Phenylketonuria, congenital hypothyroidism and haemoglobinopathies: public health issues for a Brazilian newborn screening program

Abstract

In this study, the frequency of detected congenital hypothyroidism, phenylketonuria and haemoglobinopathies in the State of Rio de Janeiro’s (Brazil) Newborn Screening Program (NBSP) was analyzed between the years of 2005 and 2007. There were two Newborn Screening Reference Centers (named NSRC A and B) with programmatic differences. In 2007, overall detection coverage reached 80.7%. The increase in the incidence of congenital hypothyroidism (1:1,030 in 2007) was attributed to the reduction of neonatal TSH value limits over time. The incidence discrepancy of phenylketonuria between NSRC A (1:28,427) and B (1:16,522) might be partially explained by the small number of cases. The incidence of sickle cell disease and its traits were uniformly high (1:1,288 and 1:21, respectively). This was coherent with the ethnic composition of the population. The differences in laboratory methods and critical values, in addition to other programmatic issues, may explain the variances in the results and limited analysis of the role of biological and environmental determinants in the occurrence of these diseases.

Neonatal Screening; Phenylketonurias; Congenital Hypothyroidism; Hemoglobinopathies

Introduction

Newborn screening programs (NBSPs), introduced in the 1970’s 1,2 have improved the early diagnosis and treatment of several congenital diseases that are asymptomatic during the neonatal period. Their early detection can improve prognosis and lead to a newborn’s full development.

Newborn screening (NBS) tests consist of the analysis of dried blood spots collected on filter paper 1. The tests have high sensitivity and specificity, but low positive predictive value for infrequently occurring diseases. The NBSP in Brazil includes testing for phenylketonuria (PKU), congenital hypothyroidism (CH) and haemoglobinopathies.

Children with PKU are unable to metabolize Phenylalanine (Phe) which accumulates in the bloodstream causing irreversible damage to the nervous system. Detection from NBS blood spots is based on high levels of plasmatic Phe and treatment consists of dietary protein restrictions 3. PKU occurrence has varied widely in Europe (between 1:3,042 and 1:35,552 newborns in 2004) 4 and Latin America (between 1:12,473 and 1:51,989 in 2005) 5.

CH is the main endocrine cause of mental retardation in the neonatal period. Early detection and hormone replacement starting no later than two weeks allow normal development 6. The frequency of occurrences of CH varies widely in Europe (incidence between 1:1,333 and 1:13,886...
in 2004) but less in Latin America (incidence between 1:1,667 and 1:3,670 in 2005).

Hereditary haemoglobinopathies affect approximately 7% of the world population. De-oxygenated haemoglobin makes erythrocytes assume a “sickle-like” shape leading to thromboembolic phenomena and haemolytic anaemia. NBS allows the introduction of prophylactic measures that reduce the disease’s morbidity and mortality. In 2005, the estimated incidence of the disease in Brazil was 1:2,043 newborns (varying from 1:1,196 to 1:39,107 across studies).

In Brazil, during the 1980s, various NBSPs were instituted in a disarticulated way until 2001, when the Government created the National NBSP. The program is aimed at providing universal access to the tests and its actions include diagnostic confirmation, treatment and follow-up for affected individuals free of charge for all citizens.

In each State, Neonatal Screening Reference Centers (NSRC) are responsible for the articulation of the laboratory with the network of collection units and management of all activities related to NBS.

The State Institute of Endocrinology and an independent philanthropic organization have managed the NBSP in the State of Rio de Janeiro since the mid-1980s. From 1994 to 2005, the municipality of Rio de Janeiro ran its own NBSP 10,11,12.

In 2001, the State of Rio de Janeiro was accredited for phase II of the governmental NBSP and has 3 NSRCs. This is a unique situation, considering that the majority of the other Brazilian states have only one NSRC. The 3 NSRCs in the State of Rio de Janeiro were: the State Institute of Endocrinology (Instituto Estadual de Diabetes e Endocrinologia – IEDE), “NSRC B” (the independent philanthropic organization), and “NSRC C” (the hospital located in the Rio de Janeiro municipality).

Bloodspots were collected on filter paper (Schleicher & Schuell 903, Schleicher & Schuell BioScience Inc., Keene, USA) by heel puncture, preferably between the child’s third and seventh day of life. They were submitted to neonatal thyrotropin (TSH) analysis using the immunofluorometric method (AutoDelfia Neonatal hTSH Kit, PerkinElmer Life and Analytical Sciences, Wallac and Oy, Turku, Finland). Laboratory A adopted a cut-off limit of 20.0µIU/mL until 2006, when it was reduced to 10.0µIU/mL. Children with levels above these limits were recalled for confirmation with serum TSH and Free-T4 (TSH and FrT4 Automated Chemiluminescence System: 180 – Siemens Medical Solutions Diagnostics, Chicago, USA) (Figure 1).

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In NSRC B, the neonatal TSH cut-off limit considered physiological variations with age. For specimens collected between the third and seventh day, with TSH levels above 15.0µIU/mL, a new bloodspot sample was obtained. If the TSH level remained higher than 10.0µIU/mL, the child was submitted to diagnostic confirmation. For NBS samples collected from children over than seven days, the critical level was 10.0µIU/mL. Any level above 25.0µIU/mL was taken for serum confirmation regardless of the child’s age at the time of the blood spot collection. Confirmation was made with serum TSH and Free-T4 using the chemiluminescence method (Symbiosys Diagnostic Ltda., Leme, Brazil). In both NSRCs, confirmed cases were treated with L-thyroxin at a dose of 10 to 15µg/kg/day.
For Phe assay, laboratory A used the fluorimetric method (Neonatal Phenylalanine Kit, Perkin Elmer Life and Analytical Sciences, Wallac and Oy, Turku, Finland), in which levels higher than 10.0mg/dL were subject to a retest with the same method. The persistence of levels higher than 10.0mg/dL indicated immediate dietary treatment (Figure 2).

Laboratory B used the colorimetric enzymatic method (NeoLisa PKU Kit; Internacional Científica Ltda., São José dos Campos, Brazil) for Phe determination. In bloodspots collected before the fifth day of life, results between 3.5 and 4.0mg/dL were subject to an analysis using another method (thin layer amino acid chromatography) to differentiate from thyrosinemia and full parenteral nutritional treatment. In specimens collected after the fifth day, Phe levels above 5mg/dL were subject to a retest on bloodspots. Levels above 10mg/dL indicated treatment.

In NSRC A, bloodspots and newborn data were sent to the State Institute of Haematology’s lab for Hb analysis using high performance liquid chromatography (HPLC – Variant – β-thalassemia Short Program, United States, Bio-Rad Laboratories, Hercules, USA) for detection of S and C hemoglobin. Normal results were returned to the main NBS laboratory and gathered in a single report with other results. Heterozygotes with AS Hb (sickle cell trait) had a note attached to the report for the family’s guidance. Homozygotes with SS Hb (sickle cell disease) were recalled for diagnostic confirmation with the same method, indicating antibiotic treatment and prophylactic management.

In NSRC B, Hb was analyzed in their own laboratory using isoelectric focusing (Resolve Hemoglobin Kit PerkinElmer Life and Analytical Sciences, Wallac and Oy, Turku, Finland) with confirmation by HPLC (Variant – Sickle Short
Program, United States Bio-Rad Laboratories, Inc., Hercules, USA) for detection of SS, FC, FSA and FSC Hbs. The procedure for heterozygotes was the same as in NSRC A. Homozygotes for SS Hb were directly recalled and sent to the State Institute of Haematology outpatient’s clinic where the same protocol for detected cases in NSRC A was followed.

In this study, the following indicators were analyzed: coverage, total recalls, recall rate and percentage of confirmed cases in relation to the overall amount of children submitted to testing in accordance with the NRSP approach, during the analyzed period.

Data were obtained by trained healthcare professionals from different sources and refers to the period 2005 to 2007 (except for data used in the calculation of coverage which relates to the period 2002 to 2007). Data were also obtained from the Live Birth Registration System (SINASC: http://www.datasus.gov.br, accessed on 24/Apr/2011), NSRC reports sent to Brazilian Ministry of Health, worksheets and forms, NSRC databases, activity reports, recall documents of second sample collections due to invalid first samples or for diagnostic confirmations, attendance monitoring spread sheets for the recall of second bloodspot collections or diagnostic confirmations. The dominant component of this data was from the public sector. Data from the complementary sector were not available.

Data were entered in MS Excel (Microsoft Corp., USA) spread sheets containing the following variables: child’s initials, specimen number, birth date, first collection date, collection unit code, registration date of the first sample, abnormal result, result release date, recall date, arrival date of the second sample/diagnostic confirmation, recall reason in case of invalid sample; reason for no-show and confirmatory results for each analyze (TSH, Free-T4, Phe e Hb).

In coverage estimates, the numerator considered the amount of tests carried out in all three NSRCs, and the denominator included the amount of births obtained from SINASC. The frequency calculations for each disease considered all confirmed cases in relation to all the tests carried out, overall and within each NSRC.
the incidence calculation, all the children with abnormal results who showed up for diagnostic confirmation were included. The calculation of annual incidence was based on children’s birth date. Double entries in the database and cases that had not been submitted to NBS by the laboratory itself were excluded. All data were inserted, checked and analyzed using MS Excel and SPSS 13.0 (SPSS Inc., Chicago, USA).

This research project was approved by the Ethics Research Committee of the Brazilian National School of Public Health, Oswaldo Cruz Foundation (Escola Nacional de Saúde Pública Sergio Arouca, Fundação Oswaldo Cruz – ENSP/ Fiocruz Rulling n°. 113/08), by the State Reference Institute in Endocrinology (IEDE Ruling n°. 48/08) and by the Board of Management of the independent philanthropic institution by means of their Terms of Agreement. The study was financed by the State of Rio de Janeiro Foundation for Research Support (Fundação de Amparo à Pesquisa do Estado do Rio de Janeiro – FAPERJ Grant n°. E-26/111.443/2008) and the National Council for Scientific and Technological Development (Conselho Nacional de Desenvolvimento Científico e Tecnológico – CNPq Grant n°. 308651/2006-5).

Results

Data were collected from October 2008 to May 2009. In NSRC A, data were collected by staff, where as in NSRC B, this work were carried out by the research team. The State Institute of Haematology did not permit access to data regarding haemoglobinopathies and analysis of this disorder was restricted to the National NBSP monitoring reports.

NBSP coverage increased from 71.6% to 80.4% during the period under analysis, with an outstanding drop to 65.4% in 2004. Since model C’s monitoring reports were not available in 2004 and results were not considered in the calculation, coverage in that year is underestimated. Both the reduction in total births (7%) and the increase in the number of tests carried out (5%) contributed to the increase in coverage. The transfer of NSRC C’s caseload to NSRC A in 2005 increased the latter’s participation from 79% in 2005, to 81% of all tests performed in the State in 2007. The remaining caseload was tested by NSRC B. The collection units that comprised NSRC B were smaller and were spread throughout the State in smaller and less populated municipalities.

In 2005, with regard to CH screenings, the newborn recall rate in NSRC A was twice as high as that in NSRC B, despite the higher TSH cut-off level in NSRC A (Table 1). In 2006, with the reduction in the cut-off level in NSRC A, the recall rate became seven times higher than that in NSRC B. Laboratory methods were different for diagnostic confirmation and the incidence rate in NSRC A increased sharply until 2007, when it was 7.7 times higher than in NSRC B. From 2005 to 2007, the global incidence rate (in both NSRCs) increased by 3.5 times.

With different laboratory methods for PKU screening and confirmation in the two NSRCs, recall rates were lower in NSRC A and detection rates were higher in NSRC B (Table 1).

Despite the different laboratory methods, recall and incidence rates of sickle cell disease and traits were similar in both NSRCs and showed a lower variation with no defined trend during the analyzed period (Table 1).

Discussion

Estimates in the frequency of the target diseases are essential for adequate planning of specific healthcare programs in addition to providing important information for assessing current early detection programs. Wide geographic and periodic variations of rare diseases such as CH and PKU may be attributed to genuine variations in diseases frequency or to operational issues such as laboratory methods or program coverage.

The common goal of all NBSPs of 100% coverage still represents a great challenge even in certain more developed countries. For instance, in 2006, Canada achieved coverage of only 76%,17 and Belgium reported coverage of only 87%.4

In Brazil, where the goal of the government program is universal access, coverage was 100% in those states with more advanced program implementation (which includes screening for CH, PKU, haemoglobinopathies and cystic fibrosis). In states in the intermediate stages of implementation (CH, PKU and haemoglobinopathies), coverage ranged from 70% to 99%, whereas in those in the initial stages (PKU and CH) coverage ranged from 3% to 47%.12,18,19,20

In 2007, coverage in the state of Rio de Janeiro (81%) was lower than that of other states in Southeast region, such as São Paulo (88%) and Espírito Santo (87%)12. It was not possible to evaluate from the available data whether the existence of more than one NSRC contributed to test accessibility. NSRC A carried out most of the tests (81% in 2007) as it covered a larger number of municipalities with larger populations.

In coverage estimates, the number of live births represents a reasonable approximation to the denominator, since all infants are eligible for NBS.
On the other hand, coverage may be inflated since it considers the total amount of bloodspots as opposed to the number of newborns tested. Second samples may have not been distinguished from initial screening tests. Moreover, registers of tests carried out in the private sector, which plays an important role in the healthcare system in the State of Rio de Janeiro, were not available and it was not possible to assess to what extent this affected coverage estimates.

The variations found in CH recall rates and incidences are explained by differences in neonatal TSH cut-off levels between laboratories and also in the same laboratory over time. The proportion of false positives (non-confirmed suspected cases) was stable in NSRC B and varied strongly in NSRC A, reaching much lower levels than in NSRC B and those reported in literature. There is no way to estimate the proportion of false negatives that are affected by other factors such as age at collection time, conservation, transportation, and sample processing.

Large variations in the frequency of CH were observed in different Brazilian states that used large variations in the frequency of CH were observed in different Brazilian states that used.

### Table 1

Critical values of the different laboratory methods, recall rates and frequency of the diseases detected in the different Newborn Screening Reference Centers (NSRC) of the Neonatal Screening Program in the State of Rio de Janeiro, Brazil between 2005 and 2007.

|                  | 2005        | 2006        | 2007        | 2006        | 2007        | 2007        |
|------------------|-------------|-------------|-------------|-------------|-------------|-------------|
|                  | NSRC A      | NSRC B      | NSRC A      | NSRC B      | NSRC A      | NSRC B      |
| Samples tested   | 139,096     | 38,098      | 153,031     | 35,845      | 142,135     | 33,043      |
| Congenital hypothyroidism
  Method (neonatal TSH)
  Limit value
  CH suspects      | 116         | 16          | 887         | 27          | 230         | 12          |
  Number of cases  | 42          | 7           | 126         | 12          | 165         | 5           |
  Recall (rate per 10,000) |
  Incidence      | 1,3,311     | 1,5,442     | 1,1,215     | 1,2,987     | 1,861       | 1,6,609     |
| Overall incidence      | 1,3,616     | 1,1,369     | 1,1,030     | 1,030       |             |             |

|                  | 2005        | 2006        | 2007        | 2006        | 2007        | 2007        |
|                  | NSRC A      | NSRC B      | NSRC A      | NSRC B      | NSRC A      | NSRC B      |
| Phenylketonuria
  Method (phenylalanine)
  Limit value
  PKU suspects      | 11          | 11          | 11          | 11          | 11          | 11          |
  Number of cases  | 5           | 2           | 9           | 3           | 5           | 2           |
  Recall (rate per 10,000) |
  Incidence      | 1,27,819    | 1,19,049    | 1,17,003    | 1,11,948    | 1,28,427    | 1,16,522    |
| Overall incidence      | 1,25,313    | 1,15,740    | 1,1,345     | 1,379       | 1,269       | 1,377       |

|                  | 2005        | 2006        | 2007        | 2006        | 2007        | 2007        |
|                  | NSRC A      | NSRC B      | NSRC A      | NSRC B      | NSRC A      | NSRC B      |
| Sickle cell disease
  Method (hemoglobin)
  Types of hemoglobin
  Number of confirmed cases *                  | 116         | 28          | 104         | 26          | 112         | 24          |
  Incidence      | 1,1,199     | 1,1,361     | 1,1,471     | 1,1,379     | 1,1,269     | 1,377       |
| Overall incidence      | 1,1,205     | 1,1,452     | 1,1,421     | 1,287       | 1,288       | 1,288       |

|                  | 2005        | 2006        | 2007        | 2006        | 2007        | 2007        |
|                  | NSRC A      | NSRC B      | NSRC A      | NSRC B      | NSRC A      | NSRC B      |
| Sickle cell trait
  Method (hemoglobin)
  Types of hemoglobin
  Number of confirmed cases *                  | 5,655       | 1,594       | 7,291       | 1,521       | 6,945       | 1,403       |
  Incidence      | 1,25        | 1,24        | 1,21        | 1,24        | 1,20        | 1,24        |
| Overall incidence      | 1,24        | 1,21        | 1,24        | 1,24        | 1,24        | 1,24        |

CH: congenital hypothyroidism; PKU: phenylketonuria.
* Number of suspected cases not available.
the same laboratory method applied in the State of Rio de Janeiro, such as Mato Grosso, with 1:9,448 20, Sergipe, with 1:6,005 19, Bahia, with 1:4,000 21 and Rio Grande do Sul, with 1:2,746 22. However, the highest incidence was found in Rio de Janeiro (1:1,030 in 2007; Table 2). Even in Ribeirão Preto 18, where the NBSP uses the same method and cut-off level as NSRC A, incidence was 1:2,595.

Frequency has been increasing over recent years in many countries due to genetic, environmental and socio-cultural factors 4,5,23. Harris & Pass 23 analyzed CH epidemiological behavior in the US over a 25-year-period and observed a continuous and pronounced increase in disease incidence that could be attributed to modifications in diagnostic criteria as well as to environmental factors (iodine intake), ethnic composition and reproductive behavior.

Large variations in PKU incidence were also verified in different states. These findings are similar to the cases of Mato Grosso, with 1:33,068 20, Bahia, with 1:22,000 21, Sergipe, with 1:23,036 19, Rio Grande do Sul, with 1:16,229 22, Ribeirão Preto (São Paulo State), with 1:19,409 18 and Rio de Janeiro with 1:25,025 in 2007 (Table 1). Loeber 4 had already demonstrated large variations between different countries in Europe. The order of magnitude of the incidence rates in Rio de Janeiro was similar to those of other Brazilian states. A few cases generated varying but consistently low rates during the period. It is interesting to note that large discrepancies in incidence and recall rates were found in Rio de Janeiro where there were two laboratories using different methodologies. It is possible that one NSRC was overlooking cases while the other, with very high testing sensitivity, was probably recalling a greater proportion of false positives, lowering its efficiency and unnecessarily increasing stress in the newborn family due to mistaken diagnosis.

In Brazil, sickle cell disease has a heterogeneous distribution and frequency is higher in populations with a greater proportion of individuals from African descent (Northeast region and the states of São Paulo, Rio de Janeiro and Minas Gerais). The frequency of haemoglobinopathies in the State of Rio de Janeiro was homogeneous in both NSRCs, and although incidence increased, it was coherent with the ethnicity of the State's population. In 2007, incidence of sickle cell disease in Rio de Janeiro, was 1:1,288 (Table 1), compared to 1:650 in the State of Bahia which has the highest incidence rate for this disease in Brazil 21. Although not directly comparable, the data regarding prevalence of sickle cell disease in Rio Grande do Sul (1:39,000) was consistent with the lower proportion of African descendants in that state 24.

**Conclusion**

Screening for rare diseases with pre-clinical phases as short as those of PKU and CH poses additional challenges to health programs. Considering the territorial extension and demographic heterogeneity of countries like Brazil, and the cost and complexity of health programs such as NBSP, analyses of this type should be regionalized to make the composition of the state disease panels more cost-effective and offer the population maximum benefits. For haemoglobinopathies, despite the less devastating impact on the newborn's development, the benefit of mass screening is a reduction in disease morbidity/mortality with a lower burden on the healthcare system.

The drawbacks of laboratory methods and the cut-off levels used for detecting suspected cases of CH show a clear need for further discussions regarding this process. In the definition of normality criteria, taking age into account has a biological basis and an empirical demonstration, but its implementation is more difficult from a practical point of view. It is also important to consider environmental, nutritional and reproductive behavior and the diversity of the ethnic composition of the population as determining factors for the variations found in the epidemiological profile as suggested by Harris & Pass 23 for the case of CH.

Considering that variations in detection frequencies may have resulted from discrepancies in laboratory tests, there is a need for further scrutiny in this process to avoid the implications of false positive and false negative results.
Resumo

Neste estudo, foi analisada a frequência de detecção do hipotireoidismo congênito, fenilcetonúria e hemoglobinopatias no Programa de Triagem Neonatal do Estado do Rio de Janeiro, Brasil, entre 2005 e 2007. Havia dois Serviços de Referência em Triagem Neonatal (designados SRTN A e B) com diferenças programáticas. Em 2007, a cobertura alcançou 80,7%. O aumento na incidência do hipotireoidismo congênito (1:1.030 em 2007) foi atribuído à redução no valor de corte do TSH ao longo do tempo. As incidências discrepantes da fenilcetonúria entre os modelos (SRTN A – 1:28.427; SRTN B – 1:16.522) podem ser parcialmente explicadas pelo pequeno número de casos. A incidência da doença falciforme e do traço falcêmico foi uniformemente elevada (1:1.288 e 1:21, respectivamente), sendo coerente com a composição étnica da população. As diferenças nos métodos laboratoriais e valores críticos, além de outras questões programáticas, podem explicar a variabilidade nos resultados e limitar a análise do papel dos determinantes biológicos e ambientais sobre a ocorrência das doenças.

Triagem Neonatal; Fenilcetonúrias; Hipotireoidismo Congênito; Hemoglobinopatias

Contributors

J. Botler contributed in all steps of research: project conception, data collection, data analysis, writing and approval of final version. L. A. B. Camacho and M. M. Cruz contributed in the research Project, analysis of results, writing, review and approval of final version.

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