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Original article

Plasma high sensitivity C-reactive protein as a marker of severity in children with diabetic ketoacidosis

Background*: Diabetic ketoacidosis (DKA) is a metabolic crisis that can precipitate other life-threatening complications. Elevated plasma level of C-reactive protein (CRP) has been reported to be marker for endothelial cell dysfunction in uncomplicated, well-controlled, type 1 diabetes mellitus.* ***Objective:*** *This study was undertaken to identify the relation between Hs-CRP and the severity of DKA in children.* ***Methods:*** *This prospective study involved pediatric patients (age <15 years) who admitted to the ICU of Zagazig University Children's Hospital with DKA during the period from May 2012 to May 2013. Blood samples were drawn at presentation before initial hydration and after resolution of DKA. Routine investigations were done. Serum IL-6 and Hs-CRP levels were measured.* ***Results:*** *Thirty patients were diagnosed as having DKA, 17 patients were males (56.7%). In most patients, DKA resolved in 24 to 48 hours. Mild DKA was diagnosed in 11 (36.7%) and moderate/severe DKA was present in 19 (63.4%) patients. Highly significant statistical difference was found between both groups as regard hs-CRP and IL6 (p value for both <0.001).As regard time factor, (at admission and after 48 hs) and its effect on hs-CRP and IL6, they did not significantly affected in mild DKA (p value for both < 0.05). Nevertheless, they showed highly significant statistical difference in moderate/severe DKA (p value for both <0.001). Hs-CRP was strongly associated with IL6 level and WBCs count in moderate /severe DKA.* ***Conclusion:*** *Hs-CRP is increased in moderate/severe DKA patients along with IL6 and leukocytes in absence of infection. This finding might clarify the role of hs-CRP in DKA crises as a marker of severity.*

Keywords: Type I DM, Diabetic ketoacidosis, hs-CRP, IL6.

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INTRODUCTION

Type 1 diabetes mellitus (T1DM) is one of the most common autoimmune diseases with several million people already affected around the globe. It is characterized by an absolute loss of insulin secretion, and results from an autoimmune process that destroys insulin producing ß cells within the pancreatic islet.[1]

Diabetic ketoacidosis (DKA) at T1DM onset is more common in children less than 4 years of age, children with no family history of diabetes, those with low socioeconomic status, and in children with no medical insurance.[2-6]

DKA is a metabolic crisis that can precipitate other life-threatening complications.[7-8] In children and adolescents, the most notable complication is clinical brain edema. [9]The pathophysiology of this life threatening event is uncertain. However, there is increasing evidence of cellular and metabolic activation during DKA and its treatment.[10]

C-reactive protein (CRP) is a type I acute phase response protein whose synthesis in the liver is regulated by the pro-inflammatory cytokines IL-6, IL-1 and TNF.[1,2] Elevated plasma levels of CRP have been reported to be marker for endothelial cell dysfunction in uncomplicated, well-controlled type 1 diabetes mellitus (TIDM).[10] Hs-CRP can also be elevated in trauma, surgery, burns, tissue necrosis and advanced cancer[11]and has been considered a marker of adverse outcome in acute coronary syndromes and atherosclerosis.[12]

Previous studies have shown that during the metabolic crisis of DKA, the pro-inflammatory cytokine levels (mainly IL-6) and anti-inflammatory cytokine IL-10 levels are increased[13] in parallel with the severity of DKA.[14] IL-6 is mainly secreted by macrophages and lymphocytes.[15] Increased IL-6 production has been

observed in patients with sepsis,[16] non-infectious systemic inflammatory response syndrome (SIRS)[17] and also during chronic inflammation and endothelial damage, such as in atherosclerosis.[18] The above pro-inflammatory cytokines and especially IL-6 induce the hepatic synthesis of plasma hs-CRP.[19]

Hs-CRP is the measure of CRP with greater accuracy with a lower detection limit of 0.02 mg/dL, which is 100 times more sensitive than the usual CRP measurement (lower detection limit 5 mg/L).[20]

The aim of this study was to investigate the role of hs-CRP in DKA children and its relation to the severity of the illness.

METHODS

Patients

This is a prospective study that involves pediatric patients age <15 years (upper limit of pediatric age admitted to our pediatric intensive care unit (PICU) during the period from May 2012 to May 2013 who presented to Pediatric ICU of Zagazig University Children's Hospital with diabetic ketoacidosis. DKA was defined as presence of hyperglycemia (blood glucose >200 mg/dL) with a venous pH <7.3 and/or bicarbonate <15 mmol/L with associated glycosuria, ketonuria, and ketonemia. DKA was categorized as mild (venous pH <7.30; bicarbonate <15 mmol/L), moderate (pH <7.2; bicarbonate <10 mmol/L), and severe (pH: <7.1; bicarbonate: <5 mmol/L).[21]

The patients had no evidence of acute pancreatitis or acute/chronic infection. Acute infection was ruled out after a thorough medical history and clinical examination by the pediatrician as well as a sequential evaluation in the pediatric department after the admission.

All studies were conducted in accordance with the guidelines approved by the local research ethics committee.

Laboratory analysis

Blood samples were drawn at 2 sessions; at presentation and after rehydration and resolution of DKA.

Laboratory tests included blood pH, glucose levels, serum electrolytes, WBC count, blood and urine cultures.

Samples for IL-6 and hs-CRP level were collected from all patients by venous puncture technique into Vacutainer tubes with EDTA. Specimens were centrifuged at 2500×g for 15 min at 4 °C. Plasma was used to measure the levels of glucose while the serum was stored at −20 °C until time of assay of IL-6 and hs-CRP levels.

The levels of serum IL-6 were measured using a commercial kit (AviBion Human IL-6 ELISA, Ani Biotech Oy Orgenium Laboratories Business Unit Tiilitie 3 FIN-07120 Vantaa Finland, ref IL06001). The level of serum hs-CRP assayed with the immunonephelometric method (Cobas 6000, automated analyzer (Roche Diagnostics, Basel, Switzerland).

Statistical analysis

The data were analyzed using *SPSS*, version 16. Quantitative variables were expressed as mean ± standard deviation (SD) and then compared using t test. Qualitative variables were expressed as frequency and percentage and compared using chi-square test. Correlations were performed using the Pearson bivariate correlation. A p value was considered significant if less than 0.05.

RESULTS

Seventeen of the studied patients were males (56.7%). In most patients DKA resolved in 24 to 48 hours. Mild DKA (1st group) was diagnosed in 11 patients (36.7%) and moderate/severe DKA (2nd group) were present in 19 patients (63.4%). Demographic and clinical data were summarized in table I. Age of patient ,sex and BMI were not significantly differ between mild and moderate/severe DKA (p value:0.254, 0.287and 0.167 respectively). As regard blood glucose at presentation it was showed non-significant statistical difference between both groups (p value: 0.737), while blood urea nitrogen and PH were significantly differ in mild DKA than moderate /severe DKA (p value for both<0.001). It was observed that hs-CRP level showed highly significant increase in moderate/severe than mild group. Also, IL6 did the same (p value for both <0.001).

As regard time factor (at admission and after DKA resolution) and its effect on hs-CRP and IL6, they did not significantly affected in mild DKA (p value: 0.892 and 0.251), nevertheless they showed highly significant statistical difference in moderate/severe DKA (p value for both<0.001).

Correlation of hs-CRP with IL6 and WBCs in moderate /severe DKA are shown in table III. So hs-CRP was strongly associated with IL6 in moderate /severe DKA (r-0.637, P-value-0.003), also it is associated with leukocytosis in moderate/severe DKA (r-0.524 P value-0.021).

Table 1. Demographic and laboratory data of study population.

	Mild DKA	Moderate/ severe DKA	t. test	p. value
Age	10.18±2.63	9.08±2.42	1.16	>0.05
Sex M **F**	5 6	12 7	0.886	>0.05
BMI	18.18±1.94	17.36±1.21	1.41	>0.05
Glucose (mg/dl)	455±69	464±77	0.339	>0.05
Ph	7.22±0.03	7.09±0.09	5.49	<0.05
BUN (mg/dl)	17.72±4.51	37.26±13.18	0.589	<0.05
hs-CRP (mg/dl)	1.29±0.72	10.3±2.79	13.31	<0.05
IL6 (pg/ml)	13±2.68	23.68±7.64	5.53	<0.05

hs-CRP: high sensitivity C-reactive protein, IL6: interleukin 6, BUN: blood urea nitrogen

Table 2. hs-CRP and IL6 among mild DKA patients before and after treatment.

	At admission	After resolution	test	P value
hs-CRP (mg/dl)	1.29±0.72	1.3±0.62	1.39	>0.05
IL6 (pg/ml)	13±2.68	14.18±2.96	1.21	>0.05

hs-CRP: high sensitivity C-reactive protein, IL6: interleukin 6

Table 3. hs-CRP and IL6 among moderate/severe DKA patients before and after treatment.

	At admission	After resolution	test	P value
hs-CRP (mg/dl)	10.3±2.79	4.83±1.07	9.18	<0.05
IL6 (pg/ml)	23.68±7.64	16.20±4.97	6.8	<0.05

hs-CRP: high sensitivity C-reactive protein, IL6: interleukin 6

Table 4. Correlation of hs-CRP with IL6 and WBCs in moderate /severe DKA.

hs-CRP	r	p-value
IL6 (pg/ml)	0.637	0.003
WBCs	0.524	0.021

WBCs: white blood cells, IL6: interleukin 6

DISCUSSION

Our results demonstrated increased hs-CRP in DKA patients at diagnosis in the absence of infection and this is in harmony with previous studies.[15,22] This increase is explained as follow: Insulin attenuates the production of the acute phase proteins (CRP and haptaglobin) in cell culture exposed to IL6,[23]also it has reported that hyperglycemia results in leukocytosis and elevation of inflammatory cytokines IL6, IL1B, TNF±, possibly through augmented production in monocytes.[24-25]

Other mechanism include hyper-triglyceridaemia observed in DKA may be a contributing factor the increased levels of hs-CRP, IL6, IL1B and TNF±, thus insulin deficiency increases level of hs-CRP by direct action and indirect action through its effects (hyperglycemia and hypertriglyceridaemia).[26]

As regard leukocytosis, it was observed in our patients without evidence of infection. This is in accordance with Dalton et al,[22] who found leukocytosis (>15,000) in 6 patients with DKA in absence of infection. Floud et al, [27]also reported that leukocytosis is commonly present in DKA, even without infection. This finding is also in harmony with Stentz et al, who confirmed the presence of leukocytosis in hyperglycemic crises without obvious infection during febrile events. This could

be due to sympathetic stimulation that occur during hyperglycemia as increased sympathetic activity in normal subjects results in leukocytosis and elevation of IL6 and TNF±.[23]

As regard IL6, it was increased in our patients at diagnosis. This also is in agreement with Karavanaki et al,[14] and Dalton et al,[22] and it is explained as IL6 is a mediator for hs-CRP and results in its increase.[24]

As regard effect of treatment on hs-CRP and IL6, we observed that their serum levels were decreased significantly in moderate/severe DKA group after treatment. This is in harmony with Hansen et al, who report that intensive insulin therapy in critically ill patients has anti-inflammatory action through reduction of CRP, IL6 and leukocytes so improve survival.[28] These changes in hs-CRP and IL6 can be attributed to the anti-inflammatory action of insulin which has been suggested by Dandona et al. [29]

Our study showed that hs-CRP has positive correlation with IL6 and WBCs in moderate /severe DKA group and this is in accordance with Dalton et al,[22] who found significant correlation between CRP and IL6 and this explained as mentioned above as IL6 is a mediator for hs-CRP.

CONCLUSION

Our study reported that hs-CRP is increased in moderate/severe DKA patients along with IL6 and leukocytes in absence of infection. These findings might clarify the role of hs-CRP in DKA crises as a marker of severity as it increase significantly at diagnosis in moderate/severe DKA group and reduced significantly after treatment.

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