

## Original Article

# Clinical significance of oxidative stress and inflammatory factors in the peripheral blood of elderly patients with diabetic ketoacidosis

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**Abstract:** Objective: To investigate the clinical significance of oxidative stress and inflammatory factors in the peripheral blood of elderly patients with diabetic ketoacidosis. Methods: Forty-eight cases of elderly patients with diabetic ketoacidosis were allocated into the observation group. At the same time, 48 cases of ordinary elderly diabetic patients were allocated into the control group (with no ketoacidosis or hyperosmolar coma). The changes in oxidative stress and inflammatory factors between the two groups were observed, so as to analyze the clinical significance and relationship of above parameters with blood ketone body in elderly patients with diabetic ketoacidosis. Results: The level of malondialdehyde (MDA) in the peripheral blood of the observation group was significantly higher than that of the control group ( $P < 0.001$ ), whereas the levels of glutathione peroxidase (GSH-Px) and superoxide dismutase (SOD) in the peripheral blood of the observation group were significantly lower than those of the control group (both  $P < 0.001$ ). The level of tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) in the peripheral blood of the observation group was significantly higher than that of the control group ( $P < 0.001$ ), whereas no significant difference was found in the expression of interleukin-10 (IL-10) and interferon- $\gamma$  (INF- $\gamma$ ) between the peripheral blood of the two groups (both  $P > 0.05$ ). The level of ketone body in the observation group was  $2.89 \pm 1.05$  mmol/L, which was positively correlated with the level of MDA ( $r = 0.7317$ ,  $P < 0.05$ ) and TNF- $\alpha$  ( $r = 0.7342$ ,  $P < 0.05$ ), but negatively correlated with the level of GSH-Px ( $r = -0.7053$ ,  $P < 0.05$ ) and SOD ( $r = -0.6541$ ,  $P < 0.05$ ). In addition, the level of ketone body in the observation group showed no correlation with the level of IL-10 and INF- $\gamma$  (both  $P > 0.05$ ). The level of ketone body in the control group was  $0.97 \pm 0.32$  mmol/L, which was positively correlated with the level of MDA ( $r = 0.7355$ ,  $P < 0.05$ ) and TNF- $\alpha$  ( $r = 0.5947$ ,  $P < 0.05$ ), but negatively correlated with the level of GSH-Px ( $r = -0.4596$ ,  $P < 0.05$ ) and SOD ( $r = -0.5773$ ,  $P < 0.05$ ). In addition, the level of ketone body in the control group showed no correlation with the level of IL-10 and INF- $\gamma$  (both  $P > 0.05$ ). Conclusion: Systemic oxidative stress and inflammation were present in elderly patients with diabetic ketoacidosis, which were correlated with the level of blood ketone bodies. Therefore, in addition to lowering the level of blood glucose, anti-inflammatory and anti-oxidative stress medications should be given in clinical applications. Such clinical practices are of great significance in the treatment of elderly patients with diabetic ketoacidosis.

**Keywords:** Diabetic ketoacidosis, oxidative stress, inflammatory factors, clinical analysis

## Introduction

Diabetes mellitus (DM) is one of the most common metabolic diseases in China. Due to the large number of DM patients, the task of DM prevention and treatment is difficult. In addition, some DM patients may develop complications, such as ketoacidosis, that are induced by infections or irregular administration of medications [1, 2]. Such patients generally present both systemic oxidative stress and inflamma-

tory response, accompanied by the aggravated imbalance of the internal environment [3, 4]. In this study, 48 cases of elderly patients with diabetic ketoacidosis were allocated into the observation group. At the same time, 48 cases of ordinary elderly diabetic patients were allocated into the control group (with no ketoacidosis or hyperosmolar coma). The changes in oxidative stress (superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), malondialdehyde (MDA)) and inflammatory factors (inter-

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**Table 1.** Comparison of general clinical data between the two groups of patients

Group	Male/Female	Age (years old)	BMI (kg/m <sup>2</sup> )	Smoking history (year)	Alcohol drinking history (year)	Blood glucose (mmol/L)	Urine sugar	
							(+)	(++)
Observation group	27/21	66.1±4.5	23.2±1.7	18	16	17.4±2.1	20	28
Control group	25/23	65.3±4.1	22.5±1.5	15	14	16.7±2.2	23	25
t/X <sup>2</sup>		0.910	0.917	0.416	0.194	1.595	1.037	
P		0.365	0.362	0.519	0.660	0.114	0.308	

Note: BMI, body mass index.

**Table 2.** Observation of oxidative stress factors in the peripheral blood of two groups of patients

Group	Case	MDA (nmol/L)	GSH-Px (μmol/L)	SOD (μU/L)
Observation group	48	24.2±8.9	153.4±30.5	28.6±8.6
Control group	48	12.3±3.9	205.6±50.4	48.2±10.1
t		8.485	6.193	10.240
P		<0.001	<0.001	<0.001

Note: MDA, malondialdehyde; GSH-Px, glutathione peroxidase; SOD, superoxide dismutase.

**Table 3.** Comparison of inflammatory factors in the peripheral blood of two groups

Group	Case	TNF-α	IL-10	INF-γ
Observation group	48	18.9±4.0	13.8±3.4	1.17±0.20
Control group	48	10.9±3.2	12.7±3.1	1.05±0.11
t		10.820	1.656	0.082
P		<0.001	0.101	0.935

Note: TNF-α, tumor necrosis factor-α; IL-10, interleukin-10; INF-γ, interferon-γ.

feron-γ (INF-γ), tumor necrosis factor-α (TNF-α), interleukin-10 (IL-10)) between the two groups were observed, so as to analyze the relationship of above parameters with the level of blood ketone body.

## Materials and methods

### General information

Forty-eight cases of elderly patients with diabetic ketoacidosis, who were treated at Shanghai Jiao Tong University Affiliated Sixth People's Hospital South Campus from June 2014 to June 2017, were allocated into the observation group. In addition, 48 cases of ordinary elderly diabetic patients with no ketoacidosis, who were treated during the same period of time, were allocated into the control group. All patients were diagnosed with type 2

DM according to the "Expert consensus for the diagnosis and treatment of elderly diabetes (2013 Edition)" [5]. For the diagnosis of patients with diabetic ketoacidosis, it was mainly based on the presence of metabolic acidosis, hyperkeratosis, and hyperglycemia: a patient could be diagnosed with diabetic ketoacidosis if all of the following conditions were met: significant increase in the level of blood glucose (general blood glucose >300 mg/dL or 16.6 mmol/L), positive in the presence of urine ketone, acidemia (pH=7.2), and the presence of clinical manifestations of ketoacidosis [6, 7]. Patients with severe liver or kidney dysfunctions or end-stage cancers were excluded from this study [8]. This study was approved by the Ethics Committee of Shanghai Jiao Tong University Affiliated Sixth People's Hospital South Campus, and all patients and their families have signed the form of informed consent.

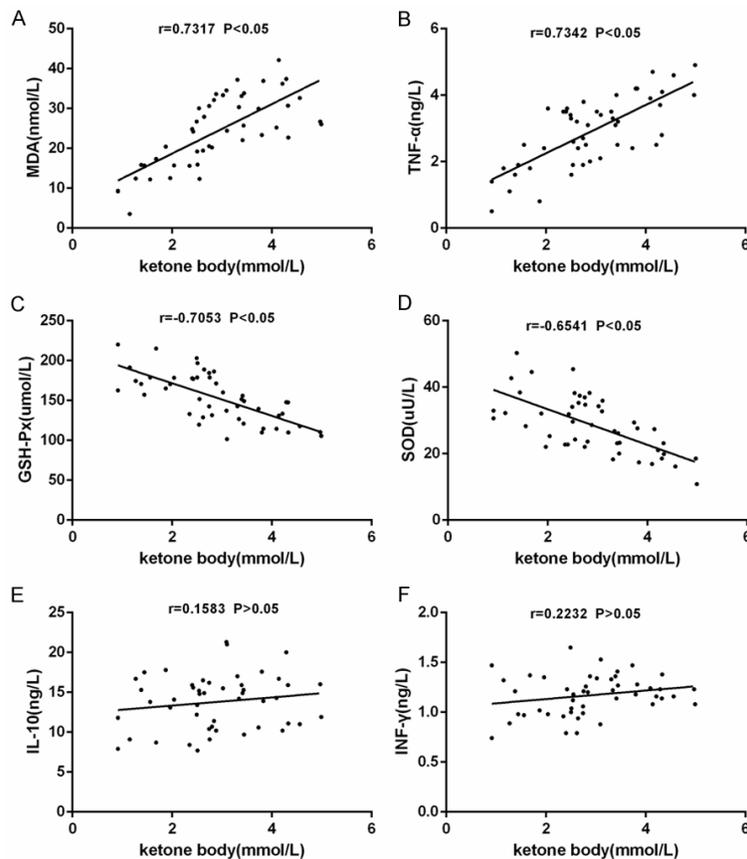
### Detection parameters

Peripheral blood (5 mL) was collected from each of the subjects in the morning under fasting conditions. The following parameters were detected: 1) Oxidative stress factors (SOD, μU/L; GSH-Px, nmol/L; MDA, nmol/L) were detected and analyzed by an automatic biochemical analyzer in Shanghai Jiao Tong University Affiliated Sixth People's Hospital South Campus; 2) Inflammatory factors (INF-γ, ng/L; TNF-α, ng/L; IL-10, ng/L) were detected by enzyme linked immunosorbent assay (ELISA), in which each sample was tested repeatedly for 3 times. The ELISA assay kits were purchased from Shanghai ELISA biotechnology Co.

### Observational parameters

In this study, the main parameters of observation were to check whether the oxidative stress

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**Figure 1.** Results of Pearson correlation analysis of ketone body, oxidative stress factors and inflammatory factors in the observation group (n=48). A: Results of correlation analysis regarding the levels of ketone body and MDA in the observation group; B: Results of correlation analysis regarding the levels of ketone body and TNF- $\alpha$  in the observation group; C: Results of correlation analysis regarding the levels of ketone body and GSH-Px in the observation group; D: Results of correlation analysis regarding the levels of ketone body and SOD in the observation group; E: Results of correlation analysis regarding the levels of ketone body and IL-10 in the observation group; F: Results of correlation analysis regarding the levels of ketone body and INF- $\gamma$  in the observation group. MDA, malondialdehyde; GSH-Px, glutathione peroxidase; SOD, superoxide dismutase; TNF- $\alpha$ , tumor necrosis factor- $\alpha$ ; IL-10, interleukin-10; INF- $\gamma$ , interferon- $\gamma$ .

(MDA, GSH-Px, SOD) and inflammatory factors (TNF- $\alpha$ , IL-10, INF- $\gamma$ ) in the diabetic patients with ketoacidosis were higher than those in ordinary diabetic without ketoacidosis. In addition, the correlation between oxidative stress/inflammatory factors and the level of blood ketone body was explored.

### Statistical methods

All data were analyzed using SPSS18.0 statistical software. The measurement data were presented as mean  $\pm$  standard deviation ( $\bar{x} \pm sd$ ) and their inter-group comparisons were carried

out using independent samples t-tests. The count data were expressed as percentages and their inter-group comparisons were carried out using chi-square tests. The correlation analysis was performed using Pearson analysis. A P value of  $<0.05$  indicated that the difference was statistically significant.

### Results

#### *Comparison of general clinical data between the two groups of patients*

The two groups of patients showed no significant difference ( $P>0.05$ ) in terms of general clinical data such as gender, age, smoking history and alcohol drinking history. Thus, the two groups were comparable. See **Table 1**.

#### *Observation of oxidative stress factors in the peripheral blood of two groups of diabetic patients*

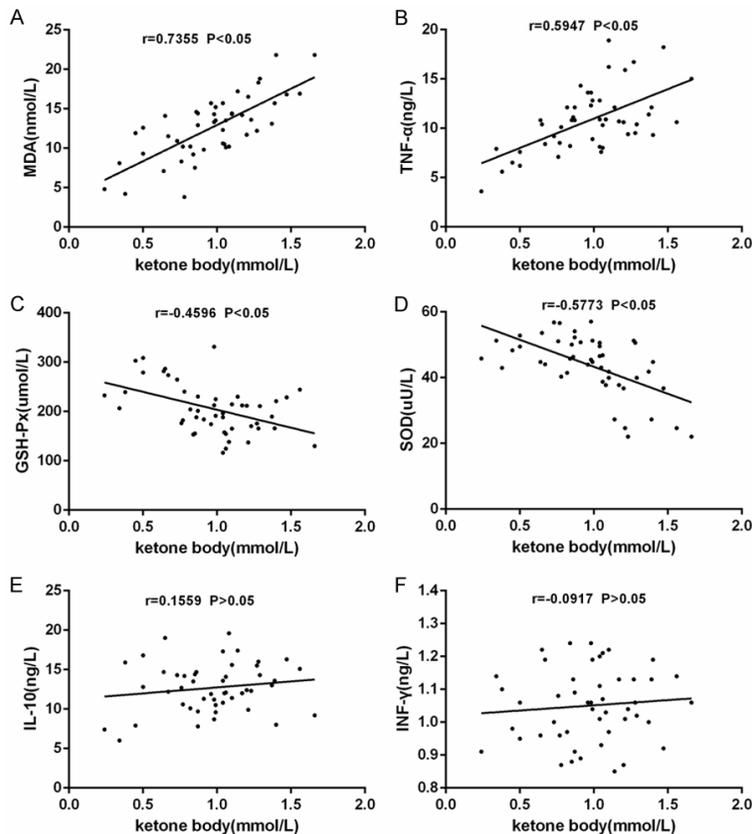
The level of MDA in the peripheral blood of the observation group was higher than that in the control group ( $P<0.001$ ), whereas the level of GSH-Px and SOD in the peripheral blood of the observation group was lower than that of the control group (both  $P<0.001$ ). The

differences were statistically significant, as shown in **Table 2**.

#### *Comparison of inflammatory factors in the peripheral blood of two groups of diabetic patients*

The level of TNF- $\alpha$  in the peripheral blood of the observation group was significantly higher than that of the control group ( $P<0.001$ ), whereas no significant difference was found in the expression of IL-10 and INF- $\gamma$  in the peripheral blood of the two groups (both  $P>0.05$ ), as shown in **Table 3**.

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**Figure 2.** Results of Pearson correlation analysis of ketone body, oxidative stress factors and inflammatory factors in the control group (n=48). A: Results of correlation analysis regarding the levels of ketone body and MDA in the control group; B: Results of correlation analysis regarding the levels of ketone body and TNF- $\alpha$  in the control group; C: Results of correlation analysis regarding the levels of ketone body and GSH-Px in the control group; D: Results of correlation analysis regarding the levels of ketone body and SOD in the control group; E: Results of correlation analysis regarding the levels of ketone body and IL-10 in the control group; F: Results of correlation analysis regarding the levels of ketone body and INF- $\gamma$  in the control group. MDA, malondialdehyde; GSH-Px, glutathione peroxidase; SOD, superoxide dismutase; TNF- $\alpha$ , tumor necrosis factor- $\alpha$ ; IL-10, interleukin-10; INF- $\gamma$ , interferon- $\gamma$ .

### *Correlation between ketone body and oxidative stress/inflammatory factors in the two groups of patients*

The level of ketone body in the observation group was  $2.89 \pm 1.05$  mmol/L, which was positively correlated with the level of MDA ( $r=0.7317$ ,  $P<0.05$ ) and TNF- $\alpha$  ( $r=0.7342$ ,  $P<0.05$ ), but negatively correlated with the level of GSH-Px ( $r=-0.7053$ ,  $P<0.05$ ) and SOD ( $r=-0.6541$ ,  $P<0.05$ ). In addition, the level of ketone body in the observation group showed no correlation with the level of IL-10 and INF- $\gamma$  (both  $P>0.05$ ). See **Figure 1**.

The level of ketone body in the control group was  $0.97 \pm 0.32$  mmol/L, which was positively correlated with the level of MDA ( $r=0.7355$ ,  $P<0.05$ ) and TNF- $\alpha$  ( $r=0.5947$ ,  $P<0.05$ ), but negatively correlated with the level of GSH-Px ( $r=-0.4596$ ,  $P<0.05$ ) and SOD ( $r=-0.5773$ ,  $P<0.05$ ). In addition, the level of ketone body in the control group showed no correlation with the level of IL-10 and INF- $\gamma$  (both  $P>0.05$ ). See **Figure 2**.

### Discussion

DM is a type of disease in which the physiological insulin cannot exert its normal functions, or the physiological secretion of insulin is reduced or even absent. In recent years, the incidence of DM has been high, while many complications are also attributed to DM. For example, diabetic ketoacidosis is one of the most common complications of DM. The predisposing factors of diabetic ketoacidosis mainly include fatigue, trauma, inadequate use of medications, infections and so on. The patients of diabetic ketoacidosis often suffer from a variety of complications, such as hyperchloremia, hypoglycemia, hypokalemia and cerebral edema [9, 10]. When the dosage of insulin

administration is insufficient or discontinued, it can lead to a sharp rise in blood glucose, abnormal decomposition and metabolism of lipids, and the accumulation of a large number of ketone bodies, which will eventually cause ketosis, positive result of ketonuria, and severe disorders in the internal environment of the body, accompanied by systemic oxidative stress and inflammatory responses [11-13]. Under physiological conditions, the free radicals produced by the body can be cleared by SOD and GSH-Px [14]. However, under conditions of diabetic ketoacidosis, the excessive amount of free radicals produced by the body

cannot be eliminated and hence will cause a damaging effect on normal cells. At the same time, the high level of blood glucose and ketone bodies can also induce systemic oxidative stress and inflammatory reactions, thus causing the degradation of the internal environment in the patient and even death in severe cases [15].

TNF- $\alpha$  plays an important role in maintaining the status of chronic inflammation and cascade amplification of acute inflammation, and hence is an important factor reflecting the degree of inflammation and infection [16]. MDA is a substance produced by the peroxidative reaction between the oxygen free radicals in the body and unsaturated fatty acids in the cells, and is also a parameter reflecting the level of oxidative stress in human body. In this study, the changes in oxidative stress and inflammatory factors in the peripheral blood between the two groups of diabetic patients were observed, so as to analyze the correlation and clinical significance of these factors with the level of blood ketone body. The results showed that the level of MDA in the peripheral blood of the observation group was significantly higher than that of the control group, whereas the level of GSH-Px and SOD in the peripheral blood of the observation group was significantly lower than that of the control group. The level of TNF- $\alpha$  in the peripheral blood of the observation group was significantly higher than that of the control group, whereas no significant difference was found in the expression of IL-10 and INF- $\gamma$  between the peripheral blood of the two groups. In addition, the level of blood ketone body was positively correlated with the level of MDA and TNF- $\alpha$ , but negatively correlated with the level of GSH-Px and SOD. Furthermore, the level of blood ketone body showed no correlation with the level of IL-10 and INF- $\gamma$ . Li et al. reported that, in patients with diabetic ketoacidosis, the fasting glucose level was positively correlated with the level of MDA and TNF- $\alpha$ , but negatively correlated with the level of SOD and GSH-Px [17, 18]. The authors also pointed out that the prognosis of diabetic ketoacidosis was closely related to oxidative stress and inflammatory factors. However, the above studies only observed the correlation between the levels of fasting blood glucose and oxidative stress/inflammatory factors, but did not observe such correlations in diabetic patients with no keto-

acidosis. Therefore, the data of this study have filled the gap of above studied and hence laid the foundation for further research.

In addition, patients of diabetic ketoacidosis suffer from a reduced capability to protect them against oxidative stress and a dramatically increased level of blood glucose, which are the leading causes of complications such as ketoacidosis [19, 20]. Therefore, in addition to lowering the level of blood glucose, anti-inflammatory and anti-oxidative stress medications should be given in clinical applications. Such clinical practices are of great significance in the treatment of elderly patients with diabetic ketoacidosis [21].

In summary, systemic oxidative stress and inflammatory response are present in elderly patients of diabetic ketoacidosis, and are related to the level of blood ketone bodies.

### Disclosure of conflict of interest

None.

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