

Investigation of the effects of biotinidase deficiency on plasma cholinesterase activity

Biyotinidaz eksikliđinin plazma kolinesteraz aktivitesi üzerindeki etkilerinin arařtırılması

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Posted date:03.09.2024

Acceptance date:11.11.2024

Abstract

Purpose: Biotinidase deficiency (BD) is a rare autosomal recessive metabolic disorder that impairs the body's ability to recycle biotin, a crucial coenzyme for carboxylase enzymes involved in various metabolic processes. This study aims to evaluate the effects of biotinidase deficiency on cholinesterase activity in plasma, hypothesizing that the metabolic disruptions caused by inadequate biotin recycling may lead to alterations in cholinesterase function.

Materials and methods: Plasma samples were collected from 73 individuals categorized into four genetic groups: wild type (n=12), heterozygous (n=30), homozygous (n=19), and compound heterozygous (n=12). Cholinesterase activity was measured using a colorimetric method.

Results: The study discovered that the cholinesterase activity of the Heterozygous group was higher than the homozygous group ($p=0.0356$). Additionally, cholinesterase activity was significantly lower in homozygous and compound heterozygous people than in wild and heterozygous groups ($p=0.0272$). The statistically significant changes suggested a relationship between biotinidase deficiency and altered cholinergic activity.

Conclusion: The findings indicate that biotinidase deficiency, particularly in its severe variants, may cause considerable reductions in cholinesterase activity, contributing to the neurological symptoms found in affected patients. More studies are needed to investigate the processes behind this association and develop strategies for reducing the effects of BD on cholinesterase activity and neurological health.

Keywords: Biotinidase deficiency, cholinesterase, metabolic disorder.

Ozcan M, Oz O, Ercan M. Investigation of the effects of biotinidase deficiency on plasma cholinesterase activity. Pam Med J 2025;18:99-104.

Öz

Amaç: Biotinidaz eksikliđi (BD), vücudun çeşitli metabolik süreçlerde yer alan karboksilaz enzimleri için kritik bir koenzim olan biotini geri dönüřtürme yeteneđini bozan nadir bir otozomal resesif metabolik bozukluktur. Bu çalıřma, yetersiz biotin geri dönüřümünün neden olduđu metabolik bozulmaların kolinesteraz fonksiyonunda deđiřikliklere yol açabileceđi hipotezini test ederek, biotinidaz eksikliđinin plazmadaki kolinesteraz aktivitesi üzerindeki etkilerini deđerlendirmeyi amaçlamaktadır.

Gereç ve yöntem: Plazma örnekleri, yabani tip (n=12), heterozigot (n=30), homozigot (n=19) ve bileşik heterozigot (n=12) olarak kategorize edilen 73 bireyden toplandı. Kolinesteraz aktivitesi kolorimetrik bir yöntem kullanılarak ölçüldü.

Bulgular: Bu çalıřmada, heterozigot grubun kolinesteraz aktivitesinin homozigot gruptan daha yüksek olduđu bulundu ($p=0,0356$). Ek olarak, yabani tip ve heterozigot gruplarına kıyasla homozigot ve bileşik heterozigot bireylerde kolinesteraz aktivitesinin önemli ölçüde azaldıđı bulundu ($p=0,0272$). Farklılıklar istatistiksel olarak anlamlıydı ve bu durum, biotinidaz eksikliđi ile deđiřmiş kolinerjik fonksiyon arasında potansiyel bir bađlantıyı işaret etmektedir.

Sonuç: Bulgular, özellikle ciddi formlarında biotinidaz eksikliđinin, kolinesteraz aktivitesinde önemli azalmalarla sonuçlanabileceđini ve bu durumun etkilenen bireylerde gözlemlenen nörolojik semptomlara katkıda bulunabileceđini düşündürmektedir. Bu iliřkiyi açıklamak ve BD'nin kolinesteraz aktivitesi ve nörolojik sađlık üzerindeki etkilerini hafifletmeye yönelik terapötik stratejiler geliřtirmek için daha fazla arařtırmaya ihtiyaç vardır.

Anahtar kelimeler: Biyotinidaz eksikliđi, kolinesteraz, metabolik bozukluk.

Özcan M, Öz Ö, Ercan M. Biyotinidaz eksikliđinin plazma kolinesteraz aktivitesi üzerindeki etkilerinin arařtırılması. Pam Tıp Derg 2025;18:99-104.

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Introduction

Biotinidase deficiency (BD) is a rare autosomal recessive metabolic disorder that impairs the body's ability to recycle the vitamin biotin, a critical coenzyme for carboxylase enzymes involved in various metabolic processes, including fatty acid synthesis, amino acid catabolism, and gluconeogenesis. The deficiency of biotinidase, an enzyme responsible for the cleavage of biotin from biocytin and other biotinyl-peptides, leads to reduced availability of free biotin, ultimately affecting the function of biotin-dependent carboxylases. If left untreated, BD can result in a range of neurological and dermatological symptoms, such as seizures, hypotonia, ataxia, alopecia, and skin rashes, which can be severe and irreversible in some cases [1].

Cholinesterase enzymes, including acetylcholinesterase (AChE) and butyrylcholinesterase (BChE), play a crucial role in the nervous system by hydrolyzing the neurotransmitter acetylcholine, thereby terminating synaptic transmission [2, 3]. These enzymes are not only vital for cholinergic signaling but also have been implicated in various non-neuronal processes, such as lipid metabolism, immune responses, and inflammation [2-4]. Alterations in cholinesterase activity have been associated with several neurodegenerative diseases, liver dysfunction, and metabolic disorders [5-7].

Recent studies have begun to explore the potential interactions between metabolic disorders and cholinergic function, particularly focusing on how metabolic imbalances might influence cholinesterase activity. Given the critical role of biotin in cellular metabolism and the potential consequences of its deficiency on overall metabolic homeostasis, it is plausible that biotinidase deficiency may also impact cholinesterase activity in the plasma [7-11]. Understanding this relationship could provide new insights into the broader metabolic implications of BD and its potential role in neurodevelopmental disorders.

This study aims to evaluate the effects of biotinidase deficiency on cholinesterase activity in plasma, hypothesizing that the metabolic disruptions caused by inadequate biotin recycling may lead to alterations in cholinesterase function. By analyzing cholinesterase activity in plasma samples from individuals with biotinidase deficiency, this research seeks to contribute to the growing body of knowledge on the systemic effects of metabolic disorders and their potential links to neurological function.

Materials and methods

Study population, sample collection and BTD gene analysis

In this study, plasma samples were collected from 73 patients diagnosed with biotinidase deficiency at the Department of Medical Genetics, Harran University Faculty of Medicine. Exclusion criteria included patients with other metabolic disorders, neurological conditions unrelated to BD, or those receiving treatment that could affect cholinesterase levels (e.g., cholinesterase inhibitors). Peripheral blood samples (2 cc) were drawn into tubes containing Ethylene Diamine Tetraacetic Acid (EDTA) for DNA isolation. Genomic DNA was then extracted, and sequence analysis was performed using primers that target the exon regions of the BTD gene. The resulting data were analyzed with the Mutation Surveyor program. The patients were classified into four genetic categories: Wild type, Heterozygous, Homozygous, and Compound Heterozygous. Each group was further stratified by gender, and age-related statistical parameters were calculated (Table 1).

Permission was obtained from the Harran University Faculty of Medicine Clinical Research Ethics Committee for the study (approval date: December 11, 2023, and number: HRÜ/23.23.26).

Table 1. Gender and age distribution of individuals across different genetic groups

Group	Gender	Patient Count	Mean Age	Age Std Dev	Median Age	Age Range
Wild type	Male	9	1.30	0.67	1	1-3
	Female	3	3.00	2.83	2	0-8
Heterozygous	Male	16	2.06	1.06	2	1-6
	Female	14	2.14	0.86	2	1-3
Homozygous	Male	11	2.82	1.34	3	1-6
	Female	8	2.20	0.79	2	1-3
Compound Heterozygous	Male	5	2.40	1.14	3	1-3
	Female	7	3.43	0.79	3	2-5

Measurement of cholinesterase activity

Cholinesterase activity in human-plasma was quantitatively determined using the Cholinesterase Gen.2 kit (Roche Diagnostics, Mannheim, Germany) on the Roche Cobas c, c 502. The test is based on a colorimetric method, where cholinesterase catalyzes the hydrolysis of butyrylthiocholine to thiocholine and butyrate. Thiocholine reduces hexacyanoferrate (III) to hexacyanoferrate (II), and the resulting decrease in absorbance at 415 nm is measured spectrophotometrically. The measurement range for cholinesterase activity was 100–14.000 U/L, with a lower detection limit of 100 U/L. The precision of the method was evaluated based on within-run and between-run coefficients of variation (CV). Within-run CVs were 0.5% for samples with mean activities of 4.887 U/L (Precinorm U) and 5.331 U/L (Precipath U). Between-run CVs were 1.0% for Precinorm U and 0.9% for Precipath U.

Statistical analysis

Statistical analysis was performed using GraphPad Prism 8.4.2. The Shapiro-Wilk test was chosen to assess data normality due to its suitability for small sample sizes [12]. Normally distributed data were analyzed using the Student's t-test, while non-normally distributed

data were evaluated with the Mann-Whitney U test. Results are reported as mean \pm standard deviation, with a *p*-value of ≤ 0.05 indicating statistical significance.

Results

Cholinesterase activities were measured in individuals with biotinidase deficiency, categorized into four groups: Wild type, Heterozygous, Homozygous, and Compound Heterozygous. The gender and age distributions of individuals in the groups are shown in Table 1. The cholinesterase activities for each group were as follows: Wild type: 9419 ± 2302 U/L, Heterozygous: 9379 ± 1561 U/L, Homozygous: 8437 ± 1326 U/L, and Compound: 8648 ± 1540 U/L (Figure 1A). A statistically significant difference was observed between the Heterozygous and Homozygous groups ($p=0.0356$, $t=2.166$). Cholinesterase activities were further analyzed by grouping individuals into two main categories: Wild type + Heterozygous and Homozygous + Compound Heterozygous. The cholinesterase activities for each group were as follows: Wild type + Heterozygous: 9391 ± 1778 U/L and Homozygous + Compound Heterozygous: 8519 ± 1391 U/L (Figure 1B). The difference between these two groups was statistically significant ($p=0.0272$, $t=2.255$).

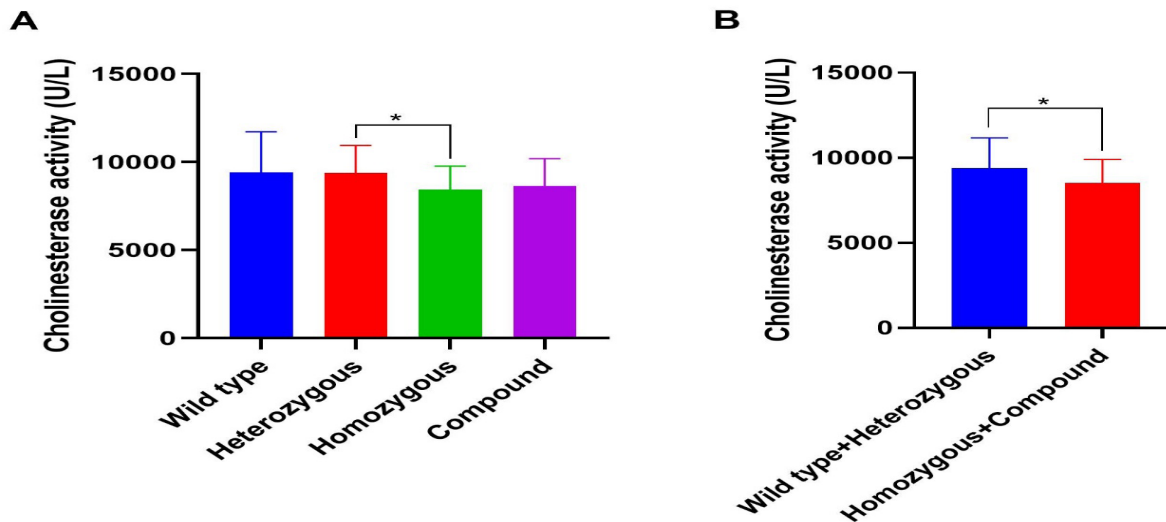


Figure 1. Effects of biotinidase deficiency on cholinesterase activity

(A) Results from four distinct genetic groups (Wild type, Heterozygous, Homozygous, and Compound Heterozygous)

(B) Results from grouping individuals into two main categories: Wild type + Heterozygous and Homozygous + Compound Heterozygous

All results are presented as mean ± standard deviation. * indicates $p < 0.05$

Discussion

This study aimed to explore the potential impact of Biotinidase Deficiency (BD) on cholinesterase activity in plasma, an area that has received limited attention in previous research. Our findings indicate that individuals with BD, particularly those classified as Homozygous or Compound Heterozygous, exhibit significantly reduced cholinesterase activity compared to their Wild type and Heterozygous counterparts. These results provide valuable insights into the broader metabolic implications of BD, highlighting its potential effects on cholinergic function and, by extension, neurological health.

The observed reduction in cholinesterase activity among Homozygous and Compound Heterozygous individuals may be attributed to the metabolic disruptions caused by inadequate biotin recycling. Biotin, a crucial coenzyme for carboxylase enzymes, plays an essential role in various metabolic processes, including fatty acid synthesis, amino acid catabolism, and gluconeogenesis [13]. A deficiency in biotinidase impairs the recycling of biotin from biocytin and other biotinyl-peptides, leading to a reduced availability of free biotin and subsequent dysregulation of biotin-dependent metabolic pathways [11].

Previous studies have suggested that alterations in cholinesterase activity are

associated with several neurodegenerative diseases and metabolic disorders [14, 15]. The reduced cholinesterase activity observed in our study may reflect a broader metabolic imbalance resulting from BD, potentially contributing to the neurological symptoms commonly seen in affected individuals, such as seizures, hypotonia, and ataxia [16, 17]. Our findings are consistent with the literature which reported that metabolic disorders could influence cholinergic function, thereby affecting neurological outcomes [16, 17].

The statistically significant difference in cholinesterase activity between the Wild type + Heterozygous and Homozygous + Compound Heterozygous groups ($p = 0.0272$, $t = 2.255$) further underscores the potential link between BD and cholinergic dysfunction. This result suggests that even partial impairment of biotin recycling, as seen in heterozygous individuals, may not significantly disrupt cholinesterase activity, whereas more severe forms of the deficiency (i.e., Homozygous and Compound Heterozygous) lead to notable alterations in enzyme function. This study adds to the growing body of evidence suggesting that BD has systemic effects beyond the well-characterized neurological and dermatological symptoms, potentially influencing broader metabolic and enzymatic processes [10, 18].

Given the critical role of cholinesterase enzymes in terminating synaptic transmission and their involvement in various non-neuronal processes, such as lipid metabolism and inflammation [4, 19, 20], the implications of reduced cholinesterase activity in BD are significant. The potential link between BD and cholinergic dysfunction could open new avenues for understanding the pathophysiology of the neurological symptoms associated with this condition. Further research is warranted to explore the mechanisms underlying this relationship and determine whether therapeutic interventions to restore biotin levels can normalize cholinesterase activity and improve neurological outcomes in affected individuals.

A limitation of this study is the relatively small sample size, which may affect the generalizability of the results. Additionally, other unaccounted factors, such as patients' nutritional status or the use of medications that could affect cholinesterase activity, were not controlled for. Future studies with larger cohorts and broader inclusion criteria are recommended.

In conclusion, our study demonstrates a significant reduction in cholinesterase activity in individuals with Biotinidase Deficiency, particularly those with Homozygous or Compound Heterozygous mutations. These findings highlight the potential for BD to impact cholinergic function, thereby contributing to the neurological symptoms observed in this condition. Future research should focus on elucidating the mechanisms driving this relationship and exploring potential therapeutic strategies to mitigate the effects of BD on cholinesterase activity and neurological health.

Funding: This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Authors contributions: M.O. and M.E. have constructed the main idea and hypothesis of the study. M.O., O.O., and M.E. developed the theory and edited the material and method section. M.O. and M.E. have evaluated the data in the Results section. The discussion section of the article was written by M.O. and corrected and approved by O.O. and M.E. In addition,

all authors discussed the entire study and approved the final version.

Conflict of interest: No conflict of interest was declared by the authors.

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