NEWBORN SCREENING OF BIOTINIDASE DEFICIENCY – RESULTS OF A PRIVATE PROJECT IN BRAZIL

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Introduction

Biotinidase (EC 3.5.1.12) is the enzyme responsible for the recycling of endogenous biotin, from biocytin or small biotinylated peptides obtained from partial or total proteolytic degradation of holocarboxylases. It also releases biotin bound to proteins of the diet, bringing it into the biologically active free form [1].

Biotin is a water-soluble vitamin required as a cofactor for the proper functioning of acetyl CoA carboxylase (EC 6.4.1.2), pyruvate carboxylase (EC 6.4.1.1), propionyl CoA carboxylase (EC 6.4.1.3), and methylcrotonoyl-CoA carboxylase (EC 6.4.1.4) [2].

Biotinidase deficiency is an autosomal recessive metabolic disorder caused by the deficient activity of biotinidase. In this condition, endogenous biotin cannot be recycled, nor biotin bound to dietary proteins can be released [3].

The clinical manifestations of the disease usually appear from the second to the fifth months of life, although subtle neurological signs may occur in the neonatal period. Some patients develop symptoms only after several years. The clinical symptoms of the disease are highly variable. When left untreated, patients develop neurological disorders, which may include seizures, hypotonia, ataxia, developmental delay, vision problems and hearing loss, and skin manifestations - alopecia, dermatitis and susceptibility to fungal infections [3].

Biotinidase deficiency meets the criteria for inclusion in newborn screening programs because: 1. the disease is not clinically recognizable at birth; 2. children with biotinidase deficiency who are not diagnosed early can have irreversible neurological damage; 3. clinical signs can be prevented by continuous oral replacement of biotin; 4. the treatment is simple, cheap and highly effective [4]. Therefore, it was included in the Brazilian National Newborn Screening Program (*Programa Nacional de Triagem Neonatal* – PNTN) in 2012 [5].

Objectives

Around 50 million Brazilians have some sort of private health insurance coverage [6]. Private laboratories offer newborn screening tests for this population. This study seeks to evaluate the rate of positive screening initial tests - recall rate, the positive predictive value and the incidence of (partial or profound) biotinidase deficiency in neonates whose samples were analyzed by a private laboratory.

Methodology

A retrospective study was conducted on newborn screening dried blood spot - DBS specimens received from January 2014 to December 2016. DBS specimens were collected from neonates by heel-prick after 24 hours of life. Biotinidase activity was determined using the PerkinElmer Neonatal Biotinidase Kit (Wallac Oy, Finland). It is a semi-quantitative fluorometric assay whose principle is the cleavage by the sample biotinidase of the substrate biotinyl-6-aminoquinoline, generating a fluorescent 6-aminoquinoline product. The conversion is measured by a fluorometer with excitation central wavelength of 355 nm and emission central wavelength of 460 nm. The measurement is expressed as enzyme units (U), where 1 U = 1 nmol of the end-product that is formed during one minute and in one deciliter of blood (1 nmol/min/dL). Newborns with biotinidase activity < 70 U were recalled for testing in a second DBS sample. The proposed cut-off was not designed to differentiate between partial and profound biotinidase deficiency.

Results

Samples from 172,520 neonates from several Brazilian states were screened between 2014 and 2016. A total of 204 (0.12%) newborns were recalled for confirmatory samples as their first DBS specimen exhibited a biotinidase activity <70 U. It was possible to obtain a second sample from 152 neonates, corresponding to a 74.5% positive response to recall. Eleven children (1 in 15,684) presented low biotinidase activity in the recall sample. No attempt was made to further characterize these children as having partial or profound biotinidase deficiency. The positive predictive value was 5.4%, considering that all neonates that had persistently low biotinidase activity had some degree of biotinidase deficiency. Moreover, assuming that all patients from whom a second sample could not be obtained as false positives, the false positive rate was 0.11%. Figure 1 summarizes these results.

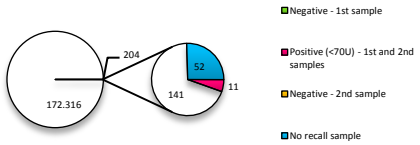

Figure 1. Biotinidase screening results of 172,520 neonates from Brazil. A total of 204 neonates exhibited suspicious results of biotinidase deficiency - <70 U in their first sample. A second DBS sample was obtained from 152 of these neonates. Eleven neonates had persistently low biotinidase activity results, while the second sample from 141 neonates revealed normal activity.

Conclusions

Our recall rate (0.12%) was comparable to that reported by other Brazilian authors - Pinto *et al.* [7] - 0.17%, in Paraná, and Neto *et al*. [8] - 0.12%, in Rio Grande do Sul, both conducted in Southern states, but higher than that calculated from the data informed by Lara *et al*. [9] - 0.07%, in Minas Gerais, Southeast Brazil. All these authors employed a colorimetric method for the initial screening of biotinidase deficiency. Furthermore, the false positive rates calculated from the data reported by Pinto *et al*. [7] - 0.12%, and Neto *et al.* [8] - 0.11% were almost identical to ours - 0.11%, but higher than that calculated for Lara *et al.* [9] - 0.07%. The prevalence rates reported by these three authors are divergent. Pinto *et al*. [7] showed a combined prevalence of profound and partial biotinidase deficiency at birth of 1:62,500; Lara *et al*. [9] reported an intermediate prevalence of 1:22,861, and Neto *et al*. [8] the highest prevalence - 1:9,000. As neither a serum quantitative confirmatory test, nor a molecular analysis were done for the 11 patients repeatedly showing low biotinidase activity, our figure of 1 in 15,684 cannot be interpreted as a true prevalence rate. Nevertheless, our results clearly support the adequacy of the inclusion of biotinidase deficiency screening in the Brazilian National Newborn Screening Program.

References

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