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Serum Calcium Levels, Erythrocyte Indices, and Associated Factors in Children with Febrile Seizures

Febril Konvülziyonlu Çocuklarda Serum Kalsiyum Düzeyleri, Eritrosit İndeksleri ve İlişkili Faktörler

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ABSTRACT

Febrile seizures (FS) are the most common seizures in childhood. In most studies, iron deficiency was associated with FS, whereas serum calcium (Ca) levels were contradictory. This study evaluated the relationships of erythrocyte indices, serum Ca levels, and related factors among children with FS. Prospectively, patients aged six months to five years were included in the complex FS (n=23), simple FS (n=22) and febrile without seizure groups (n=25). Patients with central nervous system infection, previous afebrile seizures, or chronic drug use were excluded. Total and ionized Ca, inorganic phosphate, vitamin D levels, erythrocyte count and indices, hemoglobin, hematocrit, pH, bicarbonate and lactate levels in blood were examined. The cutoff values of variables discriminating between the FS and control groups were established through receiver operating characteristic analysis. In the complex FS group, Ca and mean corpuscular hemoglobin concentration (MCHC) levels were lower than those in the control group (p=0.004 for both) and albumin levels were lower than those in the simple FS group (p=0.02). Ca levels were positively correlated with MCHC (r=0.252, p=0.039). In the FS group, the proportions of MCHC<34.1 g/dL, Ca<9.9 mg/dL, ionized Ca<1.27 mmol/L and pH<7.38 were more frequent (p=0.003, p=0.005, p=0.044 and p=0.035 respectively). Lower Ca and MCHC levels were associated with FS. The positive correlation between Ca and MCHC levels indicates a common pathogenetic mechanism. To our knowledge, the increased proportion of patients with low blood pH level in FS is a novel finding.

Keywords: Blood pH, Calcium, Febrile seizure, MCHC, Vitamin D

ÖZET

Febril konvülziyonlar (FK), çocukluk çağının en sık nöbetleridir. Çoğu çalışmada, FK ile demir eksikliği arasında ilişki bulunurken serum kalsiyum (Ca) düzeylerine ilişkin sonuçlar çelişkilidir. Bu çalışmada, eritrosit indeksleri, serum Ca düzeyleri ve bunu etkileyebilecek faktörlerin FK'daki tutulumu incelenmiştir. Prospektif olarak, altı ay-beş yaş arası hastalar komplike FK (n=23), basit FK (n=22) ve nöbet olmaksızın ateşli kontrol grubuna (n=25) alındı. Merkezi sinir sistemi enfeksiyonu, afebril nöbet öyküsü ve kronik ilaç kullanımı olanlar dışlandı. Serumda total ve iyonize Ca, inorganik fosfat, D vitamin düzeyleri, kanda eritrosit sayısı ve indeksleri, hemoglobin, hematokrit, pH, bikarbonat ve laktat düzeyleri incelendi. FK ve kontrol grubu ayrımında verilerin optimal kesme değerlerinin belirlenmesinde 'alıcı çalışma karakteristiği' analizi kullanıldı. Komplike FK grubunda Ca ve ortalama korpüsküler hemoglobin konsantrasyonu (MCHC) düzeyi kontrol grubuna göre düşük (her ikisi için p=0.004), albümin düzeyi basit FK grubuna göre düşüktü (p=0.02). Ca ve MCHC düzeyleri arasında pozitif korelasyon saptandı (r=0.252, p=0.039). FK grubunda, MCHC<34.1 g/dL, Ca<9.9 mg/dL, iyonize Ca<1.27 mmol/L ve pH<7.38 olanların oranı daha sıktı (sırasıyla p=0.003, p=0.005, p=0.044 ve p=0035). Kalsiyum ve MCHC düzeylerinin düşüklüğü ile FK arasında anlamlı ilişki saptandı. Kalsiyum ve MCHC düzeyleri arasındaki anlamlı pozitif korelasyon, bu bulguların ortak patogenetik mekanizması olabileceğini düşündürmektedir. Kan pH seviyesi düşük olanların oranındaki anlamlı artış, bildiğimiz kadarıyla FK ile ilişkili yeni bir bulgudur.

Anahtar Kelimeler: D vitamini, Febril konvülziyon, Kalsiyum, Kan pH, MCHC

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INTRODUCTION

Febrile seizures (FS) are the most common seizures in childhood, observed in 2-5% of children between 6 months and 5 years of age.[1] FSs are usually associated with viral infections. This definition excludes febrile seizures in patients who have previously experienced afebrile seizures, seizures linked to metabolic disorders, and central nervous system (CNS) infections.[2] FS is classified into two groups according to clinical features: simple and complex. Simple FS includes generalized, single seizures lasting less than 15 minutes. Febrile seizures lasting >15 minutes, with focal features, or recurrence within 24 hours are referred to as complex FS. Although most FSs end spontaneously with no long-term consequences, complex FS or those with a family history of epilepsy are at risk of developing epilepsy or afebrile seizures in the future. The complete pathophysiology of FS is unclear. However, genetic predisposition and environmental factors, such as viral infections, can trigger FSs.[2] The genetic background of FS is associated with the regulation of various processes. These include individual and familial susceptibility, immune response modulation, neuronal excitability, and interactions with exogenous agents, such as viruses.[3]

Calcium (Ca) plays a role in many physiological processes, including nerve impulse transmission.[4] There is a negative relationship between extracellular Ca and neuronal excitability.[5] More than 99% of the Ca in the body is stored in bone. Complex interactions with different actors finely regulate non-osseous Ca. Forty percent of Ca in the blood is free or ionized, fifty percent is bound to proteins, and 10 percent is ionic-bound (complexes of calcium oxalate, carbonate, and phosphate).[4] Ionized Ca is the most important element in nerve impulses and transmission.[6] Calcium is mainly bound to albumin, and albumin levels can influence ionized Ca levels. Blood pH can affect Ca-albumin complexes. Changes in serum lactate levels can also affect ionized Ca levels because they can alter pH and bicarbonate (HCO_3^-) levels.[4] Vitamin D is an important factor affecting Ca metabolism. Vitamin D deficiency decreases serum Ca levels because of decreased Ca absorption from the intestinal lumen.[7] However, studies assessing Ca levels in patients with FS have reported conflicting results. In addition to studies showing lower calcium levels than in the control group, some reported no difference in calcium levels.[6,8-11]

Serum iron and ferritin levels were also reported to be lower, and iron deficiency anemia (IDA) was more common in patients with FS.[6,12] Iron deficiency (ID) is associated with FS but not with other seizures. The mechanism underlying this association is unknown, but it may be likely due to iron's impact on immune response capacity through immune cell differentiation and growth, thus affecting cytokine activity and cell-mediated pathways.[13] IDA is a hypochromic, microcytic anemia. The evaluation of the complete blood count is one of the most important diagnostic steps, demonstrating low values of hemoglobin (Hb), hematocrit (Hct), erythrocyte count, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC), and a high value of red cell distribution width (RDW).[14]

Although some studies have evaluated the relationship of FS with iron and Ca metabolism, it is unknown whether there is a relationship between Ca metabolism and iron deficiency in FS patients. This case–control study was performed to determine the association between serum Ca levels, erythrocyte indices, and related factors and whether there is any relationship between Ca levels and erythrocyte indices in patients with FS.

METHODS

Ethical approval and study population

The prospective study was initiated after receiving approval from the local ethics committee. The study protocol was approved by the Local Ethics Committee of Karadeniz Technical University (Protocol Number: 2019/57). Written consent from each participant's parents was obtained. The study cohort included 21 patients in the simple FS (SFS) and 22 patients in the complex FS (CFS) groups. The control group comprised 25 patients with fever without seizures. FS patients aged six months to five years who presented to the emergency room were selected randomly for one year, considering seasonal distribution. Given that complex FS is less prevalent (20–35% of all FS)[15], the age and gender of the SFS group were matched to those of the CFS group. The febrile control group comprised patients who presented to the emergency room during the same period as the FS group, considering age and gender distribution in the FS groups. Those with CNS infection or a previous afebrile seizure or conditions such as electrolyte disturbances, hypoglycemia, head trauma that could lead to seizures, other systemic diseases,

serious infections such as bronchopneumonia and sepsis, and chronic drug usage were excluded from the study.

Data acquisition

Age, gender, seizure duration in FS groups, whether seizure continued or not on admission to the emergency department, seizure characteristics, recurrence within 24 hours, previous FS number, family history of FS, and epilepsy were questioned and collected using standard case report forms.

All blood samples were collected immediately after presentation to the emergency department. Erythrocyte count, Hb, Hct, MCV, MCH, MCHC, and RDW levels were studied in whole blood taken in ethylene diamine tetraacetic acid (EDTA) tubes within the first 20 minutes by cell counter; ionized Ca, pH, HCO_3^-, and lactate levels in heparinized whole blood were studied within the first 30 minutes by potentiometric-photometric method; total Ca, inorganic phosphate (Pi), and aspartate transaminase (AST) levels were studied in serum within the first 1 hour by spectrophotometric method; and vitamin D (25-OH vitamin D3) levels in EDTA plasma kept at -20 ^oC for one week were studied by high-performance liquid chromatography. Based on laboratory reference values, the lower limits were determined as 8.8 mg/dL for total Ca, 1.15 mmol/L for ionized Ca, 35 g/L for albumin, 7.35 for pH, and 20 µg/L for vitamin D. The upper limit levels were 4.5 mg/dL for Pi and 17 mg/dL for lactate. Hb<11 g/dL was considered low, given the typical range for this age group.

Statistical analysis

Continuous data were expressed as mean±standard deviation (SD), and categorical data were expressed as numbers (n) and percentages. The Kolmogorov-Smirnov test was used to assess the normality of the distribution. The normally distributed data were compared using ANOVA and post-hoc Tukey or Tamhane tests, with consideration of homogeneity. The Kruskal-Wallis and Mann–Whitney U tests were used as nonparametric tests. In the Kruskal-Wallis test, p values were adjusted using the Bonferroni method for multiple-test correction. Categorical variables were evaluated using Pearson's chi-square test or Fisher's exact test, as appropriate. Depending on whether the distribution was normal, Pearson's or Spearman's correlation analysis was used to assess the relationships between continuous variables. Receiver operating characteristic (ROC) curve analysis was used to determine the cutoff values of continuous variables that discriminated between the control and FS groups, as well as the levels of sensitivity and specificity. The Youden index was used to determine the optimal cutoff value. Logistic regression analysis was performed to identify risk factors for FS. In multivariable analysis, the backward stepwise method was used to examine variables that demonstrated statistical significance in univariable analysis. The level of statistical significance was accepted as $p<0.05$.

RESULTS

General findings and characteristics of seizures

In the SFS group, 21 patients (14 males, 7 females) had a mean age of 26.2±14.3 months; in the CFS group, 22 patients (14 males, 8 females) had a mean age of 24.4±14 months; and in the febrile control group, 25 patients (13 males, 12 females) had a mean age of 26.7±16.6 months. The age and sex distributions of the patients did not show any statistically significant difference (p=0.746, p=0.555, respectively) (Table 1). The seasonal distribution was grouped into two periods, winter-spring and summer-autumn, considering that serum vitamin D levels may vary. No significant difference between the groups was observed in the seasonal distribution (p=0.314). Upper respiratory tract infections (URTIs), including tonsillitis, were the most common infections, accounting for 82.3% of cases. Other infections included acute gastroenteritis (n=6), acute otitis media (n=2), urinary tract infection (n=2), and bronchiolitis (n=1). The distribution of URTIs and other infections was similar between the FS and control groups (p=0.127, Fisher exact test).

Focal seizures were observed in two patients in the CFS group. The total number of seizures, proportions of patients with first FS, ongoing seizures at admission, and family history of FS and epilepsy were not different between the SFS and CFS groups (Table 1). In the CFS group, seizures lasting ≥5 min were more frequent (p=0.012), and the proportion of those who had seizures recurred within 24 h was 68.2% (Table 1).

Calcium levels in six patients (9%), ionized Ca levels in three (6.5%), vitamin D levels in 17 (32.7%), albumin levels in three (7.3%), pH levels in 14 patients (28.6%), and Hb levels in 21 patients (31.3%) were below normal levels, and lactate levels in 15 patients (42.9%) were higher than normal levels.

Comparison of groups

Calcium, MCHC, and albumin levels differed between the groups (Table 1). In post-hoc analyses, Ca and

MCHC values were lower in the CFS group than in the control group (p=0.004 for both, post-hoc Tukey and Mann-Whitney U test, respectively); there was no difference with the SFS group (p=0.203, p=0.398, respectively). Albumin levels were lower in the CFS group than in the SFS group (p=0.02, post-hoc Tamhane's test) (Table 1).

The distribution of patients with abnormal laboratory values did not show a significant difference between the groups (p>0.05 for all).

Table 1. Comparison of data between the simple and complex FS and control groups

Variables (mean ± SD)	Control (n = 25)	SFS Group (n = 21)	CFS Group (n = 22)	p-value
Comparison of continuous variables				
Age (months)	26.7±16.6	26.2±14.3	24.4±14	0.746[a]
Sex (M/F)	13/12	14/7	14/8	0.555[b]
Admission in winter-spring	10/25 (60)	8/21 (38.1)	10/22 (45.5)	0.314[b]
Ca (mg/dL)	9.8±0.6	9.6±0.4	9.3±0.5	**0.005**
Pi (mg/dL)	4.6±1	4.7±0.8	4.6 ±1.1	0.905
Vitamin D (µg/L)	26±9.5	23.1±13.7	24.5±10.3	0.610[a]
AST (U/L)	44.1±13.4	42.4±11.5	42.1±13.6	0.890
Albumin (g/L)	41.1±2.3	42.3±2.2	39.4±4	**0.011**
Erythrocyte count ($\times 10^6$/µL)	4.27±0.37	4.45±0.34	4.36±0.36	0.267
Hb (g/dL)	11.6±1.1	11.6±0.9	11.3±0.6	0.355
Hct (%)	33.8±2.5	34.7±2.4	33.9±1.7	0.520[a]
MCV (fL)	78.9±4.9	78±3.6	78.1±5.4	0.943[a]
MCH (pg)	27±2.2	26.2±1.7	25.9±2.2	0.273[a]
MCHC (g/dL)	34.1±1.1	33.5±1.2	33.2±1.1	**0.045**[a]
RDW (%)	13.4±1	13.5±1.5	13.5±1.4	0.999[a]
pH	7.4±0.04	7.35±0.08	7.36±0.08	0.157
HCO_3^- (mmol/L)	22±1.7	20.5±2.5	21.4±1.7	0.120
Ionized Ca (mmol/L)	1.34±0.19	1.27±0.07	1.24±0.08	0.133[a]
Lactate (mg/dL)	15 6	21.9±10.8	18.6±9.9	0.251
Seizure-related findings (n/total, %)				
Total FS number		2.4±1.4	1.7±1	0.063[c]
Focal seizures (n/total)		0/21 (0)	2/22 (9.1)	0.256[d]
First FS (n/total)		8/21 (38.1)	13/22 (59.1)	0.169[b]
Seizure duration ≥ 5 min		3/21 (14.3)	11/22 (50)	**0.012**[b]
FS recurrence within 24 h		0/21 (0)	15/22 (68.2)	**<0.001**[b]
Ongoing seizure on admission		1/21 (4.8)	5/22 (22.7)	0.103[d]
Family history of FS		11/21 (52.4)	12/22 (54.5)	0.887[b]
Family history of epilepsy		5/21 (23.8)	5/22 (22.7)	0.608[d]
Findings associated with cutoff points (n/total, %)	**FS Group**			
MCHC <34.1 g/dL	8/25 (32)	29/42 (69)		**0.003**[b]
Ca <9.9 mg/dL	12/25 (48)	34/42 (81)		**0.005**[b]
Ionized Ca <1.27 mmol/L	3/12 (25)	20/34 (58.8)		**0.044**[b]
pH <7.38	2/12 (16.7)	19/37 (51.4)		**0.035**[b]
Lactate ≥20 mg/dL	1/10 (10)	10/25 (40)		0.089[d]
HCO_3^- ≤22.6 mmol/L	4/12 (33.3)	22/37 (59.5)		0.115[b]

AST: aspartate transferase, Ca: calcium, CFS: complex febrile seizure, FS: febrile seizure, Hb: hemoglobin, HCO_3^-: bicarbonate, Hct: hematocrit, MCH: mean corpuscular hemoglobin, MCHC: mean corpuscular hemoglobin concentration, MCV: mean corpuscular volume, Pi: inorganic phosphate, RDW: red blood cell distribution width, SD: standard deviation, SFS: simple febrile seizure. Statistical tests: [a] Kruskal-Wallis, [b] Pearson's chi-square test, [c] Mann–Whitney U test, [d] Fisher's exact test, and one-way ANOVA for other tests.

ROC and logistic regression analysis results

In the ROC analysis, area under the ROC curve (AUC) values were statistically significant for MCHC and Ca levels, and cut-off values were calculated as MCHC<34.1 g/dL and Ca<9.9 mg/dL. AUCs were ≥0.634 in the larger values for lactate levels and the smaller values for serum pH, ionized Ca, and HCO_3^-, but they were not statistically significant (Table 2, Figure

1). The proportions of patients with MCHC<34.1 g/dL, Ca<9.9 mg/dL, ionized Ca<1.27 mmol/L and pH<7.38 were significantly more frequent in the FS group than in the control group (Table 1).

Table 2. Receiver operating characteristic (ROC) curve analysis results of laboratory data with the highest area under the curve (AUC) values for the distinction between the FS and control groups

Variables	ROC curve analysis			Statistical diagnostic measures			
	AUC (95% CI)	p-value	Optimum cut-off	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
MCHC (g/dL)	0.701 (0.569-0.833)	**0.005**	<34.1	69 (54.2-81.6)	68 (48.6-83.9)	78.4 (63.5-89.5)	56.7 (38.9-73.3)
Ca (mg/dL)	0.690 (0.554-0.826)	**0.01**	<9.9	83.3 (70.3-92.5)	52 (32.9-70.7)	74.5 (60.9-85.4)	65 (43.2-83.2)
pH	0.668 (0.504-0.831)	0.083	<7.38	51.4 (35.6-67)	83.3 (56.9-97)	90.5 (73.4-98.4)	35.7 (19.8-54.1)
Ionized Ca (mmol/L)	0.656 (0.447-0.864)	0.112	<1.27	58.8 (42.1-74.3)	75 (47.2-93.1)	87 (69.6-96.6)	39.1 (21.1-59.4)
HCO_3^- (mmol/L)	0.666 (0.481-0.850)	0.087	≤22.6	59.5 (43.4-74.2)	66.7 (38.7-88.2)	84.6 (67.8-94.9)	34.8 (17.7-55.1)
Lactate (mg/dL)	0.634 (0.441-0.827)	0.221	≥20	40 (22.5-59.5)	90 (62.8-99.4)	90.9 (65.7-99.5)	37.5 (20.1-57.4)

AUC: area under the ROC curve, Ca: calcium, CI: confidence interval, HCO_3^-: bicarbonate, MCHC: mean corpuscular hemoglobin concentration, NPV: negative predictive value, PPV: positive predictive value, ROC: receiver operating characteristic.

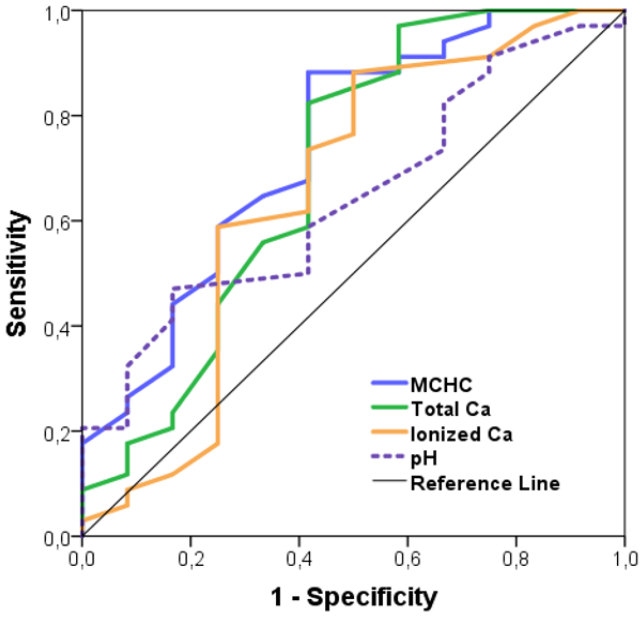

Figure 1. ROC curves of the variables with the highest AUC values among the studied parameters for distinguishing between the FS and control groups.
The ROC curves were generated by indicating that smaller values represent more positive results. AUC: area under the ROC curve, Ca: calcium, MCHC: mean corpuscular hemoglobin concentration, ROC: receiver operating characteristic.

In the logistic regression analysis summarized in Table 3, MCHC and Ca levels, MCHC<34.1 g/dL, Ca<9.9 mg/dL, and pH<7.38 showed statistical significance for FS risk in the univariable analysis. The multivariable analysis adjusted for age revealed that Ca and MCHC levels, as well as the smallness of these values, were statistically significant (Table 3).

Table 3. Logistic regression analysis of the risk factors of FS

Variables	Univariable analysis			Multivariable analysis			
				Model 1		Model 2	
	OR (95% CI)	R^2 (%)	p-value	OR (95% CI)	p-value	OR (95% CI)	p-value
Age (months)	0.993 (0.96-1.03)	0.3	0.695	0.99 (0.95-1.03)	0.523	0.99 (0.95-1.03)	0.518
MCHC (g/dL)	0.49 (0.28-0.83)	16.1	**0.009**	0.544 (0.31-0.85)	**0.032**		
MCHC <34.1 g/dL	4.74 (1.63-13.75)	16.8	**0.004**			3.31 (1.06-10.3)	**0.039**
Ca level (mg/dL)	0.24 (0.08-0.715)	15.3	**0.011**	0.262 (0.08-0.85)	**0.026**		
Ca <9.9 mg/dL	4.6 (1.53-13.82)	15	**0.006**			3.48 (1.03-11.8)	**0.045**
Ionized Ca <1.27 mg/dL	4.29 (0.98-18.7)	12.8	0.053				
Vitamin D <20 µg/L	1.73 (0.52-5.72)	2	0.368				
Albumin (g/L)	0.97 (0.8-1.17)	0.3	0.741				
pH <7.38	5.28 (1.014-27.46)	14	**0.048**				
HCO_3^- ≤22.6 mmol/L	2.93 (0.75-11.5)	7.4	0.123				

Ca: calcium, CI: confidence interval, FS: febrile seizure, HCO_3^-: bicarbonate, MCHC: mean corpuscular hemoglobin concentration, OR: odds ratio.

Correlation analysis results

There was a positive correlation between MCHC and Ca levels (r=0.252, p=0.039). MCHC levels were positively correlated with Hb levels (r=0.524, p<0.001) and negatively correlated with RDW levels (r=-0.427, p<0.001) (Figure 2).

Ca levels, in addition to MCHC, showed significant positive correlations with ionized Ca (r=0.652, p<0.001), Hb (r=0.338, p=0.005), and albumin levels (r=0.272, p=0.041) (Figure 2).

Serum HCO_3^- levels were positively correlated with both MCHC (r=0.322, p=0.024) and Ca levels (r=0.285, p=0.047). There was a negative correlation between HCO_3^- and lactate levels (r=-0.485, p=0.003) (Figure 3).

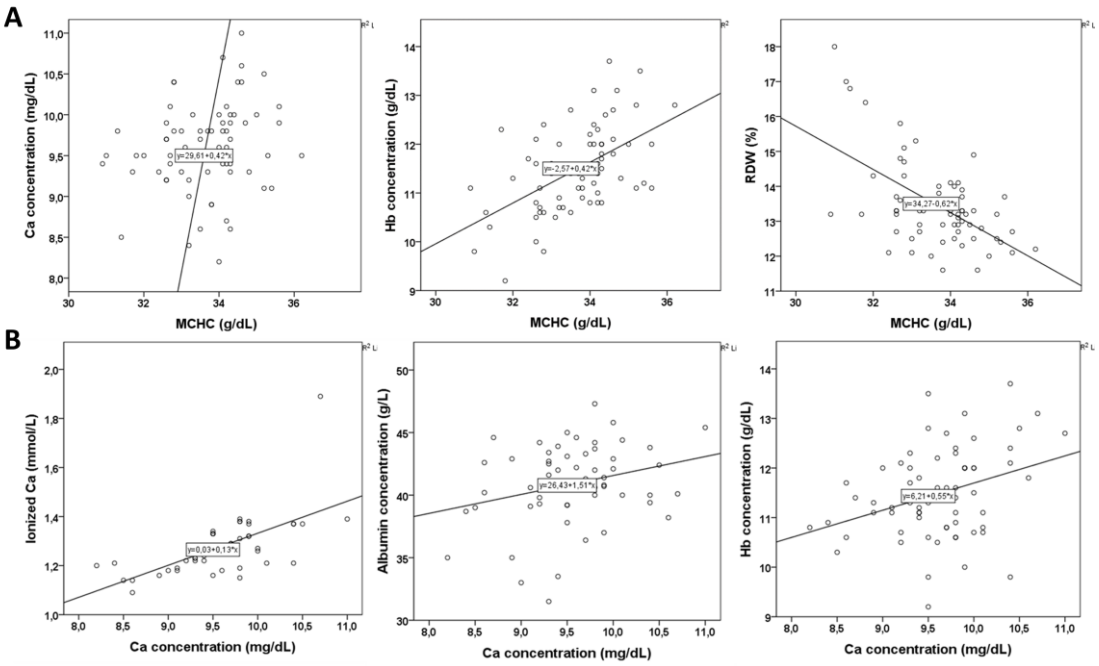

Figure 2. Correlation curves of variables significantly correlated with Ca and MCHC levels. **(A)** MCHC levels were positively correlated with Ca levels (r=0.252, p=0.039). MCHC levels were also positively correlated with Hb levels (r=0.524, p<0.001) and inversely correlated with RDW levels (r=-0.427, p<0.001). **(B)** Ca levels showed a positive correlation with ionized Ca (r=0.652, p<0.001), albumin (r=0.272, p=0.041), and Hb levels (r=0.338, p=0.005), as well as MCHC.

Ca: calcium, Hb: hemoglobin, MCHC: mean corpuscular hemoglobin concentration, RDW: red blood cell distribution width.

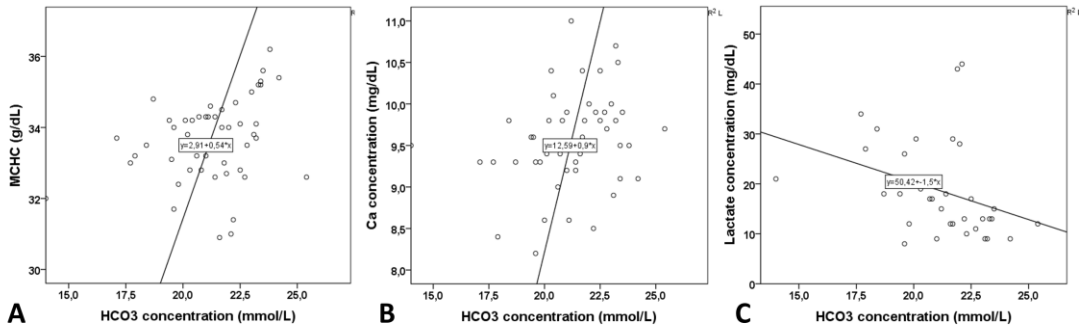

Figure 3. Serum HCO_3^- levels showed a positive correlation with both MCHC (r=0.322, p=0.024) and Ca levels (r=0.285, p=0.047), and had a negative correlation with lactate levels (r=-0.485, p=0.003).
Ca: calcium, HCO_3^-: bicarbonate, MCHC: mean corpuscular hemoglobin concentration.

DISCUSSION

In this study, the MCHC and Ca levels in the CFS group were significantly lower. Additionally, compared with the control group, a higher percentage of patients in the FS group, including all FS patients, had lower Ca and MCHC values. Calcium and MCHC below the cutoff values were independent factors associated with an increased risk of FS. The significant correlation between MCHC and Ca levels was remarkable. Although blood pH levels did not significantly differ between the groups, lower blood pH values were significantly more frequent in the FS group. In the univariable analysis, a blood pH below 7.38 increased the risk of FS by a statistically significant 5-fold.

This study's findings showed that the only CFS group had lower Ca levels than the control group, which may be why some previous studies did not find a difference in Ca levels in FS patients.[10,11] However, in most studies, Ca levels were generally reported to be lower in FS patients, even if they were within the normal range.[6,8,9,16] In our study, vitamin D levels did not show a significant difference, similar to several previous studies[17–19], suggesting that vitamin D does not play a role in the decrease in Ca levels in FS. In this study, albumin levels were significantly lower in the CFS group than in the SFS group, which may have contributed to the decrease in Ca levels. The significant positive correlation between Ca and albumin levels also supports this hypothesis. On the other hand, in another study, albumin levels were higher in the patients with their first FS episode than in the control group.[20] Although we could not find another study evaluating albumin levels, it has been frequently reported that various trace elements bound to albumin are lower in the FS group.[9,21] It is possible that these findings are related to the decrease in albumin levels. The lack of difference in AST levels excludes the association between low albumin levels and liver dysfunction. The decrease in albumin levels in this study may be related to the fact that albumin is a negative acute-phase reactant.[22] Because there is strong evidence of an association between FS and inflammation. Increased levels of proinflammatory cytokines IL-1β and IL-6 [6,21] and decreased levels of anti-inflammatory IL-10 in serum and cerebrospinal fluid[6,23] were detected in patients with FS. In addition, patients with CFS had significantly higher inflammatory indices, including neutrophil-to-lymphocyte ratio, than those with SFS.[24]

Infection-related decreases in Ca levels have been observed, especially in patients with COVID-19. Mild/moderate patients showed a significant decrease in Ca levels during the early phase of COVID-19 compared with 1 month later, whereas severe patients showed low levels during both periods. Low Ca levels were found to be associated with disease severity.[25] The FS and control groups in our study had similar infection distributions, which ruled out infection-induced changes in Ca levels. Furthermore, in another study, children with URTI had similar Ca levels as healthy controls.[26] However, there is evidence that inflammation-related proinflammatory cytokines may lower Ca levels. Experimental studies have shown that proinflammatory cytokines such as IL-1β and IL-6, upregulate calcium-sensing receptor gene (CASR) expression, causing a significant decrease in parathyroid hormone (PTH) and vitamin D levels and subsequently in Ca levels. In critically ill patients with sepsis or burn injury, hypocalcemia is associated with proinflammatory cytokine-induced upregulation of the CASR gene.[27] Although changes in CASR gene expression may also affect vitamin D levels, we found no relationship between vitamin D levels and FS. However, a population study in adults revealed a negative correlation between vitamin D levels and

recent URTI, indicating that vitamin D contributes to innate immunity.[28]

The low MCHC levels in the CFS group and the significant negative correlation between MCHC and RDW levels in this study may support the relationship between FS and IDA in previous studies. Low MCHC levels as a finding of IDA was observed in patients with FS.[29] Although different studies have reported inconsistent results on the relationship between IDA and FS, several meta-analyses and prospective studies support this relationship.[13,30,31] Iron deficiency may be a risk factor for FS even if it does not cause anemia.[32] Although some previous studies reported lower MCHC values in patients with FS[33], there were also studies in which no difference was shown or even higher MCHC was detected in patients with FS.[12,34] Hb levels may remain normal for a while in ID because there is only a decrease in iron stores in the early stages. During this period, only low ferritin and plasma transferrin saturation levels were observed.[14] This may explain why other erythrocyte indices in our study remained unchanged even though the CFS group had lower MCHC levels.

This study found a significant positive correlation between Ca and MCHC levels. Similar to MCHC, Ca levels were positively correlated with Hb levels. Another variable showing positive correlations with both Ca and MCHC levels was HCO_3^- levels, suggesting that it could explain the Ca and MCHC relationship. In support of this finding, although blood pH levels did not show a significant difference between the FS and control groups, the proportion of those with pH<7.38 was significantly higher. We may characterize this as a tendency toward metabolic acidosis in conjunction with the tendency for elevated lactate levels and decreased HCO_3^- levels observed in patients with FS in this study. Seizures can cause type-A lactic acidosis, which is caused by hypoperfusion and hypoxia.[35] Lactate elevation predicts recurrence within 24 hours in patients with simple FS.[36] However, the tendency toward acidosis associated with FS is not known. On the contrary, one study focused on the relationship between FS and respiratory alkalosis, but a statistically significant, progressive decrease in the initial alkaline pH was observed within two hours.[37] Although the precise cause of the correlation between ID and lactic acidosis is unknown, it might be important for FS. It has been reported that ID may increase lactate levels by impairing glycolysis by decreasing the levels of the iron-containing mitochondrial enzyme α-glycerophosphate oxidase.[38] Conversely, elevated lactate levels may lead to hepatic hepcidin upregulation, which could lead to iron restriction.[39] A tendency toward metabolic acidosis can also lead to hypercalciuria by decreasing calcium reabsorption in renal tubules.[40] In another study, although serum total Ca levels did not differ between the FS and control groups, a significant increase in urinary Ca excretion was detected, and hypercalciuria was observed in 23.7% of the FS patients. The lack of difference in serum Ca levels was attributed to significantly elevated PTH levels.[41] This suggests that Ca metabolism may contribute to the pathogenesis of FS. Studies evaluating ionized Ca levels in FS are limited. In one study, ionized Ca levels were significantly lower in the FS group[46], but there were no

The significant correlation between Ca and MCHC levels in this study could also be attributable to inflammation. Inflammation can lead to anemia of inflammation (AI), which was previously thought to occur only in chronic diseases. However, Hb levels can also decrease during acute infections.[42] We could not find any study evaluating AI in FS. Similar to IDA, AI also exhibits decreased reticulocyte counts, serum iron, and transferrin saturation.[43] Although erythrocyte staining indices are useful for differentiating between AI and IDA, they are insufficient. In addition to normocytic normochromic traits, AI may display microcytic hypochromic traits, such as IDA.[44] Inflammation can also lead to elevated RDW values, as increased disease activity in inflammatory diseases significantly increases RDW values.[45] Therefore, it is impossible to determine from this study whether the low MCHC values in the CFS group are caused by AI or IDA. AI patients often have normal or elevated serum ferritin levels, but distinguishing AI from IDA may be challenging because ferritin is also an acute phase reactant, and IDA is prevalent in 20-85% of AI patients.[43] Therefore, soluble transferrin receptor (sTfR) and ferritin index (sTfR/log ferritin) have been proposed as crucial markers for differentiating IDA from AI.[42,43]

In this study, ionized Ca levels were lower in both the SFS and CFS groups, but the differences from the controls were not statistically significant. However, despite the tendency toward low blood pH in patients with FS, the proportion of patients with ionized Ca<1.27 mmol/L was significantly higher. However, acidemia increases ionized Ca levels by reducing calcium binding to albumin.[40]

differences in another study.[47]A limitation of this study is the small number of patients in the groups because it was planned as a pilot study within the funding limits. The second limitation is the inability to fully match the genders of the control and study groups because the control group was selected from a smaller number of patients who came in concurrently with the FS group. However, there was no statistically significant difference in gender distribution among the groups. Third, IDA-related parameters, such as iron and ferritin, were not evaluated. The study's strengths include its prospective design, performing all blood tests immediately after emergency admission, and considering seasonal distribution in patient selection to prevent bias about serum vitamin D levels, possibly nutritional conditions, and insensible fluid losses.

CONCLUSION

This study demonstrated that both Ca and MCHC were associated with FS. The significant positive correlation between serum Ca and MCHC levels may indicate a common pathogenetic mechanism, such as inflammation. The significant correlations between HCO_3^- levels and these parameters and the tendency for lower blood pH in patients with FS imply that these results may also be related to the propensity of patients with FS for metabolic acidosis. Nevertheless, additional studies involving a larger patient population are needed to confirm these results and to clarify the pathogenetic mechanisms. Since FS regresses at approximately 5 years of age and the frequency of abnormal levels in the parameters was the same for both FS and febrile control in this study, these findings may not have an impact on prognosis. However, we hope that the findings of this study will contribute to a better understanding of the pathogenesis of FS.

Authorship contribution statement

Consept and desing: SS, AC, SCK.
Acquisition of data: NY, GE, POK.
Analysis and interpretation of data: SS, TK, AKO.
Drafting of the manuscript: SS, AC.
Critical revision of the manuscript for important intellectual content: SS, AC.
Statistical analysis: SS.

Declaration of competing interest

None of the authors have potential conflicts of interest to be disclosed.

Ethical approval

This study was approved by the Local Research Ethics Committee of Karadeniz Technical University (Protocol no: 2019/57) and performed in accordance with the ethical standards as laid down in the 1964 Declaration of Helsinki and its later amendments or comparable ethical standards.

Availability of data and materials

All data generated or analyzed during this study are included in this published article.

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