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Original Article

Evaluation of newborn screening for biotinidase deficiency from southeastern region of Türkiye

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ABSTRACT

Objective: Biotinidase deficiency (BD) is an autosomal recessive inherited metabolic disorder. Biotin plays an important role as a cofactor of carboxylases. BD is categorized into two groups as profound and partial deficiency based on serum quantitative biotinidase enzyme activity (BA). Clinical manifestations are highly variable, ranging from severe metabolic acidosis to asymptomatic.

Methods: Patients who were referred to the pediatric metabolism department due to the suspicion of BD are retrospectively retrieved. This study was conducted between 2019 to 2021 at Cengiz Gökçek Children's Hospital. The values of quantitative BA, below 30% were defined as deficiency, 10-30% were defined as partial deficiency (PBD), and below 10% were defined as profound deficiency (PFBD). Molecular analysis was performed on the patients. Quantitative analysis of the BA and BTD genes supported the diagnosis. Patients who were misdiagnosed with BD were classified as a false-positive group.

Results: A total of 255 patient files were retrospectively evaluated. 211 patients were included. The median age at presentation of the patients was 27±26,2 days (range: 10-240). 48.3% (n=102) patients in the BD group, and 51.7 % (n=109) patients in the false-positive group. Consanguinity was significantly higher in the BD group (p=0.002). The rate of patients with normal quantitative BA was 54.5% (n=115), PBD was 36.5% (n=77) and PFBD was 9% (n=19). For a variety of reasons, BTD gene analysis was carried out in 79.6% (n=168) of patients. 35.1% (n=59) of them were homozygous mutations, 13.1% (n=22) were compound heterozygous mutations, 40.5% were (n=68) heterozygous mutations, and 11.3% (n=19) were normal. Genetic analysis was consistent with BD in 26.8% (n=25/93) of patients with normal quantitative BA.

Conclusion: BA measurement may be affected by technical reasons. Because sensitivity and specificity of quantitative BA measurement methods are still controversial and inconsistent, confirmation of results by molecular analysis may reduce the risk of misdiagnosis.

Keywords: Biotinidase deficiency, biotin, newborn screening

INTRODUCTION

Biotinidase deficiency (OMIM #253260, BD) is an autosomal recessive inherited metabolic disorder. BD causes a defect in the metabolism of the vitamin biotin, resulting in a deficiency of biotin-dependent enzymes.[1] Biotinidase (EC 3.5.1.12, *amidohydrolase biotinidase*, *BTD*) is the enzyme that enables the formation of free biotin as a result of degradation and recycling of biocytin or biotinyl-peptides as well as dietary protein-bound sources. Biotin plays an important role as a cofactor of carboxylases, which are propionyl-CoA carboxylase, methyl-crotonyl-CoA carboxylase, pyruvate carboxylase, and acetyl-CoA carboxylase.[2] These carboxylases are involved in amino acid catabolism, fatty acid synthesis, and gluconeogenesis steps. All

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the mitochondrial carboxylase activities are impaired in BD. The inability of the carboxylases to function as a result of BD leads to clinical features.[3]

BD is categorized into two groups, profound and partial deficiency based on serum quantitative biotinidase enzyme activity (BA), which is less than 10% and 10-30% of regular serum activity, respectively.[4] Clinical manifestations are highly variable, ranging from severe metabolic acidosis to asymptomatic. Clinical features of symptomatic patients include neurologic manifestations such as developmental delay, seizures, hearing loss, optic atrophy, ataxia and immunologic manifestations, and cutaneous lesions such as alopecia, conjunctivitis, and skin rash.[1] In contrast to partial deficiency, where symptoms are anticipated to be mild at any age from childhood to adulthood, profound BD is predicted to present with severe symptoms early in life. Additionally, PBD is frequently asymptomatic, but may manifest symptoms in response to stressful events such as infection.[5]

Oral biotin therapy is recommended as 5-20mg/day in BD. Biotin treatment, started in the asymptomatic period, prevents the symptoms of biotinidase deficiency and also provides improvement in affected patients.[6,7] If the treatment is not administered in time, hearing and vision problems as well as neurological damage may not be fully recovered and may persist.[1,7] In Türkiye, BD has been screened under the National Newborn Screening (NBS) Program since October 2008.[8] The aim is to diagnose the patients in the asymptomatic period and to follow them without sequelae. The incidence of biotinidase deficiency ranges from 1:4500 to 1:62500 in countries that screen for this condition.[9] In some studies, the false- positivity rates of biotinidase activity screening with NBS are high. In the Netherlands, only 7% of those identified by screening were diagnosed with a profound biotinidase deficiency.[9] This study aims to identify false-positive NBS results in order to increase awareness of accurate diagnosis and follow-up of patients identified by the screening program.

MATERIALS AND METHODS

Patients who were referred to the pediatric metabolism department in Cengiz Gökçek Children's Hospital, located in the southeastern region of Türkiye, with the suspicion of BD between 2019-2021 were included in the study. Gender, age at admission, consanguinity, dried blood spot (DBS) biotinidase activity (BA), quantitative BA, and molecular analysis results were retrieved from the medical records.

The local ethics committee of Gaziantep University approved the study protocol.

Quantitative biotinidase activity and molecular analysis were performed at the first admission of patients with biotinidase activity <65 IU in DBS. DBS BA was analyzed by fluorometric immunoassay while quantitative BA was analyzed by spectrophotometric method. The determined BA values were calculated as "%" enzyme activity. Values below 30% were defined as a deficiency, below 10% defined as a profound deficiency (PFBD), and 10-30% defined as a partial deficiency (PBD). For patients with more than one value, the mean value was calculated. The diagnosis was confirmed by the quantitative BA and BTD gene analysis. BA results of DBS and quantitative and molecular analysis results were compared. Patients who were not diagnosed with BD were considered the false-positive group.

Categorical data were expressed as numbers and percentages (%), while numerical data were expressed as medians (minimum-maximum). The Chi-square test was used to compare categorical data. When comparing the numerical data from two or more independent groups, the data were first examined for parametric properties. In this study, descriptive statistics and Shapiro-Wilk tests were used. Numerical data from two or more independent groups that did not show parametric properties, were compared using the Mann-Whitney U test or Kruskal-Wallis test. All analyses were calculated with the Statistical Package for the Social Sciences 23.0 (IBM SPSS) statistical program. A p value <0.05 was considered statistically significant.

RESULTS

A total of 255 patient files were retrospectively evaluated. Patients who were previously referred to other metabolic centers, did not have a quantitative BA result, or had a suspicious diagnosis were excluded. A total of 211 patients were included, 99 (46.9%) females and 112 (53.1%) males. The median age at the presentation of the patients was 27 days (range:10-240). When the patients were evaluated with both activity and genetic results (final result), 51.7% (n=109) were normal, and 48.3% (n=102) patients were diagnosed with BD. A total of 49.8% (n=105) patients had consanguine marriages, including 60.8% (n=62) patients in the BD group and 39.4% (n=43) patients in the false-positive group. The rate of consanguinity was significantly higher in the BD group (p=0.002) (Table 1). Median BA in DBS and quantitative BA in plasma were 52.5 IU (2-65) and 33.0% (2.3-88%), respectively. When patients were categorized based on quantitative analysis, 54.5% (n=115) had normal quantitative BA, 36.5% (n=77) had PBD, and 9% (n=19) had PFBD. For a variety of reasons, BTD gene analysis was performed in 79.6% (n=168) of the patients. Homozygous mutations were found in 35.1% (n=59) of patients, compound heterozygous mutations in %13 (n=22) of patients, and heterozygous mutations in 40.4%

Table 1. Demographic findings of patients			
Final Result	**BD (n=102)**	***Normal (n=109)**	**p**
Male/female	58 (%52.9) / 48 (%47.1)	58 (%53.2) / 51 (%46.8)	0.969
Admission Age -day (median)	23.0 (11.0-85.0)	21.0 (10.0-240.0)	0.392
Consanguinity	62 (%60.8)	43 (%39.4)	0.002
*False-positive group			

(n=68) of patients. The BTD gene was normal in 11.3% (n=19) of the patients (Figure 1).

The patients were analyzed according to molecular analysis and BA. The relationships between BA and molecular analysis and consanguinity were evaluated (Table 2-3).

Despite the activity level being normal, 26.8% (n=25) of the patients had genetic data that were consistent with the diagnosis of BD. Of these, 21.5% (n=20) had homozygous mutations and 5.3% (n=5) had compound heterozygous mutations. However, heterozygous mutations were detected in 18.6% (n=14) of PBD patients, whereas no mutation was detected in 6.6% (n=5) of them. All PFBD patients had mutations in both alleles (Table 2).

DISCUSSION

BD is an autosomal recessive metabolic disorder resulting in inadequacy of biotin. Several neurological and dermatological disorders may occur. BD was first described in 1983 by Wolf et al.[10] Heard et al. described a method to measure biotinidase activity by colorimetric assessment using a DBS card in 1984.[11] The first NBS pilot NBS program for BD was carried out in US, in 1984.[12] Since 2008, NBS for BD has been launched in Türkiye. A research found that the incidence of BD was 1:60089 within the scope of the NBS program in 14 countries between 1984-1990.[13] The incidence of BD was 1:61,000 according to the 30-year screening data in Italy[14], but it was 1:5,996 in another part of the city site.[15] Although the incidence of BD in Türkiye is 1:11614[16], a recent study in the city of Şanlıurfa in southeastern Türkiye, where consanguineous marriages are more common, found the incidence to be 1:1177.[17] These results demonstrate that there are regional differences in incidence.

The main purpose of screening is to identify profound deficiencies with irreversible severe symptoms if left untreated and to prevent the onset of symptoms through early treatment. A neonatal screening program for BD is critical because of the

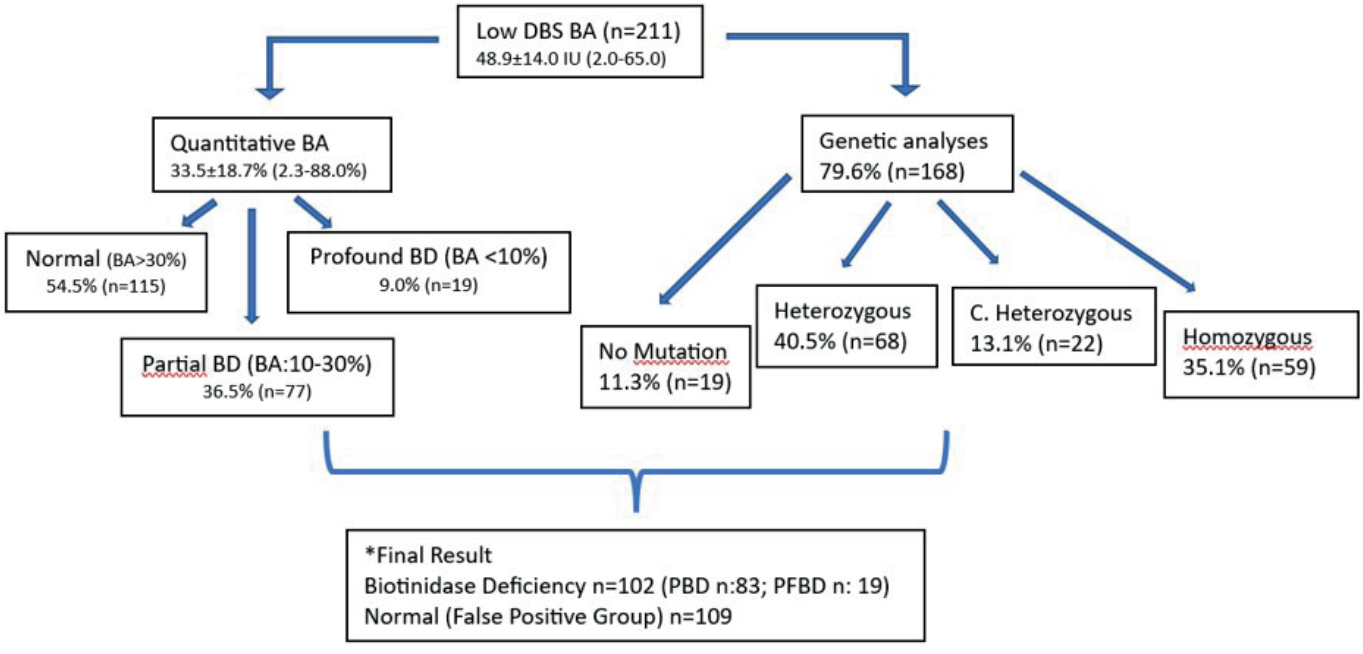

Figure 1. Evaluation scheme of patients referred from newborn screening

*The result determined after evaluating both genetic analysis and biotinidase activity of the patients

Table 2. Classification by biotinidase activity					
Quantitative BA	**Normal**	**PBD**	**PFBD**	***Combined BD**	**p**
Genetik					
N	14 (%15.1)	5 (%8.5)	0 (%0.0)	5 (%6.7)	<0.001[1]
Heterozygous	54 (%58.1)	14 (%23.7)	0 (%0.0)	14 (%18.7)	
Compound Heterozygous	5 (%5.4)	13 (%22.0)	4 (%25.0)	17 (%22.7)	
Homozygous	20 (%21.5)	27 (%45.8)	12 (%75.0)	39 (%52.0)	
**Total	93	59	16	75	
DBS Mean	55.0 (40.0-65.0)	49.0 (6.0-65.0)	14.0 (2.0-55.5)	46.8 (2.0-65.0)	<0.001[2]

*Combined BD= PBD+PFBD
**Total= The total number of quantitative BA in the genetically analyzed patients.
[1]The heterozygous mutation was more frequent in normal quantitative BA group and the homozygous mutation was more frequent in PFBD group.
[2]Normal vs PBD, Normal vs PFBD, PBD vs PFBD were significant (p<0.001)

Table 3. Classification by consanguinity				
Consanguinity	**Exist**	**No**	**Total**	**p**
Genetic				
No mutation	8 (42.1%)	11 (57.9%)	19	<0.001[1]
Heterozygous	26 (38.2%)	42 (61.8%)	68	
Compound Heterozygous	11 (50%)	11 (50%)	22	
Homozygous	44 (74.6%)	15 (25.4%)	59	
Total	89	79	168	
Quantitative type				
PBD	40 (51.9%)	37 (48.1%)		0.344
PFBD	12 (63.2%)	7 (36.8%)		
Normal	53 (46.1%)	62 (53.9%)		
Total	62 (100%)	44 (100%)		

[1]Consanguinity was detected more frequently in patients with homozygous mutations detected by genetic analysis.

early initiation of biotin therapy. Therefore, the age of admission becomes vital. In the studies conducted at Dokuz Eylül University[18] and Ege University[19] Hospitals (both located in the western region of Türkiye), the median age at presentation of patients with biotinidase deficiency who were referred from the NBS program was 15 days (5-46), 1.20 months (0.4–5), respectively. The median age of admission in this study was 22 days (10-240). Another metabolic center in the same region as our study also found the mean age of admission to be 96.30 (min-max, 2-828) days.[17] The reason for the late age at first presentation in these two studies conducted in the same period and region may be the lack of awareness about NBS, poor socioeconomic conditions, and inadequate care at the time of referral to the hospital. The patients in this study were asymptomatic, whereas the study by Kazanasmaz et al.[17], in which the age at first presentation was higher, reported the presence of symptomatic patients in the PFBD group. This highlights the importance of early diagnosis.

There was no statistically significant difference between males and females in this study. In other studies carried out in Türkiye, it has been observed that the ratio of females to males is similar.[17-21] As this is an autosomal recessive disease, there are no significant differences between the genders, as would be expected. The rate of consanguineous marriage is as high as 24% in Türkiye. The consanguinity rate was found to be 49.8% in all patients included in this study. It was 60.8% in the BD group and 39.4% in the false-positive group and was found to be statistically significant (p=0.002). It is known that the consanguinity rate in the southeastern region of Türkiye is higher than the average in Türkiye, which is 43%. In similar studies conducted in the same

region, Şanlıurfa study group detected consanguinity rate as 38.5% in BD group while 13.5% in false negative group (p=0.002), Adana group detected consanguinity rate in all enrolled patients as 61.5%. All these consanguinity rates are significantly higher than the Turkish average.[17,21,22] This situation can be explained by the autosomal recessive inheritance of the disease, as well as the higher consanguineous marriage rate in the region.

DBS BA mean is 48.9(2-65), and quantitative BA 33.5% (2.3-88%). There were 77(36.5%) patients in the PBD group and 19(9%) in the PFBD group (Figure 1). Consistent with the literature, partial and profound BD patients' rates are similar to this study.[17,19,21]

In the consanguineous marriage group, molecular analysis revealed 29.1% (n=26) heterozygous and 12.3% (n=11) compound heterozygous mutations (p<0.001). Furthermore, heterozygous mutations were detected in 40% (n=68) and compound heterozygous mutations in 13% (n=22) of the patients in this cohort. In a study conducted in the same region, compound heterozygous mutations were detected in 43% (n=89) of patients.[21] These results indicate the high carrier frequency in the BTD gene.

When the patients were evaluated with both activity and molecular analyses, it was demonstrated that 109 patients (51.7%) – false-positive group – were normal, and 102 patients (48.3%) were diagnosed with BD. In patients recalled with suspicion of BD, In Italy 17% (n=49/287) of patients[15] and 3.9% (n=18/461) of patients[14], and in the Netherlands 42% (n=111/261) of patients[9] were diagnosed as BD. In Türkiye, Kazanasmaz et al. demonstrate 57.3% false-positive results.[17] These results suggest that the laboratory measurement method is controversial and not sufficient. In Türkiye, DBS BA is analyzed by fluoroscopic immune assay and generally spectrophotometric method is used for the measurement of quantitative biotinidase activity in recalled suspicion of BD patients. However, in a study, conducted in Türkiye in 2016, spectrophotometric and fluorometric methods were compared in the evaluation of biotinidase deficiency. It was reported that the fluorometric method is more sensitive.[23] In this study, this conclusion could be reached as the samples were studied in the same center, which will increase the quality of the sample. It raises the question of whether sample quality is degraded during the transfer of DBS cards to public health laboratories.

In this study, molecular analysis confirmed BD in all PFBD. In cases of partial deficiency with molecular analysis (n=59), any mutation was detected in 8.5% (n=5) of patients, and heterozygous mutations were detected in 23.7% (n=14) of patients. In addition, the genetic result was compatible with BD in %26.8 (n=25) of the patients with normal quantitative BA levels whose molecular analysis was performed (n=93). Of these patients, 20% (n=5/25) have compound heterozygous mutations and 80% (n=20/25) have homozygous mutations. In the study conducted in the Netherlands, 54% (n=50) of the 92 patients, who were referred with suspicion of BD, were diagnosed. 66% (n=61) of 92 the patients have molecular analysis. Of these, 7 of 21 patients with normal quantitative BA were heterozygous, and the other 7 had compound heterozygous mutations.[9]

CONCLUSIONS

Regarding all these results, it may be considered that there is residual enzyme activity in patients with compound heterozygous mutation and biotinidase activity may be borderline low in carriers. These results demonstrate us that the diagnosis of BD should be confirmed via molecular analysis. Since BA may be affected by technical reasons and the sensitivity and specificity of measurement methods are still controversial and inconsistent, this study predicts that confirming results by molecular analysis will reduce the risk of misdiagnosis.

Therefore, all patients referred for suspected BD within the scope of the newborn screening program should have access to molecular analysis by healthcare providers.

Limitations of this study

Because the laboratory where the genetic analysis was performed was in a different area from the center where the patients were enrolled, the molecular analysis could not be carried out in all patients. MLPA analysis could not be performed in heterozygous patients due to technical incompetence.

Ethical approval

This study has been approved by the Gaziantep University Clinical Research Ethics Committee (approval date 04.11.2020, number 2020/367). Written informed consent was obtained from the participants.

Author contribution

Surgical and Medical Practices: EG; Concept: EG; Design: EG; Data Collection or Processing: EG; Analysis or Interpretation: EG; Literature Search: EG; Writing: EG. All authors reviewed the results and approved the final version of the article.

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Conflict of interest

The authors declare that there is no conflict of interest.

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