Establishing biotinidase reference interval: A foundation stone for newborn screening of biotinidase deficiency in Pakistan

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Abstract

Objective: To determine the reference interval of biotinidase activity in healthy neonates.

Method: The cross-sectional study was conducted at the Department of Chemical Pathology and Endocrinology, Armed Forces Institute of Pathology, Rawalpindi, Pakistan, from May to November 2019, and comprised blood samples collected from healthy neonates aged 2-6 days. The samples were collected on filter paper and analysed on genetic screening processor based on dissociation-enhanced lanthanide flouroimmunoassay. Data was analysed using SPSS 21.

Results: Of the 120 dried blood spot specimens, 81(67.5%) were from male babies and 39(32.5%) from female babies. Reference interval for biotinidase activity, based on 2.5th and 97.5th percentiles, was from 3.0 to 11.0 nmol/ml/min.

Conclusion: Screening of newborns for biotinidase deficiency is crucial to prevent irreversible neurological damage.

Keywords: Biotinidase reference interval, Biotinidase deficiency, Newborn screening in Pakistan. (JPMA 72: 97; 2022)

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Introduction

Among the genetic disorders, biotinidase deficiency (BD) is considered the most rewarding metabolic disorder to treat.[1] BD is not common worldwide, but in countries like Saudi Arabia,[2] Turkey[3] and Iran,[4] where the rate of consanguineous marriages is very high, this disorder is very common. One out of 60,000 children worldwide are born with BD.[5] BD is reported recently in multiple case reports from Pakistan[6-8] and it has also been reported in Pakistani children residing in other countries.[9] A study in Karachi reported 33 cases of confirmed BD out of 113 cases reported with signs and symptoms of BD over a 10-year period from a single centre.[10] This shows that BD is very much present in Pakistan, but, due to lack of diagnostic facilities and awareness of disease, the disease is rarely diagnosed.

BD is an inherited, autosomal recessive disorder of biotin recycling, also referred to as multiple carboxylase deficiency.[11] Biotin is an essential vitamin that serves as a coenzyme for four carboxylases, including propionyl-Coenzyme A (COA) carboxylase, 3-methylcrotonyl-COA carboxylase, pyruvate carboxylase, and acetyl-COA carboxylase, in humans. These enzymes play important roles in carbohydrate, amino acid and fatty acid metabolism. The major cause of this disease is absence or abnormal activity of a cytosolic enzyme biotinidase. Newborns having BD may or may not show symptoms at the time of birth. Based on the level of activity of biotinidase enzyme in the serum of the patients, the disease is classified as partial deficiency 10-30% enzyme activity, and profound deficiency 0-10% enzyme activity. The condition is easily treatable by biotin supplements, but, if not diagnosed timely, it can lead to severe consequences, like irreversible neurological damage, sensorineural deafness, developmental delay and seizures.[12]

The National Institute of Population Studies in 2013 reported that 48.5% of all marriages in Pakistan occur between first cousins and 7.9% between second cousins.[13] The more the consanguinity, the more is the risk of having genetic disorders.[14] The incidence of inherited metabolic disorders varies from country to country, and, compared to developed countries, there is predicted ten-fold higher incidence of inherited metabolic disorders (IMDs) in Pakistani children.[15] In Pakistan, infant mortality rate is 62 per 1000 live births.[16] It is also a fact that IMDs did not gain much importance and have been ignored by both the private sector as well as the government.

Biotinidase testing is part of newborn screening programme in most developed countries. Determining reference values for biotinidase in healthy neonates bear paramount importance for the launching of a neonatal screening programme in Pakistan. Early and prompt diagnosis can prevent irreversible neurological damage and even death of the neonates. The current study was planned to determine the reference interval of biotinidase activity in healthy Pakistani neonates.

Materials and Methods

The cross-sectional study was conducted at the Department of Chemical Pathology and Endocrinology,

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Armed Forces Institute of Pathology (AFIP), Rawalpindi, Pakistan, from May to November 2019. After approval from the institutional ethics review, the sample size was calculated in line with Clinical and Laboratory Standards Institute (CLSI) guidelines.[17] The sample was raised using non-probability convenience sampling technique. committee After written informed consent from the parents, blood samples were taken from the Paediatrics Department of the Pakistan Emirates Military Hospital (PEMH), Rawalpindi. The samples were taken from healthy neonates aged 2-6 days. . Neonates with history of sepsis or IMDs or on some management strategy, like dietary restrictions or enzyme replacement, and premature neonates as well as those who had received blood transfusions were excluded.

Demographic characteristics, family history, biochemical findings and results of biotinidase assay were recorded. Preliminary biochemical tests included complete blood count (CBC), plasma ammonia, lactate, plasma glucose, arterial blood gas analysis, urine for non-glucose reducing substances, and liver functions tests (LFTs). These tests were done to exclude sick newborns from the study. Only newborns who had normal results for all these tests were included in the study. Dried blood spot (DBS) was collected on Guthrie cards for biotinidase estimation through heel prick test. DBS samples were transported in special plastic bags protected from direct sunlight and humidity. Biotinidase enzyme analysis was carried out on a fully automated analyser with loaded microtitration plates containing DBS (Genetic Screening Processor 2021, Perkin Elmer). The sample was extracted from DBS by the extraction solution present in the reagents as a first step of the assay. Biotinidase assay (Kit product No. 3307-0010) was used containing biotinidase substrate (Europium- labelled biotin) reagent, biotinidase streptavidin reagent, assay buffer and anti-streptavidin immunoglobulin-G (IgG) microtitration strips. Biotinidase assay was based on an enzyme reaction with a solid phase time-resolved immunofluorescence assay. The amide bond in europium-labelled biotin was cleaved by the biotinidase enzyme present in the sample. The enzyme reaction was stopped by adding streptavidin and the resultant streptavidin-biotin complexes were captured by solid phase monoclonal antibodies. Dissociation-enhanced lanthanide flouro-immunoassay (DELFIA) inducer dissociated the molecules into the solution where the europium fluorescence was measured. The measured fluorescence was inversely proportional to the biotinidase activity in the sample. For the biotinidase assay, six standards were used for every analytical run. In every plate, medium and high levels of standards (six for each) were measured. Intra- and inter-assay coefficient of variation (CV) was 3.3% and 7.2% respectively. A positive abnormal and normal control was included with each batch of samples. Data was analysed using SPSS 21. Kolmogorov Smirnov test was applied

Table-1: Frequency distribution and ranks of biotinidase activity levels in 120 healthy neonates.

Value	Frequency	Rank No
2.9	1	1
3.0*	2	2-3
3.1	4	4-7
3.2	3	8-10
3.3	2	11-12
3.5	5	13-17
3.7	2	18-19
3.8	1	20
3.9	1	21
4.0	1	22
4.1	2	23-24
4.2	10	25-34
4.3	1	35
4.5	8	36-43
4.8	2	44-45
4.9	1	46
5.0	1	47
5.1	1	48
5.2	7	49-55
5.4	1	56
5.5	3	56-59
5.6	1	60
5.7	1	61
5.8	3	62-64
5.9	1	65
6.0	1	66
6.1	1	67
6.2	1	68
6.5	1	69
7.1	4	70-73
7.2	6	74-79
7.5	1	80
7.8	2	81-82
7.9	1	83
8.0	1	84
8.1	1	85
8.2	6	86-91
8.5	9	92-100
8.8	1	101
8.9	1	102
9.2	1	103
9.3	1	104
9.5	4	105-108
9.9	2	109-110
10.0	3	111-113
10.1	2	114-115
10.2	1	116
10.5	1	117
11.0*	1	118
11.1	1	119
11.2	1	120

Table-2: Non-parametric determination of biotinidase reference interval.

Calculation of Rank Numbers of Percentiles		
Lower	0.025(120+1) = 3.01	Rank No 3
Upper	0.975(120+1) = 117.97	Rank no 118
Original Values Corresponding to these Rank Numbers		
Lower Limit (nmol/ml/min)	2.5 percentile	3.0
Upper Limit (nmol/ml/min)	97.5 percentile	11.0
Rank Numbers and Values of the 0.90 Confidence Limits		
Lower Reference Limits	Rank No 1and 7	Values 2.9 and 3.1
Upper Reference Limits	Rank no 114 and 120	Values 10.1 and 11.2
Summary		
Biotinidase Lower Reference Limit (nmol/ml/min)		3.0 (2.9 to 3.1)
Biiotinidase Upper Reference Limit (nmol/ml/min)		11.0 (10.1 to 11.2)

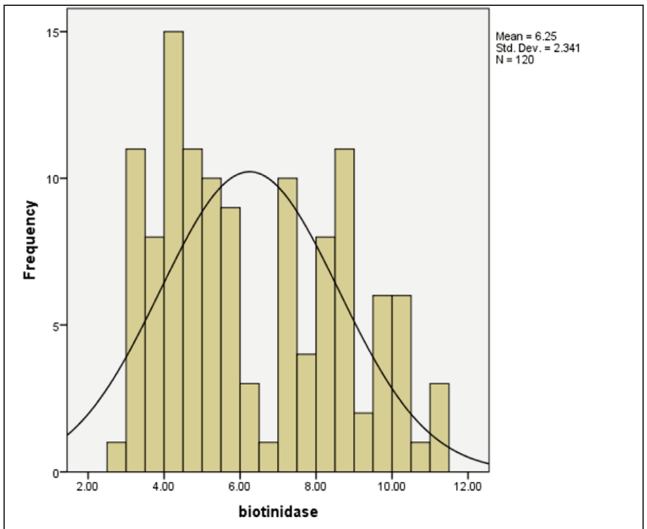

Figure: Histogram of biotinidase activity levels.

where necessary. P<0.05 was considered significant. The 2.5th and 97.5th percentiles were computed using the formula 0.025(n+1) and 0.975(n+1), which corresponded to rank number 3 and 118 respectively with 90% confidence interval (CI).

Results

Of the 120 DBS specimens, 81(67.5%) were from male babies and 39(32.5%) from female babies. Histogram showed non-parametric distribution (p<0.05) (Figure).

Data was arranged in ascending order and rank number was allotted (Table 1). Reference interval of biotinidase, established on the basis of 2.5th and 97.5th percentiles, was from 3.0 to 11.0nmol/ml/min (Table 2).

Discussion

Health-associated reference values for every laboratory analyte are universally required for the interpretation of medical laboratory results, as a decision-making process. The current study determined reference values for biotinidase activity (from 3.0 to 11.0 nmol/ml/min) in Pakistani neonates so that newborn screening for BD can be started in Pakistan.

Table-3: Comparison of reference interval of different populations.

Study	Turkey (2002)[18]	Belgium (2002)[18]	Iran (2016)[4]	India (2017)[20]	Pakistan (2020)
Sample Size (n)	328	260	30	35	120
Reference Interval (nmol/ml/min)	4.16-5.86	4.85-5.63	3.81-8.25	5.52-9.14	3.0-11.0

A mass screening study was conducted in Belgium and Turkish populations in 2002.[18] Belgian population's biotinidase reference interval was significantly higher than the Turkish population. Reference interval for biotinidase was found to be from 4.85 to 5.63nmol/ml/min for Belgian population and from 4.16 to 5.86 for Turkish population.[18]

A study in Iran reported biotinidase activity values from 3.81 to 8.35nmol/ml/min, identifying 8 patients with BD out of 47 children with neurological signs. Two mutations were detected, c.98-104del7ins3 and p. Arg79Cys, in 5 patients with profound BD, and one p. Asp444His mutation in 3 patients with partial BD.[4]

A study in Brazil reported biotinidase reference interval from 5.2 to 9.5nmol/ml/min.[19] A study from India reported biotinidase activity levels from 4.52 to 9.14nmol/ml/min.[20]

Biotinidase activity levels show slight variation among different populations. This variability depends on ethnicity, dietary patterns, genetic makeup and traditions of interfamily marriages. The reference interval in the current study showed more resemblance with Iran, India and Turkey[4,18,20] (Table 3). This is because of the of similarities in ethnicity, dietary habits and same traditions of consanguineous marriages in these communities.

Newborn screening should be established as an important tool to screen for inborn errors of metabolism at the time of birth. However, the current state of newborn screening is not very good in Pakistan. The main challenges in establishing this programme have been the lack of funds, less awareness in public, inadequate manpower and support services. Early diagnosis may save a number of children from getting disabled. In fact, pre-symptomatic evaluation of these conditions through newborn screening will definitely minimise the irreversible, life-threatening side effects of the disease. The present study put forward reference interval of biotinidase in Pakistani neonates, but many such studies are required to be done in this regard so as to establish the actual burden of BD in the country, its frequency and strategies to cope with this situation.

Appropriate measures are being taken by other neighbouring countries, like Iran and India[4,20] for progress

in this regard. Diagnostic facilities are being established and continuous improvements are being done to screen neonates at the appropriate time and to initiate treatment, if required, to stop the development of irreversible neurological damage.

Conclusion

The reference interval of biotinidase for disease-free newborns was found to range from 3.0 to 11.0nmol/ml/min. In Pakistan, the BD prevalence is not known, but is expected to be high due to high rate of consanguineous marriages.

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Conflict of Interest: One of the co-authors heads the institutional ethics review board.

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References

1. Wolf B. Biotinidase deficiency: "If you have to have an inherited metabolic disease, this is the one to have". Genet Med 2012;14:565-75.
2. Alfadhel M, Al Othaim A, Al Saif S, Al Mutairi F, Alsayed M, Rahbeeni Z, et al. Expanded newborn screening program in Saudi Arabia: incidence of screened disorders. J Paediatr Child health. 2017;53:585-91.
3. Canda E, Kalkan Uçar S, Çoker M. Biotinidase Deficiency: Prevalence, Impact And Management Strategies. Pediatric Health Med Ther. 2020;11:127-33.
4. Asgari A, Dehnabeh SR, Zargari M, Khani S, Mozafari H, Varasteh A, et al. Clinical, biochemical and genetic analysis of biotinidase deficiency in Iranian population. Arch Iranian Med. 2016; 19:774-8.
5. Hsu RH, Chien YH, Hwu WL, Chang IF, Ho HC, Chou SP, et al. Genotypic and phenotypic correlations of biotinidase deficiency in the Chinese population. Orphanet J Rare Dis. 2019;14:1-6.
6. Afroze B, Wasay M. Diagnosis, treatment and follow-up in four children with biotinidase deficiency from Pakistan. J Coll Physicians Surg Pak. 2013;23:823.
7. Fatima S, Aamir M, Bibi A. Multiple Carboxylase Deficiency Organic Acidemia as a Cause of Infantile Seizures. J Coll Physicians Surg Pak. 2021;31:95-7.
8. Shoaib M, Faraz A, Ahmed SA, Jamil M, Aijaz Z. Diagnostic Dilemma Of Biotinidase Deficiency: Case Of A Child From Pakistan. J Ayub Med Coll Abbottabad. 2016;28:821-3.
9. Mc Sweeney N, Grunewald S, Bhate S, Ganesan V, Chong WK, Hemingway C. Two unusual clinical and radiological presentations of biotinidase deficiency. Eur J Paediatr Neurol 2010; 14: 535-8.
10. Ahmed S, Ni M, DeBerardinis RJ, Habib A, Akbar F, Afroze B. Clinico-Pathological and Molecular Spectrum of Biotinidase Deficiency-Experience from a Lower Middle-Income Country. Clin. Lab. 2021:1.
11. Strovel ET, Cowan TM, Scott AI, Wolf B. Laboratory diagnosis of biotinidase deficiency, 2017 update: a technical standard and guideline of the American College of Medical Genetics and Genomics. Genet Med. 2017;19:1079.
12. Asgari A, Dehnabeh SR, Zargari M, Khani S, Mozafari H, Varasteh A, et al. Clinical, biochemical and genetic analysis of biotinidase deficiency in Iranian population. Arch Iranian Med. 2016;19(11-):0-. SAME AS REF 4
13. Sthanadar AA, Bittles AH, Zahid M. Increasing prevalence of consanguineous marriage confirmed in Khyber Pakhtunkhwa Province, Pakistan. J Biosoc Sci. 2015;48:418-20.
14. Afzal RM, Lund AM, Skovby F. The impact of consanguinity on the frequency of inborn errors of metabolism. Mol Genet Metabol Reports. 2018;15:6-10.
15. Cheema HA, Malik HS, Parkash A, Fayyaz Z. Spectrum of inherited metabolic disorders in Pakistani children presenting at a tertiary care centre. J Coll Physicians Surg Pak. 2016;26:498-502.
16. Patel KK, Rai R, Rai AK. Determinants of infant mortality in Pakistan: evidence from Pakistan demographic and health survey 2017–18. J Public Health. 2021;29:1-9.
17. Clinical and Laboratory Standards Institute. How to define and determine reference intervals in the clinical laboratory: approved guideline – second edition. CLSI document C28-A2. Wayne, PA, USA: CLSI; 2000.
18. Tanyalçın T, Eyskens F, Philips E, Lefevere M, Büyükgebiz B. A marked difference between two populations under mass screening of neonatal TSH and biotinidase activity. Accred Qual Assur 2002;7:498–506.
19. Carvalho ND, Januário JN, Felix GL, Nolasco DM, Ladeira RV, Del Castillo DM, et al. Frequency of biotinidase gene variants and incidence of biotinidase deficiency in the newborn screening program in Minas Gerais, Brazil. J Med Screening. 2020;27:115-20.
20. Mohan IK, Baba KS, Prasad C, Hussain T, Alrokayan SA, Naushad SM, et al. A Micro-Method for Biotinidase Estimation Using Dried Blood Spots and Comparison with Plasma Biotinidase. IOSR J Dental Med Sci. 2017;16:23-6.