# Ten-year evaluation of a Neonatal Screening Program for Congenital Adrenal Hyperplasia

*Avaliação de dez anos de um Programa de Triagem Neonatal para Hiperplasia Adrenal Congênita*

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## ABSTRACT

**Objective:** Evaluate the Neonatal Screening Program (NSP) for congenital adrenal hyperplasia (CAH) of the Department of Health of the State of Santa Catarina (*Secretaria de Estado da Saúde de Santa Catarina*, SES/SC), and provide information to improve the program. **Subjects and methods:** Descriptive, retrospective study of 748,395 children screened between January 2001 and December 2010. We analyzed the coverage of the NSP-SES/SC prevalence of CAH, child's age when the first sample for 17-hydroxyprogesterone (17OHP) measurement was collected, levels of 17OHP, mean age at treatment onset and main clinical manifestations. **Results:** The NSP-SES/SC covered 89% of the live newborns in the State. It diagnosed 50 cases of CAH, yielding an incidence of 1:14,967. Mean age at collection of the first sample was 7.3 days and mean level of 17OHP was 152.9 ng/mL. The most frequent manifestations were virilized genitalia with nonpalpable gonads, clitoromegaly and genital hyperpigmentation. In three girls, the genre established at birth was incorrect. The salt-wasting form was present in 74% of the cases. There was no occurrence of shock or death. Mean age at treatment onset in the salt-wasting form was 17.4 days compared with 54.9 days in those without the salt-wasting form of the disease. All children were treated with hydrocortisone, and those with salt-wasting CAH were also treated with fludrocortisone. **Conclusions:** The incidence of CAH was 1 case to 14,967 live newborns. Collection of the first sample occurred outside the recommended time, resulting in delays in treatment onset. Arq Bras Endocrinol Metab. 2014;58(7):765-71

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## RESUMO

**Objetivo:** Avaliar o Programa de Triagem Neonatal da Secretaria de Estado da Saúde de Santa Catarina (PTN-SES/SC) em relação à hiperplasia adrenal congênita (HAC) e fornecer subsídios que possibilitem seu aperfeiçoamento. **Sujeitos e métodos:** Estudo descritivo e retrospectivo de 748.395 crianças triadas no período de janeiro de 2001 a dezembro de 2010, sendo analisados a cobertura do PTN-SES/SC, a prevalência da HAC, a idade na coleta da primeira amostra para 17-hidroxiprogesterona (17OHP), os níveis de 17OHP, a idade média de início de tratamento e as principais manifestações clínicas. **Resultados:** A cobertura do PTN-SES/SC foi de 89% dos recém-nascidos vivos no Estado. Foram diagnosticados 50 casos de HAC, com incidência de 1:14.967. A média de idade na coleta da primeira amostra foi de 7,3 dias e a de 17OHP, de 152,9 ng/mL. As manifestações mais frequentes foram genitália virilizada sem gônadas palpáveis, clitoromegalia e hiperpigmentação genital. Em três meninas ocorreu erro no estabelecimento de gênero ao nascimento. A forma perdedora de sal foi encontrada em 74% dos casos. Nenhum caso de choque ou óbito foi verificado. A média de idade no início do tratamento nos perdedores de sal foi de 17,4 dias e nos não perdedores, de 54,9 dias. Todas as crianças foram tratadas com hidrocortisona e, nos casos com a forma perdedora de sal, associou-se fludrocortisona. **Conclusões:** A incidência de HAC foi de 1 caso para 14.967 recém-nascidos vivos. A coleta da primeira amostra ainda ocorreu fora do tempo preconizado, acarretando atraso no início do tratamento. Arq Bras Endocrinol Metab. 2014;58(7):765-71

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## INTRODUCTION

Deficiency of the 21-hydroxylase (21OH) enzyme as a result of mutations in the *CYP21A2* gene (chromosome 6) is the most common form of congenital adrenal hyperplasia (CAH) (1). This deficiency hinders the production of cortisol in sufficient amounts to inhibit secretion of the adrenocorticotropic hormone (ACTH) by the pituitary, resulting in accumulation of the precursor 17-hydroxyprogesterone (17OHP) and increase in synthesis of adrenal androgens (2).

Overall, the disease affects 1:10.000-15.000 newborns worldwide. The degree with which each mutation compromises the enzymatic activity is strongly associated with the severity of the clinical manifestations of the disorder (3). In the 21OH deficiency, there is an spectrum of clinical manifestations that can be divided into three forms: classic form (salt-wasting, with compromised aldosterone synthesis), classic simple-virilizing (non-salt wasting) form, and nonclassic form (3,4). In the classic form, female newborns present several degrees of virilization of the external genitalia at birth. In males, the external genitalia undergoes normal differentiation, but enlarged phallus may be present. The salt-wasting form is present in 75% of the cases and manifests early, generally after the second week, affecting both sexes. When present, adrenal crisis is characterized by volume depletion, dehydration, hypotension, hyponatremia and hypokalemia which can result in death if not treated (5). The nonclassic form manifests in both genders in the postnatal period with premature pubarche, clitoromegaly or enlarged penis, increase in growth rate with advance in bone age, and eventually, decrease in the estimated final height (6,7).

Since CAH is an autosomal recessive disease, an even distribution between both genders is expected. However, when based on a clinical diagnosis, girls predominate over boys (4:1) since in them, the changes in the external genitalia make the diagnosis more evident. In contrast, boys with the salt-wasting form of the disease generally die without a diagnosis (8).

The need to prevent an adrenal crisis and its consequences and avoid psychological damage from an incorrect gender diagnosis in female newborns lead to a search for methods to screen this pathology in neonates (9).

The main aims of the screening are: to detect severe cases of the disease in order to prevent cerebral damage and death through presymptomatic treatment and prevent in virilized girls an incorrect gender identification on civil registration, or even, reduce the time that the register remains incorrect (10,11).

A reliable and valid screening test for CAH using a blotter impregnated with a blood sample obtained from the heel of the newborn became first available in 1977 and allowed establishment of an elevated blood level of 17OHP as an indication of CAH. This technique was initially developed in Alaska and used radioimmunoassay developed by Pang and cols. (12).

After that, the method to screen CAH at birth was systematically introduced in several regions of the world. The subsequent development of methods for direct determination of 17OHP rendered the test faster and simpler and promoted the beginning of new screening programs. Strategies to increase trust in the screening are still under development worldwide (5).

Since October 2000 in Santa Catarina, neonatal screening for CAH became part of a statewide program, rendering the State the first in Brazil to conduct this type of screening.

This study aims at evaluating the Neonatal Screening Program (NSP) for CAH of the Department of Health of the State of Santa Catarina (Secretaria de Estado da Saúde de Santa Catarina, SES/SC), between January 2001 and December 2010, providing information to allow improvement of the program. It also aimed at describing clinical and laboratorial features of children diagnosed with CAH, allowing a better knowledge of these features in this population.

## SUBJECTS AND METHODS

This study was conducted at the Department of Neonatal Analyses of the Central Laboratory of Public Health of Santa Catarina (*Laboratório Central de Saúde Pública de Santa Catarina*, LACEN/SES), a reference center in neonatal screening, located in the Endocrine Pediatric Department (*Serviço de Endocrinologia Pediátrica* – SEP) of Joana de Gusmão Infant Hospital (*Hospital Infantil Joana de Gusmão* – HIJG) in Florianópolis, Santa Catarina, Brazil. We included all newborns screened for CAH by the NSP-SES/SC program between January 2001 and December 2010.

Samples for screening were obtained by collection of capillary blood in a special filter paper (Fitec®, thickness 2.24 mm, grammage 105 – 110 g/m², linear, 100% cotton – S & S 903) by puncturing the heel of the newborns in hospitals and health centers in all municipalities in Santa Catarina. Samples were sent to LACEN/SES by mail, hand or mail pouch. Serum 17OHP levels were measured by time-resolved fluoroimmunoassay

using the kit AutoDELFIA® neonatal 17OHP (B015-112) until September 2009 and, after that, with the kit GSP® neonatal 17OHP.

The cutoff values for 17OHP were based on birth weight. The approach adopted by the program before and after the change in screening kits is shown in table 1.

**Table 1.** Cutoff values according to birth weight and approach adopted by the neonatal screening program for congenital adrenal hyperplasia (CAH) in Santa Catarina

| Weight range | Reference value to recall for a second sample (ng/mL) | Risk value – urgent medical evaluation (ng/mL) |
|---|---|---|
| From October 2000 to September 3, 2009 (Kit AutoDELFIA®) | | |
| ≥ 2,500 g | 9.9 to 30 | > 30 |
| < 2,500 g | 19.8 to 40 | > 40 |
| After September 4, 2009 (Kit GSP®) | | |
| ≥ 2,250 g | 14 to 21 | > 21 |
| 1,250 – 2,249 g | 33 to 41 | > 41 |
| < 1,250 g | 56 to 73 | > 73 |

When levels of 17OHP were above the upper cutoff value, the measurements were repeated twice on the first collected sample. If levels of 17OHP were confirmed to be abnormal, children were then referred for clinical and laboratory evaluation at SEP-HIJG. Laboratory evaluation included measurement of serum 17OHP (radioimmunoassay), androstenedione (radioimmunoassay), testosterone (chemiluminescence), sodium (spectophotometry), potassium (spectrophotometry) and karyotype analysis in children with ambiguous genitalia. The reference values of these tests are summarized in table 2.

We graded the external genitalia in female patients using the scale developed by Prader (13). When the diagnosis of CAH was suspected, treatment was started immediately on the first medical evaluation with hydrocortisone 10 to 25 mg/m²/day (14). After the re-

sults of the serum tests were obtained and 17OHP was confirmed to be elevated, treatment was maintained. We considered children with serum sodium below 132 mEq/l and/or serum potassium higher than 5.0 mEq/l as having the salt-wasting form of CAH (15). In this situation, we added to the treatment oral fludrocortisone 0.1 to 0.2 mg a day. We used an ion-selective electrode to measure electrolytes and repeated the measurement to confirm when the result was abnormal.

We retrieved data on the number of live births in the State from the database of the Brazilian Integrated Health Care System (*Banco de Dados do Sistema Único de Saúde*, Datasus) (16). The geographical origin of the patients was classified according to the mesoregions of Santa Catarina determined by the Brazilian Institute of Geography and Statistic (*Instituto Brasileiro de Geografia e Estatística*, IBGE) (17).

We analyzed the following variables: number of children screened; age at screening; 17OHP level on the first measurement and, if applicable, on the second measurement; elapsed time between the first 17OHP measurement and the first appointment (time to track, refer for medical evaluation and evaluate the child); geographical origin; gender; birth weight; levels of 17OHP, testosterone, androstenedione, sodium and potassium on diagnosis confirmation; karyotype; clinical manifestations on the first appointment; stage of development according to the Prader scale; age of the child on the first appointment and on treatment onset.

The study protocol was approved by the Ethics Committee for Research on Humans of HIJG as number 002/2011 and received the decision number 005-B/2011.

To describe the results, we used absolute values of frequency, relative frequency, mean ± standard deviation (SD), median, minimum and maximum. Student's

**Table 2.** Laboratory results in children with salt-wasting and simple-virilizing CAH. Data expressed as means ± standard deviations

| Variables | Salt wasting (n = 37) | Confidence interval 95% | Simple virilizing (n = 13) | Confidence interval 95% | *p* value | Reference values |
|---|---|---|---|---|---|---|
| 17OHP (ng/mL) | 209.4 ± 196.7 | 143.5 - 274.9 | 56.3 ± 43.5 | 29.5 - 82.4 | 0.00003 | 1 to 5 dL = 0.82-4.2 |
| | | | | | | 5 to 30 dL = 0.1-16.8 |
| | | | | | | 30 to 60 dL = 1.9-9.8 |
| Androstenedione (ng/mL) | 15.6 ± 30.1 | 5.48 - 25.6 | 6.7 ± 2.9 | 4.61 - 8.32 | 0.14 | 0.1 - 0.9 |
| Testosterone (ng/dL) | 613.9 ± 647.7 | 397.8 - 829.8 | 223.8 ± 99.3 | 163.7 - 283.9 | 0.0005 | < 40[1] |
| Sodium (mEq/l) | 127.2 ± 9.2 | 124.2 - 130.3 | 135 ± 3.9 | 132 - 136.3 | 0.0004 | 132 - 146 |
| Potassium (mEq/l) | 5.4 ± 1.0 | 5.02 - 5.73 | 4.5 ± 0.7 | 4.07 - 5.0 | 0.0008 | 3.0 - 5.0 |

17OHP: 17-hydroxyprogesterone; dL: days of life.

[1] Except for boys younger than 1 year.

*t*-test was applied to compare numerical variables from subjects with salt-wasting and simple-virilized forms. The level of significance was set at 5% (p < 0.05). Statistical analysis was conducted with SPSS, version 17.0 (Chicago: SPSS Inc.).

## RESULTS

There were 884,742 live births registered in the State of Santa Catarina between January 2001 and December 2010. Of these, 748.395 (89%) were screened for CAH by the NSP-DH/SC.

In total, 129 children with abnormal 17OHP levels on screening were referred for medical evaluation. Using the first kit (AutoDELFIA®), 78 children who presented initially with elevated 17OHP levels showed normalization of these levels during follow-up. After change in kits (GSP®) and in cutoff level, only a single case of transitory elevation was observed.

There were 50 cases of CAH, yielding an incidence of 1:14.972. Of these, 27 (54%) were girls. The female to male ratio was 1:1.7. Mean birth weight was 3.213 ± 595 g and all children were born full-term. The salt-wasting form was diagnosed in 37 children (74%), 21 of which (56.7%) were girls and 16 (43.2%) were boys. In 24 children (48%), the diagnosis of salt-wasting CAH was established by the presence of hyponatremia in the first medical evaluation. Serum levels of 17OHP, androstenedione, testosterone, sodium and potassium in patients with salt-wasting and simple-virilizing forms on the first appointment are shown in table 2.

Mean age at first sample collection in patients with confirmed CAH was 7.3 ± 6.6 days (median 5.5 days) and it did not differ between patients with and without the salt-wasting form of the disease (p = 0.08). In patients with confirmed CAH mean 17OHP was 152.9 ± 151.0 ng/mL (median 139.0 ng/mL – minimum 11.9 ng/mL and maximum 788.0 ng/mL). The minimum screening value of 17OHP in patients with salt-wasting CAH was 16.4 ng/mL and among those with simple-virilizing CAH, 11.9 ng/mL. Mean age on treatment onset was 21.6 days. In patients with salt-wasting CAH, mean age on treatment onset was 17.4 days and in those with simple-virilizing CAH, it was 54.9 days.

The most frequent clinical manifestations in CAH – affected children were: virilized genitalia with nonpalpable gonads (27%), clitoromegaly (26%), genital hyperpigmentation (25%), fused labioscrotal fold (24%), enlarged phallus (23%), dehydration (16%), fused labia

majora (16%), vomit (10%), diarrhea (5%), anorexia (5%) and fever (5%). There were no cases of shock or death.

All children with virilized genitalia and nonpalpable gonads had a 46, XX karyotype. Table 3 shows the distribution of female children according to Prader stage and by clinical form. In three girls with Prader 5, there was an error in genre identification that required correction of the civil registration.

**Table 3.** Distribution of female children according to Prader stage and CAH forms

| Scale | Salt wasting (n = 21) | Simple virilizing (n = 6) |
|---|---|---|
| Prader 0 | 0 | 1 (16.6%) |
| Prader I | 0 | 3 (50%) |
| Prader II | 1 (4.8%) | 1 (16.6%) |
| Prader III | 4 (19%) | 1 (16.6%) |
| Prader IV | 13 (62%) | 0 |
| Prader V | 3 (14.2%) | 0 |

Figure 1 shows the distribution of cases of CAH according to mesoregions of origin in the State of Santa Catarina. One child with CAH (2%) was born in the State of Minas Gerais, but CAH screening, diagnosis and treatment were all conducted in Santa Catarina.
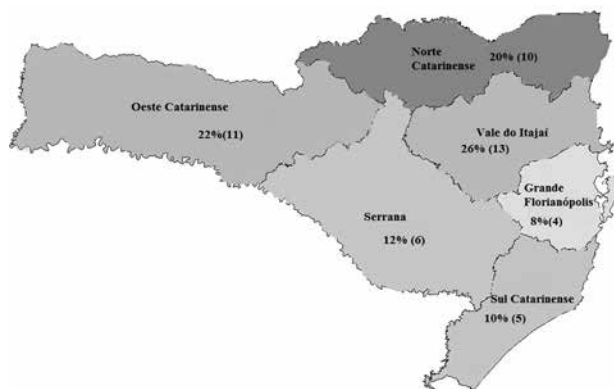


**Figure 1.** Distribution of cases of congenital adrenal hyperplasia (CAH) according to mesoregions in Santa Catarina.

## DISCUSSION

The neonatal screening program for CAH was implemented in Santa Catarina in October 2000. During that year, no cases of CAH were diagnosed. The current study started in January 2001.

One of the aims of neonatal screening programs is universal coverage, which is, coverage of 100% of the live births. A 2005 study about neonatal screening programs in Latin America showed an overall coverage of

49.3%, including 64% in Argentina, 70% in Mexico, and the best results in Chile, Uruguay and Cuba, all with 99% (18). The coverage in the present study was 89%, which is below ideal, but still, above the coverage in most Latin American countries (18).

It is possible that the coverage below 100% observed in our study may be related to lack of notification of screening results conducted in the private setting, such as clinics, maternity hospitals and laboratories. This may occur with any neonatal screening in Brazil, since the country has no legislation requiring notification of screening results or of cases diagnosed in private networks (19).

The incidence of CAH worldwide varies according to ethnic and geographic areas. In the state of Goiás in Brazil, the incidence was estimated in 1:10.325 (20) and in Minas Gerais in 1:19.939 (21). A study from Argentina reported an incidence of 1:8.900 (22). In Europe and North America, it is close to 1:15.000 and 1:16.000, reaching 1:2.100 in the French island of La Réunion and 1:280 among Yupik Eskimos in Alaska. In Japan, it is slightly lower, at 1:19.000 (9,23,24). In Santa Catarina, it was 1:14.967, which is similar to results observed in Europe.

Sample collection for measurement of 17OHP should be performed after 48 hours of life. Screening programs recommend it to be performed between the 3rd and 5th day of life and to not exceed seven days (5). The NSP-SES/SC also adopts this recommendation, but in this study we observed that the average age at first sampling was 7.3 days, which is outside the recommended period. After this result, a new training was conducted, summoning all screening centers located in the State of Santa Catarina.

One of the limitations of screening programs for CAH is the high number of false-positive results (24-27). Collection of samples before 48 hours of life can increase the rate of false positivity. Premature children and those with low weight have higher risk of false positive results in the neonatal screening test analyzed by the fluoroimmunoassay, method, which is the international standard (5,28). Adoption of more precise cut-off levels for 17OHP, according to gestational age and birth weight, decrease the rate of false positivity and identify individuals at risk of adrenal crisis from those with mild forms of the disease (28,29). Newborns with false-positive results have elevated levels of 17OHP, but are asymptomatic. Within the first months of live, 17OHP levels decrease spontaneously with subsequent measurements in these patients (26,27). All newborns with CAH in our study were born at term and most had appropriate weight for gestational age, yielding a lower possibility of false positivity. However, until September 3, 2009, there were 78 cases with initial elevation in 17OHP which normalized without treatment during follow-up. With the change in kits and adjustment in cutoff level after September 4, 2009, the frequency of false positive results decreased and only one occurrence of transitory elevation was observed. We believe that this occurred due to the change in cutoff level with increased stratification for newborns with lower weight and to the new kit having lower cross reaction with other hormones such as 11-deoxycortisol, 17α-pregnenolone, progesterone, and others.

It is very well recognized today that the age at diagnosis and treatment onset of children with CAH has decreased following establishment of neonatal screening programs. Studies demonstrate that the younger the age of the newborn on diagnosis, lower the duration of hospitalization and morbidity (30,31). In Texas, the average age at diagnosis in the screened population was 12 days (32). In Santa Catarina, the average age identified was 21.6 days. However, we observed that the average age of treatment onset in patients with the salt-wasting form of the disease was even lower. Although this is still inappropriate considering that the main objective of the screening is to start treatment as early as possible in all cases, the approach adopted by the program identified with more effectiveness the newborns at higher risk of adrenal crisis. Since there was no difference in time of first sampling between CAH forms, the time for recall and execution of the second sample in cases in which the initial screening showed no levels for urgent medical assessment may have influenced these results and should be analyzed.

Girls with classic CAH have ambiguous genitalia at birth and may be registered and raised improperly as boys (8). We observed in our study that 6% of the children had improper gender identification, hence the importance of early diagnosis and confirmation with karyotype for early correction of the error in establishing the correct gender in affected girls.

The girls in this cohort showed variable degrees of virilization of the external genitalia. Regarding the type of CAH, girls with the salt-wasting form of the disease showed a higher degree of virilization than those with the simple-virilizing form, which is consistent with the literature (6).

According to the international experience in this type of screening, the salt-wasting form comprises 66-75% of all cases when compared with the simple-virilizing form, which ranges from 15 to 32% of the cases (6,24). In this study, the salt-wasting form was responsible for 74% of cases, which is consistent with international statistics.

A study conducted in Texas observed that children with salt-wasting CAH presented more biochemical abnormalities when compared with those with the simple-virilizing form (32). This was also observed in our study in all tests but serum androstenedione, which did not differ significantly in patients with both of these forms of CAH. However, considering that the criteria used to define salt-wasting and simple-virilizing CAH was the presence of electrolyte disorder, the comparative analysis, particularly with respect to sodium and potassium, is subjected to bias.

One of the goals of screening for 21OH deficiency is to detect newborns with salt-wasting CAH before they present hypovolemic shock, endangering the life of the child. The mortality rate associated with a salt-loss crisis in CAH is 4 to 10% (8). In our study, no cases of adrenal crisis or death were observed, despite the fact that 37 children presented the salt-wasting form of the disease. However, 65% of these already had hyponatremia at initial medical evaluation (Table 3), which probably would not have occurred had the diagnosis been established earlier. Anticipation of the diagnosis in boys with the simple-virilizing form is another goal of neonatal screening for CAH (32,33). In this study, this was accomplished in seven boys.

One of the most important aspects of a neonatal screening program is to ensure medical care and treatment to all children detected with the abnormality (29). All children diagnosed in this program received treatment at SEP-HIJG.

The coverage rate of the program by mesoregion of Santa Catarina was not analyzed, but we observed that children with CAH originated from all mesoregions, which suggests that the program has a broad scope.

The results of this study indicate that the NSP for CAH of the SES/SC needs to improve even further to diagnose the disease and start treatment at an earlier age. Strategies will be needed to streamline the several stages of the program, especially the age at which blood sample for 17OHP is first collected, which was the most critical point observed in our study. To improve this problem, the first sample should be collected between three and five days of life, as already recommended by the program.

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