HOSTED BY
ELSEVIER
Contents lists available at ScienceDirect
Journal of Acute Disease
journal homepage: www.jadweb.org

Original article http://dx.doi.org/10.1016/j.joad.2016.03.011

Study on the relationship of acute ketosis intoxication and type 2 diabetes mellitus

Tian-Xiao Hu, Qing-Ying Tan, Yun Ruan, Yong Ruan, Xiu-Jing Wang, Jia-Qi Yao, Hui-Ling Wang, Jing Wang*

Department of Endocrinology, the 117th Hospital of Chinese People's Liberation Army, Hangzhou, Zhejiang, China

ARTICLE INFO

Article history:
Received 28 Feb 2016
Accepted 12 Mar 2016
Available online 2 Apr 2016

Keywords:
Type 2 diabetes mellitus
Ketosis
β-Hydroxybutyric acid
C-reactive protein

ABSTRACT

Objective: To study the change of serum C-reactive protein (CRP) levels and its correlation with ketosis in type 2 diabetes mellitus patients with acute ketosis intoxication.
Methods: A retrospective analysis was conducted for the patients with type 2 diabetes mellitus from August 2015 to January 2016. The patients combined with ketosis were included into diabetic ketosis group and the patients without ketosis were included into negative control group. The clinical data were collected from two groups including general data, blood pressure, liver function and the levels of blood fat, glycosylated hemoglobin, blood ketone, β-hydroxybutyric acid and CRP. The discrepancy of clinical data between two groups was analyzed.
Results: The levels of glycosylated hemoglobin [(11.6 ± 2.1)% *vs.* (8.3 ± 1.9)%], blood ketone [0.65 (0.3, 1.75) *vs.* 0.1 (0.1, 0.2) mmol/L], β-hydroxybutyric acid [0.595 (0.303, 1.775) *vs.* 0.08 (0.06, 0.15) mmol/L] and CRP [0.595 (0.303, 1.775) *vs.* 0.08 (0.06, 0.15) mmol/L] were significant higher than those of negative control group, while the levels of blood pressure, blood fat and aminopherase had no significant difference. The serum CRP levels showed positive correlation with blood ketone and β-hydroxybutyric acid (r = 0.490 and r = 0.478, respectively).
Conclusions: Poor blood glucose control for a long time and strengthening inflammatory response are correlated with the status of acute ketosis in type 2 diabetes mellitus patients. The CRP levels in ketosis patients were significantly elevated and could be used to evaluate the degree of ketosis.

1. Introduction

Diabetic ketosis (DK) is one of the common acute complications in diabetes mellitus patients and this disease caused by ketosis has always been considered as the important feature of type 1 diabetes mellitus [1,2]. Nevertheless, in recent years, more and more foreign scholars found that type 2 diabetes mellitus is also quite common in diabetes mellitus patients caused by ketosis and ketoacidosis. Umpierrez research showed that the ratio of type 2 diabetes mellitus is taking up more than half in Afro-American diabetes mellitus patients caused by ketoacidosis[3]. Kim study revealed that the ratio of type 2 diabetes mellitus is up to 40% in Korean diabetes mellitus patients caused by ketoacidosis[4]. Therefore, clinical scholars pay more attention to these type 2 diabetes mellitus patients combined with ketosis.

All DK patients are in an acute and inflammatory condition. Acute phase reactive protein CRP, proinflammatory cytokine IL-6 and suppression of inflammatory cytokine IL-10 have a large number of synthesis and secretion in an acute condition[5–7]. A number of studies have confirmed that CRP levels are significant higher and related with the degree of severity of ketosis in the serum of type 1 diabetes mellitus patients combined with ketosis[8–10]. However, the synthetic condition of CRP has not been reported clearly in the body of type 2 diabetes mellitus patients combined with ketosis so far. CRP is a kind of acute phase reactive protein, which is compounded and secreted from hepatic cell under inducing of proinflammatory cytokine such as IL-6 and so on and can participate in inflammatory response by activating inflammatory factors secreted from macrophage and promoting inflammatory

*Corresponding author: Jing Wang, Department of Endocrinology, Chinese PLA 117th Hospital, 14 Lingyin Road, Hangzhou 310013, PR China.
Tel: +86 0571 87348612
E-mail: Wangjing20071@126.com

The study protocol was performed according to the Helsinki declaration and approved by the ethic committee of 117th Hospital. Informed written consent was obtained from 117th Hospital.

Foundation Project: Supported by Medical Science and Technology Innovation Subject of Nanjing Region (Grant No. 14ZX30 and 11MA124).

Peer review under responsibility of Hainan Medical College. The journal implements double-blind peer review practiced by specially invited international editorial board members.

cell sticking to inflammatory site. In the following study, we analyze the change of serum C-reactive protein (CRP) levels and its correlation with ketosis in type 2 diabetes mellitus patients with acute ketosis intoxication.

2. Materials and methods

2.1. Study object

A total of 100 type 2 diabetes mellitus patients were collected randomly from Department of Endocrinology, the 117th Hospital of Chinese People's Liberation Army from August 2015 to January 2016 and were retrospectively analyzed. Inclusion and exclusion criteria of cases were as follows: (1) met the diagnostic criteria for type 2 diabetes mellitus established by World Health Organization (1999); (2) all the serum glutamic acid decarboxylase antibody (GAD-Ab), islet cell cytoplasmic antibody (ICA) and insulin autoantibody were negative; (3) type 1 diabetes mellitus, gestational diabetes mellitus and other specific types of diabetes mellitus were excluded and (4) the patients with a state of infection and fever after operation were excluded. In 100 patients, 67 cases were male and 33 cases were female. A total of 52 cases were received the treatment of oral hypoglycemic drugs, 11 cases were received insulin treatment and 37 cases were received the treatment of oral hypoglycemic drugs combined with insulin. According to the following criteria to judge ketosis: blood glucose levels were more than 13.9 mmol/L and ketonuria was more than 1+. A total of 39 cases diagnosed with ketosis were collected into DK group and 61 cases not combined with ketosis diagnosis were collected into NC group.

2.2. Clinical data collection

After admitting to hospital, all patients received a physical examination and measurement to record the height and weight and calculate body weight index (BMI). Riva-rocci sphygmomanometer was used to measure systolic pressure (SBP) and diastolic pressure (DBP). Peripheral vein blood was collected under the fasting state. Beckman fully automatic biochemical analyser was used to detect triglyceride (TG), total cholesterol (TC), alanine aminotransferase (ALT), aspartate aminotransferase (AST) and β-hydroxybutyric acid levels. Clean catch midstream urine was collected and urine acetone bodies levels were disposed with semi-quantitative analysis by chemical method. After collecting terminal blood, blood ketone levels were detected by Optium Xceed blood ketone instrument manufactured from Abbott Company. Full-automatic Afinion AS100 Analyzer (Afinion Company) was used to measure glycosylated hemoglobin levels.

2.3. Statistical methods

SPSS 20.0 software was used to input data. Measurement data were examined using homogeneity of variance of which the data accorded to homogeneity of variance were expressed as mean ± SD and were analyzed by *t*-test, while the data not accorded to homogeneity of variance were expressed as median (P25, P75) and were analyzed by nonparametric rank and inspection. Enumeration data were expressed as frequency and were analyzed by *Chi*-square test. The correlativity between two variables was analyzed by Pearson correlation. Difference was statistically significant ($P < 0.05$).

3. Results

3.1. General clinical data of two group patients

DK group included 28 male cases and 11 female cases [(47.8 ± 12.4) years, (23.9 ± 3.1) kg/m^2 BMI, (127.2 ± 13.2) mmHg SBP, (83.8 ± 8.6) mmHg DBP, (5.5 ± 1.8) mmol/L TG, (2.0 ± 0.76) mmol/L cholesterol, (24.1 ± 12.7) IU/mL ALT, (19.7 ± 11.9) IU/mL AST and 11.6% ± 2.1% glycosylated hemoglobin]. NC group included 39 male cases and 22 female cases [(55.5 ± 10.8) years, (24.7 ± 2.9) kg/m^2 BMI, (130.7 ± 19.8) mmHg SBP, (84.7 ± 12.3) mmHg DBP, (5.2 ± 1.3) mmol/L triglyceride, (2.1 ± 1.5) mmol/L cholesterol, (27.8 ± 14.5) IU/mL ALT, (21.8 ± 14.5) IU/mL AST and 8.3% ± 1.9% glycosylated hemoglobin]. According to statistic analysis, the gender, BMI, blood pressure and triglyceride, cholesterol, AST and ALT levels of DK group had no significant difference with NC group. The age in DK group was significant lower than NC group, while glycated hemoglobin levels were significant higher than those in NC group (Table 1).

3.2. Blood ketone and β-hydroxybutyric acid levels of patients in two groups

After homogeneity of variance test, blood ketone and β-hydroxybutyric acid levels of two groups were not accordant with homogeneity of variance and were expressed as median (P25, P75). In DK group, blood ketone level was [0.65 (0.3, 1.75)] mmol/L and β-hydroxybutyric acid level was [0.595 (0.303, 1.775)] mmol/L. In NC group, blood ketone level was [0.1 (0.1, 0.2)] mmol/L and β-hydroxybutyric acid level was [0.08 (0.06, 0.15)] mmol/L. After nonparametric rank sum test, blood ketone and β-hydroxybutyric acid levels of patients in DK group were significant higher than NC group (Figure 1).

3.3. Serum CRP contents of patients in two groups

According to homogeneity of variance test, serum CRP contents of patients in two groups were not accordant with homogeneity of variance and were expressed as median (P25, P75). Serum CRP contents of patients in DK group {[4.78 (2.85, 7.63)] mg/L} were significant higher than serum CRP contents in NC group {[2.45 (2.10, 3.07)] mg/L} (Figure 2).

Table 1
General clinical data of two group patients.

Parameter	DK (n = 39)	NC group (n = 61)	P
Gender (male/female)	28/11	39/22	> 0.05
Age (years)	47.8 ± 12.4	55.5 ± 10.8	< 0.01
BMI (kg/m^2)	23.9 ± 3.1	24.7 ± 2.9	> 0.05
SBP (mmHg)	127.2 ± 13.2	130.7 ± 19.8	> 0.05
DBP (mmHg)	83.8 ± 8.6	84.7 ± 12.3	> 0.05
TG (mmol/L)	5.5 ± 1.8	5.2 ± 1.3	> 0.05
TC (mmol/L)	2.0 ± 0.76	2.1 ± 1.5	> 0.05
ALT (IU/L)	24.1 ± 12.7	27.8 ± 14.5	> 0.05
AST (IU/L)	19.7 ± 11.9	21.8 ± 14.5	> 0.05
HbA1c (%)	11.6 ± 2.1	8.3 ± 1.9	< 0.01

HbA1c: Glycosylated hemoglobin.

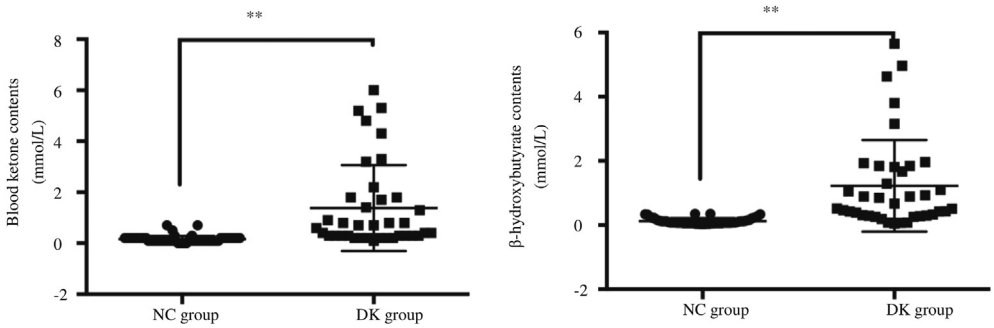

Figure 1. Blood ketone and β-hydroxybutyric acid contents in two groups.
Left: Blood ketone contents of patients in DK group {[0.65 (0.3, 1.75)] mmol/L} were significant higher than those in NC group {[0.1 (0.1, 0.2)] mmol/L}, $Z = 7.215$, $P < 0.001$; Right: β-hydroxybutyric acid contents of patients in DK group {[0.595 (0.303, 1.775)] mmol/L} were significant higher than those in NC group {[0.08 (0.06, 0.15)] mmol/L}, $Z = 6.776$, $P < 0.001$; **: $P < 0.01$. Data were expressed as median (P25, P75).

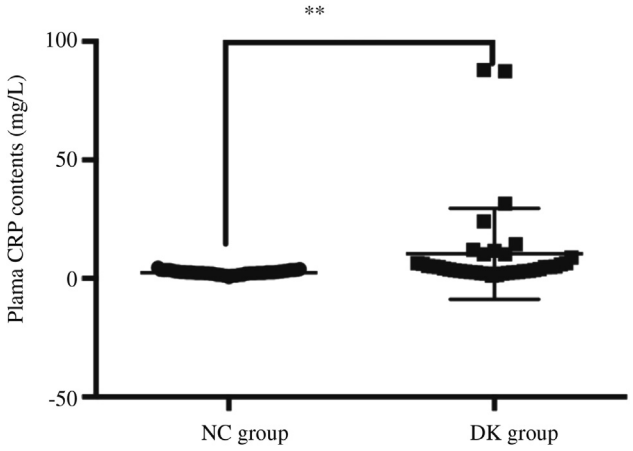

Figure 2. Serum CRP contents of patients in two groups.
Serum CRP contents of patients in DK group {[4.78 (2.85, 7.63)] mg/L} were significant higher than those in NC group {[2.45 (2.10, 3.07)] mg/L}, $P < 0.001$. Data were expressed as median (P25, P75). **: $P < 0.01$.

3.4. Results of Pearson correlation analysis

Through Pearson correlation analysis, the correlativity of CRP contents, blood ketone and β-hydroxybutyric acid contents showed that CRP contents presented positive correlation with blood ketone and β-hydroxybutyric acid contents ($r = 0.490$ and 0.478, respectively) ($P < 0.01$) (Figure 3).

4. Discussion

Ketone body is a product of incomplete oxidation in internal fat. As the aggravation of diabetes mellitus, the metabolic disorders of three major matters and the increasing of lipid mobilization and decomposition, β-oxidation can occur in a large number of fatty acids that produce a plenty of β-hydroxybutyric acid, acetoacetic acid and acetone, which are collectively referred to as the ketone body[11–13]. When the generation of ketone body increases and exceeds the oxidizing ability of extrahepatic tissue, ketone body contents are significant higher and the discharge of urine acetone body increases and becomes ketonuria, which is referred as ketosis clinically[14,15]. DK is one of the common acute complications in diabetes mellitus patients and the majority of type 1 diabetes mellitus patients are caused by ketoacidosis[16–18]. In recent years, more and more foreign scholars found that type 2 diabetes mellitus patients are also caused by ketosis and ketoacidosis[3,4]; the reasons include obesity, insulin resistance and poor control of blood glucose for a long time and so on[19–21]. In the collected type 2 diabetes mellitus patients of above research, the age of patients with ketosis is significant lower than patients without ketosis and glycosylated hemoglobin contents are significant higher than patients without ketosis, while the BMI index and blood fat content had no significant difference with patients without ketosis. Glycosylated hemoglobin content is the index to reflect blood glucose control situation of diabetes mellitus patients in previous 2–3 months. The higher content explained

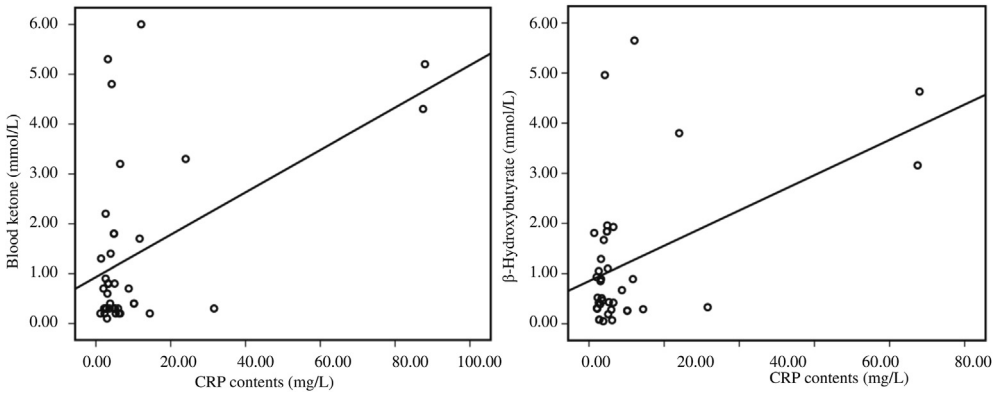

Figure 3. The correlation of serum CRP contents, blood ketone and β-hydroxybutyric acid contents.
Left: The correlation of serum CRP contents and blood ketone contents, $r = 0.490$, $P < 0.01$; Right: The correlation of serum CRP contents and β-hydroxybutyric acid contents, $r = 0.478$, $P < 0.01$.

that the blood glucose level is higher within previous 2–3 months. Combining with the analysis result showed that the occurrence of type 2 diabetic ketosis is mainly associated with poor long-term blood sugar control and the occurrence of risk of ketosis in young patients is relatively high. However, the association has not yet been found in obesity, blood lipid metabolism condition, and type 2 diabetes mellitus patients with ketosis, which is not consistent with the existing research results and may be due to smaller sample size in this study.

Diabetic ketosis is a kind of acute complication and its body is in a state of inflammation. A variety of proinflammatory factors generate a mass of synthesis and secretion in the process of quickly raising blood sugar and mass-produced blood ketone. Some studies have confirmed that in the process of the progression of diabetic ketosis, all the generation of proinflammatory cytokine (IL-6) and suppression of inflammatory cytokines (IL-10) are significantly increased[7,22]. IL-6 can promote inflammatory response and participate in the occurrence of concurrent change of ketosis such as pulmonary edema and hydrocephalus, while IL-10 is the expression of its compensation in body and can exert protective effect by reducing endothelial permeability[23,24]. In a state of inflammation, IL-6 will induce hepatic cell to compound a mass of CRP. In recent years, foreign study has reported that there is a correlation between the change of serum CRP content and type 1 diabetes mellitus patients ketosis and ketoacidosis, which showed that there is a correlation between the generation of ketone body and CRP[8–10]. Nevertheless, the change condition of serum CRP content of type 2 diabetes mellitus ketosis patients has not yet been reported clearly by far. The research of Namavar Jahromi B analyzed 68 cases in gestational diabetes mellitus (GDM) and found that GDM combined with ketosis will not increase CRP content[25]. Whereas, in view of the endocrine environment existing significant change in body in the process of pregnancy and the placenta tissue itself can synthesize and secrete a large number of CRP, the change of CRP synthesis of hepatic cell is hardly expressed in serum when diabetes mellitus combined with ketosis. We analyzed serum CRP content in type 2 diabetes mellitus patients and found that serum CRP content of patients in DK group is significant higher than that in NC group. This preliminary illustrated that type 2 diabetes mellitus patients combined with ketosis are similar with type 1 diabetes mellitus patients combined with ketosis, where body is in a state of inflammation and serum CRP content is significantly elevated.

β-Hydroxybutyric acid is the main component of ketone body and also is the main substance which causes diabetic ketosis accounting for about 78%, while acetoacetic acid and acetone account for 20% and 2% respectively[26,27]. In clinic, diabetic ketosis is diagnosed commonly by detecting urine acetone body; the method is sodium nitroprusside and can only semi-quantitative detect the acetyl acetate and acetone, while it almost didn't react with β-hydroxybutyric acid. However, in the process of the occurrence of diabetic ketosis, the production of β-hydroxybutyric acid is increased significantly, while the production of acetone and acetoacetic acid are reduced relatively. At this moment, through the test of acetone and acetoacetic acid in urine to evaluate the production situation of ketone body *in vivo* can cause false negative[28,29]. From the generation process and discharge process of ketone body *in vivo*, the change of blood ketone body is ahead of urine ketone body, while β-hydroxybutyric acid is also the main component of blood ketone body. Therefore, the generation condition of ketone body of diabetes mellitus patients with ketosis can be evaluated more accurately through detecting the blood ketone body and β-hydroxybutyric acid content[30]. In the above research, we analyzed and found that all the blood ketone body content and β-hydroxybutyric acid are elevated significantly and have a positive correlation with serum CRP content. This means that the change of CRP content in type 2 diabetes mellitus patients is closely related to the generation of ketone body. The generation condition of ketone body and the severity of patients' condition of ketosis can be evaluated through detecting serum CRP content.

In conclusion, poor control of blood glucose for a long time and strengthened inflammatory response are related to the state of acute ketosis of type 2 diabetes mellitus patients. Serum CRP content of patients with ketosis is increased significantly and can be used to evaluate the degree of ketosis.

Conflict of interest statement

The authors report no conflict of interest.

References

[1] Karges B, Rosenbauer J, Holterhus PM, Beyer P, Seithe H, Vogel C, et al. Hospital admission for diabetic ketoacidosis or severe hypoglycemia in 31 330 young patients with type 1 diabetes. *Eur J Endocrinol* 2015; **173**(3): 341-50.

[2] Amiel SA, Pursey N, Higgins B, Dawoud D; Guideline Development Group. Diagnosis and management of type 1 diabetes in adults: summary of updated NICE guidance. *BMJ* 2015; **351**: h4188.

[3] Umpierrez GE, Smiley D, Kitabchi AE. Narrative review: ketosis-prone type 2 diabetes mellitus. *Ann Intern Med* 2006; **144**: 350-7.

[4] Kim MK, Lee SH, Kim JH, Lee JI, Kim JH, Jang EH, et al. Clinical characteristics of Korean patients with new-onset diabetes presenting with diabetic ketoacidosis. *Diabetes Res Clin Pract* 2009; **85**: e8-11.

[5] Close TE, Cepinskas G, Omatsu T, Rose KL, Summers K, Patterson EK, et al. Diabetic ketoacidosis elicits systemic inflammation associated with cerebrovascular endothelial cell dysfunction. *Microcirculation* 2013; **20**(6): 534-43.

[6] Shi X, Li D, Deng Q, Li Y, Sun G, Yuan X, et al. NEFAs activate the oxidative stress-mediated NF-κB signaling pathway to induce inflammatory response in calf hepatocytes. *J Steroid Biochem Mol Biol* 2015; **145**: 103-12.

[7] Li J, Huang M, Shen X. The association of oxidative stress and pro-inflammatory cytokines in diabetic patients with hyperglycemic crisis. *J Diabetes Complications* 2014; **28**(5): 662-6.

[8] Karavanaki K, Kakleas K, Georga S, Bartzeliotou A, Mavropoulos G, Tsouvalas M, et al. Plasma high sensitivity C-reactive protein and its relationship with cytokine levels in children with newly diagnosed type 1 diabetes and ketoacidosis. *Clin Biochem* 2012; **45**(16–17): 1383-8.

[9] Ma SG, Jin Y, Xu W, Hu W, Bai F, Wu XJ. Increased serum levels of ischemia-modified albumin and C-reactive protein in type 1 diabetes patients with ketoacidosis. *Endocrine* 2012; **42**(3): 570-6.

[10] Lindroos-Jokinen K, Keltanen T, Vanhala T, Valonen T, Sajantila A. Postmortem measurement of C-reactive protein and interpretation of results in ketoacidosis. *Leg Med (Tokyo)* 2012; **14**(3): 140-6.

[11] Misra S, Oliver NS. Utility of ketone measurement in the prevention, diagnosis and management of diabetic ketoacidosis. *Diabet Med* 2015; **32**(1): 14-23.

[12] Lertwattanarak R, Plainkum P. Efficacy of quantitative capillary beta-hydroxybutyrate measurement in the diagnosis of diabetic ketoacidosis: a comparison to quantitative serum ketone

measurement by nitroprusside reaction. *J Med Assoc Thai* 2014; **97**(Suppl 3): 78-85.
[13] Akram M. A focused review of the role of ketone bodies in health and disease. *J Med Food* 2013; **16**(11): 965-7.
[14] Rosival V. Pathophysiology of diabetic ketoacidosis. *Diabet Med* 2015; **32**(11): 1527.
[15] Misra S, Oliver N. Response to Rosival: pathophysiology of diabetic ketoacidosis. *Diabet Med* 2015; **32**(11): 1527-8.
[16] Miller KM, Foster NC, Beck RW, Bergenstal RM, DuBose SN, DiMeglio LA, et al. Current state of type 1 diabetes treatment in the U.S.: updated data from the T1D Exchange clinic registry. *Diabetes Care* 2015; **38**(6): 971-8.
[17] Miller SC. Diabetic ketoacidosis and acute mountain sickness: case report and review of treatment options in type 1 diabetes mellitus. *Wilderness Environ Med* 2015; **26**(2): 185-8.
[18] Rewers A, Dong F, Slover RH, Klingensmith GJ, Rewers M. Incidence of diabetic ketoacidosis at diagnosis of type 1 diabetes in Colorado youth, 1998–2012. *JAMA* 2015; **313**(15): 1570-2.
[19] Rao PV. Type 2 diabetes in children: clinical aspects and risk factors. *Indian J Endocrinol Metab* 2015; **19**(Suppl 1): S47-50.
[20] Paoli A. Ketogenic diet for obesity: friend or foe? *Int J Environ Res Public Health* 2014; **11**(2): 2092-107.
[21] Concha LL, Durruty AP, García de Los Ríos AM. [Ketosis prone type 2 diabetes (KPD)]. *Rev Med Chil* 2015; **143**(9): 1215-8. Spanish.
[22] O'Neill S, Drobatz K, Satyaraj E, Hess R. Evaluation of cytokines and hormones in dogs before and after treatment of diabetic ketoacidosis and in uncomplicated diabetes mellitus. *Vet Immunol Immunopathol* 2012; **148**(3–4): 276-83.
[23] Karavanaki K, Karanika E, Georga S, Bartzeliotou A, Tsouvalas M, Konstantopoulos I, et al. Cytokine response to diabetic ketoacidosis (DKA) in children with type 1 diabetes (T1DM). *Endocr J* 2011; **58**(12): 1045-53.
[24] Hoffman WH, Burek CL, Waller JL, Fisher LE, Khichi M, Mellick LB. Cytokine response to diabetic ketoacidosis and its treatment. *Clin Immunol* 2003; **108**(3): 175-81.
[25] Namavar Jahromi B, Ahmadi N, Cohan N, Jahromi MR. Comparison of the umbilical artery blood gas, nucleated red blood cell, C-reactive protein, and white blood cell differential counts between neonates of diabetic and nondiabetic mothers. *Taiwan J Obstet Gynecol* 2011; **50**(3): 301-5.
[26] Yang HL, Yuan GP, Deng H, Chen C, Peng X, An ZM. [The diagnostic value of serum beta-hydroxybutyrate in diabetic ketosis or diabetic ketoacidosis]. *Sichuan Da Xue Xue Bao Yi Xue Ban* 2014; **45**(2): 270-3. Chinese.
[27] Ge CJ, Yang J, Wang Q. [Clinical significance of rapid blood ketone versus urine ketone bodies detection in diabetic ketosis diagnosis and treatment]. *Chin J Diabetes* 2014; **22**(12): 1116-8. Chinese.
[28] Compton CW, Young L, McDougall S. Subclinical ketosis in postpartum dairy cows fed a predominantly pasture-based diet: defining cut-points for diagnosis using concentrations of beta-hydroxybutyrate in blood and determining prevalence. *N Z Vet J* 2015; **63**(5): 241-8.
[29] Ferguson I, Mullins ME. Diagnostic accuracy of fingerstick β-hydroxybutyrate for ketonuria in pregnant women with nausea and vomiting. *Acad Emerg Med* 2013; **20**(9): 954-6.
[30] Mahrt A, Burfeind O, Heuwieser W. Effects of time and sampling location on concentrations of β-hydroxybutyric acid in dairy cows. *J Dairy Sci* 2014; **97**(1): 291-8.