

Pediatric Patients with Febrile Seizures: The Role of The Copeptin Assay, at Sohag University Hospital

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ABSTRACT

Background: Recognizing febrile seizures in children might be challenging due to the absence of clear postictal symptoms. In healthy children, Von Willebrand factor and copeptin levels in the blood are normal but significantly elevated after febrile seizures in young children.

Objective: To examine copeptin's utility as a serum biomarker for the early detection of febrile seizures in children.

Patients and methods: Quantitative analysis of serum copeptin levels was carried out using a sandwich enzyme-linked immunosorbent assay. A total of 90 children participated in the study, 30 each in groups 1 (children with febrile seizures), 2 (children without febrile seizures), and 3 (healthy controls).

Results: Serum copeptin levels were found to differ considerably among the groups investigated, with group I (children with febrile seizures) showing significantly greater levels than the other groups ($P < 0.001$). Serum copeptin value of ≥ 8.775 pmol/L was the most accurate threshold for predicting febrile convulsion. In a ROC study, the area under the curve was 0.986, showing good accuracy. The cutoff value was found to have a sensitivity of 93.3% and a specificity of 91.7%. Overall, the accuracy of the forecast was determined to be 92.2%, with a positive predictive value (PPV) of 84.9%, and a negative predictive value (NPV) of 96.5%.

Conclusion: Discovery of serum copeptin as a biomarker for febrile convulsions is promising. Copeptin is a postictal biomarker that shows promise for use in the emergency department, particularly in instances with equivocal clinical histories and presentations, where it might help in diagnosis and management.

Keywords: Copeptin, Febrile seizures, Pediatric fever.

INTRODUCTION

Febrile seizures (FS) are a kind of convulsion that affects 2%-5% of children aged 6 months to 6 years. Febrile seizures may seem like other fever symptoms, such as shivering or dizziness, making diagnosis challenging. Febrile seizure is a major challenge in pediatric practice because of its high incidence in young children and its tendency to recur. In recent years, there has been more awareness about the potential complications of febrile seizures and management of this condition. The Japanese Society of Child Neurology and the American Academy of Pediatrics (AAP) both released revised guidelines for the assessment and treatment of febrile seizures in 2011 and 2015, respectively. Due to the absence of an objective biomarker, post-fever diagnosis of FS may be challenging^(1,15,16).

Some possible biomarkers for use in the diagnosis or prognosis of FS have been identified thanks to recent developments in medical technology, most notably in molecular biology. Serum and cerebral fluid lactic acid levels were considerably raised in children with FS, as revealed by Imuekemhe *et al.*⁽²⁾. Arginine-vasopressin (AVP), a hormone produced in the pituitary gland, has a role in the body's thermoregulatory response to high temperatures and seizures^(3,13). However, the diagnostic use of AVP is restricted by its volatility in peripheral blood. Since the AVP precursor's C-terminal portion is stable in serum and plasma at room temperature, quantifying copeptin may be done *ex vivo* with considerable simplicity. Copeptin is a valid indicator of AVP secretion since its serum levels are

significantly higher in children with febrile seizures^(4,5,14). This study set out to ascertain whether copeptin may be used as a biomarker in the blood of children experiencing febrile seizures.

PATIENTS AND METHODS

This cross-sectional research was conducted at the Pediatric Department at Sohag University Hospital for 8 months after receiving the necessary ethical clearance from January 2022 to August 2022. Thirty children in each of the three groups comprised the study's population. Children with febrile seizures (group 1) ranged in age from 6 months to 6 years old; all were experiencing seizures. Simple febrile seizures are characterized by their short duration, generalized convulsions, and lack of focal neurological indications. and had an abnormal temperature (38°C). Complex febrile seizures are characterized by a prolonged duration, focal characteristics during the seizure, or multiple seizures within 24 hours⁽²⁾. Patients with a history of epilepsy, neurological abnormalities, inborn metabolic errors, endocrine diseases (such as diabetes mellitus), or gastrointestinal issues (such as diarrhoea) were not eligible for inclusion in this study.

Children aged 6 months to 6 years old who arrived with a fever ($>38^{\circ}\text{C}$) owing to acute infection constituted Group 2. Central nervous system infections, preexisting neurological abnormalities, Seizures, inherited metabolic problems (such as diabetes mellitus), and endocrine diseases (such as diarrhoea) were all disqualifying conditions for this population.

Children between the ages of 6 months and 6 years old and considered to be in perfect health made up Group 3. Conditions such as recent illness within the past month, high body temperature, seizures, infections of the central nervous system, or history of neurological abnormalities, as well as congenital metabolic disorders like diabetes mellitus, endocrine disorders like hypothyroidism, or gastrointestinal disorders like diarrhea, were all disqualifying conditions.

Methods of the study

All of the research participants had extensive diagnostic procedures performed on them. First, a complete medical history was taken, including the child's age, gender, length, and frequency of their febrile seizures. The next step was a detailed neurological evaluation after carefully monitoring the patient's temperature and other vitals. Complete blood count, blood glucose, C-reactive protein, serum electrolyte (potassium, calcium, sodium and magnesium) values, and liver and kidney function tests were among the laboratory studies conducted.

Serum copeptin levels were evaluated using an ELISA (enzyme-linked immunosorbent assay) sandwich test. This experiment produced a solid-phase antibody by coating a microtiter plate with a purified anti-copeptin antibody. Samples of copeptin were introduced to the wells, at which point the copeptin antibody formed a compound with labelled horseradish peroxidase (HRP). The HRP enzyme catalyzed a colour change reaction after being washed with a TMB (3,3',5,5'-tetramethylbenzidine) substrate solution. After adding a stop solution, we observed the ensuing colour shift at 450 nm and determined that the process had ended successfully. Sample optical density (O.D.) was compared to a standard curve to quantify copeptin concentration ⁽⁵⁾.

Ethical consideration

On January 7, 2022, the Institutional Review Board (IRB) of the School of Medicine at Sohag University authorized this study (IRB no. Soh-Med-22-01-07). All the children who participated in the research did so voluntarily and after their parents gave their written approval. The trial, with the identification NCT05215366, was prospectively registered in the ClinicalTrials.gov database.

Statistical analysis

IBM SPSS® software, version 20 (IBM Corp., Armonk, NY, USA), was used for the statistical analysis. Kolmogorov-Smirnov was used to check for data normality. Quantitative data with parametric distribution were given as mean and standard deviation (SD). Frequency and percentage representations of qualitative data were provided. Non-normally distributed data were given as a median.

Two sets of qualitative data were compared using the chi-square test. The Fisher exact test was employed instead of the chi-square test if the predicted number of cells was less than five. Parametric quantitative data were compared between the two groups using the Student's t-test. This study defined statistical significance as a two-tailed p-value of less than 0.05. ANOVA test and KRUSKAL-Wallis test were used to compare the mean difference between groups. Pearson correlation test were used in Correlation between variables, Tukey test was used to compare between pairs of mean.

RESULTS

The children in this research ranged in age from 6 months to 6 years; all of them had gone to the emergency room at Sohag University Hospital because of seizures. Seizures brought on by fever, epilepsy, and other conditions were all included. However, parental permission was not acquired in five instances before the research began. There were 30 children, 18 girls and 12 boys, were put into the group with febrile seizures. Conveniently, the main control group consisted of 30 children with febrile illnesses but no seizures over the same period as the trial. In addition, 30 typically developing youngsters served as a supplementary control group. Each cohort of children was selected according to its inclusion and exclusion criteria.

Febrile convulsion (FC) patients were mostly female (60%), whereas (33.3%) of febrile control patients were females and (43.3%) of healthy control patients were females ($p = 0.111$). There was no statistically significant difference in median age between the FS and non-febrile control groups ($p = 0.122$). However, the non-febrile control group was insignificantly older (1.8 ± 0.53) than the FS group (1.5 ± 0.57) and the febrile control group (1.76 ± 0.69). Children with a positive family history of febrile seizures were not significantly different from those without such a history in the control groups ($p = 0.289$).

The preponderance of children (29, or 96.7% of the cohort) suffered from simple febrile seizures. In contrast, a small percentage of the children (1, or 3.3% of the group) exhibited complex febrile seizures..

WBC, haemoglobin, and serum salt levels differed significantly across groups. Group I (febrile seizures) stood out compared to the other groups. Serum potassium, calcium, and platelet counts were similar between groups. Group II (febrile without seizures) had significantly higher mean total leukocytic count than the other 2 groups. Haemoglobin, hematocrit, and mean corpuscular volume were decreased in febrile seizures. Ninety percent of children in group II (febrile without seizures) had increased C-reactive protein (CRP) levels, compared to 56.7% in group I. Group III (healthy controls) showed negative CRP values. The control group had CRP levels of 12 mg/l, whereas febrile seizure patients had 6 mg/l (**Table 1**).

Table 1: Comparison between the studied groups regarding laboratory data

	Group I	Group II	Group III	P –value*
	N (30)	N (30)	N (30)	
	Mean ± SD	Mean ± SD	Mean ± SD	
WBCs	12.76 ± 4.77	15.23 ± 3.05	8.56 ± 1.13	<0.001***
Hemoglobin(g/dl)	10.14 ± 1.09	11.0 ± 1.14	11.27 ± 0.71	<0.001***
HCT (%)	31.25 ± 5.21	35.4 ± 4.21	39.23 ± 3.77	<0.001***
MCV (fl)	78.21 ± 11.79	87.4 ± 13.98	95.07 ± 6.09	<0.001***
Platelet count	306.17 ± 69.36	265.7 ± 74.85	289.47 ± 55.74	0.072
Sodium(mEq/L)	134.6 ± 2.93	138.5 ± 4.93	137.2 ± 2.41	<0.001***
Potassium(mmol/L)	3.91 ± 0.39	3.68 ± 0.45	3.79 ± 0.21	0.055
Calcium(mg/dl)	1.1 ± 0.1	1.15 ± 0.14	1.13 ± 0.1	0.263
CRP (mg/dl)	6 (0-6)	12 (6 - 48)		
Positive	17 (56.7%)	27 (90%)	NA 0%	<0.001***

Group I: febrile convulsions, Group II: fever without convulsions, Group III: healthy control group,

WBCs: White blood cells , MCV: Mean corpuscular volume, HCT: Hematocrit value

* ANOVA test was used to compare the mean difference between groups.

** KRUSKAL-Wallis test was used to compare the mean difference between groups as regard WBCs and platelet count

*** p<0.001 is statistically highly significant.

There was a statistically significant difference between the studied groups regarding serum copeptin , on post hoc comparison, the difference was significant between group I and each other groups, highest level was detected in group I followed by group II then lowest level in healthy control group(Table 2)

Table 2 Comparison between the studied groups regarding serum copeptin

	Group I	Group II	Group III	p
	Mean ± SD	Mean ± SD	Mean ± SD	
Copeptin	17.3 ± 5.17	5.63 ± 1.51	0.43 ± 0.19	<0.001*
	P ₁ <0.001*	P ₂ <0.001*	P ₃ <0.001*	

*p<0.05 is statistically significant

Group I: Group with febrile convulsions, **Group II:** Group fever without convulsions **Group III:** healthy control group Tukey highest significant difference test was used **p1:** difference between group I and II **p2:** difference between group II and III **p3:** difference between group I and III

Blood copeptin levels were substantially higher in male patients with febrile convulsions. There was no statistically substantial correlation between age, weight, length, or body mass index and serum copeptin in patients with febrile convulsions. Patients with febrile convulsions were likelier to have elevated serum copeptin levels, and this association was statistically substantial. In patients experiencing febrile convulsions at home, serum copeptin was significantly correlated with temperature but not with pulse rate or respiration rate (**Table 3**).

Table 3: Correlation between serum copeptin and baseline parameter among patients with febrile convulsions

	R *	P – value
Age	-0.076	0.689
Gender	-0.411	0.024**
Weight	-0.059	0.755
Length	-0.053	0.78
BMI	0.026	0.889
Duration since presentation	-0.425	0.019**
Temperature at home	0.375	0.041**
Pulse	0.295	0.113
Respiratory rate	-0.063	0.741

* R Pearson correlation coefficient , ** p<0.05 is statistically significant

Serum copeptin correlated with hematocrit and MCV in individuals with febrile convulsions. Higher serum copeptin levels were found in individuals with poor hematocrit and MCV. Patients with febrile convulsions had a serum copeptin level that was not significantly correlated with platelet count, potassium, white blood cell count, sodium, calcium, glucose, or C-reactive protein levels (**Table 4**).

Table 4: Correlation between serum copeptin and laboratory data among patients with febrile convulsions

	R*	P – value
WBCs	0.015	0.937
Haemoglobin(g/dl)	-0.274	0.143
HCT(%)	-0.371	0.044**
MCV (FL)	-0.415	0.022**
Platelet count	-0.267	0.154
Sodium(mEq/L)	-0.166	0.38
Potassium(mmol/L)	0.218	0.247
Calcium(mg/dl)	-0.084	0.66
Glucose(mg/dl)	-0.154	0.417
CRP(mg/dl)	0.008	0.975

WBCs: White blood cells , MCV: Mean corpuscular volume, HCT: Hematocrit value, * R Pearson correlation coefficient, * * p<0.05 is statistically significant.

Serum copeptin cutoff value of ≥ 8.775 pmol/L had the maximum area under the curve (0.986) for predicting febrile convulsion (Table 5).

Table 5: Serum copeptin predicts febrile convulsions in patients

Cutoff	AUC*	Sensitivity	Specificity	PPV*	NPV*	Accuracy	P - value
≥ 8.775 pmol/L	0.986	93.3%	91.7%	84.9%	96.5%	92.2%	<0.001**

*AUC area under curve PPV positive predictive value NPV negative predictive value

**p \leq 0.001 is statistically highly significant.

DISCUSSION

Research has been conducted into several biomarkers for predicting and diagnosing febrile seizures (FS). **Imuekemhe et al.** discovered substantially higher levels of lactic acid in the blood and brain fluid of newborns with FS in researches done in 1989 and 1996 (2).

B-type natriuretic peptide (BNP) and N-terminal pro-BNP (NT-proBNP) levels are often higher in children with tonic-clonic seizures, including FS, making them another biomarker linked to the disorder. This points to the neuro-cardio-endocrine axis as a potential contributor to these events (6). However, NT-proBNP levels may also increase in children with simple febrile illnesses, limiting the value of these biomarkers in reliably identifying FS in emergencies (7).

Our research aimed to compare the serum copeptin values of children with FS to those with and without fever. **Pechmann et al.** (4) and **Stöcklin et al.** (5) observed no statistically significant difference in sex between the patients and the control group. Our research found no statistically significant differences in presentation age across groups, with the mean presentation age for febrile seizure patients being 1.5 years. **Pechmann et al.** (4) and **Stöcklin et al.** (5) found mean ages of 1.6 and 1.7 years, respectively, consistent with this conclusion.

Our research showed that the mean haemoglobin (10.14 \pm 1.09), MCV (72.41 \pm 5.1), and hematocrit (31.25 \pm 5.21) of children with febrile convulsions were considerably lesser than those of the other groups. **Sharif et al.** (8) found a higher frequency of iron deficiency anaemia in the convulsions group (45%) linked to the febrile group (22%) when convulsions were not present. **Kwak et al.** (9) found that children with

febrile seizures had substantially decreased levels of haemoglobin and MCV. Our results are at odds with those of **Pechmann et al.** (4) and **Stöcklin et al.** (5) who found no abnormalities in hematocrit levels (34.3% 1% and 37.3% 2.5%) among patients with febrile seizures.

Children with febrile seizures in our research had substantially higher white blood cell count (WBC) and C-reactive protein (CRP) levels compared to healthy controls. Still, no significant difference was identified when compared to febrile controls. **Pechmann et al.** (4) and **Stöcklin et al.** (5) found similar patterns in their research.

We found that the serum copeptin levels of children with febrile convulsions were considerably greater than those of the febrile control group (5.63 \pm 1.51 pmol/L) and the non-feverish control group (0.43 \pm 0.19 pmol/L), which was our main outcome. The outcomes are reliable with those observed by **Stöcklin et al.** (5) who discovered that the median circulating copeptin level in children with febrile convulsions was 18.9 pmol/L, substantially greater than that in febrile controls (5.6 pmol/L). Serum copeptin levels in children with FS were greater than in the febrile or non-febrile control groups (4,10).

This is consistent with the results of **Stöcklin et al.** (5) and **Salam et al.** (10) who detected no significant variation in copeptin levels between the subtypes of febrile convulsions (simple and complicated). We also found that serum copeptin levels were inversely associated with the duration from the onset of febrile seizures. The roughly 45–60-minute half-life of copeptin in the peripheral circulation may account for these results, which is consistent with the work by other authors (5,11).

LIMITATIONS

Even though we measured serum copeptin levels in children with febrile seizures, we did not look into the processes that led to this increase. Studies have shown that the hormone arginine vasopressin (AVP) has a role in temperature control and the development of febrile convulsions⁽¹²⁻¹⁴⁾.

CONCLUSION

Febrile convulsions may be diagnosed using a new and promising biomarker, serum copeptin. Therefore, copeptin may be employed as a postictal biomarker in the ER, especially when the patient's medical history and clinical presentation are equivocal, further complicating an already difficult diagnostic process.

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- **Conflict of interest:** Authors do not have any conflict of interest.
- **Data availability statement:** Data are available within the manuscript and supplementary file.
- **Author Contributions:** The authors have contributed to writing, designing, compiling and editing the final manuscript.

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