ORIGINAL ARTICLE

Neonatal screening for congenital adrenal hyperplasia

Cristina Botelho Barra[1], Ivani Novato Silva[2], Isabela Leite Pezzuti[1], José Nélio Januário[3]

[1] Pediatric Endocrinologists; Students, Postgraduate Course, Faculdade de Medicina, Universidade Federal do Minas Gerais (FM-UFMG), Belo Horizonte, MG, Brazil
[2] PhD; Associate Professor, Department of Pediatrics, FM-UFMG; Coordinator of the Pediatric Endocrinology Division, Hospital das Clínicas da Universidade Federal de Minas Gerais (UFMG), Belo Horizonte, MG, Brazil
[3] PhD Student, FM-UFMG; Assistant Professor, Internal Medicine Departament, FM-UFMG, Belo Horizonte, MG; Coordinator of Diagnostic Support Action and Research Center (NUPAD), FM-UFMG, Belo Horizonte, MG, Brazil

Correspondence to:
Ivani Novato Silva
Divisão de Endocrinologia Infantil e do Adolescente
Departamento de Pediatria
Faculdade de Medicina
Hospital das Clínicas
Universidade Federal de Minas Gerais
Av. Alfredo Balena, 190 - s/267
Santa Efigênia
Belo Horizonte – MG, Brazil
CEP: 30130-100
Phone: +55 (31) 3409-9773
Fax: +55 (31) 3409-9770
ivanins@medicina.ufmg.br

Conflict of interest: None.

SUMMARY

Objective: The effectiveness of neonatal screening for reducing morbimortality in children with congenital adrenal hyperplasia (CAH) is the main justification for its implementation. One of the challenges for its implementation is to determine the cutoff value for laboratory measurement of 17-hydroxyprogesterone (17OHP) with appropriate cost-effectiveness. This study identified factors affecting the results of the pilot project of newborn screening for CAH, performed in the state of Minas Gerais, Brazil. **Methods:** Neonatal screening performed between September, 2007 and May, 2008, with 17OHP measurements performed in blood samples taken from the heel (filter paper), on the 5th day of life, processed by the UMELISA 17-OH Progesterona NEONATAL® method. The cutoff value was 80 and 160 nmol/L for healthy children or not, respectively. **Results:** The incidence of CAH was 1:19,939 in 159,415 children screened. The 99th percentile (p99) of 17OHP in the first sample was 108 nmol/L. In 13,298 newborns whose weight had been reported, the p99 of 17OHP were, respectively: 344 nmol/L for weight < 1,500 g; 260 nmol/L for weight between 1,500 and 1,999 g; 221 nmol/L for weight between 2,000 and 2,499 g; 109 nmol/L for weight ≥ 2,500g. The rate of recall for medical consultation was 0.31%. The test sensitivity was 100%, specificity was 99.6%, and the positive predictive value was 2.2%. By adjusting the cutoff values of 17OHP to 110 nmol/L and 220 nmol/L, a 76% decrease in consultation referrals was projected. **Conclusion:** The use of 17OHP cutoff values, considering birth weight, was a cost-effective measure to reduce false positives. The results of this pilot study suggest that screening for CAH might benefit the pediatric population.

Keywords: Congenital adrenal hyperplasia; neonatal screening; early diagnosis.

INTRODUCTION

Neonatal screening tests have changed the course of several diseases, by allowing children to be treated even before the onset of clinical manifestations, thus providing significant improvement in prognosis. For the inclusion of a disease in a neonatal screening program, certain requirements must be met: the disease in question must be relatively frequent in the screened population, have significant morbidity and mortality if not treated early, and show a beneficial response to treatment[1-3].

Congenital adrenal hyperplasia (CAH) due to 21-hydroxylase (21-OH) enzyme deficiency is related to alterations in cortisol biosynthesis, and has a worldwide incidence of 1:15,000 live births[4]. Approximately 65% to 75% of these children have the classic salt-wasting form, which is characterized by higher morbidity and mortality if not treated early; newborn females have ambiguous genitalia at birth, which may lead to incorrect social gender assignment, depending on the severity of the condition. In males, however, the first manifestation observed is the salt-loss crisis, with risk of death within the first weeks of life. An early diagnosis is, therefore, essential to reduce these occurrences.

The screening programs for CAH aim primarily at the early diagnosis of the salt-wasting form, which is more severe and harmful. The accumulation of the 17-hydroxyprogesterone (17OHP) metabolite, which occurs due to a deficiency of 21-OH, can be identified by its measurement on filter paper blood spots. What is observed, however, is that increases in this metabolite may occur in newborns without CAH under stress situations due to maternal or neonatal factors. Premature infants without CAH also may have elevated levels of 17OHP at the screening, due to higher concentrations of conjugated steroids, and relative renal system immaturity with insufficient excretory function[5,6].

Thus, the main challenge to screening for CAH is to determine a cutoff value for 17OHP that will result in adequate cost-benefit. The use of different reference values according to gestational age or birth weight and age at collection are strategies often adopted to make the screening process feasible, mainly regarding the reduction of false-positive results[7-10].

Aiming to contribute to the advancement of CAH screening programs, data from a pilot CAH project, conducted in the state of Minas Gerais (MG), were analyzed. The results can contribute to the discussion on the feasibility of establishing new neonatal screening programs to be implemented as routine.

METHODS

Screening for CAH was included in the newborn screening program of the state of Minas Gerais (Programa de Triagem Neonatal – PETN-MG) as a pilot project, taking advantage of the already consolidated structure for neonatal screening. The Action and Research Center for Diagnostic Support (Núcleo de Ações e Pesquisa em Apoio Diagnóstico - NUPAD), a complementary organ of the School of Medicine of Universidade Federal de Minas Gerais (UFMG) was accredited in 2001 by the Ministry of Health as a reference service for neonatal screening in Minas Gerais. Since 2003, the PETN-MG has been responsible for the screening of the four selected diseases - phenylketonuria, congenital hypothyroidism, cystic fibrosis, and hemoglobinopathies, for 853 municipalities[11].

The study was approved by the Ethics in Research Committees of the UFMG and of the Health Secretariat of Belo Horizonte City Council. The families gave their informed consent for participation in the project.

The collection of blood samples was performed on the heel of newborns, in the basic units of the public health network of municipalities as part of the Guthrie or "heel prick" test, preferably on the 5th day of life.

A total of 159,415 children were screened between September, 2007 and May, 2008, corresponding to nearly all newborns in the period, as the program covers nearly 100% of the municipalities of the state of Minas Gerais.

The measurements of 17OHP on filter paper samples, sent to NUPAD by the municipalities, were performed using the UMELISA 17OH Progesterone NEONATAL® (Immunoassay Center, Havana, Cuba). The cutoff value reported by the kit manufacturer (55 nmol/L) was modified after analysis of preliminary results to assess the adequacy of this value in the studied population. The 99th percentile (p99) of 17OHP values was established as the reference for this population of 15,000 initial samples. The value of 80 nmol/L was adopted as the normal range based on these results (p99 = 82.21 nmol/L). Children with basal 17OHP levels > 160 nmol/L were immediately called for medical consultation. Children with levels between 80 and 160 nmol/L were retested, also on filter paper. The finding of a second sample > 80 nmol/L automatically generated a request for medical consultation (Figure 1).

Based on the assessment of the results obtained until November 29, 2007 with a large number of positive tests in children maintained in maternity wards, a specific cutoff of 160 nmol/L was adopted for non-healthy newborns classified as low birth weight (weighing less than 2,500 grams) or preterm (gestational age less than 37 weeks). Information on birth weight was provided at the time of collection. In the absence of the reported weight, newborns were evaluated using the cutoff of 80 nmol/L.

All children with a positive screening were referred for follow-up at the pediatric outpatient endocrinology clinic at the Hospital das Clínicas of the UFMG in Belo Horizonte (HC-UFMG).
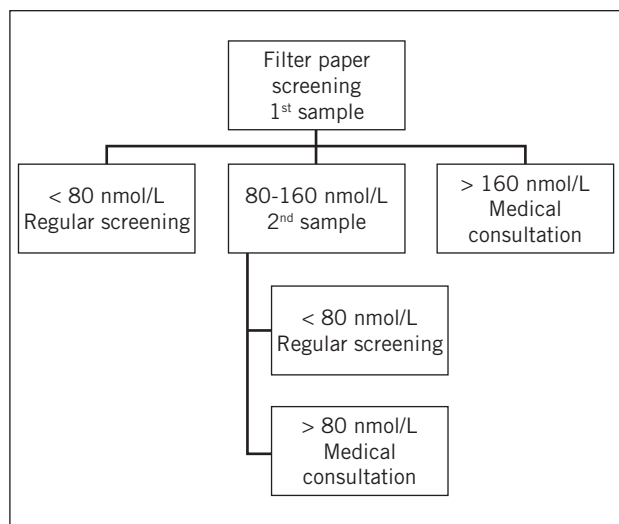
Figure 1 – Flow chart of the screening pilot project for congenital adrenal hyperplasia in Minas Gerais with cutoff values for 17OHP (17-hydroxyprogesterone) measurements on filter paper.

The confirmation of 17OHP level alteration in a peripheral blood sample was obtained by measurement in a reference laboratory, by radioimmunoassay (reference value for the first month: 17-204 ng/dL for females and 53-186 ng/dL for males). Children with elevated serum 17OHP, suggestive clinical features, and alterations in other complementary tests (ionogram and androgens: androstenedione and testosterone) had the diagnosis of 21-OH deficiency confirmed. These and the indeterminate cases were followed at the abovementioned outpatient pediatric endocrinology clinic. Children with positive screening who did not have CAH were referred to pediatric follow-up in their own municipalities after normalization of hormone levels.

The information on weight and gestational age at birth of patients undergoing follow-up were obtained through medical records.

Throughout the project, information was provided to the primary care teams, and parents and families were instructed about the inclusion of CAH screening in the routine Guthrie test as a pilot study by several means of communication, including video conferences. NUPAD employees were responsible for reporting positive test results, requesting additional blood samples, setting up medical appointments, arranging transportation for timely consultations, and forwarding samples and supplying medications, when necessary, in addition to welcoming and housing children and families at a support center.

STATISTICAL ANALYSIS

For data analysis Microsoft SQL Management Studio Express from Microsoft SQL Server 2000 software was used. The disease incidence was estimated by the number of children with confirmed disease divided by the total number of neonates screened in the period. The values of 17OHP did not show a normal distribution, and were presented as medians and analyzed by nonparametric tests: Mann-Whitney and Kruskal-Wallis test were used to compare two and four independent groups in relation to the median, respectively. For multiple comparisons, the correction of significance level was performed. The significance level was set at 5% ($p < 0.05$). Percentiles of 17OHP values were calculated from the first sample on filter paper of all children, and separately according to the birth weight ($<1,500$ g, 1,500 to 1,999 g, 2,000 to 2,499 g, and $\geq 2,500$ g) using Microsoft Excel 2007 software. The positive predictive value (PPV) of the evaluation, the recall rate (RR), and the sensitivity and specificity of the test were calculated.

RESULTS

A total of 159,415 newborns were screened for CAH between September 2007 and May 2008 by the PETN-MG. The age at screening was 8 ± 14 days, with a median of six days. Sixteen children started treatment for CAH, ten of whom were males. During clinical follow-up, eight children had no confirmation of the diagnosis of CAH and therapy was discontinued, which resulted in an incidence of disease for this population of 1:19,939. Of the eight patients, five were females and three were males, and 75% had the salt-wasting form. The mean age of the affected children at the screening was 7 ± 2 days. The median age at start of treatment was 39 days (range 13-581).

Between September 1, 2007 and February 26, 2008 106,476 newborns were screened and seven children were considered to be affected at the follow-up (incidence of 1:15,211). According to the established criteria, 103,269 newborns were evaluated by the cutoff value of 80 nmol/L and 1% of them (n = 1,036) had the first sample above the reference value. During the same period, the birth weight of 12.5% of all newborns was registered. Of 13,298 records, 23% were reported as low birth weight. Of the 3,207 newborns with low weight, assessed by the cutoff value of 160 nmol/L, 11% (n = 354) had the first sample above the reference value.

A total of 380 children were recalled (RR = 0.36%) for medical consultation. Of these, 315 children were followed, of which 63% were premature and/or had low birth weight, according to the study of the medical files.

The test sensitivity, considering the absence of false-negative records, was 100%, with a specificity of 99.6%. The positive predictive value of the assessment was 2.2%.

The 99th percentile (p99) of 17OHP of all newborns screened in the first sample on filter paper was 108 nmol/L.

The 17OHP distribution of the first sample on filter paper, in percentiles, was calculated for each weight group at birth: < 1500 g (n = 748), 1,500 to 1,999 g (n = 971),

2,000 to 2,499 g (n = 1,357) and ≥ 2,500 g (n = 10,222), and this distribution is shown in Figure 2. The percentiles p50 and p99, were respectively 96 and 344 nmol/L, 58 and 260 nmol/L, 47 and 221 nmol/L, 35 and 109 nmol/L for each weight group. There was a statistically significant difference of the medians (p50) of 17OHP between the four weight groups (p < 0.001) and also when the groups were compared two by two (p < 0.001).

By adjusting the cutoff value for 17OHP, in accordance with the p99 for newborns with appropriate weight, to 110 nmol/L and that of newborns with low birth weight (< 2500 g) to 220 nmol/L, it was calculated that there would be a 76% decrease in referrals for medical consultation after the first sample collection.
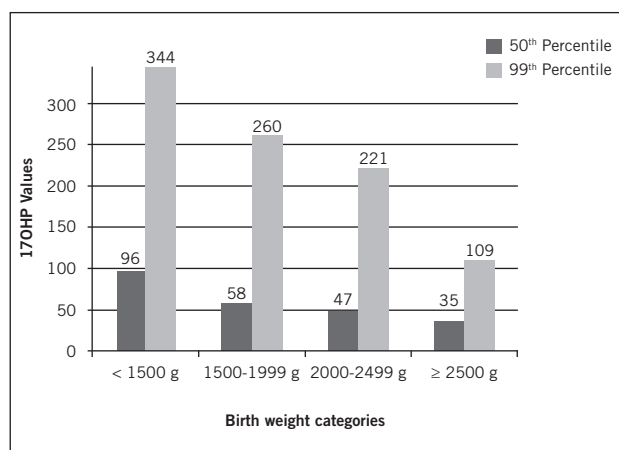
Figure 2 – 50th and 99th percentiles of 17OHP on filter paper (17-hydroxyprogesterone – nmol/L) for groups of children distributed according to birth weight.

DISCUSSION

Neonatal screening for CAH is not yet universally accepted, and is included in the group that generates controversy regarding its inclusion in screening programs. The diseases screened in each country, or even in different regions of a country, depend on health policy decisions based on epidemiological, ethnic, social, economic, and ethical aspects[5].

Although the cost-effectiveness of screening for CAH is still debated, the benefits must be considered, especially in developing countries with incompletely consolidated health systems that do not favor the performance of accurate and fast diagnoses, as required by this disease.

It has been reported that screening for CAH is effective to promote a 74% to 86% decrease in mortality in patients with the disease[12]. Moreover, the families of sick children describe a more stressful experience when the diagnosis is made based on the clinical manifestations and not by neonatal screening. Parental discomfort with screening occurs when the goal of early detection of potentially affected children is not understood[13].

Screening for CAH is part of screening programs in France, Sweden, Switzerland, New Zealand, Israel, Iceland, Austria, Germany and in several U.S. states, including Alaska, a pioneer in this screening[4] and, more recently, in Argentina[14].

In Brazil, routine screening for CAH is performed only in Goiás and Santa Catarina. The incidence of CAH found in Goiás was 1:10,325, lower than that previously found in southern Brazil, of 1:7,533[15].

The accomplishment of the pilot program for CAH in Minas Gerais was possible due to the already established PETN structure implemented in 1993 and coordinated by the NUPAD[16].

The PETN-MG is linked to the National Neonatal Screening Program (PNTN) of the Brazilian Ministry of Health[17], implemented in 2001, aiming at 100% coverage of live births for phenylketonuria, congenital hypothyroidism, sickle-cell anemia, and cystic fibrosis.

The PNTN defines the process of newborn screening in five steps, as recommended by the American College of Medical Genetics[18], i.e., laboratory testing, investigation of suspected cases, diagnostic confirmation, treatment, and follow-up by a multidisciplinary team. The implementation is performed in three phases, according to the level of organization and coverage of each state. In phase I, the diseases screened for are phenylketonuria and congenital hypothyroidism. In phase II, sickle cell anemia is included, and in phase III, cystic fibrosis is included[19]. Currently, only four Brazilian states are in phase III (Minas Gerais, Santa Catarina, Paraná, and Espírito Santo). According to 2007 data, the population coverage of neonatal screening in Brazil is 78.9% and some states report coverage > 95% for the four diseases. PNTN still has room for improvement and the possibility of expanding the range of diseases to be screened is considered, if there is a proven benefit for the population[20].

The PETN-MG has a population coverage of 94.6% (2007 data), and it has been in phase III since 2003, showing the feasibility of implementing new screening programs, as other Brazilian states.

The available tests for CAH neonatal screening include radioimmunoassay (RIA), initially described by Pang et al.[21], fluorimetry, and tandem mass spectrometry (TMS). RIA is currently underutilized due to large interference of other metabolites, especially 17-hydroxypregnenolone[22]. On the other hand, TMS is not widely used, despite its higher specificity, due to the need for large capital investment. Fluorimetry is the most often used technique, including in the Brazilian programs.

In the pilot study conducted in MG, measurements were performed using the UMELISA 17-OH Progesterona NEONATAL® assay, resulting from the combination of the high sensitivity of the microElisa tests (ELISA

ultramicro-Umelisa) and the use of ultra-microvolumes for samples and reagents (Micro Ultra Analytic System, SUMA). Studies on filter paper showed that the technique provides superior results to RIA, with less interference, good precision, and accuracy, allowing the study of a large number of samples at the lowest possible cost[23,24]. These characteristics, combined with cost-effectiveness for large-scale screening and the use of the technique with good results for tests, e.g., the congenital hypothyroidism program in MG, were responsible for the choice of this method for the pilot study. The use of an immunoenzymatic assay in pilot programs of screening for CAH has been previously described in China[25].

The great challenge related to neonatal screening for CAH is the definition of an appropriate cutoff for 17OHP in the population. Interference caused by other steroids, especially sulfates[26] and transient elevations in 17OHP levels in preterm newborns or those with low birth weight may generate large numbers of false positive results, causing increased costs and parental stress, affecting the real perception of parents regarding the child's health[27].

During the pilot project in MG, it was observed that the cutoff value initially used resulted in a great number of false positive results.

The categorization of 17OHP reference values according to birth weight and gestational age is a strategy used to reduce the false-positive rate. In the state of Wisconsin, USA (1997), division into four weight categories increased the positive predictive value for screening by ten times, without impairing test sensitivity, with a decrease of 50% in the recall rate[8].

Olgemöller, in the German region of Bavaria, also proposed a screening categorization, based on five ranges of birth weight and according to age adjusted at the collection for weight > 2,500g. Thus, the specificity was 99.3%, the positive predictive value increased from 0.84% to 1.29% (1.5 times), and the false-positive rate decreased from 1.12% to 0.73%[7].

In Brazil, rates of prematurity and low birth weight are highly variable, depending on the region evaluated. According to the Interagency Network for Health and Health Information (Rede Interagencial de Saúde e Informação para a Saúde – RIPSA/DATASUS-2005), the Brazilian total rate of prematurity was 6.6% and of these, 37.4% also had low birth weight. The worldwide trend is of stabilization of the percentage of children with low birth weight and increased prematurity due to the improvement of perinatal and neonatal care. In 2007, an overall rate of 9.1% was recorded for prematurity in the country (Instituto Nacional de Estatística)[28], showing the importance of adjusting the cutoff values of 17OHP for this population.

The reference adjustment for sick children and information obtained regarding birth weight were necessary for the discussion of a cutoff value suitable for each group. Although the screening program in Minas Gerais is well established, information on birth weight was not compulsory until the pilot project for CAH was started. During the study period, information on weight was obtained for 12.5% of children, of whom 23% had low birth weight and only 0.5% were premature, which possibly represents an inadequate quality of information.

Nevertheless, it was observed that while only 1% of healthy children with normal weight at birth had a positive first sample, 11% of premature newborns and or those with low birth weight showed this result, suggesting inadequacy of the reference value for 17OHP and the need to change the cutoff value for the project's feasibility.

The principles postulated by Wilson-Jungner were observed during the pilot project, which suggest the appropriate cost-effectiveness of the program[2,29,30]: high incidence of disease (< 1:20,000), test with good sensitivity and specificity (approximately 100%), recall rate that is feasible for the program organization, and prospect of significantly reducing mortality of the salt-wasting form[31].

These findings help to reinforce the possibility of including congenital adrenal hyperplasia in the PNTN announced at a hearing held on June 6, 2011 by the Ministry of Health[32].

The results justify the performance of newborn screening for CAH and suggest the possibility of extending the screening to other Brazilian states. The use of the cutoff values suggested for 17OHP, according to birth weight, showed to be a cost-effective measure for program implementation.

References

1. Leão LL, Aguiar MJ. Newborn screening: what pediatricians should know. J Pediatr (Rio J.). 2008;84(Suppl 4):S80-90.
2. Wilson JMG, Jungner G. Principles and practice of screening for disease. Geneva: WHO; 1968. Public Health Papers, 34. Available from: http://www.who.int/bulletin/volumes/86/4/07-050112BP.pdf.
3. White PC. Neonatal screening for congenital adrenal hyperplasia. Nat Rev Endocrinol. 2009;5:490-8.
4. Honour JW, Torresani, T. Evaluation of Neonatal Screening for Congenital Adrenal Hyperplasia. Horm Res. 2001; 55:206-11.
5. van der Kamp HJ, Wit JM. Neonatal screening for congenital adrenal hyperplasia. Eur J Endocrinol. 2004;151(Suppl):U71-5.
6. Ballerini MG, Chiesa A, Scaglia P, Gruñeiro-Papendieck L, Heinrich JJ, Ropelato MG. 17alpha-hydroxyprogesterone and cortisol serum in neonates and young children: influence of age, gender and methodological procedures. J Pediatr Endocrinol Metab. 2010;23:121-32.
7. Olgemöller B, Roscher AA, Liebl B, Fingerhut R. Screening for congenital adrenal hyperplasia: adjustment of 17-hydroxyprogesterone cut-off values to both age and birth weight markedly improves the predictive value. J Clin Endocrinol Metab. 2003;88:5790-4.

8. Allen DB, Hoffman GL, Fitzpatrick P, Laessig R, Maby S, Slyper A. Improved precision of newborn screening for congenital adrenal hyperplasia using weight-adjusted criteria for 17-hydroxyprogesterone levels. J Pediatr. 1997;130:128-33.

9. National Newborn Screening Information System 2009. Disorder Report for Congenital Adrenal Hyperplasia (CAH). Available from:http ://www2.uthscsa.edu/nnsis/.

10. Papendieck LG, Prieto L, Chiesa A, Bengolea S, Bergadá C. Congenital hyperplasia and early newborn screening: 17 alpha-hydroxyprogesterone (17 alpha-OHP) during the first days of life. J Med Screen. 1998;5:24-6.

11. Triagem neonatal. Available from: http://www.nupad.medicina.ufmg.br.

12. Yoo, BK, Grosse SD. The cost effectiveness of screening newborns for congenital adrenal hyperplasia. Public Health Genomics. 2009;12: 67-72.

13. Waisbren SE, Albers S, Amato S, Ampola M, Brewster TG, Demmer L, et al. Effect of expanded newborn screening for biochemical genetic disorders on child outcomes and parental stress. JAMA. 2003;290:2564-72.

14. Gruñieiro-Papendieck L, Chiesa A, Mendez V, Prieto L. Neonatal screening for congenital adrenal hyperplasia: experience and results in Argentina. J Pediatr Endocrinol Metab. 2008;21:73-8.

15. Silveira EL, Santos EP, Bachega TA, van der Linden Nader I, Gross JL, Elnecave RH. The actual incidence of congenital adrenal hyperplasia in Brazil may not be as high as inferred - an estimate based on a public neonatal screening program in the state of Goiás. J Pediatr Endocrinol Metab. 2008;21:455-60.

16. Souza CF, Schwartz IV, Giugliani R. Triagem neonatal de distúrbios metabólicos. Ciênc Saúde Coletiva. 2002;7:129-37.

17. Brasil. Ministério da Saúde. Secretaria de Atenção à Saúde. Departamento de Atenção Especializada. Manual de normas técnicas e rotinas operacionais do programa nacional de triagem neonatal/ Brasil. 2a ed. Brasília (DF): Ministério da Saúde; 2005.

18. American College of Medical Genetics. Maternal and Child Health Bureau. Health Resources and Services Administration. US Department of Health and Human Services. Newborn screening: toward a uniform screening panel and system. 2005. Available from: http://www.acmg.net//AM/Template.cfm?Section=Home3.

19. Carvalho TM, Santos HP, Santos IC, Vargas PR, Pedrosa J. Newborn screening: a national public health programme in Brazil. J Inherit Metab Dis. 2007;30:615.

20. Programa Nacional de Triagem Neonatal. Available from: http://portal.saude.gov.br/portal/saude/area.cfm?id_area=1061.

21. Pang S, Hotchkiss J, Drash AL, Levine LS, New MI. Microfilter paper method for 17 alpha-hydroxyprogesterone radioimmunoassay: its application for rapid screening for congenital adrenal hyperplasia. J Clin Endocrinol Metab. 1977;45:1003-8.

22. Caulfield MP, Lynn T, Gottschalk ME, Jones KL, Taylor NF, Malunowicz EM, et al. The diagnosis of congenital adrenal hyperplasia in the newborn by gas chromatography/mass spectrometry analysis of random urine speciemens. J Clin Endocrinol Metab. 2002;87:3682-90.

23. Maeda M, Arakawa H, Tsuji A, Yamagami Y, Isozaki A, Takahashi T, et al. Enzyme-linked immunosorbent assay for 17-hydroxyprogesterone in dried blood spotted on filter paper. Clin Chem. 1987;33:761-4.

24. González EC, Marrero N, Pérez PL, Frómeta A, Zulueta O, Herrera D, et al. An enzyme immunoassay for determining 17 alpha-hydroxyprogesterone in dried blood spots on filter paper using an ultramicroanalytical system. Clin Chim Acta. 2008;394:63-6.

25. Chu SY, Tsai WY, Chen LH, Wei ML, Chien YH, Hwu WL. Neonatal screening for congenital adrenal hyperplasia in Taiwan: a pilot study. J Formos Med Assoc. 2002;101:691-4.

26. Fingerhut R. False positive rate in newborn screening for congenital adrenal hyperplasia (CAH)-ether extraction reveals two distinct reasons for elevated 17-alpha-hydroxyprogesterone (17-OHP) values. Steroids. 2009;74:662-5.

27. Gurian EA, Kinnamon DD, Henry JJ, Waisbren SE. Expanded newborn screening for biochemical disorders: the effect of a false-positive result. Pediatrics. 2006;117:1915-21.

28. Rugolo LM. Growth and developmental outcomes of the extremely preterm infant. J Pediatr (Rio J). 2005;81(Suppl 1):S101-10.

29. Pollit RJ. International perspectives on newborn screening. J Inherit Metab Dis. 2006;29:390-6.

30. Andermann A, Blancquaert I, Beauchamp S, Déry V. Revisiting Wilson and Jungner in the genomic age: a review of screening criteria over the past 40 years. Bull World Health Organ. 2008;86:317-9. Available from: http://www.who.int/bulletin/volumes/86/4/07-050112/en/index.html.

31. Gruñeiro-Papendieck L, Prieto L, Chiesa A, Bengolea S, Bossi G, Bergadá C. Neonatal screening program for congenital adrenal hyperplasia: adjustments to the recall protocol. Horm Res. 2001;55:271-7.

32. Brasil. Ministério da Saúde. Audiência pública para discutir os avanços e possibilidades do Teste do Pezinho. Available from: http://www.senado.gov.br/noticias/tv/programaListaPadrao.asp?COD_VIDEO=86302.