Research Article

Early Sepsis Markers in Patients Admitted to Intensive Care Unit with Moderate to Severe Diabetic Ketoacidosis

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Abstract
Background: Diabetes is chronic endocrine/metabolic disease with heterogeneous etiologies, clinical presentations and associated complications.Its biochemical hallmark is hyperglycemia (American Diabetes Association., 2019)caused mainly by insulin deficiency and/or insulin resistance. **Aim of the work:** To test the validity of procalcitonin versus other sepsis markers in detection of early sepsis in diabetic ketoacidosis patients and it will impact on thyroid function. **Patient and methods:** This is a study will be performed in the ICU of Minia University Hospital from May 2020 to May 2021. It will include 30 patients aged from 19 to 42 years old admitted in ICU of elminya university hospital who have diabetic ketoacidosis. The patient include 15 male and 15 female. DKA will be defined as a glucose concentration>300mg/dL, pH≤7.25 or a serum bicarbonate concentration<15mmol/L, and the presence of ketones acetoacetate(either in the blood or the urine). **Results:** This case control study was performed in the ICU of Minia University Hospital from May 2020 to May 2021. It included 30 DKA patients admitted to ICU of Minia University hospital and 30 controls. The patients age ranged from 19 to 42 years old and included 15 males and 15 females. Table 1 shows clinical and demographic data of studied groups.
Keywords: Diabetes, chronic endocrine/metabolic, hyperglycemia

Introduction
Diabetes is chronic endocrine/metabolic disease with heterogeneous etiologies, clinical presentations and associated complications. Its biochemical hallmark is hyperglycemia[(1)] caused mainly by insulin deficiency and/or insulin resistance.

Diabetic ketoacidosis (DKA) accounts for 4–9% of all hospital discharge summaries among diabetic patients, Despite a 50% drop in mortality since 1980 due to standardized protocols**,** recent studies still report a mortality rate of about 2–15%, mostly depending on the age of the patients[(2)]**.**

Sepsis is a life-threatening condition that arises when the body's response to infection causes injury to its own tissues and organs. This initial stage is followed by suppression of the immune system[(3)]. Common signs and symptoms include fever, increased heart rate, increased breathing rate, and confusion. There may also be symptoms related to a specific infection, such as a cough with pneumonia, or painful urination with a kidney infection. The very young, old, and people with a weakened immune system may have no symptoms of a specific infection, and the body temperature may be low or normal instead of having a fever. Severe sepsis causes poor organ function or blood flow. The presence of low blood pressure, high blood lactate, or low urine output may suggest poor blood flow. Septic shock is low blood pressure due to sepsis that does not improve after fluid replacement[(4)]**.**

Pro calcitonin (PCT) is actually one of the major relevant markers for the diagnostic of bacterial infections. The typical laboratory tests for sepsis diagnosis include determining erythrocytes sedimentation rate (ESR), C-reactive protein (CRP), and white blood cell (WBC) count or the percentage of neutron-phils, and performing polymerase chain reaction (PCR), which are slow and not sensitive and specific enough[(5)]**.** In fact, except for the Procalcitonin (PCT), other tests cannot confirm the existence of blood infection if used alone. In severe sepsis, WBC count may reduce, and CRP may

remain low; hence, the availability of a more sensitive and specific biomarker can be helpful in the diagnosis of sepsis.

Aim of the work

To test the validity of procalcitonin versus other sepsis markers in detection of early sepsis in diabetic ketoacidosis patients and it will impact on thyroid function.

Patient and methods

This is a study will be performed in the ICU of Minia University Hospital from May 2020 to May 2021. It will include 30 patients aged from 19 to 42 years old admitted in ICU of elminya university hospital who have dia-betic ketoacidosis. The patient include 15 male and 15 female.

DKA will be defined as a glucose concentration >300mg/dL, pH≤7.25 or a serum bicarbonate concentration<15mmol/L, and the presence of ketones acetoacetate (either in the blood or the urine)

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Ethical aspects:

The study protocol was approved by the Institutional Ethics Committee All patients and control gave informed consent before partici-pating in this study. The study was conducted in accordance with the ethical guidelines of the Declaration of Helsinki and International Conference on Harmonization Guidelines for Good Clinical Practice.

Exclusion criteria: subjects had any one of the following criteria were excluded from study:

1- medullary thyroid carcinoma
2- small cell lung cancer
3- cardiac arrest .
4- heat stroke.
5- pancreatitis.
6- malaria, notion of fungal infection, severe trauma.
7- Received any local, systemic treatment for hyper or hypothyroidism or other causes of increased RBS, received any hormonal treatment, etc….

All included patients were subjected to the following:

I) Thorough History Taking.

Subject answered a standard questionnaire that included:

- Personal history with special attention to name, age, sex, residence, marital status, occupation and special habits of medical importance as cigarette smo-king and daily alcohol intake in the past 6 months
- Current history of symptoms sugges-tive of Diabetes and risk facters for DKA as acidosis (increase respiratory rate), thirst sensation abdominal pain, vomiting, fever, cough, expectration, diarrhea, burning micturition, dysuria, frequency and disturbed level of cons-ciousness.
- All women of our study were in menopausal status, menopausal status was considered according to self-reported cessation of menses for ≥ 12 consecutive months not due to pregnancy or medical treatment.

II) Thorough Clinical Examination:-

- Measurement of vital signs (body temperature, pulse, blood pressure and respiratory rate).
- General examination with special emphasis on stigmata of dehydration, thyroid status, lower limb edema, and conscious level.
- Careful abdominal examination with special reference to the status of the liver and spleen as regards size, surface, edge, consistency and tende-rness on examination.
- Systemic examination to search for sepsis signs.

- Other body systems were examined as well.

(III) Laboratory Investigations:

Blood Sampling Protocol:

After 8-hour overnight fasting blood sample was drawn via sterile venipuncture at 8 A.M. Samples were divided into three blood collecting tubes: first tube containing ethylene diaminetetraacetic acid (EDTA) was used for complete blood count, the second one containing tri sodium citrate was used for determination of prothrombin time and concentration and lastly a plane tube.

The plane tube was left to clot then centrifuged; separated serum was put into aliquot tube used for routine biochemistry tests including liver function test, renal function, FBS, thyroid function tests, reversed T3and PCT.

- **Complete blood count (CBC)** was determined by automated cell counter, Sysmex Counter K-1000, TAO Medical Incorpo-ration, Japan according to the method of [9].
- **Prothrombin time and concentration** were done using option 2 Biomerieux, Vitek, Inc.595, USA, according to method of [10].
- **Routine biochemical analyses** that involved fasting blood glucose, liver and renal function tests were done by auto-analyzer Kone-lab (2011), Thermo-electro, Clinical chemistry automation systems, Finland, using comercially available kits following instruction depicted in the enclosed pamphlets.
- **Liver function test** included liver enzymes (ALT, AST), serum bilirubin and serum albumin.
- **Renal function test** composed of blood urea and serum creatinine.
- **arterial blood gases** detect metabolic acidosis that present in DKA.
- **urine analysis** detect infection ,acetone in urine, albumin,RBS ,…
- **Thyroid profile include T3,T4,TSH**.
- **inflammatory markers** as ESR and CRP

Specific laboratory Investigation;

1- **Serum plasma procalcitonin** was performed by enzyme linked immunosorbent assay (ELISA) (**bioassay Technology laboratory, 228 Niangua Rd. yangpu Dist shinghai. china.**):

Assay Principle

This kit is an Enzyme-Linked Immuno-sorbent Assay (ELISA). The plate has been pre-coated with human PCT antibody. PCT present in the sample is added and binds to antibodies coated on the wells. And then biotinylated human PCT Antibody is added and binds to PCT in the sample. Then Streptavidin-HRP is added and binds to the Biotinylated PCT antibody. After incubation unbound Streptavidin-HRP is washed away during a washing step. Substrate solution is then added and color develops in proportion to the amount of human PCT. The reaction is terminated by addition of acidic stop solution and absorbance is measured at 450 nm.

Results

This case control study was performed in the ICU of Minia University Hospital from May 2020 to May 2021. It included 30 DKA patients admitted to ICU of Minia University hospital and 30 controls. The patients age ranged from 19 to 42 years old and included 15 males and 15 females. Table 1 shows clinical and demographic data of studied groups.

Table (1): Clinical data of studied groups.

	Case (N=30)	Control (N=30)	p value
	Mean±SD Median (Range)	Mean±SD Median (Range)	
Age(years) [a]	32±8 34 (19-43)	30±6 30 (21-41)	0.149
Sex [b]			
Female	15 (50.0%)	15 (50.0%)	>0.99
Male	15 (50.0%)	15 (50.0%)	
Pulse [a]	93.2±8.3 92 (78-108)	80.9±8.1 81.5 (68-92)	<0.001*
SBP (mmHg) [a]	118.4±14.3 117 (90-150)	116.4±11.5 111 (100-138)	0.397
DBP (mmHg) [a]	78.2±10.8 80 (60-100)	75.6±7.7 79 (60-88)	0.173
Temp [a]	38.7±0.8 38.7 (37.4-40)	37±0.2 37 (36.6-37.3)	<0.001*
RR	24.7±5 24 (16-36)	13.1±1.1 13 (12-15)	<0.001*
CXR [b]			
Chest infection	13 (43.3%)	0 (0.0%)	<0.001*
No	17 (56.7%)	30 (100.0%)	
UTI [b]			
UTI	16 (53.3%)	0 (0.0%)	<0.001*
No	14 (46.7%)	30 (100.0%)	

SBP=systolic blood pressure,**DBP=**diastolic blood pressure, **Temp=**temperature**,RR=**respiratory rate, **CXR=** chest x ray, **UTI**= urinary tract infection

a= normally distributed quantitative data are expressed as mean ± standard deviation and compared by independent sample t test

b= qualitative data are expressed as number (percentage) and compared by Chi square test

Table (1) shows clinical data of studied groups: DKA and healthy control groups.

Diabetic ketoacidosis patients had comparable age, sex, pulse, blood pressure, Temp, RR, CXR, Urinary tract infection as compared to control group, meanwhile DKA group has higher pulse, temperature, respiratory rate than control group with clinical significance p value < 0.001.

About half of diabetic ketoacidosis patients (53.3%) had UTI and 43.3% had chest infection and that was higher compared to control group with significant difference (p value <0.001).

Table (4): laboratory data of studied groups: DKA and healthy control groups.

Lab	Case (N=30)	Control (N=30)	p value
	Mean±SD Median (Range)	Mean±SD Median (Range)	
Hb (gm/dl)	13.2±1.6 13 (10.3-15.9)	12.2±0.9 12.1 (11-15)	0.001*
WBC(x10^3)	11.87±5.22 12.45 (4.3-20)	7.57±2.37 6.95 (4.9-11)	0.003*
Plt(x10^3)	282±105 255 (117-521)	247±104 219 (20-385)	0.267
INR	1.2±0.27 1.1 (0.8-1.92)	1.06±0.07 1.02 (1-1.2)	0.044*
RBS(mg/dl)	368±107.9 371.5 (0.5-600)	149.8±28.3 140 (116-198)	<0.001*
Cr (mg/dl)	1.79±2.61 1.1 (0.5-15)	0.87±0.19 0.9 (0.5-1.1)	0.012*
Urea (mg/dl)	71.4±50.9 46 (19-216)	34.1±13.5 33 (22-69)	0.001*
Na (mmol/L)	138.9±5.9 137 (126-151)	140.4±3.7 141 (134-145)	0.118
K (mmol/L)	4±0.7 3.9 (2.3-5.3)	3.9±0.3 3.9 (3.6-4.8)	0.789
Ica (mmol/L)	1.05±0.15 1.07 (0.6-1.32)	1.05±0.06 1.06 (0.98-1.14)	0.689

Hb= Hemoglobin, WBC= white blood cell, PLT=Platelet, INR= international normalized ratio,RBS= random blood sugar, Cr=Creatinine,Na =sodium, K=potassium, I.CA=ionized calcium

DKA patients had comparable Hemoglobin, WBC, Platelet, INR, RBS, renal functions and electrolytes as compared to control group in which there is significance higher level of Hemoglobin, WBCs, INR and RBS DKA cases more than control group with significance p value (p<0.001, p=0.003, p=0.044, p<0.001 respectively).

Renal function tests urea and creatinine were higher among DKA patients compared to control group (71.4 and 1.79 compared to 34.1 and 0.87 respectively) and this difference was statistically significant.

Discussion

This case control study was performed in the ICU of Minia University Hospital from May 2020 to May 2021. It included 30 DKA patients admitted to ICU of Minia University hospital and 30 controls. The patients' age ranged from 19 to 42 years old with mean and standard deviation in cases 32±8 and 30±6 in control and included 15 males and 15 females and pulse is93.2±8.3 which is clinically significant with p value<0.001 and temp is 38.7±0.8 which is clinicily significant with p value<0.001 and respiratory rate is 38.7±0.8 which is clinically significant with p value<0.001 and 13 patient have chest infection and 16 of patients has urinary tract infection.

In our study we found that Hemoglobin in DKA Cases is 13.2±1.6 which is clinically significant with p value is 0.001and WBCS are 11.87±5.22 which are clinically significant with p value is 0.003.

this results similar to Małachowska et al., 2020 which studied Changes in hematological parameters during first days of diabetic ketoacidosis treatment in children with type 1 diabetes mellitus where The DKA group was characterized by significantly higher values of baseline RBC (p = 0.0026), Hct (p = 0.0019), Hb (p = 0.0235), PLT (p = 0.0427) and WBC count (p < 0.0001) vs. patients without DKA[(11)].

And similar to Westerberg., 2013 that found Leukocytosis can occur even in the absence of infection; bandemia more accurately predicts infection. One study showed that an elevated band count in persons with DKA had a sensitivity for predicting infection of 100 percent (19 out of 19 cases) and a specificity of 80 percent, An elevated hemoglobin level caused by dehydration may also exist[12].

Some studies described White Blood Cell (WBC) count changes in DKA. Karavanaky et al., (2016), found average number of WBC was $15.2*10^2$ mm^3 in hospital arrival that was high more than normal ranges[13]. Another study illustrated a significant increase in level of WBC in DKA compared to patients without DKA (13325 mm3 against 6008 mm3). Meanwhile, the increase of infection led to higher level of WBC significantly in DKA patients than non-infected DKA (16910 mm3 against 10310 mm3)[14]. The results of researcher showed a direct relationship between blood pH and level of WBC. In other words, high level of WBC is correlated with increase of blood acidity. Wasif et al., (2012) examined predictors of infection in DKA patients and found leukocytosis is a more accurate predicator for DKA severity rather than infection, In a case report conducted by Kayshyma et al., (2017), leukemoid reaction (increased leukocytes over $25 * 10^5$ without leukemia) occurred in patients with DKA absences of infection[14].

In several studies, leukocytosis in DKA has been attributed to various factors. many of researcher believed lack of insulin can stimulate production of Neutrophil in bone marrow, upon insulin administration and fluid therapy induced to decrease leukocyte count. after 120 hours, furthermore, secretion of adrenaline, cortisol and inflammatory mediators can lead to increase of leukocytes amount[15]. In another study, it evaluated correlation Granulocyte Colony Stimulating Factor (GCSF) level and leukocytosis in DKA that results showed no relationship both of them[16].

In general, leukocytosis in DKA can linked to different factors such as infections, insulin deficiency, dehydration and stress hormones secretion. At first, medical team should determine infection with take a history, physical examinations and laboratory tests. These results can be useful for make a proper decision about antibiotic initiation. Therefore, decision for antibiotic initiation based on leukocytosis as a sign of infection isn't reliable[17].

In our study we found About half of diabetic ketoacidosis patients (53.3%) had UTI and 43.3% had chest infection and that was higher compared to control group with significant difference (p value <0.001).

And this similar to Poovazhagi Varadarajan and Saradha suresh .,2020 in their study Role of Infections in Cases with Diabetic Ketoacidosis-A Study from South India who found that Sepsis, UTI, Bronchopneumonia and skin and soft tissue infections are the common infections in children with DKA. Infections are significantly associated complications like shock, cerebral oedema and renal failure and mortality in DKA in univariate anlysis. Infections are significantly associated with severe and persistent acidosis, persistent higher osmolaity, and increased hospital stay in DKA in univariate analysis, Multivariate analysis has revealed infections complicating DKA to be significantly associated with mortality and increased duration of hospital stay, Empirical antibiotics have to be initiated in children with fever with or without a focus of infection in cases with DKA and persistent acidosis despite 6 hours of therapy in the absence of renal failure, Cases with infection can present without fever in DKA. Consider to start antibiotics in all DKA episodes until infections have been ruled out based on units protocol, as infections are significantly associated with mortality in DKA in developing countries[18].

also Mohammed et al., 2018 found that the major precipitating factor for DKA was infection (67.8%), most commonly pharynx-gitis, chest infection, tonsillitis, urinary tract infections, hepatitis A and gastroenteritis. This was in agreement with a study, which reported that the major precipitating factor for DKA was infection (most commonly viral fever, peritonitis, pneumonia and urinary tract infections)[19].

Summary and Conclusion

This is a study will be performed in the ICU of Minia University Hospital from May 2020 to May 2021. It will include 30 patients aged

from 19 to 42 years old admitted in ICU of Elminya University hospital who have diabetic ketoacidosis. The patient include 15 male and 15 female.

DKA will be defined as a glucose concentration >300mg/dL, pH≤7.25 or a serum bicarbonate concentration<15mmol/L, and the presence of ketones acetoacetate (either in the blood or the urine). Sepsis is a life-threatening condition that arises when the body's response to infection causes injury to its own tissues and organs. This initial stage is followed by suppression of the immune system. Common signs and symptoms include fever, increased heart rate, increased breathing rate, and confusion. There may also be symptoms related to a specific infection, such as a cough with pneumonia, or painful urination with a kidney infection. The very young, old, and people with a weakened immune system may have no symptoms of a specific infection, and the body temperature may be low or normal instead of having a fever. Severe sepsis causes poor organ function or blood flow. The presence of low blood pressure, high blood lactate, or low urine output may suggest poor blood flow. Septic shock is low blood pressure due to sepsis that does not improve after fluid replacement.

All participates had conventional assess-ments including thorough history taking, physical examination, and abdominal ultra-sonography. They underwent routine hema-tologic and biochemistry tests.

Exclusion criteria: subjects had any one of the following criteria were excluded from study: medullary thyroid carcinoma, small cell lung cancer, cardiac arrest, heat stroke, pancreatitis, malaria, notion of fungal infection, severe trauma, Received any local, systemic treatment for hyper or hypothyroid-dism or other causes of increased RBS, received any hormonal treatment, etc….

Blood sampling and biochemical assays:
Fasting venous blood samples were collected in the morning at 9 AM. EDTA containing blood sample tube was used for complete blood count, renal function and fasting blood sugar. Citrated blood sample used to separate plasma for prothrombin time assay and calculation of international normalized ratio (INR), arterial blood gases to detect meta-bolic acidosis that present in DKA, urine analysis detect infection, acetone in urine, albumin, RBS. inflammatory markers as ESR and CRP and Thyroid profile include T3,T4,TSH.
Serum plasma procalcitonin was performed by enzyme linked immunosorbent assay (ELISA) (bioassay Technology laboratory, 228 Niangua Rd. yangpu Dist shinghai. china.)
Serum Reverse Tri-iodothyronine (RT3) was performed by (ELISA) (bioassay Technology laboratory, 228 Niangua Rd. yangpu Dist shinghai. china.).

Results

Demographic and Clinical Characteristics of studied groups:
Atotal of 30 DKA patients admitted to ICU of Minia University hospital and 30 controls. The patients' age ranged from 19 to 42 years old and included 15 males and 15 females.
Diabetic ketoacidosis patients had compar-able age, sex, pulse, blood pressure, Temp, RR, CXR, Urinary tract infection as com-pared to control group, meanwhile DKA group has higher pulse, temperature, respire-tory rate than control group with clinical significance p value < 0.001.
About half of diabetic ketoacidosis patients (53.3%) had UTI and 43.3% had chest infection and that was higher compared to control group with significant difference (p value <0.001).
DKA patients had comparable Hemoglobin, WBC, Platelet, INR, RBS, renal functions and electrolytes as compared to control group in which there is significance higher level of Hemoglobin , WBCs, INR and RBS DKA cases more than control group with significance p value (p<0.001, p=0.003, p=0.044, p<0.001 respectively).
Renal function tests urea and creatinine were higher among DKA patients compared to control group (71.4 and 1.79 compared to 34.1 and 0.87 respectively) and this differ-rence was statistically significant.
AS regard Thyroid profile TSH, FT3 and FT4 were significantly lower in patients compared to control groups (p <0.001). The mean reverse T3 is 780.1±55.6 in DKA patients higher than control group (520±67.5) with p value p<0.001.
As regard inflammatory markers, DKA patients had significantly higher level of PCT, CRP and ESR compared to control group (p value <0.001).

Receiver operator curves were generated to determine the cut-off value for optimal sensitivity and specificity for PCT. The area under the PCT curve was 0.993 (P<0.001) with sensitivity and specificity of 96.7% and 93.3%, respectively, when the critical value was 50.

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