Research Article

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Investigation of alanine, propionylcarnitine (C3) and 3-hydroxyisovalerylcarnitine (C5-OH) levels in patients with partial biotinidase deficiency

Kısmi biyotinidaz eksikliği olan hastalarda alanin, propiyonil karnitin (C3) ve 3-hidroksi izovaleril karnitin (C5-OH) seviyelerinin araştırılması

Abstract

Background: Biotinidase deficiency is a treatable metabolic disease that can be seen with various neurological and dermatological complications. Biomarkers such as alanine, propionylcarnitine (C3) and 3-hydroxyisovalerylcarnitine (C5-OH), which are used to diagnose biotinidase deficiency, are also present.

Materials and methods: In cases with partial biotinidase deficiency and normal biotinidase activity, alanine, C3 and C5-OH levels were compared in the field by liquid chromatography-tandem mass spectrometry.

Results: There was no significant difference between subjects with partial biotinidase deficiency and those with normal biotinidase activity between C3 and C5-OH levels. The mean alanine levels in heel blood and plasma were significantly higher than those with normal biotinidase activity in patients with partial biotinidase deficiency.

Conclusion: In cases with partial biotinidase deficiency, the heel blood alanine level that can be detected in the neonatal screening program may be a leading marker in diagnosis.

Keywords: Alanine; Biotinidase; Carboxylase; Propionylcarnitine; 3-Hydroxyisovalerylcarnitine.

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Öz

Amaç: Biyotinidaz eksikliği, çeşitli nörolojik ve dermatolojik komplikasyonlarla görülebilen tedavi edilebilir bir metabolik hastalıktır. Biyotinidaz eksikliğini teşhis etmek için kullanılan alanin, propiyonil karnitin (C3) ve 3-hidroksi izovaleril karnitin (C5-OH) gibi biyobelirteçler de mevcuttur.

Materyal ve metod: Kısmi biyotinidaz eksikliği ve normal biyotinidaz aktivitesi olan olgularda alanin, C3 and C5-OH düzeyleri sıvı kromatografi-tandem kütle spektrometresi yöntemi ile karşılaştırıldı.

Bulgular: Kısmi biyotinidaz eksikliği olan kişiler ile normal biyotinidaz aktivitesi olanların C3 ve C5-OH düzeyleri arasında anlamlı fark yoktu. Kısmi biyotinidaz eksikliği olan hastalarda normal biyotinidaz aktivitesi olanlara göre topuk kanında ve plazmada ortalama alanin düzeyleri anlamlı derecede daha yüksekti.

Sonuç: Kısmi biyotinidaz eksikliği olan olgularda yenidoğan tarama programında tespit edilebilecek topuk kan alanin düzeyi tanıda yol gösterici bir belirteç olabilir.

Anahtar Kelimeler: Alanin; Biyotinidaz; Karboksilaz; Propiyonil karnitin; 3-hidroksi izovaleril karnitin.

Introduction

Biotin is a water-soluble vitamin that is mostly found as protein-bound and in small quantities in natural food products. The classical role of biotin is to act as a coenzyme of four important carboxylases which play a role in gluconeogenesis, fatty acid synthesis, and catabolism of various aminoacids [1, 2]. Covalent binding of biotin to four inactive apocarboxylases, which are catalyzed by holocarboxylase synthetase (HCS), is required for the production of active holocarboxylases. These inactive apocarboxylases

are: acetyl-CoA carboxylase (ACC), 3-methylcrotonyl-CoA carboxylase (MCC), pyruvate carboxylase (PC) and propionyl-CoA carboxylase (PCC) [1, 2]. These apocarboxylases have various functions within the body.

MCC catalyzes the carboxylation of 3-methylcrotonyl-CoA to 3-methylglutaconyl-CoA in the leucine catabolic pathway. The accumulation of 3-methylcrotonyl-CoA as a result of MCC deficiency also results in the accumulation of 3-hydroxyisovaleryl-CoA due to hydration reaction. The final product that accumulates in conjugation with carnitine is 3-hydroxyisovalerylcarnitine (C5-OH) [1, 3]. In biotin deficiency, C5-OH can be analyzed with liquid chromatography-tandem mass spectrometry (LC-MS/MS) method, using the heel blood sample [4–7].

PC is a biotin-dependent enzyme in the gluconeogenesis, and catalyzes the transformation of pyruvate to oxaloacetate in the citric acid cycle [8]. Decreased activity of this enzyme can lead to pyruvate accumulation. Accumulating pyruvate can be transformed into lactic acid as well as to alanine by aminotransferase (ALT) enzyme [9] (Figure 1). At present, alanine level can be determined by LC-MS/MS method both from the heel blood sample and from plasma.

PCC is a biotin-dependent carboxylase and its deficiency can cause an increase in the propionylcarnitine (C3), an intermediate metabolite. Elevations in C3 suggest propionic acidemia, methylmalonic acidemia, or other hereditary disorders of intracellular cobalamin metabolism [7]. At present, C3 level can be easily detected by LC-MS/MS method using the heel blood sample in the newborn screening programs [1, 7].
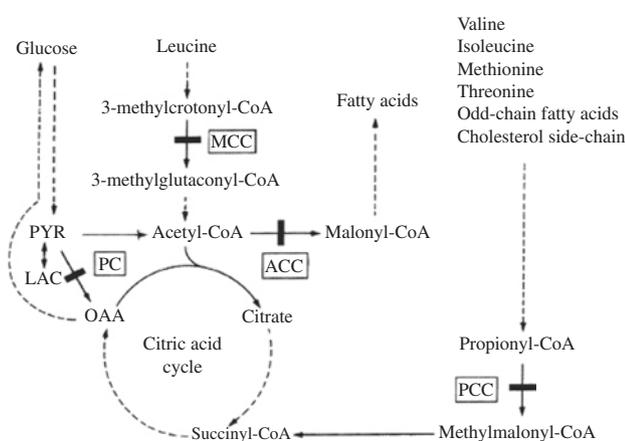
In this study, the aim is to investigate the levels of alanine, C3 and C5-OH obtained using the heel blood sample in cases with various degrees of biotinidase (BTD) deficiency.

Materials and methods

Patients, who referred to our clinic from the primary care health institutions with the suspicion of BTD deficiency (due to the results of the colorimetric method using heel blood sample) within the scope of the Turkish National Newborn Screening Programme (NNSP), were included in the study [10]. According to the classification based on serum BTD activity, those with a BTD activity <10% were classified as severe, and those with a BTD activity between 10 and 30% were classified as partial BTD deficiency (PBD) group [1, 11]. Those with a BTD activity >30% were classified as normal BTD activity (NBA) group. The C3 and C5-OH levels from heel blood were measured with LC-MS/MS method, using SHIMADZU LC-MS/MS 8040. Standard levels obtained from heel blood were 143–439 µmol/L for alanine, 0.8–2.9 µmol/L for C3, and 0–0.57 µmol/L for C5. Plasma alanine levels were measured with LC-MS/MS method using SHIMADZU LC-MS/MS 8045. Standard levels obtained from plasma was 144–400 µmol/L for alanine. Written consent of the volunteers have been obtained from parents.

Determination of biotinidase enzyme activity

Two to five milliliter venous blood sample was collected into BD Vacutainer SST II advance gelous tube (Becton, Dickinson and Company, Franklin Lakes, NJ, USA). The collected blood sample was centrifuged at 10,000 rpm for 5 min. One hundred microliter biotinidase was added on each well, and then, to determine biotinidase enzyme activity using colorimetric method (ODAK Neonatal Biotinidase Assay), 10 µL of the supernatant obtained by centrifuging the blood sample was added on each well. Samples were incubated in the incubator for 2 h at 37°C. After the addition of 100 µL TCA reagent on each well with sample, the samples were centrifuged at 4000 rpm for 5 min. Of the supernatant obtained after the centrifugation, 100 µL was added on the new wells. Thirty microliter Reagent-1 was added on the wells, the mixture was incubated at room temperature for 5 min and was slowly and manually mixed for 30 s during this period. After this, 30 µL Reagent-2 was added and the mixture was incubated for 5 min and was slowly and manually mixed for 30 s

Figure 1: Location of the biotin dependent carboxylases in intermediary metabolism.

ACC, acetyl-CoA carboxylase; CoA, coenzyme A; HCS, holocarboxylase synthetase; LAC, lactate; MCC, 3-methylcrotonyl-CoA carboxylase; OAA, oxaloacetate; PC, pyruvate carboxylase; PCC, propionyl-CoA carboxylase; PYR, pyruvate [1].

during this period. Finally, 30 μL Reagent-3 was added and the mixture was incubated at room temperature for 5 min, during which it was slowly and manually mixed for 30 s. The resulting absorbances were read at 570 nm wavelength using a Thermo Scientific Multiskan GO spectrophotometer (ThermoFisher Scientific).

Statistical analysis

Statistical analysis of the data was performed using the SPSS 24.0 software package (SPSS Inc., Chicago, IL, USA). Visual (histogram and probability plots) and analytical techniques (Kolmogorov-Smirnov, Shapiro-Wilk tests) were used to evaluate whether the variables follow normal distribution. Continuous variables were analyzed with either Student t-test or Mann-Whitney U-test depending on distribution and homogeneity of the data. Categorical variables were analyzed using Pearson χ^2 or Fisher's exact test (when any of the theoretical values observed on a 2×2 table was <5). Statistical significance level was accepted as $p < 0.05$ in all statistical analyses.

Results

Seventy-eight patients, who are pre-diagnosed with biotinidase enzyme activity deficiency, were included in the study. The mean age of the patients was 21.17 ± 6.34 days (range 4–38 days). Of the cases included in the study, 60.3% were male and 39.7% were female. 44.9% of the cases were in the PBD group, and 55.1% were in the NBA group. It was found that the PBD and NBA groups were similar in terms of the percentile distribution of gender, average age, height (cm) and weight (kg) ($p > 0.05$) (Table 1). Physical examination of the cases were normal, but only one case in the PBD group had skin eruptions. None of the cases had history of unexplained sibling death at birth. Only four cases had history of low BTD enzyme activity in sibling, and all these cases were in the PBD group. Among the cases in PBD group, consanguineous marriage rate was 74.3%, whereas this rate was 18.6% in NBA group. Among the cases in PBD group, consanguineous marriage rate was significantly higher than the NBA group ($p < 0.0001$).

The mean plasma alanine level of the cases in PBD group was 322.51 ± 70.08 μmol/L, while the mean plasma alanine level of the cases in NBA group was 192.52 ± 76.13 μmol/L. The mean plasma alanine levels of the cases in PBD group was significantly higher than the NBA group ($p < 0.0001$). The mean heel blood alanine level of the cases in PBD group was 314.45 ± 90.98 μmol/L, while the mean plasma alanine level of NBA group was 177.98 ± 59.55 μmol/L. The mean heel blood alanine level of the PBD group was significantly higher than the NBA group ($p < 0.0001$) (Table 2). Pearson correlation analysis revealed a negative correlation between plasma alanine levels and BTD activity ($r = -0.717$, $p < 0.0001$). Similarly, in the Pearson correlation analysis between the heel blood alanine level and BTD activity, a negative correlation was detected ($r = -0.646$, $p < 0.0001$).

The mean C3 level of the cases in the PBD group was 1.50 ± 0.68 μmol/L, while the mean C3 level of the cases in the NBA group was 1.73 ± 0.88 μmol/L, and the difference between the mean C3 levels of the two groups was not statistically significant ($p = 0.213$). The mean C5-OH level of the cases in PBD group was 0.23 ± 0.10 μmol/L,

Table 1: Sociodemographic characteristics of the cases.

	PBD (n=35)	NBA (n=43)	p-Value
Gender M/F	13/22	18/25	[a]0.672
Age/day-mean±SD (min-max)	22.31±6.57 (11−38)	20.23±6.07 (4−31)	[b]0.151
Weight percentile (n)			
10−25 p	8	12	
25−50 p	13	15	[a]0.951
50−75 p	8	10	
75−90 p	6	6	
Length percentile (n)			
10−25 p	7	11	
25−50 p	14	17	[a]0.936
50−75 p	8	9	
75−90 p	6	6	
Parental consanguineous marriage, yes/no (n)	26/9	8/35	[a]<0.0001

PBD, partial biotinidase deficiency; NBA, normal biotinidase activity; SD, standard deviation. [a]Pearson χ^2 test. [b]Independent sample t test.

Table 2: The average laboratory values of the groups.

Mean ± SD	PBD (n = 35)	NBA (n = 43)	p-Value[a]
Biotinidase enzyme activity/% (min-max)	21.68 ± 6.45 (10–30)	55.71 ± 16.22 (30–95)	<0.0001
Plasma alanine (μmol/L)	322.51 ± 70.08	192.52 ± 76.13	<0.0001
Heel blood alanine (μmol/L)	314.45 ± 90.98	177.98 ± 59.55	<0.0001
C3 (μmol/L)	1.50 ± 0.68	1.73 ± 0.88	0.213
C5-OH (μmol/L)	0.23 ± 0.10	0.25 ± 0.16	0.481

C3, propionylcarnitine; C5-OH, 3-hydroxyisovalerylcarnitine; SD, standard deviation; PBD, partial biotinidase deficiency; NBA, normal biotinidase activity. [a]Independent sample t test.

Table 3: Relationship between alanine, C3 and C5-OH levels with BTD activity.

	(r)	p-Value
Plasma alanine level-BTD activity	−0.717	<0.0001
Heel blood alanine level-BTD activity	−0.646	<0.0001
Heel blood C5-OH level-BTD activity	−0.021	0.856
Heel blood C3 level-BTD activity	0.221	0.078

r, Pearson correlation coefficient; C3, propionylcarnitine; C5-OH, 3-hydroxyisovalerylcarnitine; BTD, biotinidase.

while the mean C5-OH level of the cases in NBA group was 0.25 ± 0.16 μmol/L, and the difference between the mean C5-OH levels of the two groups was not statistically significant (p = 0.481). In the Pearson correlation analysis, it was found that there was no significant correlation between C3 and C5-OH and BTD activity (p < 0.05) (Table 3).

Discussion

BTD deficiency is a treatable metabolic disease which can manifest with a variety of neurological and dermatological complications [1, 11, 12]. In regions such as Turkey where consanguineous marriage is common, this autosomal recessive disease has a higher incidence [13, 14]. Indeed, in our study, we found that the rate of consanguineous marriage was higher in the PBD group than the NBA group. In our study, BTD activity level of the cases in the PBD group was between 10 and 30%, and there were no cases with severe BTD deficiency (<10%). Thus, it was thought that the reason for not having any pathological findings either neurological or dermatologic (except for one) in the physical examination may be due to the absence of a case with severe BTD deficiency in the study.

There are auxiliary laboratory tests used for the diagnosis of BTD deficiency. For PCC deficiency, C3; for MCC deficiency, C5-OH can be used for diagnosis [1, 6, 15, 16].

Clinical studies have shown that, particularly in severe BTD deficiency, plasma C3 levels can be useful in diagnosis [6, 15]. In this study, the mean C3 and C5-OH levels of the PBD and NBA groups were compared and the difference between them was considered insignificant. Similarly, in the correlation analysis between C3 and C5-OH and BTD, no significant correlation was detected between the BTD activity and C3 and C5-OH (Table 3). It was thought that the reason for not having a significant difference between the PBD and NBA groups in terms of mean C3 and C5-OH levels could be the absence of a case with severe BTD.

In our study, mean alanine levels in both plasma and heel blood in the PBD group were significantly higher than the NBA group (Table 2). However, there was a negative correlation between the BTD activity and plasma and heel blood alanine level. PC is a biotin-dependent carboxylase whose activity can decrease in the case of BTD deficiency, and this was considered potentially responsible for the higher mean alanine levels in the plasma and heel blood in the PBD group. Accumulating pyruvate is converted to lactate, and clinically can cause lactic acidose [17, 18]. However, in this study, the emphasis is on the conversion mechanism of pyruvate, which can accumulate as a result of biotin deficiency, into alanine via ALTs. In addition, it was found that the mean alanine levels in both PBD and NBA groups were within the specified reference range. In our study, the absence of a case with severe BTD deficiency was considered responsible for the level of alanine accumulating in the body to remain within the reference range. In cases with PBD; alanine levels, which can be measured using heel blood within the scope of NNSP, were considered as a helpful parameter in diagnosis. With meta-analysis studies that include cases with severe BTD deficiency, the relationship between alanine level and BTD activity can be demonstrated more clearly.

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