



Short Communication

Diagnosis and management of symptomatic profound biotinidase deficiency in a tertiary care center in Lebanon

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ABSTRACT

Neonatal screening for biotinidase deficiency is still lacking in several countries worldwide, although this neurocutaneous disorder is treatable and preventable. Therefore, unscreened patients are diagnosed when symptomatic; treatment with Biotin is known to reverse cutaneous symptoms and improve neurological outcome. We describe a series of five symptomatic patients diagnosed with profound biotinidase deficiency and followed at a tertiary care center in Lebanon, for a variable period from 16 months to 11 years. Adjustment of Biotin therapy is correlated to clinical response and biochemical profile including 3-hydroxyisovalerylcarnitine on dried blood spots and urine organic acids. A previously unreported mutation is also reported in a patient who displayed an unusual outcome with reversible hearing loss on Biotin therapy.

Clinical responsiveness to Biotin may be related to the underlying genetic mutation, although no clear genotype-phenotype correlation in biotinidase deficiency is proven. Furthermore, in the absence of systematic newborn screening for this disorder in several countries, identification of a reliable blood biomarker of Biotin responsiveness is warranted for better management of late diagnosed symptomatic patients.

1. Introduction

Biotinidase deficiency (BTD), OMIM #253260, is an autosomal recessive metabolic disorder with a worldwide prevalence is 1:40,000 to 1:60,000, or even higher in countries with elevated consanguinity rates [1]. Untreated BTD patients present with neurological and/or cutaneous symptoms including developmental delay, seizures, hypotonia in addition to alopecia, and skin rash, neurosensory hearing loss (NSHL) and optic atrophy may also be observed [2]. Metabolic decompensation with ketoacidosis, hyperammonemia and lactic acidosis can also occur. In asymptomatic patients, BTD diagnosis is suggested by the detection of elevated hydroxyisovalerylcarnitine (C5OH) level on dried blood spots, by tandem mass spectrometry (TMS) while urine organic acid chromatography (OAC) may show an accumulation of lactate, 3-methylcrotonylglycine, 3-hydroxypropionate, 3-hydroxyisovalerate and methyl citrate [3].

Confirmatory diagnosis is based on direct enzymatic assay in serum or plasma, through quantitative colorimetric method utilizing N-biotinyl-p-aminobenzoate as a substrate [4] or recently, by fluorometry [5].

Profound BTD is defined by an activity of less than 10% of normal range while partial BTD is considered for an enzymatic activity between 10 and 30% of normal range. Further confirmatory molecular testing of BTD may be performed [3].

Biotin supplementation is known for its remarkable efficiency in preventing clinical symptoms when BTD is detected in the neonatal period. However, if started late in symptomatic patients its effect on developmental delay, hearing and vision remains controversial with irreversible damage in most cases [1,6].

Newborn screening (NBS) for BTD, first started in the United States in 1984 [3], is currently adopted in several countries worldwide such as Canada [7], Australia, and some European countries; however, many others still do not screen for this treatable disorder [8], mainly developing countries [9].

In Lebanon, since 2008, NBS is offered in few private hospitals; therefore, diagnosis of patients presenting with symptomatic profound BTD and delayed management is still challenging.

In this retrospective study, we report the diagnosis and management of a series of patients with profound BTD, diagnosed and followed at a

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Table 1

Profile and outcome of patients with profound biotinidase deficiency. C5OH: 3-hydroxyisovalerylcarnitine, NSHL: neurosensory hearing loss, bid: twice daily, ND: not done.

Patient		1	2	3	4	5
<i>Family</i>	Origin	Iraqi	Syrian	Syrian	Lebanese	Lebanese
	Consanguinity	yes	Yes	Yes	Yes	Yes
	History	Cousin: delay, seizures	Cousin: delay	Negative	Sibling BTD	Sibling BTD
<i>Clinical presentation</i>	Age at first symptoms	2 m	6 m	3 m	15 m	13 m
	Age at diagnosis	20 m	16 m	3 m	36 m	13 m
	Seizures	+	+	+	+	+
	Hypotonia	+	+	+	+	+
	Coma	–	+	–	–	–
	Speech delay	+	–	–	+	+
	Skin rash	–	+	+	+	+
	Alopecia	–	+	+	+	+
	Hearing	Bilateral NSHL 90 dB	Normal	Normal	Bilateral NSHL 85 dB	Bilateral NSHL 60 dB
	Vision	Optic atrophy	Optic atrophy	Optic atrophy	Normal	Normal
<i>Diagnosis</i>	C5OH (N:0.08–0.47 mol/L)	1.14	1.28	7.38	3.20	2.68
	Organic aciduria at presentation	+	+	+	+	+
	Biotinidase activity (N: 3.3–100 IU)	Null	0.5	0.3	Null	Null
	Gene sequencing	Homozygous c.1420G > T in exon 4	ND	ND	c.203_206dupTCCT in exon 2	c.203_206dupTCCT in exon 2
<i>Outcome</i>	Age at last visit	36 m	34 m	34 m	14 y	10 y
	Number of C5OH tests	4	4	7	24	16
	Mean C5OH(N:0.08–0.47 mol/L)*	1.3	1	6.7	2.8	1.2
	Biotin dosage/compliance	10 mg bid/yes	10 mg bid/yes	10 mg bid/no	10 mg bid/yes	10 mg bid/yes
	Neurological	Improved	Improved	No improvement	Mild cognitive delay	Normal
	Rash/alopecia	Normal	Resolved	Resolved	Resolved	Resolved
	Hearing	NSHL stable 90 dB	normal	Stable at 60 dB	Stable at 85 dB	60 dB, reversed to 30 dB
	Vision	Optic atrophy	Optic atrophy	Optic atrophy	Normal	Normal

major tertiary care center in Lebanon.

2. Methods

A retrospective chart review of patients diagnosed with symptomatic profound BTB between January 2008 and January 2019 at the Inherited Metabolic Diseases Program -American University of Beirut Medical Center (AUBMC) was performed. This study was approved and conducted in accordance with the Institutional Review Board requirements at the American University of Beirut, Lebanon. Collected data included: age at presentation and at diagnosis, clinical manifestations, family history, biochemical and genetic testing. Initial and subsequent blood levels of 3-hydroxyisovalerylcarnitine (C5OH), checked every 4 to 6 months on dried blood spots were recorded. During the follow-up period, number of C5OH testing varied between 4 and 24 times for each patient. Since 2008, acylcarnitine profile along with expanded newborn screening including biotinidase activity were referred to Hamburg University Medical Center, Germany then to Archimed Life Science GmbH Laboratories, Austria, starting 2017.

Acylcarnitine profile was tested by liquid chromatography–tandem mass spectrometry (LC-MS/MS) in the reference laboratories following effective extraction of the analytes from blood spots with subsequent derivatization by butylation to minimize interferences. Biotinidase activity was measured using fluorometric method (Perkin Elmer kit, Perkin Elmer Life and Analytical Sciences, Wallac Oy, Turku, Finland) in dried blood spots.

In addition, urine organic acid chromatography (OAC) was performed using Gas Chromatography/Mass Spectrometry (GC/MS) either in-house at AUBMC or in other established laboratories. At AUBMC, qualitative and quantitative analysis of trimethylsilyl ester/ether derivatives with an internal standard for quantitation [10] and Agilent

5975C VL MSD Triple Axis Detector with 6890 GC instruments (Agilent, US) were used. The within-run imprecision was less than 2% and between-run precision was less than 17% for various organic acids. Checked urinary metabolites for each patient included: lactate, 3-methylcrotonylglycine, 3-hydroxypropionate, 3-hydroxyisovalerate, methylmalonate and methyl citrate.

The BTB gene was analyzed by PCR and sequencing of both DNA strands of the entire coding region and the highly conserved exon–intron splice junctions was performed by Centogene, Germany. Furthermore, treatment and outcome of each patient were also recorded.

3. Results

Between January 2008 and January 2019, 3 cases of partial BTB out of 15,000 newborns delivered at AUBMC were detected and treated. During the same period, five patients with symptomatic profound BTB were diagnosed at the Inherited Metabolic Diseases Program- AUBMC (Table 1).

Patient 1, a 20-month-old Iraqi male presented for developmental delay and intractable seizures despite treatment with several anti-epileptics, since 2 months of age. He had optic atrophy and severe bilateral neurosensory hearing loss (NSHL) but no cutaneous manifestations. Metabolic work-up revealed an elevated blood C5OH and elevated levels of lactate and OH isovalerylcarnitine by qualitative urine OAC. Serum biotinidase activity was null. Gene sequencing detected a previously unreported homozygous mutation c.1420G > T, likely disease-causing variant in exon 4. Biotin therapy was started at 5 mg twice daily with normalization of urine OAC, however mean C5OH levels remained elevated. Biotin dosage was increased to 10 mg twice daily to achieve seizure control and better muscle tone. At 36 months, seizures recurred with poor compliance to Biotin therapy; nevertheless,

Table 2

Profound biotinidase deficiency patients with reversible hearing loss. NSHL: neurosensory hearing loss.

Study	Origin	Age at diagnosis	Clinical presentation				Daily Biotin dosage	Age at last follow-up	Outcome	BTD activity nmol/min/mL	Genetic study
			Neurological	Cutaneous	Hearing	Vision					
Nothjunge 1989	Not available	13 m	Hypotonia	Rash Alopecia	NSHL	Normal	10 mg	19 m	Hearing: significant improvement	0.05	Not done
Dabbagh 1994	Saudi	28 m	Coma Seizures	Alopecia	NSHL	Optic atrophy	10 mg	3.5y	Hearing and vision: significant improvement	null	Not done
Dabbagh 1994	Saudi	4y	Spastic quadriparesis Dysarthria	None	NSHL	Optic atrophy	10 mg	4y7m	Hearing and vision: significant improvement	null	Not done
Straussberg 2000	Palestinian	3 m	Seizures	Rash	Profound NSHL	Normal	20 mg	1y	Hearing: marked improvement.	null	Not done
Tsao 2002	Not available	17 m	Ataxia	None	Moderate NSHL	Normal	5 mg	10y	Hearing back to normal	null	Not done
Current	Lebanese	13 m	Delay Seizures	Rash Alopecia	Moderate NSHL	Normal	20 mg	10y	Hearing back to normal	null	Homozygous c.203_206dupTCCT in exon 2

no skin rash nor alopecia were noted.

Patient 2, a 16-month-old Syrian male patient, presented for global delay and sudden hair loss with positive family history of BTD in a cousin. He was treated since 6 months of age with Biotin 5 mg once daily without improvement. Two months later, he went into coma for 3 days during an intercurrent respiratory infection. Biotin was stopped during his hospitalization, causing sudden hair loss along with a diffuse rash. He presented to AUBMC at 16 months of age, hearing screen was normal but he had partial optic atrophy. Metabolic work-up showed an elevated C5OH with a transient elevation of methylmalonic acid at 386 mmol/mol creatinine (Normal: less than 5) on urine OAC. Biotinidase activity was almost null. Biotin dosage was increased to 20 mg daily which corrected the cutaneous manifestations and normalized urine OAC. On follow-up at 34 months, vision and hearing were stable with improved neurodevelopment.

Patient 3, a three-month-old Syrian male presented for seizures and hypotonia along with diffuse rash and alopecia. He had normal hearing and vision. He had elevated blood level of C5OH. Several urine organic acids were elevated as well, including 3-Hydroxyisovaleric acid = 380 mmol/mol creatinine (Normal: less than 5) and 3-Methylcrotonyl glycine = 28 mmol/mol creatinine (Normal: less than 2). Biotinidase activity was almost null at 0.3 IU (normal range: 3.3–100). Biotin therapy was started at a dose of 10 mg/day. One month later, he was able to hold his head and skin manifestations resolved. Due to poor compliance to therapy, he developed global delay, seizures and bilateral NSHL which stabilized after resuming Biotin.

Patients 4 and 5 were male siblings from a Lebanese family. Patient 4 presented to AUBMC at 36 months. He was noted to have speech delay since 15 months of age, diagnosed with bilateral NSHL with normal vision. He developed seizures at 2 years of age, with alopecia. Biotinidase activity was almost null. He was treated with Biotin which controlled seizures and anti-epileptics were stopped. Patient was lost to follow-up for 2 years. Afterwards, his brother, patient 5- presented at 13 months of age for global delay and hair loss. He had bilateral NSHL at 60 dB and normal vision. Biotinidase activity was not checked at birth despite medical advice. He had high C5OH and suggestive qualitative urine OAC with elevated 3-hydroxyisovalerate. Biotinidase activity was null. Genetic testing revealed a previously unreported homozygous c.203_206dupTCCT mutation in exon 2 of biotinidase gene. Biotin therapy was started at 5 mg once daily, normalizing urine OAC. then increased to 10 mg daily to correct alopecia. At 3 years of age, he had normal speech and development. Repeated auditory brain stem response test was normal with hearing at 30 dB in both ears. Biotin was increased

at 4 years of age to 10 mg twice daily for recurrent rash with good compliance to therapy. At last follow-up with the family, patient 4 had mild cognitive delay and understandable speech at 14 years of age, however, NSHL remained stable at 85 dB while patient 5, had normal development and hearing at 10 years of age.

4. Discussion

BTD is a treatable and preventable neurocutaneous disorder for which NBS was implemented almost two decades ago, in several countries. In the absence of newborn screening, prompt diagnosis and early treatment of BTD can be challenging, as clinical presentation may include a variable constellation of neurological symptoms with or without suggestive skin manifestations. This is the first study of profound BTD patients from Lebanon, Syria and Iraq. In our series, patients were diagnosed between 3 and 36 months of age. Reversible skin manifestations, improvement of psychomotor development and seizures control were observed in treated compliant patients. Biotin therapy dosage was adjusted based on clinical response and normalization of urine organic acids. The abnormal accumulation of urine metabolites in BTD usually reflects the impairment of pyruvate carboxylase, propionyl-CoA carboxylase and 3-methylcrotonyl-CoA carboxylase activities, resulting in increased lactate, propionate, 3-OH propionate, methyl citrate as well as 3-methylcrotonylglycine and 3-hydroxyisovalerate.

In our series, initial UOA showed an elevation of some but not all of these compounds: mainly 3-hydroxyisovalerate (patient 1, 4 and 5), transient elevation of methylmalonic acid (patient 2), 3-hydroxyisovalerate and 3-Methylcrotonyl glycine (patient 3). Normalization of initially elevated urinary levels of these metabolites was observed with Biotin initiation and maintained throughout follow-up duration.

While propionylcarnitine (C3) levels by acylcarnitine profile were normal at diagnosis and during follow-up period, mean blood levels of C5OH were elevated (Table 1) and did not seem to correlate with Biotin dosage, nor with clinical response. In our series, Biotin dosage was solely based on clinical deterioration since urine OAC remained normal throughout the study duration. To date, adjustment of Biotin dosage is still challenging since no biomarkers are constantly correlated with responsiveness; some centers rely on normalization of metabolites in urine OAC, however, excretion of these metabolites is variable among patients and in about 20% of cases urine OAC was found to be normal, even in symptomatic patients [3].

Treatment with Biotin stabilized hearing in 3 patients while, interestingly, moderate hearing loss was reversed back to 30 dB and

remained normal in patient 5. To the best of our knowledge, only one other child reported by Tsao et al. [11] had a complete reversal of her hearing impairment. Significant hearing improvement was also reported in four other patients [12–14] but unfortunately, no genetic studies were performed to any of these children (Table 2). Molecular studies of the Iraqi patient and the Lebanese family revealed previously unreported mutations. The Iraqi patient carried a homozygous c.1420G > T variant in exon 4 (p.Gly474*), causing a premature stop codon associated to a nonsense mutation of the BTM gene. The Lebanese family had a duplication of four nucleotides c.203_206dupTCCT (p.Ser70Profs*23) creating a frame shift starting at codon Ser70 that ends in a STOP codon 22 positions downstream. Although patients 4 and 5 are siblings, they exhibited different hearing response to Biotin therapy. This could be partially explained by the difference in age at Biotin initiation, but mostly by the inconsistency of genotype-phenotype correlation [15]. It is noteworthy that among the six reported profound BTM patients with reversible hearing loss, four were of Arab origin (Table 2). This might indicate a genetic predisposition for Biotin responsiveness of NSHL in late-diagnosed patients. Further delineation of the genotypic-phenotypic variability of BTM among different populations while controlling for other confounders such as delay in diagnosis and Biotin responsiveness is needed. In the absence of worldwide NBS for BTM, identifying a reliable biomarker of Biotin responsiveness may help in better therapeutic management of symptomatic patients affected by this devastating disorder.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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