Annals of Medical Research
DOI: 10.5455/annalsmedres.2019.01.023 2019;26(4):616-20

Original Article

The value of the capillary blood ketone measurement in rating of dehydration: An experimental study

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Abstract

Aim: In this study, we aimed to determine the value of blood ketone levels in diagnosing and classifying dehydration.

Material and Methods: A total of 40 rats were included into the study. Rats were categorized into four groups according to their weight loss: control group (no weight loss), mild dehydration group (3 – 5% weight loss), moderate dehydration group (5 – 10% weight loss) and severe dehydration group (>10% weight loss). The blood samples taken from the rats were analyzed for capillary blood ketone levels, venous blood ketone levels and the other biochemical parameters.

Results: There was no significant difference between four study groups according to the venous blood ketone levels and capillary ketone levels. Only Na levels were significantly different between study groups among all the metabolic parameters. (p=0.044). After categorizing the study groups as control and dehydration groups according to the weight loss, a borderline significance was established for Na (146±6 vs 151±2.5; difference: 4.2 mmol/L, %95 CI: -0.2 to 8.6, respectively; p=0.06) and capillary blood ketone (0.4 (IQR:0.3-0.5) vs 0.6 (IQR:0.4-0.7), respectively; p=0.097), while other parameters did not differ significantly. The capillary blood ketone had a sensitivity of 96.7% (95% CI: 82.8 -99.9), specificity of 10% (95% CI: 0.3-44.5) for detecting dehydration.

Conclusions: This study showed that there was no significant difference for the development of ketosis in dehydration. However, the borderline significance for the capillary ketone levels indicates the necessity of human studies.

Keywords: Dehydration; Ketosis; Blood Ketone; Rat.

INTRODUCTION

Dehydration is defined as loss of body fluid with variable degree of electrolyte loss. The term of dehydration is used to define most of the conditions with fluid loss (1,2).

Dehydration is classified as mild, moderate and severe. This classification is often done by the clinical findings occurred by the severity of fluid loss. However, this classification cannot be done accurately due to individual differences of adaptation mechanisms to fluid loss. Hence, the clinicians are confronted with uncertainties about the appropriate time, application route and amount of the fluid replacement. Today, most widespread method to determine dehydration is to calculate the rate of the loss of body weight to normal body weight. The most important limitation of this method in adult patients is not always being able to know their previous weights. Beside clinical findings and weight changes, biochemical parameters (blood urea nitrogen (BUN), creatinine (Cr), sodium (Na), osmolarity of serum and urine) are also conventional methods to classify the dehydration (3). Although it seems that the most objective finding to reveal the fluid loss is actual weight change, because of the mentioned disadvantage its use is highly limited (4). In most of the diseases with dehydration, vomiting and decreased oral carbohydrate intake results with increased free fatty acid oxidation and also increased serum and urine ketone levels. Even if dehydration doesn't directly cause ketosis, the level of oral intake and vomiting might be associated with the degree of dehydration (3).

The aim of this experimental study carried out in rats is to investigate the value of the capillary blood ketone measurement in rating of dehydration.

MATERIAL and METHODS

This study was performed in the animal laboratory of a university hospital and it was approved by the institutional

Received: 14.01.2019 **Accepted:** 04.02.2019 **Available online:** 07.02.2019
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ethics review board for the animal studies. 40 Rattus norvegicus (Wistar) female rats obtained from the animal laboratory unit were used. Rats were kept in the cages with constant 250C individually. Night and day cycle was 12 hours. Rats were fed ad libitum (food available all the time to consume) before and during the study with commercial pellets for rats.

Study protocol

In order to establish a study protocol a preliminary study was done and with this preliminary work it was figured out how much time is needed to develop a mild, moderate and severe dehydration in anhydrous rats.

In this preliminary work, 3 rats divided in 3 groups were used. First, the weights of these rats were determined. They were kept in separate cages and fed with pellets throughout the preliminary study. In the meantime, a dehydration model was established as the rats were kept anhydrous for the predetermined time and their weights were recorded intermittently.

As a result of this preliminary work it was determined that rats must be kept dehydrated; seven hours for Group I: mild dehydration (loss of 2-5% of body weight), fourteen hours for Group II: moderate dehydration (loss of 5-10% of body weight), twenty-one hours for Group III: severe dehydration (loss of more than 10% of body weight).

Four groups with ten rats in each group were created for the study due to the findings of the preliminary work. Each rat was kept in separate cages.

Group A was determined as control group. Rats in this group were not dehydrated. Group B was designated as mildly dehydrated group. Rats in this group were dehydrated for seven hours. Group C was designated as moderately dehydrated group. Rats in this group were dehydrated for fourteen hours. Group D was designated as severely dehydrated group. Rats in this group were dehydrated for twenty-one hours and severely dehydrated. All rats in each group were put to sleep with ether and weighted. Then, 2 ml blood was taken from tail vein; 0,5 ml for blood Ph and HCO3 levels, 0,1 ml for capillary blood ketone level, 1,4 ml for venous blood ketone, BUN, Cr, Na, chlorine (Cl) and hematocrit (hct) level measurements. 0.1 ml blood sample was dropped to blood chamber of Medisense Optıum ß-Ketone Test Strip® for the measurement of capıllary blood ketone level. Then the findings of the Optium Xceed® blood ketone measurement device were recorded. Blood samples for blood pH and HCO3 were transported in heparinized insulin injector in the iced container to blood gas center and analyzed.

Blood samples for venous blood ketone, BUN, Cr, Na, Cl, hct were kept in biochemistry tubes. Plasmas were separated after centrifuged at 3000 turnovers for 5 minutes. Plasmas were kept in Eppendorf tubes at -800C. After obtaining all plasmas, they were taken from -800C and liquefied. Then, the measurements for the venous ketone level with Cayman Ketone Assay Kits®, for the other biochemical parameters with standard biochemical methods were performed. After blood sampling process, rats were followed to awaken from ether anesthesia. Then, they were put to their cages and they maintained their normal life circle.

Outcomes

The primary aim of this study was to investigate the relationship of blood ketone level and capillary blood ketone level in rating of mild, moderate, severe dehydration. On the other hand, the secondary follow-up aim of this study was to investigate the characteristics of the rats, determining the Ph, HCO3, BUN, Cr, Na, Cl and hct levels and their value in rating of dehydration.

Statistical Analyses

SPSS analysis was performed using SPSS software (version 15.0; SPSS Inc., Chicago, IL, USA) and the parameters were expressed as mean and standard deviation. For the comparison of two groups, Student-t test was used for normal distribution data and Mann Whitney U test was used for those who did not. Comparison of three and more groups One Way ANOVA was used for the variables that fit the normal distribution and Kruskal Wallis test was used for those who did not. Normal distribution analysis was performed with Kolmogorow Smirnov test. Sensitivity, selectivity, positive likelihood ratio (PLR) and negative probability ratio (NLR) were used to determine the diagnostic value of metabolic parameters studied in the determination of dehydration. The statistical significance was accepted as $\alpha = 0.05$.

RESULTS

Demographic features of the study population

The mean primary weights of the rats were 254.075 ± 11 gr. The mean weight loss of rats in study groups after dehydration was 0% in first group, 3.3% in second group, 6.7% in third group and 10% in fourth group. Weight loss of the study groups were significantly different ($p<0.001$). Weight loss of all groups was significantly different also in post hoc analyses (Table 1).

Main results

The primary follow-up finding of this study is to investigate the value of relationship of blood ketone level and dehydration, and capillary blood ketone measurement in rating of mild, moderate, severe dehydration. When four study groups were compared, there was no statically significant difference for venous blood ketone and capillary blood ketone levels (0.23±0.17 vs. 0.24±0.05 vs. 0.26±0.1 vs. 0.19±0.1, respectively; p=0.357), (0.40±0.17 vs. 0.45±0.24 vs. 0.6±0.40 vs. 0.65±0.29 respectively; p=0.334). Only Na levels among the metabolic parameters were significantly different (144.3±6.1 vs 150.7±2.2 vs 151.4±2.3 vs 151.7±2.1 respectively; p=0.044). Na levels of control group and moderate, severe dehydrated group was significantly different in post hoc analyses. All findings between four groups are shown in table 1.

Study groups were categorized as control and dehydrated groups according to weight loss (group 1 and the others),

comparisons between normal group and dehydrated groups determined borderline significance for Na levels (146±6 vs 151±2.5; difference: 4.2 Mmol/lt, %95 CI: -0.2 - 8.6; p=0.06) and capillary blood ketone levels (0.4 (IQR:0.3-0.5) vs 0.6 (IQR:0.4-0.7); p=0.097), but no significant difference for the other parameters (Table 2).

Table 1. The comparison of study groups in terms of body weights and metabolic parameters

	Control Group	Mild dehydration	Moderate dehydration	Severe dehydration	P
Initial weight (gr)	241.5±26.5	260.5±31.1	266±33	255±38	0.764
Body weight change %	0±0	3.3±1	6.9±1	10±0.5	0.001*
Blood ketone (mMol/L)	0.23±0.17	0.24±0.05	0.26±0.1	0.19±0.1	0.357
Capillary blood ketone(mMol/L)	0.40±0.17	0.45±0.24	0.6±0.40	0.65±0.29	0.334
Hb (gr/dL)	15.1±2.7	14.3±2.3	14.2±2	14.5±5.1	0.913
Hct	44.1±7.9	42.8±6.9	42.7±6.0	43.6±15.4	0.950
HCO3	25.6±3.1	25.2±1.6	24.9±2.4	24.4±2.2	0.283
K+	4.06±0.52	3.42±0.22	3.85±0.55	3.69±0.81	0.074
Na+	144.3±6.1	150.7±2.2	151.4±2.3	151.7±2.1	0.044*
pH	7.327±0.075	7.360±0.052	7.381±0.052	7.337±0.068	0.278

Abbreviations: Hb- hemoglobin; hct- hematocrit; HCO_3- bicarbonate; K- potassium; Na- sodium

Table 2. The comparison of the study groups according to weight loss as control group and dehydration group

	Control Group	Dehydration group	Difference (95% CI)	P
Blood ketone (mMol/L)	0.27±0.17	0.23±0.09	0.03 (-0.05 – 0.02)	0.429
Stick ketone (mMol/L)	0.4 (0.3-0.5)	0.6 (0.4-0.7)	NA	0.09
pH	7.338±0.075	7.360±0.058	0.02 (-0.07 – 0.02)	0.334
Hb	14.5±2.72	14.0±3.46	0.53 (-1.94 – 3)	0.666
Hct	43±7.9	41.9±10.4	1.02 (-6.35 – 8.4)	0.780
Na	146.8±6.1	151.0±2.5	-4.21 (-8.62 – 0.21)	0.060
HCO_3	26.2±3.1	24.7±2.1	1.47 (-0.3 – 3.2)	0.100

Abbreviations: Hb- hemoglobin; hct- hematocrit; Na- sodium; HCO_3- bicarbonate;NA- Not Available

When the weight changes of laboratory animals were used as gold standard in determining dehydration Na levels has a sensitivity of 96.7 (95% CI: 82.8 - 99.9), specificity of 10% (95% CI: 0.3 - 44.5), PLR 1.1 (0.9 – 1.3), NLR 0.3 (0 – 4.9); capillary blood ketone levels has a sensitivity of 96.7% (95% CI: 82.8 - 99.9), specificity of 10% (95% CI: 0.3 - 44.5), PLR 1.1 (0.9 – 1.3), NLR 0.3 (0 – 4.9) .

When the venous blood ketone levels were used as gold standard, (blood ketone threshold value was taken 0.2 mMol/L), in determining dehydration capillary blood ketone levels have a sensitivity of 96% (95% CI: 80 -100), specificity of 6.7% (95% CI: 0.2 – 32); PLR 1.03 (0.88 – 1.2), NLR 0.6 (0.04 – 8.9) (p=0.357). Na+ levels have a sensitivity of 16% (95% CI: 5 – 36%), specificity of 80% (95% CI: 52 – 96%); PLR 0.8 (0.21 – 3.1), NLR 1.05 (0.77 – 1.43). HCO3- levels have a sensitivity of 16% (95% CI: 5 – 36%), specificity of 80% (95% CI: 52 – 96%); PLR 0.8 (0.21 – 3.1), NLR 1.05 (0.77 – 1.43) (p=0.251). pH has a sensitivity of 16% (95% CI: 5 – 36%), specificity of 80% (95% CI: 52 – 96%); PLR 0.8 (0.2 – 3.1), NLR 1.05 (0.77 – 1.43) (p=0.422).

Correlation between capillary blood ketone levels and venous blood ketone levels was analyzed in Bland Altman and the mean measurement difference between two methods was 0.32mmol/L. The results of Bland Altman analysis are shown in Figure 1 and 2.

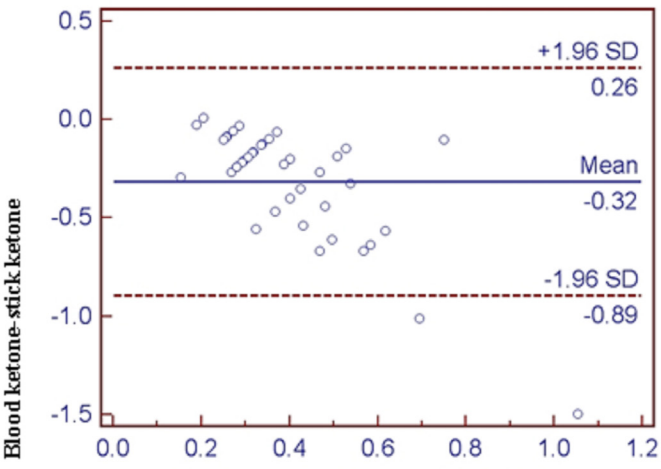

Figure1. Bland-Altman analysis to determine consistency between venous blood ketone and capillary blood ketone

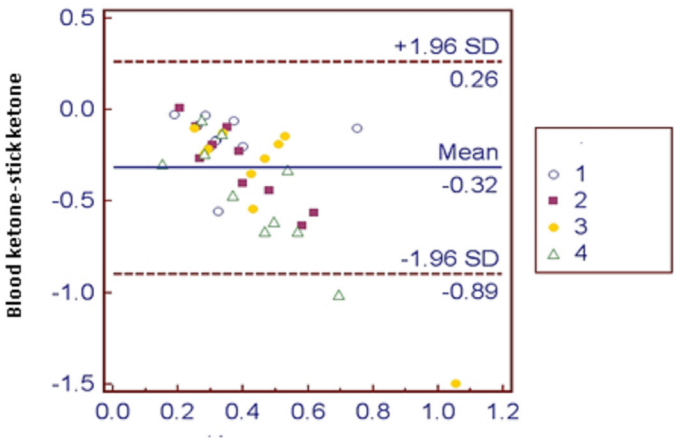

Figure 2. Presentation of Bland-Altman analysis performed to determine the consistency between venous blood ketone and capillary blood ketone

DISCUSSION

With this dehydration model only designed by fluid restriction, it is determined that blood ketone measurement has no value in rating dehydration, however when control group and dehydrated groups are compared increased capillary blood ketone levels can be used to detect the presence of dehydration. There was no clinical study to compare our results in medical literature. Also, it is necessary to conduct well- designed, prospective human trials to determine the ketonemia in dehydrated patents.

Dehydration can be determined by measurement of fluid amount in body compartments. Fluid compartment measurement is not possible in routine clinical practice. For this reason intravascular volume can be evaluated by physical examination, laboratory results or complex hemodynamic observation methods. To observe only one parameter is inaccurate and dangerous, because all parameters are indirect and nonspecific volume measurements (5). History and physical examination is the mostly used parameter to observe the presence of dehydration; also clinical parameters such as patient consciousness, capillary refill time, skin turgor, dryness of mucous membranes, changes in heart rate and blood pressure, abnormal respiratory pattern, urine output and weight changes are often used (5). There are some laboratory findings of dehydration such as increased hct, progressive metabolic acidosis, urine specific gravity more than 1010, urine Na more than 20mEq/L, urine osmolality more than 450 mosm/L, hypernatremia, BUN/Cr more than 10/1 which can be used in emergency room (5). There is no priority of all these clinical and laboratory parameters in evaluating dehydration. Moreover, in our study, there was no significance in laboratory findings except serum Na levels showing that dehydration is actually a multifactorial problem. Our dehydration model was designed by fluid restriction so hypertonic dehydration was developed in rats. Thus, Na+ levels were significantly high as a result of study methodology.

Dehydration is a condition that causes serious health problems especially in children and elderly patients. The degree of dehydration is crucial in evaluating the current status of the patient and planning the management. However, the technical shortcomings even in today's technological level urge us to determine the objective degree of dehydration. Today, the widespread technique used in the evaluation of the degree of dehydration is determining the percentage of the weight loss and grading by that value (4). According to current rating method %2 – 5 of weight loss is defined as mild, 5 – 10% moderate and >10% is defined as heavy dehydration (3) The main limitation of this technique is the inability to exactly predict the previous weight of the patients (6). In the study of Yilmaz et al. which they investigated the extent of dehydration status in children with acute gastroenteritis, blood pH turned out to be successful in determining the moderate dehydration (7). The most important cause of this phenomenon might be the diminished capillary circulation leading to decreased supply of blood to tissues and the resulting lactic acid synthesis or the increased use of keton bodies. However, in our study, we didn't find any significant correlation of blood pH with rating of dehydration (p=0.278).

Ketone bodies are the molecules which are used as source of energy in all organ systems except liver which are derived by the breakdown of fatty acids in the liver (8,9). It is well known that ketone bodies can be found in blood in increased amounts in conditions like prolonged fasting, diabetes, hyperemesis gravidorum, alcohol overuse. The importance of ketosis associated with diabetes and ketone bodies is associated with the possible complications. Among well-known complications, diabetic ketosis, diabetic ketoacidosis, hyperglycemic hyperosmolar nonketotic syndrome, infections, hypoglycemia, micro vascular complications (retinopathy, nephropathy) and macro vascular complications (hypertension, atherosclerosis) can be stated. In the pregnancy, it may lead to termination of pregnancy and without appropriate therapy it may lead to serious complications like death (10). Byrne et al. showed in a study published in 2000 that, bedside capillary ketone measurement can be used to determine diabetic ketosis (DK) and ketoacidosis (DKA) in diabetics (11). In this study, blood samples were drawn from 19 patients with DKA and the blood β-hydroxybutyrate (βHBA) levels were compared with 156 diabetic patients which have regulated blood glucose levels with low calorie diets without any complaints. In the study, capillary and venous blood samples were taken simultaneously on bedside and blood βHBA levels were separately measured on laboratory. As a result, bedside capillary measurements were found to be consistent with the reference measurements. This study was not clinical in design by its methodology using blood tests in the laboratory. However it turned out to be a good reference study for the future studies to be conducted in the diabetic patients. First time in the literature, the value of the capillary blood ketone in revealing the DKA and DK complications was studied in patients admitting to emergency departments without any traumatic reasons.

In this study, hyperglycemia and venous blood βHBA value>4.39 mg/dl and pH < 7.35 were accepted as gold standard values. Sensitivity and specificity of determining DK and DKA with capillary blood βHBA measurement in diabetic patients admitting to emergency departments with medical complaints was found 45% and 91% respectively while the sensitivity and specificity of urine analyses in determining the same patients was found to be 54% and 82% (12).

The most important limitation of this study is its being an animal trial. The results of animal trials cannot reflect the daily practice, but can be a guide to further trials. The other limitation is the difference of reference values of the rats and the humans. In this case, especially when comparing quantitative values the differences of human values must be kept in mind. The diagnostic values that are classified by using reference values would be more directive. Eventually, beside the differences of the metabolic parameters of the rats and the humans, the metabolic parameters can also be different because of the different body surface areas.

CONCLUSION

This dehydration model which is defined by only with fluid restriction detected by measuring blood ketone value doesn't contribute the classification of dehydration; however when control group is compared to dehydrated group, increased capillary blood ketone level can be a laboratory parameter that shows the presence of dehydration. These results must be verified with prospective, well-designed, large scale clinical studies.

Competing interests: The authors declare that they have no competing interest.
Financial Disclosure: There are no financial supports
Ethical approval: This research was carried out with the permission of Akdeniz University, Clinical Researches Ethics Committee

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