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Diagnostic Utility of Serum Copeptin Level in Children with Febrile Seizures

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ABSTRACT

Introduction: Febrile seizures are the most frequent convulsive episode in children, involving 2-5% of those between the ages of six months and five years. Arginine-vasopressin (AVP), a pituitary hormone, has been shown to play a role in response to fever and convulsions, affecting the thermoregulatory system. Copeptin's C-terminal region has been identified as a reliable predictor of AVP production. **Objective:** The aim of the current study was to assess serum copeptin's diagnostic utility in febrile seizures.

Patients and methods: A case-control study was conducted at Zagazig University's Emergency Room and Neurology Unit of Children's Hospital. A total of 46 patients were recruited and were divided into two groups; Febrile seizure group included 23 patients and febrile without seizures group included 23 patients.

Results: There is significant higher copeptin value in febrile seizures group compared to febrile control without seizures group ($P<0.05$). Copeptin in diagnose seizure patients in febrile patients revealed a sensitivity of 82.6%, a specificity of 78.3% and 80.4% accuracy at a cutoff value >75 pg/ml.

Conclusion: Serum copeptin can tell the difference between a febrile seizure and a febrile without a seizure. Serum copeptin at cut off value >75pg/ml was a good marker for detecting seizures in febrile patients. Serum copeptin performed adequately as a diagnostic tool, highlighting its potential role in the febrile seizure diagnostic algorithm.

Keywords: Serum Copeptin, Febrile Seizures, Children, Case control study, Zagazig University**.**

INTRODUCTION

Febrile seizures (FS) are seizures that occur during childhood and are not caused by a central nervous system infection. They are the most common convulsive episode in childhood and affect 2-5% of kids between the ages of six months and five **[1,2].** Although the precise etiology of febrile convulsions is unknown, it is generally accepted that a complex interaction of hereditary and environmental variables is to exist. The extracranial origin of fever in febrile convulsions and the elevated temperature that goes along with it are both typical physiological reactions to infection. Some potential mechanisms for these convulsions include the release of cytokines during fever, which results in momentary aberrant brain electrical activity **[3-5].** It has been demonstrated that the pituitary hormone arginine-vasopressin (AVP) contributes to the thermoregulatory response to fever and convulsions. The C-terminal region of copeptin has been found to be a reliable indication of AVP synthesis despite the fact that AVP is unstable in peripheral blood and therefore inappropriate for diagnostic usage **[1].** Copeptin can be used as a substitute for arginine vasopressin to indicate an overactive arginine vasopressin system because it is a more durable molecule in plasma, and the kidneys help to partially eliminate it **[6,7].** The aim of the current study was to assess serum copeptin's diagnostic utility in febrile seizures.

PATIENTS AND METHODS

A case-control study was conducted at Zagazig University's Emergency Room and Neurology Unit of Children's Hospital. A total of 46 patients were recruited and were divided into two groups: A) Febrile seizure group included 23 patients whose parents/guardians or reported a convulsive episode accompanied by a high body temperature (>38°C), without prior afebrile seizure history. B) Febrile without seizures group included 23 patients who were age and Gender matched to case group, without a prior history of febrile or afebrile seizures.

Inclusion criteria: Age ranged from six months to six years. Both genders.

Exclusion criteria: Patients aged less than 6 months or more than 6 years. Children with infections of the central nervous system. Seizures associated with hypoxic ischemic encephalopathy. Disorders of the neurocutaneous system. Metabolic inborn error.

Every patient was subjected to comprehensive **history taking and clinical examination**. **Neurological examination** included pupil, level of consciousness, motor, sensory, cranial nerves, gait, as well as Glasgow coma scale (GCS), a measurement tool for assessing consciousness in critically unwell or traumatized patients. The subsequent ratings are added and categorized: A minor brain injury is worth 13 to 15 points, a moderate brain injury is worth 9 to 12 points, and a severe brain injury is worth 3 to 8 points **[7]**.

Laboratory investigations included:

CBC (complete blood count): CBC sample was drawn from venous blood that had been thoroughly mixed and anticoagulated with ethylene diamine tetraacetic acid (EDTA). The test was carried out within 6 hours of receiving the blood specimen. The experiment was

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carried out on an automated cell counter "Sysmex XN-2000™ Hematology System" to calculate eosinophils, use (Japan's Sysmex Corporation) in conjunction involving the evaluation of peripheral blood smears stained with Leishman for a differential leucocytic count.

C-reactive protein (CRP): Quantitative determination performed by tarbidimetry on Cobas 6000 (c501). Autoanalyser (Roche Diagnostics, Germany) used detreated reagents supplied by manufacturer, according to manufacturer recommendations.

Serum electrolytes included Na, K, and Ca, and were measured by indirect poteutrometry on Cobas 8000 (ISE unit) Autoanalyser (Roche Diagnostics, Germany) using dedicated reagents supplied by manufacturer, according to manufacturer's recommendations.

Arterial blood gases were performed on Cobas 221 blood gas analyzer (Roche Diagnostics, Germany).

Test for serum copeptin: Concentrations of serum copeptin were assessed using an enzyme-linked immunosorbent test, and were quantified using ELISA technique and DL-CPP-Hu Company provided the kit. The foundation of the double antibody sandwich is a target assay that has more than two possible epitopes that the pre-coated capture antibody and the detection antibody can simultaneously recognize. This product uses Double Antibody Sandwich ELISA technique. The pre-coated antibody is an anti-Human copeptin monoclonal antibody, while the detection antibody is a biotinylated polyclonal antibody. The standard curve concentrations for the ELISAs were 1000 pg/mL, 500 pg/mL, 250 pg/mL, 125 pg/mL, 62.5 pg/mL, 31.2 pg/mL, and 15.6 pg/mL. In terms of sensitivity, the least detectable dose of copeptin is frequently lower than 5.3 pg/mL.

Standard venipuncture methods were used to collect the blood and serum was extracted, as soon as possible derived from blood cells. Samples were centrifuged for 10 minutes to remove the serum after being allowed to clot for an hour at room temperature. Samples were prepared to minimize bioactivity loss and contamination kept at -20°C. Cycles of freeze-thaw were avoided. All of the chemicals, serum references, and controls were warmed to room temperature (20 to 27°C) before the test began.

Ethical Approval:

This study was ethically approved by the Institutional Review Board of the Faculty of Medicine, Zagazig University (ZU IRB # 9690-3-7-2022). Written informed consent was obtained from all participants. This study was executed according to the code of ethics of the World Medical Association (Declaration of Helsinki) for studies on humans.

Statistical Analysis

The collected data were introduced and statistically analyzed by using the Statistical Package for Social Sciences (IBM SPSS Statistics for Windows, Version 23.0. Armonk, NY: IBM Corp. 2015). Qualitative data were defined as numbers and percentages. Chi-Square test and Fisher's exact test were used for comparison between categorical variables as appropriate. Quantitative data were tested for normality by Kolmogorov-Smirnov test. Normal distribution of variables was described as mean and standard deviation (SD), and independent sample t-test/ Mann-Whitney U test was used for comparison between groups. P value ≤ 0.05 was considered to be statistically significant.

RESULTS

There was no statistically significant difference between febrile seizures group and febrile control group, regarding to sociodemographic studied (P>0.05), except body temperature was significantly higher in seizure febrile group (**Table 1**).

Table 1: Sociodemographic characteristics of the studied groups.

	Variable	Studied groups		Test of Sig.	P-value
		Febrile Seizures group (n. 23)	Febrile control without seizures group (n. 23)		
Age (year)	Mean ± SD Median (range)	2 ± 1.4 1.6 (6 month-5 years)	2.5 ± 1.6 2 (6 months-5 years)	**U** 1.3	0.19
Sex	Females Males	11 (47.8%) 12 (52.2%)	5 (21.7%) 18 (78.3%)	**χ 2** 3.2	0.063
Body weight(kg)	Mean ± SD Median (range)	11.8 ± 3.2 11 (7-18)	13.5 ± 4.1 13 (8-26)	**U** 1.6	0.12
Body temperature (°C)	Mean ± SD Median (range)	39.2 ± 0.22 39 (38-39.7)	38.2 ± 0.16 38 (38-38.4)	**U** 17.1	0.0001*
Consanguinity	Negative Positive	21 (91.3%) 2 (8.7%)	18 (78.3%) 5 (21.7%)	**χ 2**	0.41
Duration of seizure (minute)	Mean ± SD Median (range)	3.7 ± 1.8 4 (1-10)	-----		

U: Mann-Whtiney test of significant. $\chi 2$: Chi square test of significant. P>0.05: insignificant, *P≤0.05: Significant.

Table 2 showed that there was significant higher copeptin value in febrile seizures group compared to febrile control group without seizures (P<0.05).

Table 2: Plasma level of copeptin in studied febrile seizures group and febrile control group.

Variable	Studied groups		U	P-value
	Febrile seizures group (n. 23)	Febrile control without seizures group (n. 23)		
Copeptin (pg/ml) Mean ±SD	110.8 ± 8.07	57.7 ± 3.02	3.9	0.0001*

U: Mann-Whtiney test of significant. P>0.05: insignificant, *P≤0.05: Significant.

In seizure febrile group there was significant direct relation between copeptin value and RDW, CRP ABG (P<0.05), otherwise there was no relation between other parameters (**Figure 1**).

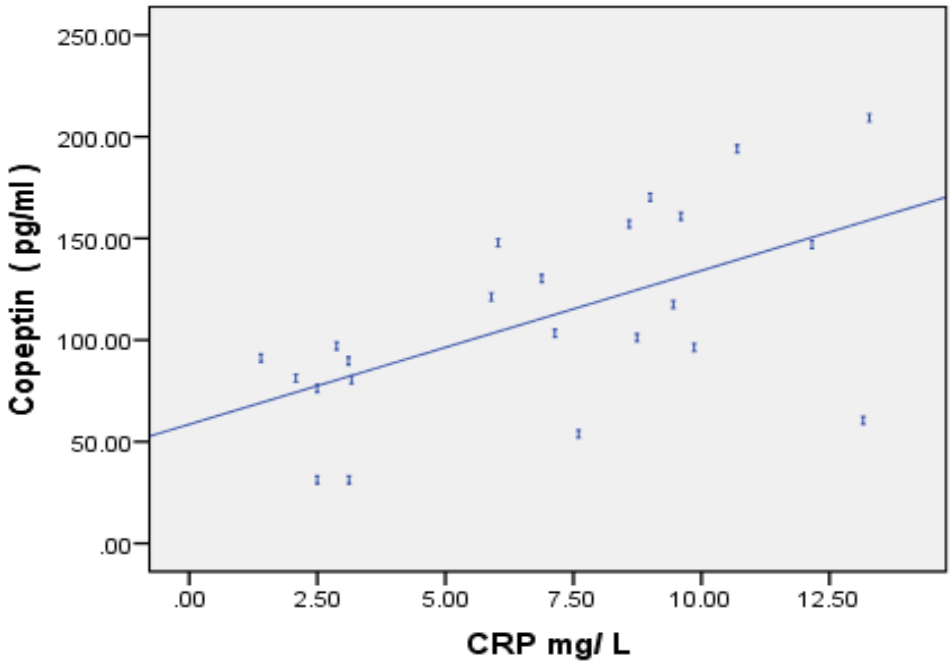

Figure 1: Scatter dot shows direct correlation between CRP and copeptin in febrile seizure group.

In febrile group: there was no relation between copeptin and age, weight, temperature, WBCs, Hb, MCV, MCH, RDW, PLT, CRP, Na, K, Ca, PH, in studied febrile group (**Figure 2**).

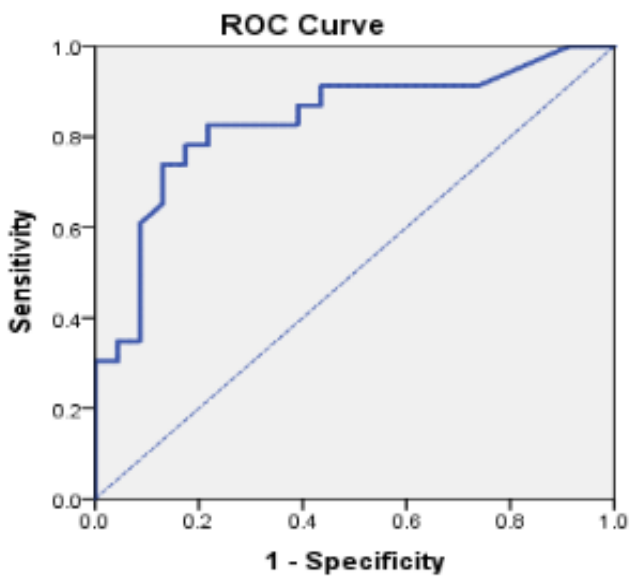

Figure 2: Receiver operating characteristic (ROC) curves of copeptin for diagnosis of febrile seizure. The area under the curve (AUC) is 0.835 with 95%CI 0.71-0.96, P<0.0001.

Table 3: Performance copeptin in diagnose seizure in febrile patients.

Cut off level (copeptin)	Sensitivity	Specificity	PPV	NPV	Accuracy
>75	82.6%	78.3%	79.2 %	81.8 %	80.4%

Copeptin in diagnose seizure patients in febrile patients revealed a sensitivity of 82.6%, a specificity of 78.3% and 80.4% accuracy at a cutoff value of > 75 (pg/ml) Explored that copeptin at cut off value >75pg/ml good marker for detecting seizure in febrile patients (**Table 3**).

DISCUSSION

Regarding demographic data of the studied groups, it was discovered that the group experiencing fever seizures and the febrile control group did not differ significantly (without Seizures) regarding to age and sex (P>0.05).

In agreement with our study, **Abd El-Moneim *et al.*** [6] in a case control study investigated the utility of copeptin idiopathic convulsions and their relationship to febrile convulsions as a biomarker. The study included 35 kids in 3 groups: 35 kids with febrile convulsions, 35 kids with idiopathic epilepsy, and 35 kids in the fever without convulsions group. The study found that neither the studied groups' age nor sex differences were statistically significant.

As regard clinical data among the studied group, it was found that there was a statistically significant difference between the febrile seizures group and the febrile control group. In contrary, there was no between the febrile seizures group and the febrile control group regarding to weight and consanguinity (P>0.05).

Also, in consistency with the current study **Heydarian *et al.*** [9] and **Evers *et al.*** [1] reported that between the febrile control group and the febrile group with convulsions, there was no appreciable weight difference.

Body temperature in the febrile seizures group compared to the febrile control group the febrile group without seizures (P<0.05). Mean duration of seizure per minute was 3.7 (SD 1.8) and range from one minute to 10 minutes.

Regarding plasma level of copeptin in studied groups, it was revealed that a significantly higher level of copeptin in febrile seizures group compared to febrile control group without seizures (P<0.05).

Abd El-Moneim *et al.* [6] showed that copeptin levels increased significantly when febrile convulsions and idiopathic epilepsy were compared with fever without convulsions. Furthermore, **Abdullah *et al.*** [10] discovered that copeptin and prolactin levels were statistically significantly higher in the febrile seizure and epileptic groups than in the fever without seizure and control groups.

Additionally, **Stöcklin *et al.*** [11] discovered that there was no difference between febrile and epileptic seizures, and that circulating copeptin levels were significantly higher in children with febrile seizures (Median 18.9 pmol/L [interquartile range 8.5-36.6]) compared to febrile controls (Median 5.6 pmol/L [interquartile range 4.1-9.4]). Moreover, in consistency with current study **Salam *et al.*** [12] showed that copeptin levels was significantly higher in febrile seizure (FS) patients than in febrile seizure control subjects without seizure patients.

Regarding the correlation between copeptin and age, weight, temperature seizure, duration, WBC, Hb, MCV, MCH, RDW, PLT, CRP, Na, K, Ca and PH, in studied febrile seizure group, it was revealed that there was significant direct relation between copeptin value and RDW, CRP and ABG. Otherwise, there is no relation between other parameters.

However, in febrile group, there was no relation between copeptin and age, weight, temperature, WBC, Hb, MCV, MCH, RDW, PLT, CRP, Na, K, Ca and PH.

In contrast to our findings, **Abdullah *et al.*** [10,13,14] found a significant correlation between the serum copeptin level and the amount of time since the febrile seizure event.

To evaluate the serum's ability to serve as a diagnostic tool, ROC curve analysis was done copeptin to discriminate febrile seizure, the current study showed that at a cutoff value of copeptin >75 pg/ml revealed a sensitivity of 82.6%, a specificity of 78.3%% and 80.4%

accuracy. The area under the curve (AUC) is 0.835 with (95%CI: 0.71-0.96), P<0.0001).

Consequently, the current study showed that copeptin at cut off value > 75pg/ml was a good marker for detecting seizure in febrile patients.

In agreement with our study **Abd El-Moneim *et al.*** [6] showed that serum Copeptin produced results for differentiating between convulsive fever and non-convulsive fever with a sensitivity of 90% and a specificity of 60%. Additionally, Idiopathic epilepsy and febrile convulsions showed a sensitivity of 86% and a specificity of 54% for serum copeptin.

Also, **Abdullah *et al.*** [10] showed that 90% sensitivity and 60% specificity of serum prolactin at a cut-off point>20.8 ng/ml for the an episode of febrile seizures is anticipated, while serum copeptin had 97% sensitivity and 70% specificity at a cut-off point>304 pg/ml. In contrast to prolactin, copeptin showed a greater overall ability to distinguish between children experiencing febrile seizures and controls (AUC 0.853, 97% vs. 0.757, 90%; p 0.001).

The current study was being a single center study, having a small sample size, and relatively short follow up period. Future studies may examine whether copeptin can differentiate between different whether the degree of the copeptin rise is related to clinical outcomes or epileptic syndromes, specifically the risk of developing epilepsy and the recurrence of febrile seizures. Further comparative it will take more extensive research to verify our findings and to identify risk factors of febrile seizure.

CONCLUSION

A novel and promising biomarker for febrile convulsions is serum copeptin. According to the current study, serum copeptin significantly distinguish between both febrile seizures and seizure-free febrile episodes. Serum copeptin had a passable diagnostic performance, underscoring its potential contribution to the diagnostic algorithm for febrile seizures.

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