Clin. Lab. 2025;71:XXX-XXX

ORIGINAL ARTICLE

Determination of Urine Netrin-1 and Beta-Hydroxy Butyrate Levels in Diabetic Ketoacidosis Cases: a Preliminary Report

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SUMMARY

Background: **Diabetes mellitus (DM) is a chronic metabolic disorder characterized by high blood glucose levels, leading to severe complications over time. Diabetic ketoacidosis (DKA) is a critical acute complication of DM marked by hyperglycemia and acidosis due to ketone body accumulation, often seen in younger patients. The pathophysiology involves insulin deficiency and the effects of counter-regulatory hormones, leading to increased gluconeogenesis and lipolysis. This study investigated the relationship between urine netrin-1 and β-hydroxybutyrate (β-OHB) levels in DKA patients.**

Methods: **The study included 40 patients diagnosed with DKA and 40 healthy controls. Urine samples were collected, centrifuged, and stored at -80°C. Netrin-1 and β-OHB levels were measured using BTlab quantitative ELISA kits. Data were analyzed using SPSS, with tests for normality and appropriate statistical comparisons conducted.**

Results: **No significant demographic differences were found between the patient and control groups. Urine ketone and glucose positivity were significantly higher in DKA patients. Blood glucose, urea, lactate, and metabolic acidosis markers were also elevated in DKA patients. No significant difference was found in urine β-OHB and netrin-1 concentrations between the groups. However, a moderate positive correlation between β-OHB and netrin-1 was observed, along with various significant correlations between these markers and other biochemical parameters.**

Conclusions: **This study highlights significant biochemical differences between DKA patients and healthy controls, emphasizing the importance of monitoring biochemical parameters for managing DKA. Although no significant differences in urine β-OHB and netrin-1 concentrations were found, their correlation suggests a potential role in DKA pathophysiology, warranting further research with larger sample sizes.**

(Clin. Lab. 2025;71:xx-xx. DOI: 10.7754/Clin.Lab.2024.240805)

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Manuscript accepted September 10, 2024

KEYWORDS

diabetic ketoacidosis, netrin-1, beta-hydroxybutyrate

INTRODUCTION

Diabetes mellitus (DM) is a chronic metabolic disease characterized by high levels of blood glucose, which over time can lead to serious damage to the heart, blood vessels, eyes, kidneys, and nervous system. DM is expected to become a leading cause of morbidity and mortality globally due to its increasing incidence. Diabetic

ketoacidosis (DKA) is an acute complication of diabetes and is considered a fatal metabolic change characterized by the accumulation of ketone bodies, resulting acidosis, and hyperglycemia. DKA is observed more frequently in younger patients. The pathophysiology of DKA consists of absolute or relative insulin deficiency and the combined effects of counter-regulatory hormones such as glucagon, growth hormone, corticosteroids, and catecholamines. While glucose uptake and consumption by cells decrease with insulin deficiency, glycogenolysis and gluconeogenesis in the liver increase in response to opposing hormones [1,2]. Muscle proteins also begin to break down, and amino acids emerge. These amino acids act as substrates for gluconeogenesis, thus increasing plasma glucose concentration. When plasma glucose exceeds 170 mg/dL, glucosuria occurs [3]. Osmotic diuresis also accompanies glucosuria, causing the loss of water and electrolytes. Dehydration contributes to acidosis. In diabetic patients, when the liver's ability to oxidize fatty acids to carbon dioxide is exceeded during the DKA process, diabetic ketosis begins to occur due to the increase in β-hydroxybutyrate (β-OHB) and acetoacetate levels. When the formation rate of ketone bodies exceeds their utilization rate, their levels increase in the blood (ketonemia) and then appear in the urine (ketonuria) [4].

In patients with uncontrolled Type 1 DM, there is absolute insulin deficiency, leading to a dominance of anti-insulin hormones, such as glucagon and epinephrine, in the general circulation. Consequently, metabolic lipolysis increases, raising plasma free fatty acids. The oxidation of free fatty acids increases the ketone bodies in the blood, resulting in acidemia. The excretion of ketone bodies and glucose in urine also causes water loss, leading to ketoacidosis, which is defined by increased acidity in plasma with reduced volume. Nitroprusside-based tests are used in the laboratory to evaluate the level of ketoacidemia and ketoaciduria by detecting acetoacetate and acetone from ketone bodies. However, the predominant ketone compound in DKA is β-hydroxybutyrate (β-OHB). This is significant because, in cases of circulatory collapse, β-OHB levels rise alongside acetoacetate due to the effect of the redox potential increasing with lactic acidosis. Therefore, nitroprusside-based ketone tests are not reliable when β-OHB is the main factor in acidosis [5-8].

Netrin-1 is a conserved family of laminin-related proteins originally identified as axonal guidance cues. Many studies have demonstrated netrin-1 expression in various organs, including the nervous system. In both *in vitro* and *in vivo* systems, netrin-1 has been shown to promote angiogenesis, cell migration, tissue morphogenesis, and regulate inflammation [9]. The use of netrin-1 levels as a biomarker is effective in diagnosing complex diseases, which has piqued researchers' interest in studying netrin-1 in diabetic patients. As an inflammation modulator marker, netrin-1 may play a role in diabetes. The relationship between netrin-1 and diabetes is controversial. Netrin-1 levels have been examined in several studies concerning microvascular and macrovascular complications of diabetes, including diabetic retinopathy, neuropathy, and microalbuminuria [10]. However, no study has examined netrin-1 levels in DKA, the most mortal complication of DM requiring urgent intervention. The present study is the first to evaluate netrin-1 and β-OHB levels in urine samples of DKA patients admitted to the emergency service.

MATERIALS AND METHODS

Forty patients admitted to Gulhane Training and Research Hospital Emergency Service with the diagnosis of DKA during the period between June and July 2024 were included in the study. The study was performed in accordance with the Declaration of Helsinki guidelines, and informed consent was obtained from all participants. The study was approved by the local ethical committee, with the number 2024 - 238. Patients having any other acute or chronic inflammatory disease and/or malignancy, diabetes mellitus, chronic renal/hepatic failure, cirrhosis, and patients younger than 18 years and older than 75 years were excluded from this study. The control group was composed of 40 healthy individuals with no signs of acute or chronic illness.

The demographic features of all participants were recorded. Routine blood and urine test results were retrieved from the Hospital Laboratory Information System. Ten milliliters of urine samples were collected from all participants. Urine analysis was carried out semiquantitatively with Roche cobas u 701 urine analyzer. Blood pH, lactate, bicarbonate, and tCO_2 were measured with Radiometer ABL800 Blood gas analyzer in the emergency laboratory. Blood glucose, creatinine, urine, and total protein were measured with Roche cobas c 702 clinical chemistry analyzer. Urine samples were centrifuged at 1,000 rpm for 5 minutes for measurement of netrin-1 and β-OHB. Separated supernatants were aliquoted into Eppendorf tubes and stored at -80°C until the time of analysis. BTlab quantitative enzyme-linked-immunosorbent assay (ELISA) kits were used for human β-OHB and netrin-1 detection. The study principle was double-antibody sandwich ELISA. All samples were studied in the same batch, according to the instructions of the manufacturer. An eight (8) point calibration curve was constructed with the serial dilution of calibrators provided in the study kits, and concentrations of samples were extrapolated with the linear regression equation generated by the calibrators.

Statistical analysis

Data analysis was carried out using SPSS for Windows 15 program. The normality tests were performed using the Kolmogorov-Smirnov test. Descriptive statistics of normally distributed continuous variables are illustrated as mean ± standard deviation (SD). Descriptive statistics of non-normally distributed continuous variables are illustrated as median (interquartile range) values.

Among-group differences of normally distributed variables were analyzed with Student's *t*-test, while non-normally distributed variables were analyzed using the Mann-Whitney U test. The Kruskal-Wallis test was used for the comparison of more than two groups. Correlation analyses were performed using Pearson's test.

RESULTS

When demographic data were examined, no significant difference was found between the patient and control groups in terms of age and gender. Urine and blood parameters of the patient group are summarized in Table 1. No significant difference was found in the gender distribution between the patient and control groups ($p > 0.05$). The average ages of the groups were also similar ($p > 0.05$).

In patients with diabetic ketoacidosis (DKA), urine ketone positivity was quite high; 62.5% had ketone levels of +2 or higher. In the control group, all samples were negative for urine ketones. In DKA patients, urine glucose was found to be positive in 90% of cases, and glucose was detected at a level of +4 in 45% of patients. In the control group, all samples were negative for urine glucose. Urine protein positivity was detected in 52.5% of DKA patients, while all samples in the control group were negative. Urine nitrite positivity was found in 7.5% of the DKA group, whereas all samples in the control group were negative. The average urine pH was 6 in the DKA group and 7 in the control group ($p < 0.05$).

There were also statistically significant differences between the patient and control groups in terms of blood glucose, urea, creatinine, pH, lactate, bicarbonate, and tCO_2 levels.

Urine β-hydroxybutyrate (β-OHB) and netrin-1 concentrations were compared between the patient and control groups by Mann-Whitney U test; the results are illustrated in Figure 1 and 2. No statistically significant difference was found between the patient and control groups in terms of β-OHB and netrin-1 concentrations ($p > 0.05$ for both comparisons). In the correlation analysis conducted between study parameters and biochemical laboratory parameters, a moderately significant correlation was found between β-OHB and netrin-1 concentrations ($r = 0.576$, $p < 0.05$). A moderately significant negative correlation was observed between urine ketone levels and β-OHB ($r = -0.492$, $p < 0.05$), and a low-level negative correlation was observed between netrin-1 and urine ketone levels ($r = -0.395$, $p < 0.05$). Low-level positive correlations were found between blood urea concentrations and β-OHB ($r = 0.316$, $p < 0.05$) and netrin-1 ($r = 0.328$, $p < 0.05$). Additionally, moderately significant positive correlations were found between netrin-1 and blood bicarbonate (HCO_3^-) ($r = 0.420$, $p < 0.05$) and total carbon dioxide (tCO_2) ($r = 0.392$, $p < 0.05$) concentrations. The results are summarized in Table 2.

DISCUSSION

In this study, various demographic and biochemical parameters of patients with diabetic ketoacidosis (DKA) were compared to those of a healthy control group. Table 1 includes data such as gender distribution, average age, and urinary and blood biochemical parameters for both groups. There was no significant difference in gender distribution between the patient and control groups ($p > 0.05$). The mean ages were also found to be similar between the groups ($p > 0.05$). These data indicate that the demographic characteristics are homogeneously distributed between the two groups, allowing the analysis to focus on biochemical parameters.

In patients with DKA, significant metabolic changes were observed, including positive urinary and blood glucose, ketonemia, low urine pH, and low blood pH, HCO_3^-, and tCO_2 levels. These findings align with the characteristic features of DKA, namely hyperglycemia and metabolic acidosis, which result from insulin deficiency and increased gluconeogenesis and lipolysis. Upon examination, these results are consistent with laboratory findings for DKA reported in the literature. This study demonstrates that the expected biochemical changes are present in patients with DKA and that these changes play a key role in the diagnosis and management of the condition. Close monitoring of biochemical parameters, such as blood glucose, ketone, and pH levels, is crucial in managing the systemic effects of DKA. Additionally, monitoring renal function and assessing patients' nutritional statuses are important for preventing complications of DKA and effectively managing the treatment process. These findings are consistent with the existing literature on DKA and contribute to a better understanding of the pathophysiological processes of this condition [11-19].

In our study, the lack of a significant difference between urinary β-hydroxybutyrate (β-OHB) and netrin-1 concentrations may be attributed to the limited sample size. Therefore, studies with a larger sample size are needed to more accurately assess the potential interactions between β-OHB and netrin-1. Such studies could provide deeper insights into the pathophysiology of DKA and further support the clinical utility of these markers.

The moderately significant positive correlation ($r = 0.576$, $p < 0.05$) between urinary β-OHB and netrin-1 concentrations suggests that these markers may play an interactive role in the pathophysiology of DKA. This finding is consistent with studies by Otal and colleagues, which demonstrated that oxidative stress markers and inflammatory responses increase concurrently during DKA [20]. Similarly, research on the anti-inflammatory effects of β-OHB further supports the role of this metabolite in DKA [21]. In this context, the effects of β-OHB on inflammatory responses may be important for understanding both the metabolic and inflammatory processes of DKA.

The moderately significant negative correlations between acetoacetate levels, as measured by urinary ke-

Table 1. Demographic features and laboratory results of study groups.

	Patient group	Control group	p
Gender			
Female	**22**	**23**	**0.82** [#]
Male	**18**	**17**	
Age (year) (mean ± standard deviation)	**51.85 ± 14.98**	**51.85 ± 14.65**	**0.99** [##]
Urine ketone, n (%)	**40 (%)**	**40** **40 (100)**	
Negative	**5 (12.5)**		
+1	**15 (37.5)**		
+2	**10 (25)**		
+3	**10 (25)**		
Urine glucose, n (%)		**40** **40 (100)**	
Negative	**4 (10)**		
+1	**4 (10)**		
+2	**11 (27.5)**		
+3	**3 (7.5)**		
+4	**18 (45)**		
Urine protein, n (%)		**40 (100)**	
Negative	**19 (47.5)**		
+1	**12 (30)**		
+2	**5 (12.5)**		
+3	**2 (5)**		
+4	**2 (5)**		
Urine nitrite, n (%)	**40 (100)**	**40 (100)** **40 (100)**	
Negative	**37 (92.5)**		
Positive	**3 (7.5)**		
Urine pH, median (25 - 75)	**6 (5 - 6)**	**7 (6 - 7)**	**< 0.001** [*]
Blood glucose, mg/dL, median (25 - 75)	**318 (256 - 400)**	**80 (75 - 86.3)**	**< 0.001** [*]
Blood urea, mg/dL, median (25 - 75)	**40 (28.8 - 61)**	**24 (19.5 - 28.5)**	**< 0.001** [*]
Blood creatinine, mg/dL, median (25 - 75)	**0.93 (0.73 - 1.14)**	**0.95 (0.9 - 1)**	**0.422** [*]
Blood total protein, g/dL, median (25 - 75)	**5.2 (4.8 - 6.35)**	**7.8 (7 - 7.9)**	**< 0.001** [*]
Blood albumin, g/dL, mean ± standard deviation	**3.45 ± 0.62**	**4.28 ± 0.61**	**< 0.001** [##]
Blood pH, median (25 - 75)	**7.25 (7.21 - 7.29)**	**7.41 (7.40 - 7.42)**	**< 0.001** [*]
tCO_2, mmol/L, median (25 - 75)	**33 (28.9 - 35.1)**	**38 (38 - 41)**	**< 0.001** [*]
Blood HCO_3^-, mmol/L, median (25 - 75)	**16 (13.1 - 17.6)**	**24 (24 - 25)**	**< 0.001** [*]
Blood lactate, mmol/L, median (25 - 75)	**2.8 (2.2 - 3.32)**	**1.3 (1.2 - 1.33)**	**< 0.001** [*]

[#] - Pearson´s chi-squared test, [##] - Student´s *t*-test, [*] - Mann-Whitney U test, PCO_2 - partial pressure of carbone dioxide.

tone tests, and β-OHB and netrin-1 represent another noteworthy finding. These negative correlations suggest that increased production and utilization of ketone bodies may have a regulatory effect on netrin-1 levels. This finding underscores the importance of carefully monitoring ketone bodies in the management of DKA as indicators of their anti-inflammatory and metabolic effects [21].

Furthermore, the positive correlations between β-OHB and blood urea levels, as well as between netrin-1 and blood urea, bicarbonate, and carbon dioxide levels, indicate the influence of these metabolites on renal function and acid-base balance. These results highlight the necessity of evaluating the systemic effects of DKA from a broader perspective and the importance of developing treatment strategies that consider these metabolic pro-

Table 2. Correlation analysis between study parameters and laboratory tests.

	β-OHB	Netrin-1	Urine ketone	Urine glucose	Blood glucose	Blood urea	Blood HCO_3^-	Blood tCO_2	Blood lactate
β-OHB correlation coefficient									
p	**1.000**	**0.576 ****	**-0.492 ****	**0.001**	**-0.242**	**0.316 ***	**0.117**	**0.155**	**0.208**
n	**- 40**	**0.000 40**	**0.001 40**	**0.996 40**	**0.132 40**	**0.047 40**	**0.471 40**	**0.339 40**	**0.199 40**
Netrin-1 correlation coefficient									
p	**0.576 ****	**1.000**	**-0.395 ***	**-0.223**	**-0.132**	**0.328 ***	**0.420 ****	**0.392 ***	**0.193**
n	**0.000 40**	**- 40**	**0.012 40**	**0.166 40**	**0.415 40**	**0.039 40**	**0.007 40**	**0.012 40**	**0.233 40**

*** - Correlation is significant at the 0.05 level (2-tailed).** - Correlation is significant at the 0.01 level (2-tailed).**

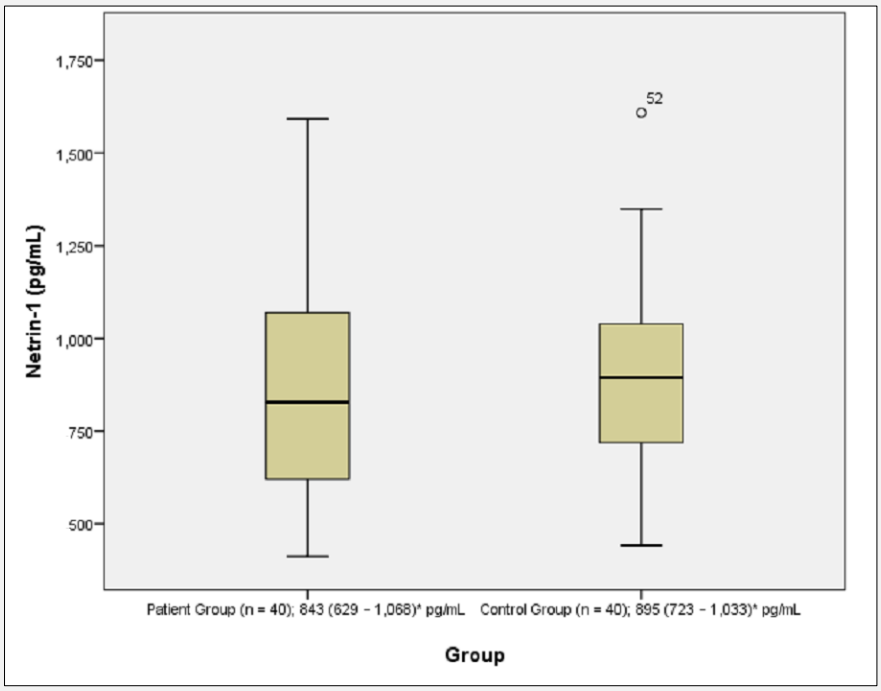

Figure 1. Netrin-1 concentrations in patient and control groups.

The test performed Mann Whitney U Test. * - Median (25 - 75) (p > 0.05).

cesses [22].

This study does pose some limitations that should be acknowledged. Firstly, the sample size of 40 patients and 40 controls is relatively small, which may limit the generalizability of the findings. A larger sample size might have provided more robust statistical β power and potentially revealed more subtle differences between groups. Secondly, the study is observational and cross-sectional, providing a snapshot of biochemical parameters at a single point in time.

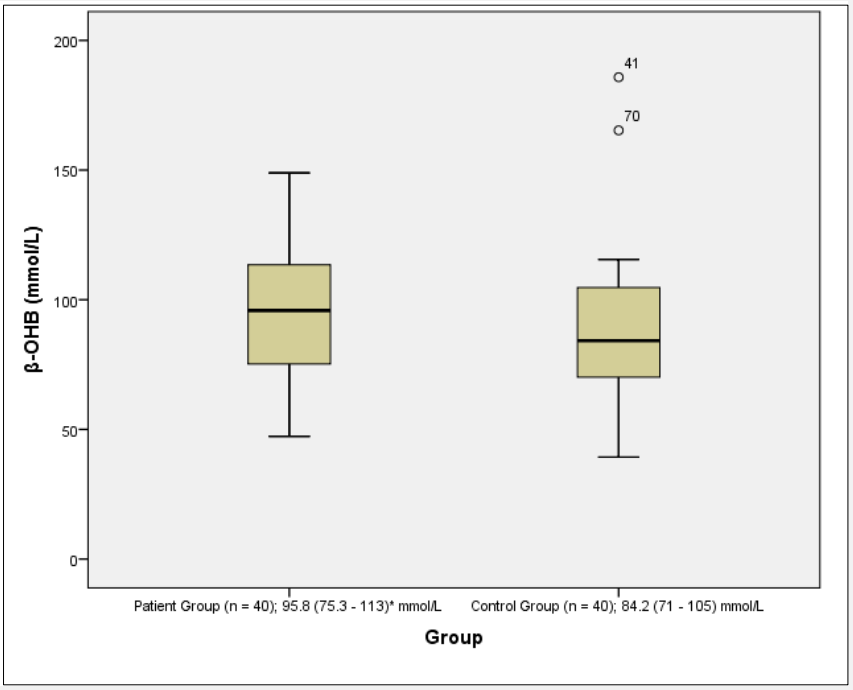

Figure 2. B-hydroxybutyrate concentrations in patient and control groups.

The test performed Mann Whitney U Test. * - Median (25 - 75) (p > 0.05).

CONCLUSION

This study highlights the significant biochemical and clinical differences between diabetic ketoacidosis (DKA) patients and a healthy control group. These findings emphasize the potential role of biomarkers such as β-OHB and netrin-1 in understanding the complex effects of DKA on metabolic and inflammatory processes. In this context, more comprehensive future studies could provide valuable information to better understand the clinical use of these biomarkers and to develop more effective strategies for the management of DKA.
Overall, while this study provides valuable preliminary insights, further research with larger, more diverse populations and longitudinal designs is needed to confirm and expand upon these findings.

Acknowledgment:
The authors would like to thank the personnel in the emergency department and the clinical biochemistry laboratory who supported them throughout the study.

Sources of Support:
The study was supported and granted by The Scientific and Technological Research Council of Turkey (TUBITAK) - 1002 programme (project number: 124S 431).

Declaration of Interest:
None.

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