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Original Article

Diagnostic value of TNF-α and IL-10 for diabetic ketoacidosis with acute pancreatitis

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Abstract: Objective: Our aim was to assess the diagnostic value of tumor necrosis factor α (TNF-α) and interleukin 10 (IL-10) for diabetic ketoacidosis (DKA) with acute pancreatitis (AP). Methods: A total of 314 patients with DKA were enrolled in this study. Ninety-eight patients with concurrent AP were assigned to the test group and the remaining 216 patients were assigned to control group. Enzyme-linked immunosorbent assays were performed to measure serum levels of TNF-α and IL-10 in both groups. Sensitivity, specificity, positive and negative predictive values, and diagnostic accuracy of TNF-α and IL-10, alone or in combination, were analyzed for diagnosis of DKA with AP. Results: Serum TNF-α levels in the test group were significantly higher than in the control group ($P < 0.05$). At TNF-α and IL-10 cutoff levels of 1.80 ng/L and 21.10 pg/L, respectively, the diagnostic accordance rate, sensitivity, specificity, negative predictive value, and positive predictive value of serum TNF-α plus IL-10 tests in diagnosing DKA with AP were 86.62% (272), 83.12% (261), 80.57% (253), 89.35% (193), and 88.78% (87), respectively. Receiver operating characteristic curve analysis revealed that, regarding diagnosis of DKA with AP, area under the curve for TNF-α alone, IL-10 alone, and in combination was 0.654, 0.699, and 0.814, respectively. This indicates that the diagnostic value of TNF-α plus IL-10 was higher than that of either cytokine alone ($P = 0.011$; $P = 0.014$). Conclusion: Expression levels of TNF-α and IL-10 are increased in patients with DKA and AP and an assay of TNF-α plus IL-10 has diagnostic value for this dual condition.

Keywords: Diabetic ketoacidosis, acute pancreatitis, TNF-α, IL-10, diagnosis

Introduction

Diabetes is a group of metabolic disorders involving proteins, glucose, lipids, and other molecules and is caused by relative or absolute deficiency of insulin. In ~20% of patients with diabetes, the first symptom is diabetic ketoacidosis (DKA) [1, 2]. Acute pancreatitis (AP), which denotes spontaneous degradation of pancreatic tissue, is caused by abnormal activation of pancreatic enzymes in patients due to certain factors and in severe cases may result in pancreatic necrosis [3, 4]. With continuous development of the economy, living standards of people have continued to improve and dietary habits have changed. Therefore, the number of patients with AP is increasing annually. Although effectiveness of medical treatment continues to improve, mortality of patients with AP is still as high as ~10% and can even reach 50% [5, 6] for those with severe forms of the disease. Therefore, proper diagnosis of AP is an urgent problem to be solved. Nonetheless, studies have shown that DKA and AP have some similar clinical features. Abdominal pain, a typical symptom of AP, also manifests itself as upper abdominal pain in patients with DKA and even nonspecific increases in serum and urine amylase levels are common to both diseases. These similar clinical features increase the difficulty of diagnosing cases where DKA and AP are comorbid. Hence, frequency of misdiagnosis and missed diagnosis increases too, thereby greatly affecting patient survival [7, 8]. Therefore, there is a need to find more specific indicators to assist in diagnosis of DKA with AP.

Many studies have found that tumor necrosis factor alpha (TNF-α) and interleukin 10 (IL-10) play an important role in prognosis of patients with AP but not with DKA [9, 10]. This situation provides an opportunity for us to reliably use inflammatory-cytokine quantification to help diagnose DKA with AP.

Table 1. General information on patients in test and control groups

	Control group	Test group	t/χ^2	*P* value
Number of patients	216	98		
Males/females	101/115	50/48		
Age (years)	51.4 ± 11.7	50.1 ± 10.6	0.547	0.744
Abdominal pain [n (%)]	31 (14.35)	93 (94.90)	11.48	0.007
Serum glucose (mmol/L)	24.6 ± 7.1	27.3 ± 8.7	3.14	0.036
Serum amylase (U/L)	351.4 ± 63.4	462.3 ± 104.5	4.05	0.031
pH	7.19 ± 0.11	7.11 ± 0.10	7.96	0.028
Urine ketone positive [n (%)]	216 (100)	98 (100)		
Urine glucose positive [n (%)]	216 (100)	98 (100)		
Residence [n (%)]			0.845	0.381
Urban	107 (49.54)	57 (58.16)		
Rural	109 (50.46)	41 (41.84)		

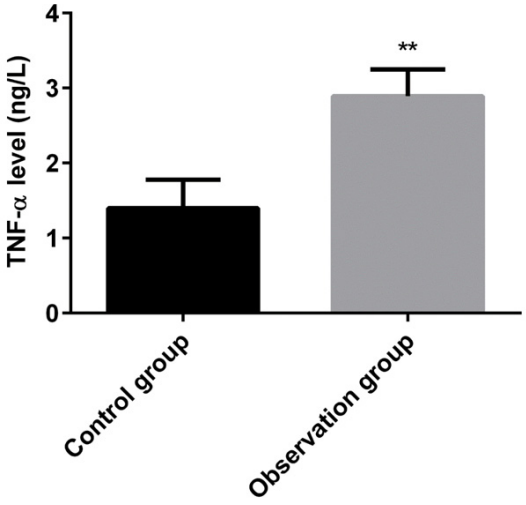

Figure 1. ELISA results on TNF-α in test and control groups. Expression levels of TNF-α in the control group were significantly lower than those in the test group (1.40 ± 0.38 vs. 2.89 ± 0.36 ng/L, P < 0.05).

In this study, serum levels of TNF-α and IL-10 were measured in patients with DKA to determine diagnostic value of these two cytokines for DKA with AP.

Materials and methods

Clinical information

A total of 314 patients with DKA, admitted to the Department of Endocrinology at our hospital, were enrolled in this study. Among them, 98 patients with concurrent AP were assigned to the test group and the remaining 216 patients to control group. All patients met the diagnostic criteria for type 2 diabetes as described in the Guidelines on Prevention and Treatment of Type 2 Diabetes in China, 2010 edition. Key criteria for diagnosis of DKA were as follows [11]: pH < 7.35 or serum bicarbonate < 15.0 mmol/L, blood glucose > 11.1 mmol/L, and ketonuria or ketonemia. Key diagnostic criteria for DKA with AP were as follows [12]: elevated amylase or lipase levels > 3 times normal value, imaging evidence of AP, and persistent abdominal pain. All of the cases were diagnosed by four experienced senior physicians of the Department of Endocrinology with a double-blind method. Patients with specific diabetes, diabetic peripheral neuropathy, cardiovascular diseases, tumors, liver or kidney failure, diabetic microangiopathy, endocrine disorders, or incomplete data were excluded as were minors and those with mental or learning dysfunction. Our study's protocol was approved by the Ethics Committee of the hospital. All of the patients or their families signed an informed consent form.

Methods

Each patient fasted overnight prior to blood sample collection by nurses in the morning. Concentrations of serum TNF-α and IL-10 were measured by enzyme-linked immunosorbent assays (ELISAs). TNF-α test kit was purchased from Beijing Huaxia Yuanyang Technology Co., Ltd., whereas the IL-10 test kit was purchased from Shanghai Jingkang Biological Engineering Co., Ltd.

Outcome measures

Sensitivity, specificity, positive predictive value, negative predictive value, and accuracy of TNF-α and IL-10, alone or in combination, for diagnosis of DKA with AP were analyzed.

Statistical analysis

SPSS 19.0 (AsiaAnalytics, formerly SPSS China) was used for statistical analysis of data. χ^2 test was carried out to compare the number of patients, gender, and outcome measures. Age,

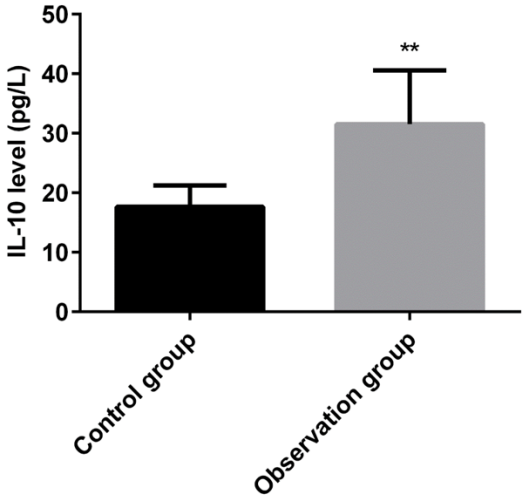

Figure 2. ELISA results on IL-10 in test and control groups. Expression levels of IL-10 in the control group were significantly lower than those in the test group (17.66 ± 3.58 vs. 31.52 ± 9.06 pg/L, P < 0.05).

cytokine concentrations, and other continuous variables are presented as mean ± SD. Nonparametric Kolmogorov-Smirnov test was conducted for comparison between the groups. Data with P < 0.05 were considered statistically significant.

Clinical information

Of the 314 patients with DKA enrolled in this study, 98 with concurrent AP (comprising 50 males and 48 females; age: 50.1 ± 10.6 years) were assigned to the test group. The remaining 216 patients (including 101 males and 115 females; age: 51.4 ± 11.7 years) were assigned to control group. There were no significant differences between the two groups in terms of gender, weight, age, or other general characteristics (P > 0.05). The proportion of test patients with abdominal pain was higher than in the control group (P < 0.05). Serum amylase concentrations in the test group were higher than in control group (P < 0.05). In the control group, 41 patients had elevated serum amylase, with 6 having levels 3-fold higher than normal value. All patients in both groups yielded positive results in urine ketone and urine glucose tests (**Table 1**).

ELISA results on TNF-α in test and control groups

Expression levels of TNF-α in the control group were significantly lower than in the test group (1.40 ± 0.38 vs. 2.89 ± 0.36 ng/L, P < 0.05; **Figure 1**).

ELISA results on IL-10 in test and control groups

ELISA was carried out to test IL-10 levels in both groups and test results were statistically analyzed. Expression levels of IL-10 in the control group were significantly lower than in test group (17.66 ± 3.58 vs. 31.52 ± 9.06 pg/L, P < 0.05; **Figure 2**).

Diagnostic value of TNF-α and IL-10 for DKA with AP

Receiver operating characteristic curve analysis revealed that in diagnosis of DKA with AP, area under the curve (AUC) was 0.654 (95% confidence interval [CI], 0.517-0.932) for TNF-α, 0.699 (95% CI, 0.601-0.792) for IL-10, and 0.814 (95% CI, 0.517-0.932) for TNF-α plus IL-10. Diagnostic value of TNF-α plus IL-10 was, thus, higher than that of TNF-α or IL-10 alone (P = 0.011, P = 0.014; **Figure 3**).

At serum TNF-α level of 1.80 ng/L, its diagnostic accordance rate, sensitivity, specificity, negative predictive value, and positive predictive value for diagnosing DKA with AP were 75.16% (236), 72.61% (228), 69.75% (219), 78.24% (169), and 76.53% (75), respectively. In the case of IL-10, at serum level of 21.10 pg/L, its diagnostic accordance rate, sensitivity, specificity, negative predictive value, and positive predictive value for diagnosing DKA with AP were 73.25% (230), 71.34% (224), 67.20% (211), 76.39% (165), and 74.49% (73), respectively. Diagnostic accordance rate, sensitivity, specificity, negative predictive value, and positive predictive value of serum TNF-α plus IL-10 test in diagnosis of DKA with AP were 86.62% (272), 83.12% (261), 80.57% (253), 89.35% (193), and 88.78% (87), respectively, higher than values obtained for either cytokine alone (P < 0.05; **Table 2**).

Value of TNF-α in combination with IL-10 for diagnosis of DKA with AP, 24 hours and 48 hours after admission

ROC curve analysis results showed that AUCs of TNF-α in combination with IL-10 for test of DKA with AP 24 hours after admission and 48 hours after admission were 0.725, (95% CI, 0.525-1.013), and 0.905, (95% CI, 0.836-

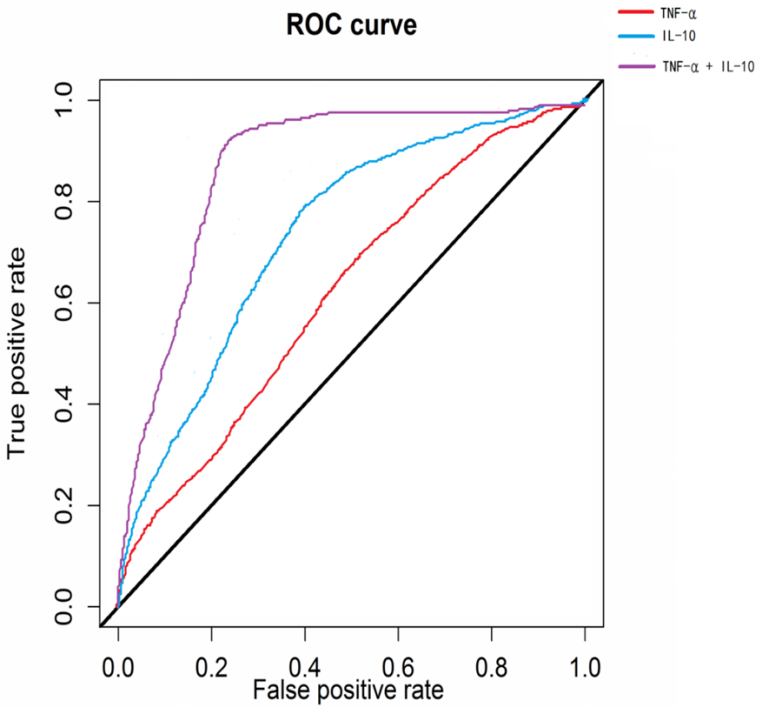

Figure 3. Receiver operating characteristic curve analysis of area under the curve (AUC) for the cytokines regarding diagnosis of DKA with AP. AUC was 0.654 (95% CI, 0.517-0.932) for TNF-α, 0.699 (95% CI, 0.601-0.792) for IL-10, and 0.814 (95% CI, 0.517-0.932) for TNF-α plus IL-10. The diagnostic value of TNF-α plus IL-10 was higher than that of TNF-α or IL-10 alone (P = 0.011; P = 0.014).

Table 2. Diagnostic performance of TNF-α and IL-10 for DKA with AP (%)

	TNF-α	IL-10	TNF-α plus IL-10	P1	P2
Diagnostic accordance rate	75.16	73.25	86.62	0.032	0.027
Sensitivity	72.61	71.34	83.12	0.041	0.038
Specificity	69.75	67.20	80.57	0.036	0.033
Negative predictive value	78.24	76.39	89.35	0.029	0.022
Positive predictive value	76.53	74.49	88.78	0.035	0.024

Note: P1, Comparison between TNF-α and TNF-α plus IL-10; P2, Comparison between IL-10 and TNF-α plus IL-10.

1.421), respectively. The diagnostic value of TNF-α in combination with IL-10 for test of DKA with AP 24 hours after admission was higher than 48 hours after admission (P = 0.001) (**Figure 4**).

Diagnostic coincidence rate, specificity, sensitivity, negative predictive value, and positive predictive value of serum TNF-α in combination with IL-10 for test of DKA with AP 24 hours after admission were 85.42% (41), 77.42% (36), 89.80% (22), 83.67% (41), and 90.00% (31), respectively. Diagnostic coincidence rate, specificity, sensitivity, negative predictive value, and positive predictive value of serum TNF-α in combination with IL-10 for testing of DKA with AP 48 hours after admiss-ion were 92.00% (46), 95.70% (46), 79.59% (20), 91.84% (45) and 84.29% (29), respectively. These were higher than that of the combined test 24 hours after admission (P < 0.05) (**Table 3**).

Discussion

DKA is a common complication of diabetes that can cause dysfunction of multiple organs and even death, in severe cases [13, 14]. Approximately 25% of AP cases are clinically critical and overall mortality is ~10% [15]. Approximately 15% of patients with DKA have comorbid AP, which has additional adverse effects on survival [16]. DKA and AP have many similar clinical signs and symptoms such as abdominal pain, nausea, and shock [7, 8] which greatly influence diagnosis made by clinicians and increase incidence of misdiagnosis and missed diagnosis.

In our study, we tested serum TNF-α and IL-10 concentrations in patients with DKA and assessed diagnostic value of these two cytokines for DKA with AP. Our aim was to provide a reference for diagnosis of this dual condition and to improve diagnostic accordance and survival rates.

Our study revealed that overall levels of serum TNF-α and IL-10 in patients with both conditions were higher than in patients with DKA only (P < 0.05). There have been few studies on levels of serum inflammatory cytokines in

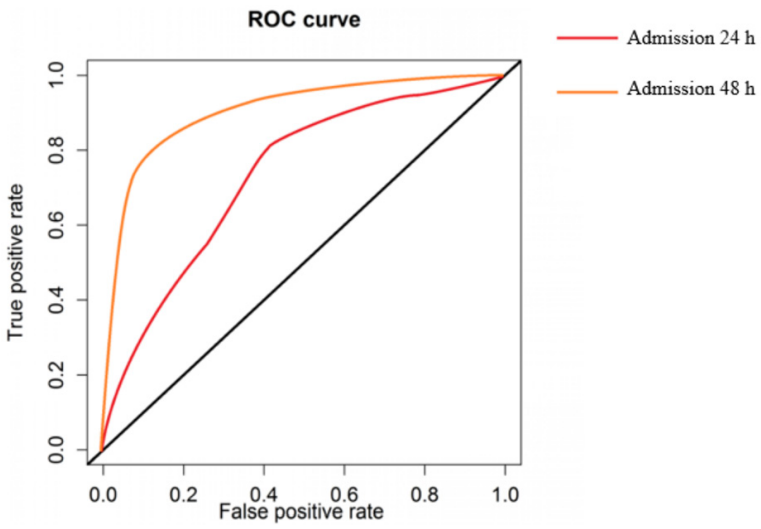

Figure 4. Receiver operating characteristic curve analysis of diagnostic value of TNF-α and IL-10 at 24 and 48 hours after admission for DKA with AP.

Table 3. Value of TNF-α in combination with IL-10 for diagnosis of DKA with AP 24 h and 48 h after admission (%)

	24 h after admission	48 h after admission	*P* value
Diagnostic coincidence rate	85.42	92.00	0.024
Specificity	77.42	95.70	0.013
Sensitivity	89.80	79.59	0.042
Negative predictive value	83.67	91.84	0.047
Positive predictive value	90.00	84.29	0.045

patients with DKA and AP. Hoffman et al. [17] reported that IL-10 concentration is significantly higher in patients with DKA before treatment. Both Concepción-Martín [18] and Vasseur [19] have demonstrated that IL-10 can serve as a predictor of AP because it is closely related to severity of the disease. Due to differences among study subjects, we could not assess their levels of IL-10 in these studies. Nevertheless, our present study shows that it is possible to distinguish between DKA alone and DKA with AP by measuring serum IL-10 levels in patients. In a study by Nett et al. [20], serum levels of TNF-α was higher in patients with DKA as well as in patients with AP. This cytokine is deeply involved in AP with multiple organ dysfunction [21]. Balog et al. [22] also found that TNF-α is associated with risk of severe AP. In our results, the AUC for IL-10 in diagnosis of DKA with AP was 0.699 (95% CI, 0.601-0.792), indicating good diagnostic value. AUC for TNF-α plus IL-10 in diagnosis of DKA with AP was 0.814 (95% CI, 0.517-0.932), higher than that of TNF-α or IL-10 alone ($P < 0.05$).

In this study, among patients in the control group, 31 (14.35%) had abdominal pain and 41 (18.98%) showed elevated serum amylase levels. Among them, 6 patients had serum amylase levels that were 3-fold higher than normal value. Fifteen patients had abdominal pain with elevated serum amylase. Generally, a serum amylase level that is 3 times the normal value is considered a specific indicator of pancreatitis [12]. Therefore, for such patients, diagnosis should be made with caution, even though elevated serum amylase levels in many patients is only temporary. In our study, among patients with DKA, only 47 had TNF-α levels above 1.80 ng/L and 51 had IL-10 levels above 21.10 pg/L. Among patients with the dual condition, 23 had TNF-α levels below 1.80 ng/L and 25 had IL-10 levels below 21.10 pg/L. Greater sample size is required for further analysis of association between changes in these levels.

We further analyzed the value of TNF-α in combination with IL-10 for diagnosis of DKA patients with AP 24 hours and 48 hours after admission. We found that the value of TNF-α in combination with IL-10 for diagnosis of DKA patients with AP 48 hours after admission was higher than that of 24 hours after admission, while AUC of TNF-α in combination with IL-10 for diagnosis of DKA patients with AP 24 hours after admission was 0.725. Our results are lacking early diagnostic efficiency for DKA patients with AP. Therefore, further study is needed regarding the value of TNF-α in combination with IL-10 for early diagnosis of DKA patients.

In summary, because serum levels of TNF-α and IL-10 are higher in patients with DKA and AP, a test based on both inflammatory cyto-

kines could have good diagnostic value for patients with the dual condition. Clinicians should be cautious with differential diagnosis of simple DKA and DKA with AP.

Disclosure of conflict of interest

None.

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