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NEONATAL SCREENING FOR HEMOGLOBIN S

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ABSTRACT

The aim of this manuscript was evaluate the Neonatal Screening Program for sickle cell disease from 2011 to 2015 in relation to its coverage and the prevalence of hemoglobin S. Methods: Cross-sectional observational study with results of Newborn Screening for sickle cell disease belonging to the database of the Research Institute, Study and Diagnostics of the Association of parents and friends of the exceptional. The variables studied were: number of children born alive; total screening; prevalence; time elapsed between the birth of the child and the collection of the screening; age at the time of the screening result. Prevalences were estimated by point and 95% confidence interval by Wald method adjusted using the Z distribution. Results: The numbers of live births was 213,739, but only 182,398 were screened for hemoglobinopathies, with a mean coverage index of 85.34%. In relation to the triage, 33 cases of sickle cell anemia were identified, 11 with FSC result and 3,328 had the result of sickle cell trait. Between the 3rd and 5th day of life were detected 25.5% of the collections. Conclusion: The average coverage index of the Neonatal Screening Program in the state of Mato Grosso do Sul / Brazil from 2011 to 2015 was 85.34%. Sickle cell anemia and FSC increased in number of cases. Sickle trait cases show gradual increase.

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INTRODUCTION

Sickle cell disease is a generic term used to define a group of hematological changes characterized by the predominance of hemoglobin S (Hb S). These changes include the homozygous form of Hb S (Hb SS), the heterozygous form composed with the association of Hb S with other variant hemoglobins and the interactions of Hb S with thalassemic syndromes (Bonini-Domingos, 2013). Sickle cell trait Hb AS is the asymptomatic form of the disease. Every year in Brazil we have about 3,500 children born with sickle cell disease, with an incidence of 1: 1000 live births, concomitantly, 200 thousand children with sickle cell trait are born per year (Brasil, 2009). The distribution of the disease in the Brazilian population is heterogeneous and a higher prevalence in the north and northeast regions (Cançado; Jesus, 2007). In Brazil, was established the National Neonatal Screening Program (PNTN) as a government program from the Ministry of Health (Ordinance No. 822/2012). This program is articulated by the Ministry of Health and state and municipal health secretariats and is characterized by four phases of implementation. Screening for sickle cell disease and other hemoglobinopathies were included in 2nd phase (Brasil, 2001). Prior to Ordinance No. 822/2001, State Law No. 2,079/2000 made it compulsory to perform tests for the detection of hemoglobinopathies in children born in the state of Mato Grosso do Sul, Brazil (Holsbach *et al.*, 2008). Research on the occurrence of hemoglobin S in the State of Mato Grosso do Sul in the period

2000 to 2010 shows that the Hb S group became predominant in the state with 36 cases of sickle cell anemia and 14 cases of Hb SC. Sickle traits (Hb AS) presented 5,613 cases out of 372,782 live births (Holsbach *et al*., 2008; Araujo, 2014). The social relevance of conducting this study was to continue to establish a systematic periodic monitoring of the Program in order to reach the target of 100%. It intends to make available the results to the competent public authorities, allowing the improvement of the Program in a continuous way. The present study aims to evaluate the Neonatal Screening Program in the state of Mato Grosso do Sul (Brazil) in relation to its coverage and the prevalence of hemoglobin S in the period from 2011 to 2015.

MATERIALS AND METHODS

This is a cross-sectional study that analyzed information about the Neonatal Screening Program for hemoglobinopathies, belonging to the database of the State Reference Laboratory - Institute of Research, Study and Diagnostics of the Association of Parents and Friends of the Exceptional (IPED-APAE). This study involved 182,392 children screened in the state of Mato Grosso do Sul in Brazil. The exams came from the 1,585 collection points registered in 79 municipalities of the state, from 2011 to 2015. The following variables were the number of children born alive; total screening; prevalence; time elapsed between the birth of the child and the collection of the screening; age at the time of the screening result. For the organization of the reference year of the examinations, the period from December 16 of the previous year to December 15 of the reference year was considered. The neonatal screening process of IPED-APAE / MS was carried out in accordance with the technical criteria established in the technical manual called Neonatal Biological Screening (Brasil, 2016). Neonatal screening begins with the collection of blood on filter paper, model Scheleicher & Schuel 903, after the filling of the blood collection card provided by the IPED-APAE laboratory. The collections are performed by puncture on the side of the heel of the newborns in the basic health units (UBS), Family Health Strategy units (ESF), maternity hospitals and hospitals. Nursing professionals or trained technicians perform the puncture. After the collection and drying of blood, the sample is packed in the appropriate envelope for biological material, with postage paid and posted to the reference laboratory by the Post Office. Municipalities can also send the samples by their own vehicles directly to the reference laboratory. The material to be received in the laboratory is analyzed and classified as to the quality of the blood sample, the filling of the data in the collection card and the age of the child at the time of collection.

Samples are discarded and canceled when they present the following characteristics: inadequate (saturated, wet and wrong material for examination), insufficient blood quantity, collected in duplicate with more than 30 days of collection (denaturation of hemoglobin) and sample without biological material. For canceled samples, new samples are requested from the person responsible for the original collection point. The screening of hemoglobinopathies is performed in an automated manner by the VARIANTnbs system through the High Performance Liquid Chromatography (HPLC) method. This screening identifies on the filter paper in blood disk eluates, the presence and concentration of normal hemoglobins F and A and variant hemoglobins S, D, C and E.

In case of problems with the processing of the sample, the other one may also be requested by the laboratory for the following reasons: insufficient or inadequate, not eluted, altered and hemolyzed. The request flow is the same as that used for samples in the material receiving sector. The reports containing the results of the examinations are available in three forms according to agreement with the collection points: online report available on the reference laboratory page, printed report sent via mail to the units and report via email. In cases of unaltered outcome, the reports are released to the patient as a normal result. For patients with sickle cell trait, the results are released along with informative material on hemoglobinopathies. For children with altered examination results for sickle cell disease, new samples are requested from the source collection station through the IPED-APAE Active Search section. Confirmed results for sickle cell disease are available from the laboratory to the Social Work sector. Children with confirmed outcomes for sickle cell disease are scheduled for the first consultation with a hematologist at IPED-APAE. Children with sickle cell disease are accompanied by a multiprofessional team until the first year of life. After confirmation of the diagnosis by Hemoglobin Electrophoresis, they are referred to the Regional Hospital of Mato Grosso do Sul. There are exceptions, which, according to medical criteria, are sent to other services in the state before completing one year of life. This research was approved by the Committee of Ethics in Research with Human Beings of the Federal University of Mato Grosso do Sul (CEP/UFMS), through opinion number 1469,166. In the calculation of program coverage, the following formula was used: coverage = (number of screened / number of live births) x 100. The number of live births in 2015 was estimated using data from the Brazilian Institute of Geography and Statistics (IBGE, 2005). An additional 10% was added in the Gross Birth Rate (DATASUS, 2010). Prevalences were estimated by point and confidence interval (CI) of 95% by the adjusted Wald method and using the Z distribution.

RESULTS

In the present study, the coverage index varied during the period from 2011 to 2015, standing out the year 2011 with the highest index (86.98%) than in 2014 (83.40%) (Table 1). In the period from 2011 to 2015, of the total of 213,739 live births, only 182,398 were selected (Table 1). Out of this amount, 3,399 results were identified with different genotypic associations of hemoglobin S: 33 cases of FS, 26 had FSA, 11 had FSC, and 3,328 had FAS (sickle cell trait) result. When analyzing the prevalence among 182,398 live births (Table 2), it is verified that Hb S is the most frequent variant hemoglobin, with the prevalence of FAS from one case for 55 live births. As for the FS genotype sickle cell anemia, the result was 0.0191%, with a case prevalence of 5,527 live births. Table 3 shows the time intervals between birth and the collection of the screening test. Regarding the children screened with Hb S, 497 (14.6%) were submitted to the test before the 3rd day of life; 866 (25.5%) were between the third and fifth day; and 1,906 (56.1%) between the sixth and twenty-eighth day of life. Of the total, 13 (0.4%) presented FAS results, one presented FSA and one for FSC, but did not present age records at the time of collection. The data referring to the occurrence of trait cases and sickle cell disease among those selected according to age in days and time of the selection result indicate that 2,856 were neonates and 543 were more than 28 days old. Among the newborns, 23 presented FS

genotype, 21 with FSA and 07 with FSC and 2.805 sickle cell trait genotypes. With regard to those selected over 28 days at the time of the result, 10 presented FS, 05 FSA, 04 FSC and 01 SCF and 523 genotypes of sickle cell trait.

Table 1. Total triage and coverage index in children screened at IPED-APAE, according to live births, Mato Grosso do Sul – period from 2011 to 2015

Year	Live births	Total triage	Coverage index (%)
2011	41.805	36.363	86,98
2012	41.876	35.750	85,37
2013	41.879	35.816	85,52
2014	43.588	36.351	83,40
2015	44.591	38.118	85,48
Total	**213.739**	**182.398**	**85,34**

Source: Information System of Live Births of the Department of Information and Informatics of the Unified Health System of Mato Grosso do Sul (SINASC/DATASUS/MS).
Institute for Research, Teaching and Diagnostics of the Association of Parents and Friends of the Exceptional (IPED-APAE).

Table 2. Prevalence of sickle cell trait and sickle cell disease estimated by point and 95% confidence interval in children screened at IPED - APAE, Mato Grosso do Sul - period from 2011 to 2015

FAZ				
Year	**Children screened**	**Frequency**	**Prevalence (%)***	**IC (95%)****
2011	36.363	638	1,7596	1,7100-1,8084
2012	35.750	662	1,8569	1,8026-1,9105
2013	35.816	669	1,8730	1,8188-1,9265
2014	36.351	676	1,8647	1,8073-1,9214
2015	38.118	683	1,7967	1,7451-1,8475
Todo	182.398	3328	1,8256	1,8019-1,8491
FS				
Year	**Children screened**	**Frequency**	**Prevalence (%)***	**IC (95%)****
Total	182.398	33	0,0191	0,0165-0,0216
FSA				
Year	**Children screened**	**Frequency**	**Prevalence (%)***	**IC (95%)****
Total	182.398	26	0,0153	0,0130-0,0175
FSC				
Year	**Children screened**	**Frequency**	**Prevalence (%)***	**IC (95%)****
Total	182.398	11	0,0071	0,0054-0,0086
SCF				
Year	**Children screened**	**Frequency**	**Prevalence (%)***	**IC (95%)****
Total	182.398	1	0,0016	0,0007-0,0023

* Estimation of prevalence per point; ** prevalence estimate by 95% confidence interval.
Note: FAS: sickle cell trait; FS, FSA, FSC, SCF: standard for sickle cell disease.

Table 3. Occurrence of trait and sickle cell disease in children born alive, according to age in days, at the time of collection of the neonatal screening test, Mato Grosso do Sul - period from 2011 to 2015

Age (days)	FAS	FS	FSA	FSC	SCF	Total
00|---|02	489	5	4	0	0	497
03|---|05	851	8	5	2	0	866
06|---|28	1.865	18	15	7	0	1.906
29|---|60	96	2	1	1	0	100
61|---|90	12	0	0	0	0	12
91|---|200	1	0	0	0	1	2
> 200	1	0	0	0	0	1
No registry	13	0	1	1	0	15
Total	3.328	33	26	11	1	3.399

Source: Institute of Research, Teaching and Diagnostics of the Association of Parents and Friends of the Exceptional (IPED-APAE). Note: FAS: sickle cell trait; FS, FSA, FSC, SCF: standard for sickle cell disease.

DISCUSSION

The results of this study showed that the average coverage rate of the PNTN in the period from 2011 to 2015 was 85.34%. In the Brazilian states with greater governmental involvement the coverage of the program is greater. The full success of the program requires a broad and complex structure with the link between public and non-public network institutions (Botler *et al*., 2010). In addition, constant technological innovations for the investigation of congenital diseases can interfere in the coverage, since they entail in the continuous necessity of qualification for the health professionals for the orientations to the parents and relatives on the importance of the neonatal screening (Marqui, 2016; Strefling *et al*., 2014). The average coverage index of the PNTN in the state of the MS was similar to that of other Brazilian states. The average index in Santa Catarina in 2011 was 89.3% and 78% in 2012 (Eller; Silva, 2016). In the state of Tocantins in 2011 coverage was 84.2% (Mendes; Santos, Bringel, 2013). For the state of Rio Grande do Sul it was 83% in 2014 (Kopacek *et al*., 2015). It should be noted that the national goal of the PNTN is to reach 100% (BRASIL, 2001). In 2014 Brazil achieved more than 83% coverage of live births in the public network. In the same year, countries like Costa Rica and Panama presented coverage of 98% and 75% respectively (Therrell *et al*., 2015). In the analysis of global neonatal screening, there was heterogeneity between regions. Overall coverage in Europe in thirty-eight countries was estimated at 69 per cent in 2004. In countries where the infant mortality rate is less than 10 per 1,000 live births, such as China, Indonesia, Bangladesh, India and Pakistan, levels of achieved were above 90%. In Latin America, coverage in 14 countries was 49% in 2005 (Botler *et al*., 2010).

Regarding the prevalence of hemoglobinopathies in this study, the results showed that of the 182,398 children selected by the PNTN in Mato Grosso do Sul, in 3,399 was identified to Hb S in different genotypic associations, reaching a percentage of 1.86% among children triaged. Hb S, among hemoglobinopathies was identified as the most common hemoglobin variant in the Brazilian population (Freitas; Ivo; Figueiredo, 2016). Corroborate with this finding the epidemiological researches developed in Mato Grosso do Sul, from 2000 to 2005 and from 2006 to 2010, which obtained 2,624 (1,38%) and 3,040 (1,67%) respectively, with identification of Hb S in different genotypic associations (Holsbach *et al*., 2008; Araujo *et al*., 2014). In the period of this research, the prevalence of FAS was 1.83%, with a prevalence of one case for 55 children selected. For other Brazilian states, this result is similar to that of Rio Grande do Sul with 1 case for 65. However, it differs from the Bahia results with 1 case for 17 children selected (Cançado; Jesus, 2007). In the period from 2011 to 2015, 33 cases of FS were registered, so there was an increase in the frequency of FS cases. Previous studies carried out in the state of Mato Grosso do Sul identified 16 cases in the period from 2000 to 2005 and 20 cases from 2006 to 2010 (Holsbach *et al*., 2008; ARAUJO *et al*., 2014). Regarding the occurrence of trait cases and sickle cell disease, according to the age of the children on days at the time of collection, it was verified that 25.5% occurred between the 3rd and 5th day, as recommended by the Manual Biological neonatal screening technician (Brasil, 2016). In Brazil, the results of research concerning the age of the child at the time of collection are described in a diversified way. A study carried out in 2013 in Piauí shows that 36.8% of the collections were performed between the 3rd and the 7th day of life (SALES *et al*., 2015). In 2014 in Rio Grande do Sul, 83% of the collections performed in children were during neonatal screening in the first week of life (Kopacek *et al*., 2015)

The result referring to the intervals between birth and neonatal screening shows 14.6% of precocious collection. An increase in this percentage was observed when compared to the study carried out in Mato Grosso do Sul from 2006 to 2010, which revealed 5.3% of the collections between zero and one day of life. In this study, 78% of the early collections were performed in hospitals and maternities (Araujo *et al*., 2014) The precocious collection of neonatal screening before 48 hours of life of the child mainly compromises the diagnosis of Phenylketonuria, since it is necessary that the child ingest sufficient amount of protein so that phenylalanine is detected by screening (Kopacek *et al*., 2015; Menezes *et al*., 2014). As a result of the analysis of the time elapsed between birth and the collection of the screening was observed in Mato Grosso do Sul an increase in the percentage of collection above the age range recommended by the PNTN - from 49.7% from 2006 to 2010 for 59.5% in the period from 2011 to 2015. According to Botler *et al*. (2010) in the Brazilian states where the coverage is not close to the targets, the collections are made later. These data suggest the need for increased investment in local measures to facilitate access to neonatal screening. With regard to the occurrence of trait cases and sickle cell disease among the triage, according to the age in days at the time of the screening result, of the total of triage (3,399), 543 (16%) were more than 28 days old. In addition, 2,856 (84%) children received the results in the period up to 28 days as recommended by the PNTN for presumptive diagnosis and initiation of follow-up. According to Araujo *et al.* (2014) in Mato Grosso do Sul in the period from 2006 to 2010, 87.8% of the results were delivered up to 28 days of the child. In fact, neonatal diagnosis and timely treatment have been shown to increase survival and improve the quality of life of people with sickle cell disease (Cançado; Jesus, 2007). The limiting factors of this study are related to the coverage rates found in Mato Grosso do Sul that may not reliably reflect the screening coverage of all live births, since some children may have been screened in the private network. Private network screening records are not integrated into the state program. From 2015 the neonatal screening database is available in PDF format, resulting in the need for research in the reports of exams for the construction of spreadsheet necessary for statistical treatment.

Conclusion

According to the evidence indicated in this manuscript, it is concluded that the average coverage index of the Neonatal Screening Program in the state of Mato Grosso do Sul of 85.34% still resembles other Brazilian states. It should be remembered that the goal of the PNTN is 100%. Sickle cell anemia and FSC increased in number of cases when compared to previous studies performed in the state. Sickle trait cases show a gradual increase in the period from 2011 to 2015. The indicators related to the time of collection at precocity and above the recommended period, as well as the presumptive diagnosis above 28 days need to be adjusted in order to comply with the recommendations recommended by the PNTN. We suggest research that identifies the determinants of obtaining universal and effective PNTN coverage in Mato Grosso do Sul.

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