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Newborn screening for congenital adrenal hyperplasia in Cuba: Six years of experience

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ABSTRACT

Background: Since 2005, a newborn screening program for congenital adrenal hyperplasia (CAH) by measuring 17-alpha-hydroxyprogesterone (17OHP) in dried blood spots was introduced in Cuba.
Methods: The hormone was measured by the 17OHP Neonatal UMELISA method, in samples collected on the 5th day as average. Confirmatory test was performed to those neonates with 17OHP values above 55 nmol/l. Some perinatal factors that can influence on 17OHP levels were studied.
Results: From January 2005 to December 2010, 621,303 newborns were screened and 39 CAH cases were detected. Coverage of the program reached 98%. The incidence of CAH in Cuba was 1:15,931, similar to that reported by other programs. A recall for suspected CAH was performed in 10,799 cases (1.74%). Therapy in classical CAH patients was started at the mean age of 22 days. 17OHP levels were significantly higher in newborns with lower birth-weight (BW) and/or gestational age (GA). In addition, 17OHP values were affected by the gender, twin status or mode of delivery.
Conclusions: In Cuba, the nationwide newborn screening program has allowed the early detection of CAH. The use of an optimized cut-off level for BW or GA could lead to a reduction in the percentage of recalled babies.

1. Introduction

Congenital adrenal hyperplasia (CAH) represents a family of autosomal recessive endocrine disorders caused by different enzyme deficiencies affecting the adrenal steroid biosynthesis of cortisol and aldosterone, and increasing secretion of 17-hydroxyprogesterone (17OHP) and androgens. The most common form of CAH, which accounts for >90% of classical forms, is the 21-hydroxylase deficiency [1]. The estimated worldwide incidence of classic CAH is around 1:15,000 [2].

Over the past 30 years, newborn screening programs for CAH have been established in many countries [3–12]. The benefits of newborn screening programs for CAH include reductions in adrenal crises, life-threatening complications, and incorrect sex assignments.

In the 1980s, a technology that allowed the study of a great number of samples with the lowest possible cost was developed in Cuba. Starting from these initial efforts, the Ultra Micro Analytic System (SUMA) and the ultramicro-ELISA (UMELISA) techniques were developed. UMELISAs combine the high sensitivity of the current microELISA tests with the use of ultramicro-volumes of samples and reagents [13]. Since January 1st, 2005, a Cuban national screening program for CAH using the 17OHP Neonatal UMELISA was initiated [14]. This work shows the major results of the first 6 years of Cuban newborn CAH screening program.

2. Materials and methods

2.1. Screening strategy

Newborn screening program for CAH is managed by the Ministry of Public Health of Cuba through its Program for Comprehensive Care for Women and Children (PAMI, Spanish acronyms) and the National Institute of Endocrinology; which coordinate the actions of the health institutions in charged for the diagnosis, confirmation, control of the cases, treatment, follow-up of patients and attention to families.

Newborn screening tests are conducted by a national laboratory network made initially up by 32 laboratories. In the year 2010, the newborn screening program was decentralized, increasing up to 200 the number of laboratories. The algorithm for laboratory procedure in the Cuban newborn screening for CAH is shown in Fig. 1.

Dried blood on filter paper (DBS) samples are collected in the municipal collection centers preferably between the 5th and 7th days of life and sent to the laboratories in <72 h. Specimens are analyzed quickly after arrival in order to have the results ready in <24 h. 17OHP is

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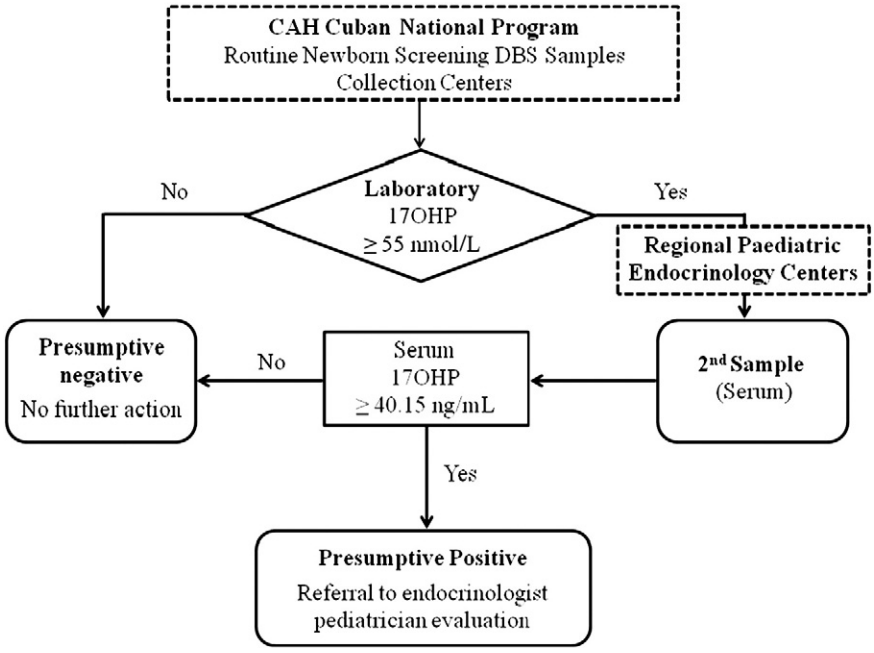

Fig. 1. CAH Cuban National Newborn Screening Program algorithm.

measured by the 17OHP Neonatal UMELISA method as previously described [14]. Three millimeter DBS disks of calibrators and samples are placed into wells of the elution microplates, followed by the addition of 40 μl of the diluted 17OHP–alkaline phosphatase conjugate in phosphate-buffered saline solution with danazol to displace 17OHP from steroid-binding proteins. After the elution for 30 min at room temperature, 10 μl of eluate are transferred into the wells of the reaction opaque polystyrene ultramicroplates (Tecnosuma International S.A., Havana, Cuba) coated with the specific polyclonal rabbit anti-17OHP antibodies. The competitive reaction occurred for 2 h at room temperature in a humid chamber and then, the plates are washed with Tris solution. The fluorogenic reaction is performed by adding 10 μl of the substrate solution (4-methylumbelliferil phosphate, pH 9.6). After 30 min at room temperature, the fluorescence is measured automatically in a fluorimeter–photometer reader (excitation/emission: 355/450 nm) from SUMA technology (Immunoassay Center, Havana, Cuba). Automatic validation and interpretation of the results is done using a specific-assay software.

The intra and inter-assay imprecision, established at 3 different concentrations (21, 44 and 87 nmol/l blood), is inferior to 10%. Analytical recovery using blood spot controls prepared by adding known amounts of commercial 17OHP, or CDC quality controls, is inferior to 20%. Detection and quantification limits for the determination of 17OHP are 2.2 and 4.6 nmol/l, respectively [14].

A unique cut off value (COV) of 55 nmol/l (98th percentile) is currently used in the Cuban program. All newborns with a positive screening test are referred immediately for biochemical and clinical confirmation and follow up at the Regional Paediatric Endocrinology Centers. Infants with serum 17OHP levels of ≤40 ng/ml are considered as simple false positives and no other further investigations are made. Neonates with serum 17OHP levels of ≥40 ng/ml without clinical signs are considered as hyper-17-hydroxyprogesteronemia infants and are submitted to clinical and biochemical follow-up monitoring until the normalization of hormone values. Treatment with hydrocortisone is begun in all newborns with 17OHP levels of ≥40 ng/ml and clinical or biochemical signs of CAH. Patients with suggestive salt wasting crises are given fludrocortisone doses.

Every result in a screening procedure that is above the applicable cut-off value is considered a recall. Therefore, in this study the recall rate (RR) represents the percentage of newborns in which serum samples were required.

2.2. DBS sample population

17OHP levels in 12,615 DBS samples from the Maternity Hospital "Ramón González Coro", Havana, over the period January 2009–September 2010, were retrospectively studied. None of the newborns was confirmed with CAH. Infant demographic and clinical data were obtained from the DBS collection card which included gestational age (GA), birthweight (BW), time of sampling, mode of delivery, gender and single or multiple gestation status. The protocol was approved by the Hospital Ethical Committee and prior to participating in the study; infant identifications were removed.

2.3. Statistical analysis

Statistical analysis was performed by using the Statistica program, ver. 6.0 (StatSoft Inc.). Data were presented as the mean ± SD or median plus interquartile range (IQR) as appropriate. 17OHP values did not show a normal distribution and nonparametric tests were used. Comparison of 17OHP levels among groups was performed using the Mann–Whitney test. Linear regression analysis was performed to evaluate the relationship between the levels of 17OHP-BW and 17OHP-GA. Percentiles were calculated using the Microsoft Excel 2007 software. A p-value of <0.05 was taken as statistically significant and all tests were 2-sided.

3. Results

3.1. Screening program results

From January 2005 to December 2010 a total of 621,303 newborn samples were studied for CAH with a screening coverage of >98% of total births and continued increasing progressively. Average age of sampling was 6 days with results available at 11 days of life. Thirty-nine patients with CAH were detected within the first 2 weeks of life and were classified by type and sex. Thirty-one neonates classical CAH forms (79%) were detected, 15 patients were diagnosed as simple virilizing