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research article

The Study of Serum Neuron-Specific Enolase and S100 calcium-binding protein B in Pediatric Diabetic Ketoacidosis

Running title: NSE and and S100 B in DKA

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What is already known on this topic?
In the literature, there are only 3 studies which have evaluated brain injury markers in children with DKA. They reported increased plasma levels of Neuron-specific enolase (NSE) and S100 calcium-binding protein B (S100 B) in patients with DKA.
Cerebral edema is the most serious and devastating event in diabetic children during the DKA period.

What does this study add?
We have unique findings in contrast to other studies as we have a simultaneous recording of two brain injury markers (NSE and S-100 protein B) and we include a diseased control group from children with type 1 diabetes mellitus without DKA. We reported that serum NSE is elevated in DKA, correlated with its severity, and could be an indicator of neuronal injury. Our study demonstrated that the severity of acidosis was the main reason responsible for the increase in NSE levels.

Abstract
Background: Neuron-specific enolase(NSE) and S100 calcium-binding protein B (S100 B) are markers of different neurological disorders.
Objectives: We aimed to investigate the relationship between brain injury markers (NSE and S-100 protein B) and severity of DKA in children.
Materials and Methods: Eighty children with DKA**,** 40 with T1DM without DKA and 40 controls were enrolled. Serum NSE and S100 B. were measured in all participants. Among children with DKA, serum NSE and S100 B were measured at 3-time points of admission, 12hr, and 24 hr after starting treatment.
Results: Children with DKA showed significantly higher serum levels of NSE at the 3-time points compared to children with T1DM without DKA and controls ($p< 0.01$) while serum S100 B levels were not different from controls and children with T1DM without DKA ($P > 0.05$). Patients with low Glasgow Coma Scale score(GCSS) and those with moderate and severe DKA had significantly higher levels of NSE at the 3-time points ($P< 0.01$for all) compared to patients with normal GCSS and those with mild DKA. No significant differences were found in serum S100 B levels according to the severity of DKA and GCS ($P > 0.05$). Younger age, lower GCSS, higher glucose and HA1c, lower pH, and bicarbonate serum levels were the risk factors associated with higher NSE in our patients.
Conclusion: Serum NSE is raised in patients with Type 1 DM and correlates with disease severity in patients with DKA. However, serum S100 B did not show any significant changes.
Keywords: Neuron-Specific Enolase- ketoacidosis- brain injury- S100 B- children.

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Introduction

Type 1 diabetes mellitus (T1DM) is the most common chronic metabolic disorder in childhood. Twenty-five to forty percent of children with the T1DM present with diabetic ketoacidosis (DKA) at the time of diagnosis [1]. DKA is a life-threatening complication of diabetes, which remains the leading cause of morbidity and mortality in children with T1DM characterized by a triad of severe hyperglycemia, metabolic acidosis and hyperketonemia [2].

Cerebral edema is the most serious and devastating event in diabetic children during the DKA period. It is relatively rare and can lead to confusion during its management [3]. A large percent of pediatric DKA patients show neurologic injury or dysfunction without manifest cerebral edema in the form of a headache, dizziness, depressed mentality and/or muscle weakness [2, 4]. Some studies reported subclinical cerebral edema in most cases of pediatric DKA. Ghetti et al. [2] and other studies reported that, in the absence of symptomatic cerebral edema, the overall cognitive functions are affected by even one episode of DKA [5,6]. Taking into consideration that most data on pediatric DKA and brain injury were derived from animal studies, small observational studies, and case reports [7, 8]. Many risk factors are involved in the development of cerebral edema [9,10, 11]. Most patients with pediatric DKA suffer an acute neurologic decompensation several hours after the start of treatment indicating a potential relationship with treatment strategies. The identification of an early marker that can be easily measured in the blood and either precedes or coincides with the clinical decompensation of diabetic children might be of value to readjust the management protocol to minimize any possible neurologic injury as far as possible[12,13]. Recent studies had shown that the estimation of neuronal derived proteins in biologic fluids (serum and cerebrospinal fluid (CSF)) can be used for the evaluation of the neurologic injury. Specifically, the proteins neuron-specific enolase (NSE), myelin basic protein (MBP), S-100b, and glial fibrillary acidic protein (GFAP) have been studied [14]. These brain injury biomarkers have been studied in traumatic brain injury. However, the studies assessing the value of these biomarkers in pediatric DKA have been limited to a few studies[15,16,17].

We aimed to investigate serum levels of NSE and S100B in children with DKA and children with type 1 diabetes mellitus without DKA and its relation to the different clinical, radiological, and laboratory variables of the studied patients.

Materials and Methods

This study was a cross-sectional case-control study. The study was conducted on 80 children with DKA. They were 55 males and 25 females. Their ages ranged from 5 to15 years with a mean age of 10.4+3.6 years. They were recruited from the pediatric intensive care unit (PICU), Al Hada and Taif military hospitals, Saudi Arabia. The study included 40 children with T1DM without DKA. They were 26 (65%) males and 14(35%) females. Their ages ranged from 4 to15 years with a mean age of 10.7 +3.2years. They were recruited from the pediatric outpatient clinic, Al Hada and Taif military hospitals, Saudi Arabia. Forty apparently healthy children of matched age and sex who visited the general pediatric outpatient clinic for purposes of immunization and/or routine health monitoring were included as controls.

Diagnosis of DKA was based on hyperglycemia (>200 mg/dL [11.1 mmol/L]) and metabolic acidosis (serum pH <7.3, bicarbonate <15 mEq/L [15 mmol/L]), with evidence of how ketoacids accumulate in the blood (measurable serum or urine ketones, increased anion gap) [18]. The diagnosis of diabetes was confirmed according to the World Health Organization diagnostic criteria [19]. Children were subdivided into (1) included children with newly diagnosed T1DM with DKA as a first presentation (no.60 [75%]) and (2) included children with a known diagnosis of T1DM and who developed DKA as a complication (no.20 [25%]). Cerebral edema was diagnosed clinically when patients developed sudden changes in the mental state associated with a severe headache, recurrence of vomiting, seizures, hypertension, inappropriate slowing of the heart rate, and/or signs of increased intracranial pressure. However, subclinical cerebral edema was defined as minor changes in mental status with or without being given mannitol therapy, but not developing into overt cerebral edema [3]. The study was conducted in the period from July 2015 to March 2018 after informed consent from all participants in the study. A local ethical committee for a research study in Al Hada and Taif military hospitals, Taif, Saudi Arabia approved the study (approval number: 53131370). The study protocol conforms to the ethical guidelines of the 1964 Declaration of Helsinki and its later amendments. Exclusion criteria were:

- Children less than one year of age.
- Preexisting medical condition apart from T1DM.
- History of recent head trauma
-Preexisting neurologic or neurodevelopmental abnormality documented by brain

CT or MRI.
- Other known complications of type T1DM (e.g., neuropathy, retinopathy, and nephropathy).
- Hypoglycemic attacks.
- Administration of insulin or intravenous fluids before enrollment.
- T1DM with a hyperosmolar hyperglycemic state.
The CONSORT flow diagram of DKA patients is shown in Figure (1).
All patients were subjected to:
- Complete history taking with stress on age, gender, the age of onset, duration of illness, the dose of insulin, insulin regimen, proper compliance to treatment, history of any episodes of DKA and clinical manifestations of DKA.
- Thorough clinical examination included anthropometric (weight, height), vital measures (temperature, respiratory rate, heart rate, and blood pressure), mental state, assessment of conscious level using Glasgow Coma Scale (GCS) for age, and assessment of cranial nerves [20].
- Laboratory investigations included complete blood count, random blood sugar, serum electrolytes (sodium, potassium, and calcium), blood urea, serum creatinine, HbA1c, osmolality, arterial blood gases analysis (pH, PO2, PCO2, and HCO3) and urine analysis for detection of ketone bodies.
DKA ranges from mild to severe and will influence the treatment and disposition of the patient. The classification is made based on two variables; pH and HCO3. Pediatric DKA is classified as mild DKA (arterial pH < 7.3 and HCO3<15), moderate (pH < 7.2 and HCO3<10), or severe (pH < 7.1 and HCO3<5) [21].
According to the GCS score, patients with DKA were divided into (1) patients with GCS score=15 and (2) patients with GCS score<15.
All patients with DKA were treated according to the standard guidelines [22]. None of our patients received any sedation.
According to the metabolic control, children with T1DM and without DKA were divided into (1) Well controlled patients with HbA1c values <7.5and (2) Poor controlled patients with HbA1c values >9.0% [23].
- In patients with DKA, a venous blood sample of 4ml for the assay of NSE and S100B concentration were withdrawn at the time of admission before the initial saline bolus. Subsequent samples were taken at 12hr and 24hr after the start of treatment.
- In the healthy controls and children with T1DM and without DKA, only baseline blood samples were taken.
All blood samples were centrifuged and stored at - 80°C until the time of assay when dissolved and analyzed. NSE and S100B were tested individually by enzyme-linked immunosorbent assays (ELISA)(EMD Millipore, the division of Merck, Germany) based on the principle of sandwich enzyme immunoassay, according to the instruction of the manufacturer. Volumes of 220 and 60 µL were used with sensitivities of 0.19 ng/mL and 50 pg/mL, respectively. The detection limit of the assay was 0-100 ng/mL and 0.25-25 pg/mL for NSE and S100 B respectively [24].
The accuracy of serum neuron-specific enolase (NSE) measurement is paramount. Hemolysis was avoided as far as possible during the procedure. Established pre-analytical precautions were followed to ensure the proper quality of returned results. However, NSE measurements are compromised by even slight hemolysis, as it is abundant in red blood cells. We derived and validated an individualized hemolysis correction equation in an attempt to reduce the sample rejection rates and overall health care costs [25].
-Brain imaging: Brain MRI was performed in all patients with DKA, after stabilization and hemodynamic stability, to demonstrate any brain injury.
Statistical Analysis: Data analysis was carried out using the SPSS software package (version 10.0, Chicago, IL, USA). Homogeneity of the data was assessed with the Kolmogorov-Smirnov test. Data were presented as means ± standard deviation. ANOVA test was used to test the significance of means. The correlation between different variables was assessed using Pearson correlation test. The determination of risk factors that were significantly associated with increased levels of NSE was done using multiple logistic regression analysis tests. The odds ratios (ORs) and significance at 95% confidence intervals CIs) were calculated.
The relationship of NSE with GCS, PH, and HCO3 was re-evaluated by partial correlation after controlling for age, duration of diabetes, and metabolic control (well controlled/poor controlled) using Spearman's rho test. $P < 0.05$ was considered significant.

Results

A total of 176 patients with DKA was admitted to pediatric endocrinology clinics.
Of these patients, 96 were excluded due to different reasons (Figure 1).
The study was conducted on 80 children with DKA and 40 children with T1DM without DKA. The mean age of children with DKA was 10.4 +3.6 years and 68.75% of patients were males. Seventy-five percent of children with DKA were newly diagnosed T1DM and their first presentation was DKA. However, 25% had an established diagnosis of T1DM, and they developed DKA as a complication.
History of earlier episodes of DKA was reported in 11(44%) among children with DKA and established T1DM (Table 1). However, the mean age of children with T1DM and without DKA was 10.7 + 3.2years and 65 % of

patients were males. Duration of illness < 5 years was reported in 32.5% and 27.5% in children with DKA and T1DM without DKA respectively. Duration of illness ≥ 5 years was reported in 67.5% and 72.5% in children with DKA and T1DM without DKA respectively (Table 1).

The clinical manifestations of DKA among our patients were rapid acidotic breathing, acetone breath, repeated vomiting, polyuria, polydipsia, enuresis, and acute abdomen. Disturbing level of consciousness was reported in 60% of children with DKA at the time of admission as indicated by GCS score < 15. This suggests cerebral edema.

PH, serum bicarbonate level, corrected serum sodium, blood urea nitrogen, and serum creatinine was all consistent with the diagnosis of DKA. Mild and moderate DKA was reported in 31.25% while severe DKA was reported in 37.5% (Table 1).

Children with DKA showed significantly higher serum levels of NSE at the 3-time points of admission (13.9 ± 2.8 ng/ml), 12hr (27.8 ± 2.3 ng/ml) and 24 hr(36.7 ± 5.6 ng/ml) after starting treatment compared to children with T1DM without DKA (10.2 ± 2.2 ng/ml, $p < 0.01$).

Also, children with DKA showed significantly higher serum levels of NSE at the 3-time points of admission (13.9 ± 2.8 ng/ml), 12hr(27.8 ± 2.3 ng/ml), and 24 hr (36.7 ± 5.6 ng/ml) after starting treatment compared to controls(5.17 ± 1.5 ng/ml, $p < 0.01$). Meanwhile, children with T1DM without DKA showed significantly higher serum levels of NSE compared to controls (respectively, 10.2 ± 2.2 ng/ml & 5.17 ± 1.5 ng/ml, $p < 0.01$).

While, there was no significant difference between the studied groups about S100B levels, $p > 0.05$. In the DKA group, serum S-100B did not change significantly at the 3-time points of admission (53.2 ± 6.7 pg/ml), 12hr(52.4 ± 7.2 pg/ml) and 24 hr (50.6 ±7.7 pg/ml) after starting treatment ($P > 0.05$) (Table 2).

Patients with low GCS score had significantly higher serum levels of NSE at the 3-time points of admission (16.7 ± 7.4 ng/ml), 12hr (30.9 ± 4.8 ng/ml), and 24 hr (22.7 ± 7.1 ng/ml), compared to patients with normal GCS score (6.42 ± 2.9 ng/ml), (5.18 ± 2.5 ng/ml), and (7.17 ± 0.6 ng/ml) respectively, $p < 0.01$ (Table 3).

Also, patients with low GCS score had significantly higher serum levels of NSE at the 3-time points (admission, 12hr, and 24hr) compared to patients with T1DM without DKA (10.2 ± 2.2 ng/ml, $p < 0.01$) (Table 3).

Regards the duration of illness, serum levels of NSE were significantly higher among DKA patients with a duration of illness ≥ 5 years than those with duration of illness < 5 years (respectively, 11.17 ± 3.2 ng/ml & 7.96 ± 2.7 ng/ml, $P < 0.05$). Also, T1DM without DKA patients with duration of illness ≥ 5 years have significantly higher serum levels of NSE than those with a duration of illness < 5 years (respectively, 10.88 ± 3.2 ng/ml & 6.23 ± 2.3 ng/ml, $P < 0.05$).

Regards the metabolic control, serum levels of NSE were significantly higher among diabetic children without DKA and with poor control than those with well control (respectively, 12.36 ± 3.3 ng/ml & 6.37 ± 2.4 ng/ml, $P < 0.05$).

Patients with severe DKA had significantly higher serum levels of NSE at the 3-time points, (admission, 12hr, and 24hr), (19.6 ± 8.4 ng/ml), (37.7 ± 5.3 ng/ml), and (28.3 ± 9.3 ng/ml) respectively, compared to patients with moderate DKA (17.3 ± 7.8 ng/ml), (33.7 ± 5.3 ng/ml), and (25.3 ± 7.2 ng/ml) respectively, and compared to patients with mild DKA (9.12 ± 3.2 ng/ml), (6.22 ± 2.9 ng/ml), and (9.13 ± 0.9 ng/ml) respectively, $p < 0.01$. The NSE level does not significantly different from the control group at the 12th hour of the mild DKA group (respectively, 6.22 ± 2.9 ng/ml & 5.25 ± 1.2 ng/ml, $P > 0.05$) (Table 4).

Patients with low GCS score showed no significant changes in the serum levels of S100B at the 3-time points(53.7 ± 5.4 pg/ml), (44.9 ± 4.9 pg/ml), and (42.7 ± 8.4 pg/ml) respectively, compared to patients with normal GCS score (51.2 ± 4.6 pg/ml), (45.6 ± 4.5 pg/ml), and (47.8 ± 5.6 pg/ml) respectively, $P > 0.05$ (Table 5).

Also, patients with low GCS score showed no significant changes in the serum levels of S100B at the 3-time points (admission, 12hr, and 24hr) compared to patients with T1DM without DKA (51.6 ± 6.8 pg/ml) respectively, $P > 0.05$. No significant changes in the serum levels of S100B irrespective of the duration of illness among children with DKA and T1DM without DKA (Table 5).

Patients with severe DKA showed no significant changes in the serum levels of S100B at the 3-time points (50.9 ± 7.4 pg/ml), (48.2 ± 6.1 pg/ml), and (46.3 ± 6.3 pg/ml) respectively, compared to patients with moderate DKA (50.1 ± 6.2 pg/ml), (46.2 ± 6.1 pg/ml), and (43.3 ± 6.3 pg/ml) respectively, and patients with mild DKA (47.1 ± 3.7 pg/ml), (46.2 ± 4.9 pg/ml), and (46.1 ± 5.2 pg/ml) respectively, $P > 0.05$ (Table 6).

When the relations between S100B levels (at admission or at any time point) and other laboratory and demographic data were investigated, we did not find any significant correlation.

Serum levels of NSE at 24 hr after starting treatment of DKA showed significant negative correlations with age ($P = 0.0001$), GCS score ($P = 0.0001$), pH ($P = 0.02$), and bicarbonate serum level ($P = 0.04$). However, there were significant positive correlations between serum levels of NSE at 24 hr after starting treatment of DKA and serum levels of NSE at baseline ($P = 0.0001$), duration of illness($P=0.03$), random blood sugar ($P = 0.0001$) and HbA1c ($P = 0.001$) (Table 7). For determination of different risk factors associated with increased serum levels of NSE at 24 hr after starting treatment, the test of multiple regression analysis was used showing significant

association with age (P = 0.001), GCS score (P = 0.007), serum levels of random blood sugar (P = 0.008), HbA1c (%) (P = 0.03), pH value (P = 0.04), and bicarbonate serum level (P = 0.003) (Table 8).
The serum NSE level with DKA was further assessed, controlling for covariables that may potentially influence the NSE level, which included age, duration of diabetes, and metabolic control (well controlled/poor controlled). After adjustment, the serum NSE level was still independently associated with GCS, PH, and HCO3.
Regarding brain imaging of our patients, MRI brain showed no significant abnormalities.

Discussion

This study provides evidence on NSE and S100B serum levels, for fluctuation, before and over the course of treatment of pediatric DKA, and according to the severity of DKA.
NSE, a soluble protein of 45 kDa, is a glycolytic enzyme present almost exclusively in neurons and neuroendocrinal cells. NSE is also present in platelets and erythrocytes. NSE in erythrocytes is of clinical importance as mild hemolysis of only 2% may increase the serum levels of NSE by five-folds [26]. When neuronal membranes are injured, NSE and S100B can diffuse to the extracellular fluid compartment and CSF. Therefore, the estimation of those markers in the CSF may be an attractive way of assessment. Indeed, we have ethical issues which hinder the lumbar puncture and CSF collection in our patients. Measurement of serum NSE and S100B was used as evidence points towards the alteration of the BBB in certain instances of DKA and NSE and S100B can reach the serum compartment. Hence, the interpretation of results using only serum levels could be beneficial [27].
In recent literature, there are only 3 studies which have evaluated brain injury markers in children with DKA. In that study, Hamed et al. (2017) compared serum NSE levels among DKA patients without documented cerebral edema (GCS<15 & GCS=15) and healthy controls. They found DKA patients with GCS<15 had significantly higher serum NSE levels than both the DKA patients with GCS=15 and healthy controls, while the difference between DKA group with GCS=15 and healthy controls was not significant. This study indicates that serum NSE is elevated in DKA and correlated with hyperglycemia, ketosis, and acidosis. [17]. In our study, we have a simultaneous recording of 2 brain injury markers (NSE and S-100 protein B) and we include a diseased control group from children with type 1 diabetes mellitus without DKA.
Also, our study showed children with DKA had significantly higher (P< 0.05) serum levels of NSE at the 3-time points of admission, 12hr, and 24 hr after starting treatment compared to children with T1DM without DKA as well as controls. However, children with T1DM without DKA showed significantly higher serum levels of NSE (P< 0.01) compared to controls. This might suggest brain damage begins early at the cellular level in the context of T1DM without DKA and associated with cognitive impairment.
We observed for the 1st time the NSE level does not significantly different from the control group at the 12th hour of the mild DKA group. This suggests that the severity of acidosis was the main factor responsible for the increase in NSE levels.
We did not observe a significant elevation in the level of S100B in the DKA group. This finding can be attributed to a quantification error of ELISA kit that plays a role in the false negative results during the assay [28].
Çatli et al. (2018) studied NSE, S-100B and Glial Fibrillary Acidic Protein (GFAP) levels in 29 patients with DKA, 30 with T1DM, and 35 healthy children. They found S100B was significantly higher in the DKA group than the healthy control and T1DM groups, while GFAP and NSE levels were not different from controls and T1DM patients. No significant differences were found in GFAP, NSE and S100B levels according to the severity of DKA, diabetes duration and GCS [29].
Kaya et al. (2015) aimed to check the pre-treatment and post-treatment oxidant capacity, antioxidant capacity and S-100B levels in cases of DKA. They believe that long-term exposure to high blood glucose concentrations leads to an increase in the oxidative stress in patients with DKA that lead to an increase in S100B levels, which is an indicator of neuronal damage [16].
In our study, we observed for the 1st time, significantly increased the level of serum NSE in diabetic patients without DKA and without detectable CNS disorders, neuropathies, and retinopathy. Hyperglycemia-induced pericyte loss contributes to blood-brain barrier disruption[30].
Among diabetic children without DKA, serum levels of NSE were significantly higher in diabetic children with poor metabolic control than those who showed well control.
Long-standing or chronic hyperglycemia may result in a brain injury with a specific vulnerability to areas of memory and learning. The neuroanatomical changes observed in experimental models of diabetes may accurately reflect what is occurring in the clinical setting[31]. It was reported that cognitive dysfunction in T2DM appears due to permanent brain damage with significant elevations in NSE level and correlated with the level of glycemic control[32].
Linda et al. (2009) reported a disturbance in the cognitive functions in school-aged children with T1DM by repeated attacks of hyperglycemia [33]. A previous study, conducted by Antenor-Dorsey et al., who observed changes in the brain imaging in the form of increased diffusivity in the superior parietal lobule and hippocampus due to repeated attacks of hyperglycemia associated with ketosis with or without academia [34]. Also,

experimental and human studies indicate that chronic hyperglycemia associated with DM results in a brain injury with specific vulnerability to areas of memory and learning abilities. The mechanisms underlying brain injury as observed in experimental models, include; disruption of blood-brain barrier (BBB), alteration of insulin transporter and decrease in insulin receptors which are expressed in discrete neuronal populations in the CNS, reduction in the uptake of glucose into the neurons, impairment of energy metabolism and impairment of brain's capacity to generate the connections vital to memory and learning [35]. Other investigators reported raised concentrations of NSE in diabetic patients with and without overt neurologic complications [36, 37]. It is well-known that T1DM has long-term complications on cognitive functions [38]. An understanding of the nature and onset of the neurological insults associated with diabetic children is essential to prevent these complications. Among diabetic patients, high glucose levels have been associated with elevated concentrations of serum NSE. In addition to central nervous system disorders, hyperglycemia-induced pericyte loss contributes to disruption of BBB[35].

Another important finding was the elevation in serum levels of NSE during DKA and its correction after starting treatment. Our findings support the hypothesis that during the critical time-period where acute complications of DKA have been reported, the levels of NSE remain high [17]. In our study, we reported significantly higher serum levels of NSE in patients with GCS score <15 compared to patients with normal GCS score at the 3- time points. Also, we found significantly higher serum levels of NSE in patients with moderate to severe DKA compared to patients with mild DKA at the 3-time points. We observed the concentrations of NSE remain high at 24hr measurement coinciding with the initial recovery of clinical manifestations of DKA. Therefore, repeated episodes of DKA can carry the risk of progressive neuronal injury. Also, we observed some patients with normal GCS score had a significant elevation in the serum concentrations of NSE after improvement of patients' condition. This suggests neuronal injury may occur in the absence of brain edema in children with DKA. Our data were consistent with previous studies that reported evidence of neuronal injury without brain edema. Wootton-Gorges et al. reported a progressive decrease in N-acetyl aspartate/creatine ratio as evidence of permanent brain injury in a teenager with T1DM and repeated episodes of DKA without clinically apparent cerebral edema [39].

S100B is a relatively small protein, 9–14 kDa synthesized largely by glial cells and a small portion is synthesized by neurons, Schwann cells, and non-neuronal peripheral sources (cardiomyocyte, alveolar cells, chondrocyte, and adipocyte) [40].

In our study, S100B protein did not show significant changes in the DKA, T1DM, and healthy control groups. In research studies, statistical power is generally calculated with 2 main objectives. 1) It can be calculated before data collection to decide the sample size needed for the current study based on information from previous studies. 2) It can also be calculated after data analysis. When the result turns out to be non-significant, statistical power is calculated to verify whether the non-significance result is due to lack of relationship between the groups or due to the lack of statistical power[41].

Power of our study was calculated for the comparison of NSE and S100B between children with DKA, children with T1DM without DKA and controls using G power software version 3.1.2. 9. We take into consideration the mean values of NSE and S100B in the studied groups, Alpha level was kept at 0.05. Power calculated was 1.0 and 0.845 for NSE and S100B respectively. We can confirm that the non-significant result of S100B between the three groups is concrete as no difference was detected although having enough power (0.845) to detect the difference if present.

There are few controversial studies showing that S100B can be used as a marker for cerebral edema in pediatric DKA[15, 16, 42]. Experimental studies reported low levels of S100B in the DKA group. This finding was attributed to glial cell dysfunction and not glial cell loss and that S100B is not a reliable marker of neuronal injury[15].

In addition, we did not notice any significant increment in S100B during follow-up of children with DKA(12hr-24hr) to predict neuronal injury. Also, when investigating the relations between S100B levels (at admission or at any time point) and other laboratory and demographic data, we did not find any significant correlation.

It was reported that S100B levels were not raised in subclinical cerebral edema in children with DKA. However, Kaya et al. found significantly higher S100B levels in children with DKA but no cerebral edema than controls but did not find a significant difference in S100B levels before and after initiation of therapy [16].

In our study, we found the risk factors and early predictors of higher serum concentrations of NSE in children with DKA were younger age, lower GCS score, higher degrees of hyperglycemia, longer duration of illness, more acidosis and ketosis. Previous studies have established many factors are suitable predictors of higher levels of NSE during DKA, but none of these variables has been singled out as the most important determining factor [17, 43, 44]. Regarding clinical risk factors, the degree of acidosis and younger age appeared to be the greatest risk factors for alterations in cerebral structure. However, the degree of acidosis was the most important determining factor of an impaired level of consciousness in children with DKA without cerebral edema [45]. Different biomarkers reflecting inflammation (tumor necrosis factor-alpha [TNF-α], interleukin-6 [IL-6]) and cerebral dysfunction and/or possible injury (S100B, glial fibrillary acidic protein [GFAP]), as well as genetic

markers of brain injury risk in children with DKA, were studied by Nett et al. They demonstrated the potential importance of these markers in the pathophysiology of CNS dysfunction and/or possible injury in DKA [47]. Under normal conditions with an intact BBB, brain-derived proteins of different molecular weights (such as S100B and NSE) do not cross BBB [24, 45, 46]. With the disruption of BBB, blood levels of these proteins can be used as a marker for brain injury [47].

During the treatment of DKA, It was observed that the whole brain and regional BBB permeability increased in most patients [31].

Although the mechanisms underlying the increase in BBB permeability is still unclear, it is suggested that DKA can disrupt the tight endothelial junctions through inflammatory and immunologic responses [48], Furthermore, many factors as matrix metalloproteinase activity, hyperglycemia, and insulin administration are associated with increased permeability of BBB [31].

Study Limitations

We did not measure NSE in the CSF of our patients for ethical issues. CSF levels are more sensitive to CNS damage. We did not repeat MRI to detect subclinical cerebral edema at the time of diagnosis and during clinical follow-up. Also, the neurocognitive function was not assessed which is a good marker of brain dysfunction. This is explained by the fact that our study focused on determining the serum levels of NSE and S100B in children with DKA and its relationship with different clinical and laboratory variables. Lastly, we did not proceed for calculation of sample size before conducting our study.

Conclusions

Serum NSE is slightly raised in children with T1DM. This might suggest a degree of neurologic dysfunction in the absence of DKA. In our study, cerebral edema is absent in brain imaging in children with DKA, elevated NSE levels in patients with abnormal GCS and the positive correlation between NSE and severity of acidosis suggest that NSE might be a reliable marker of neuronal injury. However, S100B did not show a simultaneous increase with NSE. This can be attributed to a quantification error of ELISA kit.

To clarify subclinical brain injury related to pediatric DKA, further studies are recommended to assess neurocognitive functions.

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Figure(1): CONSORT flow diagram of patients with DKA