing order of significance. Regression analysis defined high serum copeptin and IL-6 as the persistently significant predictors for FS among febrile patients and XFS among FS patients, respectively.
Conclusion: FS are associated with significantly altered immune and neuroendocrinal responses to infection. Extent of induced alterations correlates with severity of infection. Elevated serum levels of copeptin and IL-6 could discriminate febrile children susceptible to develop seizure. Elevated serum IL-6 could discriminate patients liable to develop XFS.

Keywords: Febrile seizures; copeptin; IL-6; CRP; hemoglobin concentration.

1. INTRODUCTION

Febrile seizures (FS) are the most common seizures of childhood with common family history [1]. Febrile seizure was observed predominantly in children below age of two years and simple FS was the commonest variety. However, recurrence of FS was common and significantly associated with the first episode of FS at the age of one year or below [2].

Febrile seizures may be simple (SFS) which is defined as single episode of generalized seizures occurring during the same febrile event, lasting less than 10 min and have a benign prognosis in almost all cases. Simple febrile seizure (SFS) plus is defined as SFS occurring as more than one convulsive episode in 24 h and also have benign prognosis. Complex FS is defined as focal semiology, lasting more than 10 min, occurring more than one episode during the same febrile event and need more detailed evaluation. Febrile status epilepticus is the most severe type of complex FS even its morbidity and mortality are extremely low [3].

Multiple studies provided various assumptions for risk factors associated with the development of FS such as decreased serum ferritin concentration and low iron status [4]. Increased total oxidant level and decreased total anti-oxidant level resulting in increased oxidative stress associated with FS patients may increase the risk of recurrent FS [5]. Melatonin levels were lower in pediatric patients prone to either febrile or afebrile seizures than in healthy children and increased during seizures [6]. Long-term effect on energy metabolism via histone methylation was suggested as an underlying mechanism for FS development [7].

Peripheral inflammation appears to work synergistically with hyperthermia to potentiate seizures and to exacerbate seizure-induced immune responses [8]. Certain alleles, genotypes, and haplotypes in tissue growth factor-β genes [9], IL-1β [10] and IL-4 [11] were over represented in patients with FS and could predispose individuals to this disease.

Arginine vasopressin (AVP), produced by hypothalamic neurons, is stored and released from the posterior pituitary gland following different stimuli especially change in plasma osmolality and stress [12]. Reliable measurement of AVP is hindered by several factors; its short half-life in serum, its instability in withdrawn blood samples and over 90% of AVP is tightly bound to platelets [13].

Copeptin, a 39-aminoacid glycopeptide, is a C-terminal part of the precursor pre-pro-vasopressin. Activation of the AVP system stimulates copeptin secretion into the circulation from the posterior pituitary gland in equimolar amounts with AVP. Therefore, copeptin directly reflects AVP concentration and can be used as a surrogate biomarker of AVP secretion [14]. The American Academy of Pediatrics practice parameter on Febrile Seizures states that EEG should not be a part of routine investigation after a simple febrile seizure in neurologically normal children due to its lack of ability in predicting recurrence risk or future epilepsy [15].

1.1 Aim of the Study

The current prospective comparative study aimed to evaluate serum levels of C-reactive protein (CRP), interleukin-6 (IL-6) and copeptin in children with febrile seizure (FS) and their ability to discriminate between simple and complex FS.

2. PATIENTS AND METHODS

The current study was conducted at Departments of Neurology, Pediatrics and Clinical Pathology at Saudi German Hospital, KSA; from Jan 2014 till Oct 2015. The study protocol was approved by The Local Ethical Committee, parents of enrolled patients signed written fully informed consent for study participation and undergoing the assigned investigations.

The study include 40 children presented to emergency department (ED) with fever and developed seizures. Patients with febrile seizure (FS) were categorized as having simple FS or complex FS (FS group). Simple FS was defined as a short (below 15 min) generalized seizure, not recurring within 24 h, that occurs during a febrile illness not resulting from an acute disease of the nervous system in a child aged between 6 months and 5 years, with no neurologic deficits and no previous afebrile seizures [16]. Patients missed any of diagnostic criteria of simple FS (SFS) were considered to have complex FS (XFS).

Exclusion criteria included neonates younger than one month of age and children with central nervous system infections, seizures due to hypoxic-ischaemic encephalopathy, neurocutaneous disorders and inborn error of metabolism because these disorders may cause prolonged or uncontrolled seizure, loss of consciousness and a poor general condition. Also, other co-morbid ill children or those who are not alert were also excluded of the study.

The study also included another forty, age- and sex-matched; children presented to ED with fever; but were free of any neurologic manifestations suggestive of having FS and were considered as febrile control (FC) group. Ten healthy age- and sex-matched children free of infection, immune-related disorders, and neurologic disease were enrolled as negative control (NC).

Clinical evaluation included full history taking, general examination and neurological examination to evaluate patients' general conditions and to confirm inclusion and exclusion criteria. At time of admission body temperature was determined and a 5-ml of venous blood sample was obtained under complete aseptic conditions.

Collected blood samples were divided into two parts: one was put in EDTA tube (about 1.8 mg trik EDTA/ 1 ml blood) for at once CBC estimation using cyanomethemoglobin method [17]. The other part was allowed to clot to separate serum by centrifugation, then set over pyrogen-free Eppendorf tubes and was kept at -80℃ until assayed for ELISA for serum CRP estimation (abcam Inc., San Francisco, USA) [18], IL-6 (Pelikine™ Inc., Concord, USA) [19] and copeptin (Biomérieux, Marcy-l'Étoile, France) [20].

2.1 Statistical Analysis

Got information were introduced Concerning illustration mean±SD, ranges, numbers and proportions. Results were analyzed utilizing One-way ANOVA with post-hoc Tukey HSD Test and Chi-square test (X^2 test). Sensitivity & specificity of the biomarkers given were assessed utilizing the collector operating characteristic (ROC) curve analysis judged by the area under the curve (AUC) compared versus the null hypothesis that AUC=0.05. Regression analysis (Stepwise method) was used for stratification of studied parameters as specific predictors. Statistical analysis was done by using the SPSS (Version 15, 2006). P value <0.05 is of statistically significance value.

3. RESULTS

The current study included 80 febrile children; 40 children developed FS and other patients were 40 free of FS. Male frequency was significantly ($p<0.05$) higher among FS children compared to both FC and NC children. None of NC children had family history of FS, while 9 febrile patients had family history of FS; 2 in FC and 7 in FS groups with significantly higher frequency among FS patients ($p<0.05$). At admission body temperature was higher in FS compared to NC and FC children with statistical significance ($p<0.05$). There was non-significant ($p>0.05$) difference between studied groups as regards age and body weight constitutional data, (Table 1). Eleven FS patients did not fulfill the inclusion criteria for SFS and were grouped as complex FS (XFS) group, while the remaining 29 patients had SFS.

Table 1. Patients admission data

Group parameter		NC group (n=10)	FC group (n=40)	FS group (n=40)
Age (months)		2.4±1.2	2.6±1	2.5±0.9
Gender	Males	6 (60%)	23 (57.5%)	29 (72.5%)
	Females	4 (40%)	17 (42.5%)	11 (27.5%)
	M:F	1.5:1	1.35:1	2.64:1*†
Body weight (kg)		12.8±2.4	13.2±2	13±1.8
Positive family history of FS		0	2 (5%)	7 (17.5%)†
At admission body temperature (℃)		37.2±0.1	38.6±0.55 *†	38.9±0.57*†

*Data are presented as mean±SD, Numbers and ratios; Percentages are in parenthesis; NC: Negative control; FC: Febrile control; FS: Febrile seizure; *: Significant difference between FS and FC groups versus NC groups; †: Significant difference between FS and FC groups*

Estimated laboratory parameters were significantly ($p<0.05$) disturbed in febrile patients contrasted with control children. Mean TLC number was significantly ($p<0.05$) higher in FS patients especially XFS patients contrasted with FC patients. However, mean TLC in SFS patients was non-significantly ($p>0.05$) higher compared to FC patients, but was non-significantly ($p>0.05$) lower compared to XFS patients. Mean serum CRP level was significantly ($p<0.05$) higher in all FS patients compared to FC patients and was significantly ($p<0.05$) higher in XFS patients compared to SFS patients (Fig. 1). Mean serum levels of IL-6 (Fig. 1) and copeptin (Fig. 2) were significantly ($p<0.05$) higher in all FS children in relation to FC patients with statistical significance ($p<0.05$) higher levels in XFS patients compared to SFS patients. Interestingly, haemoglobin concentration (Hb. Conc.) in febrile patients was significantly ($p<0.05$) lower compared to NC children. Mean Hb. Conc. in XFS patients was significantly ($p<0.05$) lower compared to FC and SFS patients (Fig. 3). Details of levels of estimated parameters are shown in Table 2.

Evaluation of gender, at admission temperature and estimated levels of laboratory parameters as predictors for FS using ROC curve analysis defined elevated serum co-peptin, IL-6 and CRP, high at admission body temperature, low Hb. Conc. and high TLC as predictors for FS, in

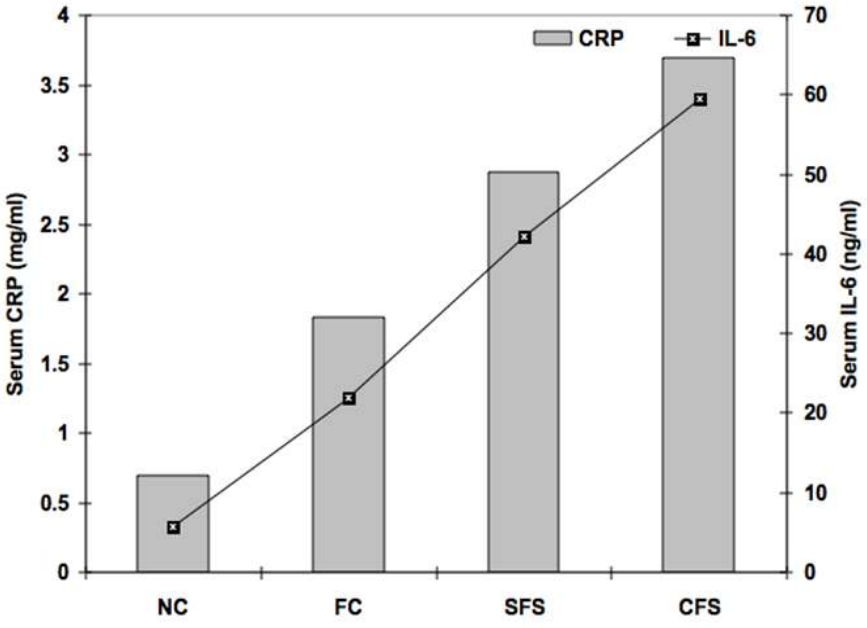

Fig. 1. Mean serum levels of CRP and IL-6 estimated in patients and controls

decreasing order of significance (Fig. 4). Moreover, ROC curve analysis defined elevated serum IL-6, high TLC, elevated serum copeptin, high at admission body temperature, high serum CRP and low Hb. Conc. as predictors for complex FS, in decreasing order of significance (Table 3, Fig. 5).

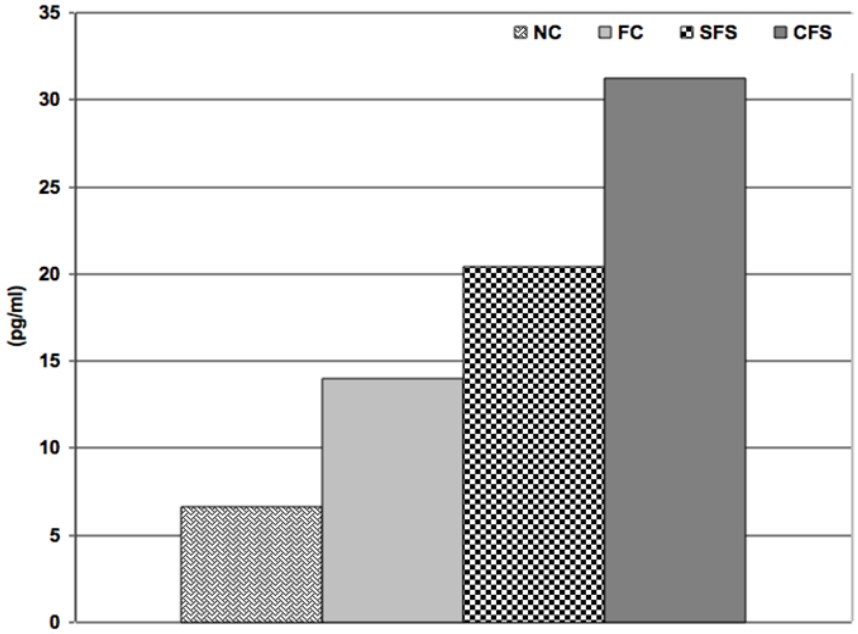

Fig. 2. Serum co-peptin estimated in studied patients comared to control children

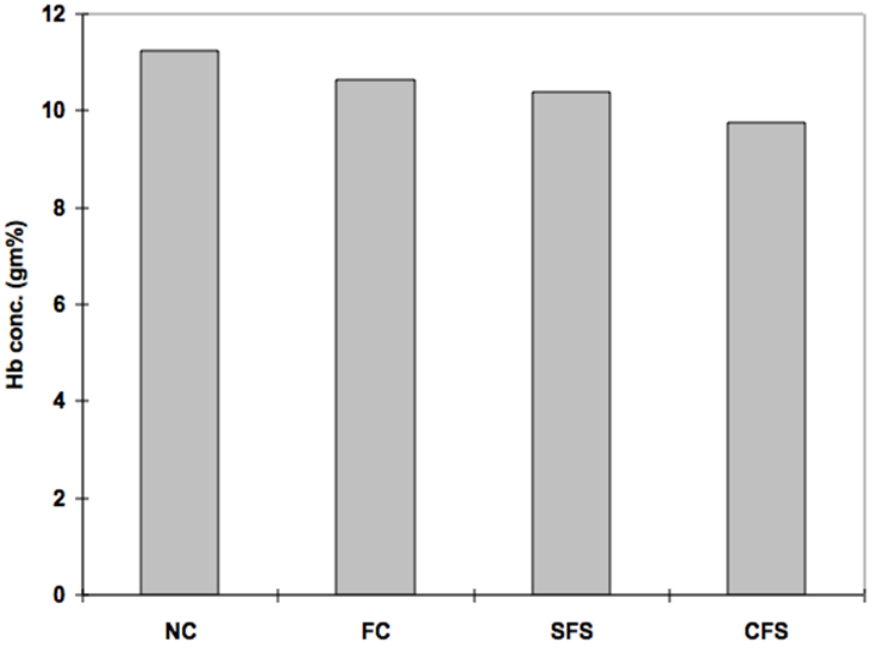

Fig. 3. Mean hemoglobin concentration estimated in patients and controls

Table 2. Estimated levels of studied parameters in FC patients and FS patients as a total and differentiated as simple and complex FS compared to NC children

Group parameter	NC group (n=10)	FC group (n=40)	FS group (n=40)		
			SFS (n=29)	CFS (n=11)	Total FS
TLC (10^3 cell/ml)	6.47±1.6*	8.59±1.49*	8.97±1.44*	10.2±1.42*†	9.31±1.52*†
CRP (mg/ml)	0.69±0.41	1.83±0.69*	2.87±1.14*†	3.7±0. 84*†‡	3.1±1.12*†
IL-6 (ng/ml)	5.77±1.6	21.9±3.68*	42.26±12.44*†	59.5±18.5*†‡	47±16.11*†
Co-peptin (pmol/ml)	6.67±3.23	14±4.89*	20.4±5.56*†	31.25±11.58*†‡	23.37 ±8.98*†
Hb. conc. (%)	11.24±0.7	10.65±0.8*	10.4±0.73*	9.75± 0.83*†‡	10.22±0.8*†

*Data are presented as mean±SD; NC: Negative control; FC: Febrile control; FS: Febrile seizure; SFS: Simple FS; XFS: Complex FS; TLC: Total leucocytic count; IL-6: Interleukin-6; Hb. Conc.: Hemoglobin concentration; *: Significant difference versus NC groups; †: Significant difference between FS and FC groups; ‡: Significant difference between SFS and CFS groups*

Table 3. ROC curve analysis for the predictors for FS and XFS among febrile patients

	FS		XFS	
	AUC	p	AUC	p
Male gender	0.575	0.248	0.690	0.067
Body temperature	0.684	0.005	0.735	0.023
Hb conc.	0.348	0.020	0.281	0.034
TLC	0.641	0.030	0.785	0.006
Serum CRP	0.794	0.0009	0.732	0.025
Serum IL-6	0.826	0.0005	0.785	0.006
Serum Copeptin	0.958	0.0003	0.777	0.007

AUC: Area under curve; FS: Febrile seizure; XFS: Complex FS; Hb. Conc.: Hemoglobin concentration; TLC: Total leucocytic count; CRP: C-reactive protein; IL-6: Interleukin-6

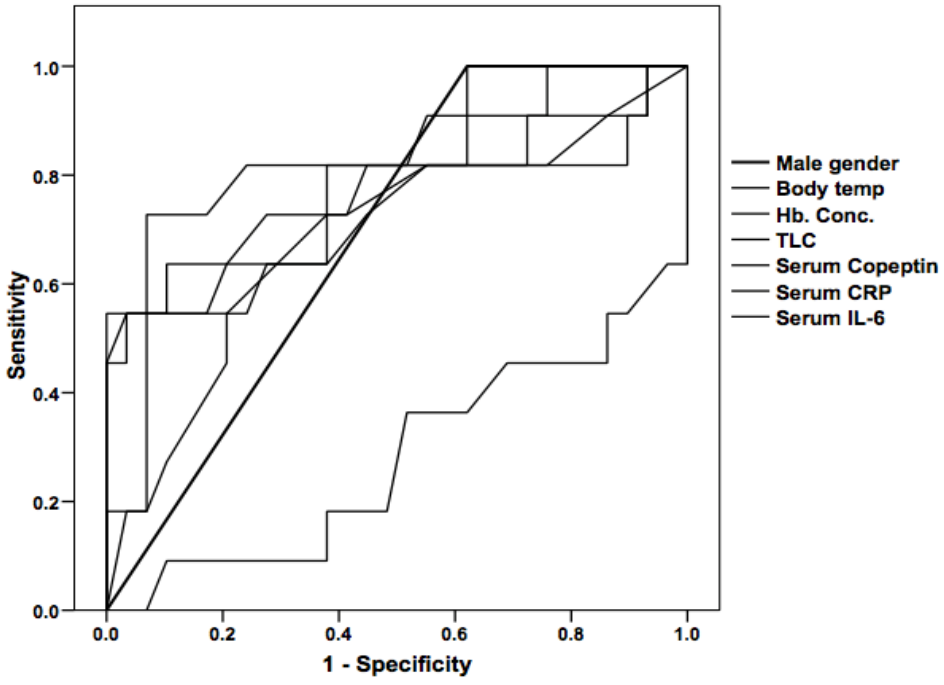

Fig. 4. ROC curve analysis of studied parameters as predictors for FS among febrile patients

Regression analysis for parameters determined by ROC analysis defined high serum copeptin is the persistently significant predictor for FS among febrile patients in three analysis models, followed by high TLC in two analysis models and male gender in one analysis model. Regression analysis for parameters determined by ROC analysis defined high serum IL-6 as the persistently significant predictor for XFS among febrile patients developed FS in three analysis models, followed by high serum CRP in two analysis models and copeptin in one analysis model. Details of outcome of regression analysis are shown in Table 4.

4. DISCUSSION

In our study; 40 child did not develop febrile seizure (FC), 29 febrile children (72.5%) developed simple febrile seizure (SFS) and 11 febrile children (27.5%) developed FS not fulfilling the criteria of SFS and were considered as complex FS (XFS). In line with these figures, Hesdorffer et al. [21] out of their multicenter study found 64.2% of studied febrile children had a first simple FS, 26.4% had a first complex FS that was not FS epilepticus (FSE), and 9.4% had FSE. Also, Shrestha et al. [2] detected simple and complex FS in 76.7% and 23.3% of their

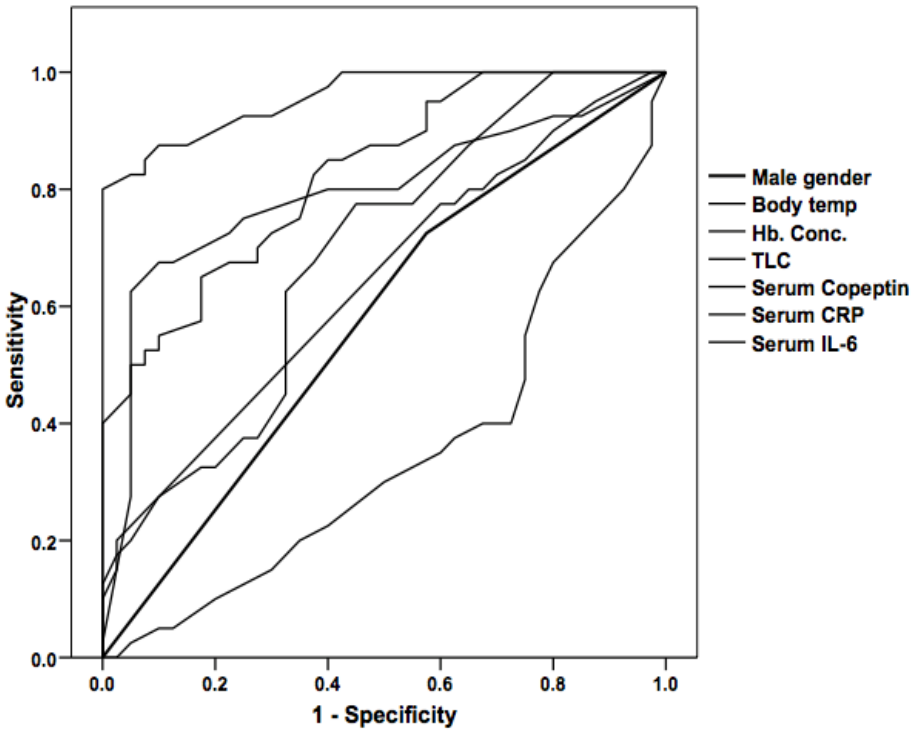

Fig. 5. ROC curve analysis of studied parameters as predictors for complex FS among FS patients

Table 4. Regression analysis models for the prediction of FS and XFS among febrile patients

Prediction for FS				Prediction of complex FS			
	Predictor	**β**	**p**		**Predictor**	**β**	**p**
Model 1	High serum copeptin	3.338	0.002	Model 1	High serum IL-6	0.577	0.0007
	High TLC	2.307	0.027		High serum CRP	0.304	0.0009
	Male gender	2.030	0.050		High serum copeptin	0.187	0.014
Model 2	High serum copeptin	0.503	0.0009	Model 2	High serum IL-6	0.622	0.0004
	High TLC	0.286	0.036		High serum CRP	0.382	0.0006
Model 3	High serum copeptin	0.548	0.0003	Model 3	High serum IL-6	0.736	0.0002

FS: Febrile seizure; XFS: Complex FS; Hb. Conc.: Hemoglobin concentration; TLC: Total leucocytic count; CRP: C-reactive protein; IL-6: Interleukin-6

studied febrile children, respectively. However, Nguefack et al. [22] reported a frequency of XFS of 41.3% of their series of febrile children.

Male gender was the predominant among the studied FS patients with a male-to-female ratio of 2.64:1. Moreover, the frequency of children had family history of FS was 17.5%. These finding emphasis on male gender and positive family history as risk factors for developing FS. Similarly, Li et al. [23] detected male/female ratio was 2.27:1 among FS patients and Elshana et al. [24] detected a male-to-female ratio of 1.57:1 and documented that male gender and 1st degree relative with FS are related to FS recurrence in a statistically significant way. Also, Hussain et al. [25] reported a frequency of male gender and family history of FS in 68% and 30%, respectively among their series of patients with FS. Recently, Sharawat et al. [26] detected a male: female ratio of 2:1, and a positive family history in 31.4% and 11.4% of 1st and 2nd degree relatives of children developed FS and concluded that male gender and family history of FS are risk factors associated with the occurrence of first episode of FS.

At admission body temperature was significantly higher in febrile patients in comparison to negative control (NC) children with significantly higher temperature in FS patients compared to FC patients. This indicated that high temperature could be considered as a risk factor for developing FS. In support of this assumption, multiple studies detected that height of peak temperature is related to FS occurrence [23,25,26] and recurrence [24] in a statistically significant way.

Interestingly, hemoglobin concentration of febrile patients was significantly lower compared to NC children with significantly lower concentration in FS patients compared to FC patients. Multiple recent studies assured such observation; wherein, Fallah et al. [27,28] reported lower hemoglobin levels, and serum iron and ferritin levels in first unprovoked afebrile seizure patients and FS patients than in healthy children. Also, Güven et al. [29] detected significantly lower serum hemoglobin levels in febrile than in healthy children. Recently, Sharawat et al. [26] documented that microcytic hypochromic anemia is a risk factor associated with the occurrence of first episode of FS and Köksal et al. [30] reported that ferritin level was significantly lower in FS group than controls and considered low plasma ferritin level as a risk factor for the development of FS.

Estimated serum levels of CRP, IL-6 and copeptin and TLC were significantly higher in febrile patients in relation to control children and in FS children in comparison to FC children. These findings highlight on the association between high levels of inflammatory markers and development of fever as a body response to underlying infection and may underlie and/or be associated with the development of seizure. Similarly, Virta et al. [31] obtained results that supported the hypothesis that the cytokine network is activated and could have a role in the pathogenesis of FS.

In support of this assumption, statistical analysis defined high levels of studied parameters as significant predictors for development of seizure among febrile patients with copeptin was the persistently significant predictor for seizure development among febrile patients in three statistical analysis models. This finding is concomitant with Stöcklin et al. [32] who reported that circulating copeptin was significantly higher in children with FS compared to febrile controls and in a multivariable regression model, seizures were the major determinant of serum copeptin independently of clinical and baseline laboratory indices with a significantly higher area under the ROC curve compared to prolactin.

The reported higher levels of copeptin in febrile patients than in NC children could be a manifestation of body neuroendocrinal response to infection because copeptin was considered as a surrogate marker for the released posterior pituitary hormone; vasopressin [12]. Increased secretion of vasopressin could be secondary to sepsis-related hypotension resulting from the direct effect of sepsis or due to concomitant fever-induced peripheral and cutaneous vasodilatation. Thus, serum copeptin correlates with severity of septic process; as documented previously by multiple studies [33,34,35,36].

Concerning differentiation between patients liable to develop simple or complex seizure, IL-6 was found to be the persistently significant predictor for development of complex seizure among feverish patients, while CRP and copeptin were significant predictors in two and one analysis models, respectively.

In line with these findings; Ichiyama et al. [37] detected significantly higher serum IL-6, IL-10, soluble tumor necrosis factor (TNF) receptor-1 and CSF IL-6 levels in patients with prolonged FS and acute encephalopathy after prolonged FS

(AEPFS) compared with control subjects and suggested that serum IL-6, IL-10, TNF-α, and CSF IL-6 are part of the regulatory system of cytokines in AEPFS.

In support of the diagnostic accuracy of IL-6; Hu et al. [38] using multivariate analysis of multiple laboratory markers found IL-6 was significantly increased in the plasma of the FS patients compared to those with severe acute encephalitis and suggested that IL-6 is activated during the acute stage of a FS. Recently, Azab et al. [39] detected significantly higher serum IL-6 levels in FS patients than febrile and healthy controls and on multivariate logistic regression analysis, high serum IL-6 level was the most significant risk factor associated with FS among studied children. Moreover, Azab et al. [39] found serum and CSF IL-6 levels were significantly higher in patients with complex FS than those with simple FS and concluded that there is a significant association between high CSF IL-6 level and susceptibility to complex FS.

The reported decreased discriminative ability of copeptin for prediction of complex FS could be attributed to the rapid decline of plasma levels of vasopressin due to depletion of its stores [40] with subsequent decreased release of copeptin, which is coreleased in an equimolar ratio with vasopressin [12].

5. CONCLUSION

Febrile seizures are associated with significantly altered immune and neuroendocrinal responses to infection. Extent of induced alterations correlates with severity of infection. Elevated serum levels of copeptin and IL-6 could discriminate febrile children susceptible to develop seizure. Elevated serum levels of IL-6 could discriminate patients liable to develop complex FS.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Mittal R. Recent advances in febrile seizures. Indian J Pediatr. 2014;81(9): 909-16.
2. Shrestha D, Dhakal AK, Shakya H, Shakya A, Shah SC, Mehata S. Clinical characteristics of children with febrile seizure. J Nepal Health Res Counc. 2014; 12(28):162-6.
3. Mastrangelo M, Midulla F, Moretti C: Actual insights into the clinical management of febrile seizures. Eur J Pediatr. 2014;173(8):977-82.
4. Zareifar S, Hosseinzadeh HR, Cohan N: Association between iron status and febrile seizures in children. Seizure. 2012;21(8): 603-5.
5. Abuhandan M, Calik M, Taskin A, Yetkin I, Selek S, Iscan A. The oxidative and antioxidative status of simple febrile seizure patients. J Pak Med Assoc. 2013; 63(5):594-7.
6. Dabak O, Altun D, Arslan M, Yaman H, Vurucu S, Yesilkaya E, Unay B. Evaluation of plasma melatonin levels in children with Afebrile and febrile seizures. Pediatr Neurol. 2016;57:51-5.
7. Heng D, Wang Z, Fan Y, Li L, Fang J, Han S, Yin J, Peng B, Liu W, He X. Long-term metabolic alterations in a febrile seizure model. Int J Neurosci. 2016;126(4):374-80.
8. Eun BL, Abraham J, Mlsna L, Kim MJ, Koh S. Lipopolysaccharide potentiates hyperthermia-induced seizures. Nutr Rev. 2015;73(11):760-79.
9. Shahrokhi A, Zare-Shahabadi A, Soltani S, Soleimani F, Vameghi R, Konjkav AR, Karimi P, Katibeh P, Vafaei M, Zoghi S, Ashrafi MR, Rezaei N. Association of TGFB, but not IL10, single nucleotide polymorphisms with febrile seizures. Seizure. 2015;29:148-52.
10. Soltani S, Zare-Shahabadi A, Shahrokhi A, Rezaei A, Zoghi S, Zamani GR, Mohammadi M, Ashrafi MR, Rezaei N. Association of interleukin-1 gene cluster and interleukin-1 receptor polymorphisms with febrile seizures. J Child Neurol. 2016; 31(6):673-7.
11. Zare-shahabadi A, Soltani S, Ashrafi MR, Shahrokhi A, Zoghi S, Pourakbari B, Zamani GR, Mohammadi M, Rezaei N. Association of IL4 single-nucleotide polymorphisms with febrile seizures. J Child Neurol. 2015;30(4):423-8.
12. Bolignano D, Cabassi A, Fiaccadori E, Ghigo E, Pasquali R, Peracino A, Peri A, Plebani M, Santoro A, Settanni F, Zoccali C. Copeptin (CTproAVP), a new tool for understanding the role of vasopressin in pathophysiology. Clin Chem Lab Med. 2014;52(10):1447-56.
13. Jarosz-Lesz A, Maruniak-Chudek I. Copeptin - stable C-terminal fragment of

pre-provasopressin as a new stress marker in newborns. Postepy Hig Med Dosw (Online). 2015;69:681-9.
14. Struck J, Morgenthaler NG, Bergmann A: Copeptin, a stable peptide derived from the vasopressin precursor, is elevated in serum of sepsis patients. Peptides. 2005; 26(12):2500-4.
15. Practice parameter: The neurodiagnostic evaluation of the child with a first simple febrile seizure. American Academy of Pediatrics. Provisional Committee on Quality Improvement, Subcommittee on Febrile Seizures. Pediatrics. 1996;97:769-72.
16. Capovilla G, Mastrangelo M, Romeo A, Vigevano F. Recommendations for the management of "febrile seizures": Ad hoc task force of LICE guidelines commission. Epilepsia. 2009;50(Suppl 1):2-6.
17. International committee for standardization in Hematology: Recommendations for hemoglobin-ometry in human blood. Br. J. Hematology, 1967;13(Suppl.1):71.
18. Ridker PM, Hennekens CH, Buring JE, Rifai N. C-reactive protein and other markers of inflammation in the prediction of cardiovascular disease in women. New England J Medicine. 2000;342(12): 836–43.
19. Gaines-Das RE, Poole S. The international standard for interleukin-6—evaluation in an international collaborative study. J Immunol Methods. 1993;160:147–53.
20. Morgenthaler NG, Struck J, Alonso C, Bergmann A. Assay for the measurement of copeptin, a stable peptide derived from the precursor of vasopressin. Clin Chem. 2006;52:112–9.
21. Hesdorffer DC, Shinnar S, Lewis DV, Moshé SL, Nordli DR Jr, Pellock JM, MacFall J, Shinnar RC, Masur D, Frank LM, Epstein LG, Litherland C, Seinfeld S, Bello JA, Chan S, Bagiella E, Sun S. FEBSTAT study team: Design and phenomenology of the FEBSTAT study. Epilepsia. 2012;53(9):1471-80.
22. Nguefack S, Ngo Kana CA, Mah E, Kuate Tegueu C, Chiabi A, Fru F, Tchokoteu PF, Mbonda E. Clinical, etiological, and therapeutic aspects of febrile convulsions. A review of 325 cases in Yaoundé. Arch Pediatr. 2010;17(5):480-5.
23. Li N, Chen YZ, Zhou KY. Changing trends and clinical characteristics of febrile seizures in children. Zhongguo Dang Dai Er Ke Za Zhi. 2015;17(2):176-9.
24. Elshana H, Özmen M, Aksu Uzunhan T, Uzunhan O, Ünüvar E, Kılıç A, Tatlı B, Aydınlı N, Çalışkan M. A tertiary care center's experience with febrile seizures: Evaluation of 632 cases. Minerva Pediatr; 2015. [Epub ahead of print]
25. Hussain S, Tarar SH; Moin Ud Din Sabir: Febrile seizrues: Demographic, clinical and etiological profile of children admitted with febrile seizures in a tertiary care hospital. J Pak Med Assoc. 2015;65(9):1008-10.
26. Sharawat IK, Singh J, Dawman L, Singh A. Evaluation of risk factors associated with first episode febrile seizure. J Clin Diagn Res. 2016;10(5):SC10-3.
27. Fallah R, Tirandazi B, Akhavan Karbasi S, Golestan M. Iron deficiency and iron deficiency anemia in children with febrile seizure. Iran J Ped Hematol Oncol. 2013; 3(1):200-3.
28. Fallah R, Tirandazi B, Ferdosian F, Fadavi N. Iron deficiency and iron deficiency anemia in children with first attack of seizure and on healthy control group: A comparative study. Iran J Child Neurol. 2014;8(3):18-23.
29. Güven AS, Icagasioglu FD, Duksal F, Sancakdar E, Alaygut D, Uysal EB, Akkar I, Oflaz MB, Kaya A. Serum adiponectin, leptin, and interleukin 6 levels as adipocytokines in children with febrile seizures: The role of adipose tissue in febrile seizures. Hum Exp Toxicol. 2015; 34(9):878-83.
30. Köksal AO, Özdemir O, Büyükkaragöz B, Karaömerlioglu M, Bulus AD. The association between plasma ferritin level and simple febrile seizures in children. J Pediatr Hematol Oncol; 2016. [Epub ahead of print]
31. Virta M, Hurme M, Helminen M: Increased plasma levels of pro- and anti-inflammatory cytokines in patients with febrile seizures. Epilepsia. 2002;43(8):920-3.
32. Stöcklin B, Fouzas S, Schillinger P, Cayir S, Skendaj R, Ramser M, Weber P, Wellmann S. Copeptin as a serum biomarker of febrile seizures. PLoS One. 2015;10(4):e0124663.
33. Morgenthaler NG, Müller B, Struck J, Bergmann A, Redl H, Christ-Crain M. Copeptin, a stable peptide of the arginine vasopressin precursor, is elevated in hemorrhagic and septic shock. Shock. 2007;28(2):219–26.
34. Seligman R, Papassotiriou J, Morgenthaler NG, Meisner M, Teixeira PJZ. Copeptin, a

novel prognostic biomarker in ventilator-associated pneumonia. Critical Care. 2008;12:(1, article R11).

35. Jiang L, Feng B, Gao D, Zhang Y. Plasma concentrations of copeptin, C-reactive protein and procalcitonin are positively correlated with APACHE II scores in patients with sepsis. Journal of International Medical Research. 2015; 43(2):188–95.
36. Battista S Battista S, Audisio U, Galluzzo C, Maggiorotto M, Masoero M, Forno D, Pizzolato E, Ulla M, Lucchiari M, Vitale A, Moiraghi C, Lupia E, Settanni F, Mengozzi G. Assessment of diagnostic and prognostic role of copeptin in the clinical setting of sepsis. Biomed Res Int. 2016; 2016:3624730.
37. Ichiyama T, Suenaga N, Kajimoto M, Tohyama J, Isumi H, Kubota M, Mori M, Furukawa S. Serum and CSF levels of cytokines in acute encephalopathy following prolonged febrile seizures. Brain Dev. 2008;30(1):47-52.
38. Hu MH, Huang GS, Wu CT, Lin JJ, Hsia SH, Wang HS, Lin KL. CHEESE study group: Analysis of plasma multiplex cytokines for children with febrile seizures and severe acute encephalitis. J Child Neurol. 2014;29(2):182-6.
39. Azab SF, Abdalhady MA, Almalky MA, Amin EK, Sarhan DT, Elhindawy EM, Allah MA, Elhewala AA, Salam MM, Hashem MI, Soliman AA, Akeel NE, Abdellatif SH, Elsamad NA, Rass AA, Arafat MS. Serum and CSF adiponectin, leptin, and interleukin 6 levels as adipocytokines in Egyptian children with febrile seizures: A cross-sectional study. Ital J Pediatr. 2016; 42:38.
40. Russell JA. Bench-to-bedside review: Vasopressin in the management of septic shock. Critical Care. 2011;15: 226-45.

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