BIOTINIDASE DEFICIENCY – DIAGNOSIS BY ENZYME ASSAY AND A FOLLOW-UP STUDY

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ABSTRACT

A 3 month old male child was brought to the hospital with complaints of skin rashes, developmental delay, seizures, seborrheic dermatitis, alopecia and mild acidosis. The child was subjected to a simple metabolic screening protocol. The result of the screening and the clinical symptoms provided an index pointing towards biotinidase deficiency, a rare autosomal recessive, inherited metabolic disorder. The enzyme was then assayed by using n-biotinyl *p*-aminobenzoate as substrate and the diagnosis confirmed. A follow-up of the case indicated the efficacy of biotin supplementation in biotinidase deficiency.

KEY WORDS

Biotin, biotinidase, n-biotinyl para aminobenzoate, alopecia.

INTRODUCTION

Biotinidase (E.C. 3.5.1.12) catalyses the cleavage of the biotinyl moiety from the ϵ -amino group of lysine, which is the terminal step in the degradation of carboxylases. The result of the catalysis is the release of free biotin for utility in the cellular needs. The deficiency of the enzyme biotinidase is a rare, autosomal recessive, inherited metabolic disorder, whose incidence is estimated at 14 per million live births, as assessed by a Neonatal Screening Program conducted worldwide (1). However, reports regarding its frequency of occurrence in developing countries are not available.

Individuals with biotinidase deficiency cannot recycle endogenous biotin and cannot release dietary protein bound biotin. As the brain begins to depend upon the biotin shuttled across the blood-brain barrier, a decreased activity of pyruvate carboxylase ensues, resulting in lactic acidosis and consequent neurologic manifestations (2-4).

Biotinidase deficiency can be diagnosed by demonstrating the decreased activity of the enzyme in serum of suspected individuals (5). The condition has been incorporated as a part of the Neonatal Screening Program along with other conditions such as phenylketonuria (6).

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The features of biotinidase deficiency include seizures, hypotonia, ataxia, breathing problems, hearing loss, skin rashes, alopecia and developmental delay (7). The mean age of onset has been considered as 3 – 5 ½ months. Most children affected show organic aciduria with elevated levels of α hydroxy isovaleric acid, lactic acid, α hydroxypropionate and methyl citrate.

Many assay methods have been developed to measure the activity of biotinidase (8,9). The colorimetric method of estimation is based on measurement of the color developed upon the release of para aminobenzoate from the artificial substrate N-biotinyl para aminobenzoate by the biotinidase present in the serum of the suspected subject (5).

CASE : A 3 month old male child, delivered at term was brought to the hospital with complaints of skin rashes, developmental delay, seizures, seborrheic dermatitis, alopecia and mild acidosis. History revealed that the pregnancy was uneventful and the marriage non-consanguineous. Birth weight was recorded as 2.6 kg, he was discharged on the fifth postnatal day after all basal investigations, and examinations were normal.

It was decided to perform a screening for metabolic disorders among other investigations since the symptoms and presentation provided an index of suspicion.

MATERIALS AND METHODS

The artificial substrate n-biotinyl para aminobenzoate

was procured from SIGMA Inc., USA. Chemicals used for the other analyses were of Analytical Grade purchased from Qualigens, India.

Blood was drawn from the suspected child following aseptic conditions. Plasma and serum were separated. 20 ml each of three early morning and mid-day void urine samples were collected. Basal biochemical investigations were performed using autoanalyser. The results are shown in Table 1.

The urine samples were subjected to a simple Metabolic Screening for detection of aminoacidurias, glycosuria, organic aciduria and ketonuria. Quantitative thin layer chromatography of plasma and urine were not indicative of any pathological aminoaciduria. Urinary organic aciduria was noted prominently. Subsequently lactic acid was identified and quantified at 28 mmol/day by partition chromatography. Serum biotinidase activity was estimated colorimetrically using n-biotinyl para aminobenzoate as substrate (5). The results are as shown in Table 2. The control chosen for the study was age and sex matched child in the same hospital approaching for treatment of a respiratory tract infection.

TREATMENT AND FOLLOWUP

The child was treated with 10 mg biotin per day (7). The cutaneous manifestations resolved quickly

followed by a regression of seizures. Repeat estimations at discharge, of basal parameters as shown in Table 1 were all normal. Follow up for 6 months indicated that developmental delay and achievement of milestones returned to normal. No cutaneous manifestations were noted again in the period of follow-up.

DISCUSSION

The mammalian enzyme biotinidase, has been demonstrated in most tissues, the highest activity being noted in the liver, kidney and adrenals (10). The human enzyme is a monomeric glycoprotein with a molecular weight of about 67 kDa (11). The Km value for the artificial substrate ranges from 5 μ M to 10 μ M and is active over the pH range 5.0–7.0 (12). Its activity in brain and CSF are very low. Non-specific clinical symptoms of biotinidase deficiency include vomiting, hypotonia and seizures. Characteristic symptoms such as alopecia, skin rashes could also manifest in zinc deficiency. Biotin deficiency can usually be excluded unless a known history of dietary indiscretion exists. After differential diagnosis of the above conditions and when associated with hyperammonemia and ketoacidosis, the presence of an inborn error of metabolism must be considered (7). Prompt diagnosis and appropriate treatment may be life saving.

Table 1

Results of basic biochemical investigations on admission

PARAMETERS IN SERUM	RESULTS
Urea	2.6 m mol/L
Uric acid	0.36 m mol/L
Lactate	1.9 m mol/L
Blood Glucose (Random)	3.1 m mol/L
Ammonia	70 μ mol/L
Aspartate aminotransferase	53 U/L
Alanine aminotransferase	11 U/L
Anion Gap	22 m mol/L

Table 2

Activity of biotinidase in nmol/min/ml serum

ASSAY	PATIENT	CONTROL
Biotinidase activity*	1.3	7.6

* The value provided is the mean of three trials.

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