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Neonatal screening - its importance and impact in Latin America

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ABSTRACT

Global research on neonatal screening began in 1962 when Dr. Robert Guthrie[1] discovered the disease at birth and with its subsequent treatment prevented irreparable consequences; he obtained the blood sample from the heel, which has proved to be harmless, safe and easy to execute. However, it was not until the 1990´s, when some Latin American countries began their programs and research on neonatal screening. Uruguay was quick in obtaining a wide coverage, this was probably due to the small number of child births or the already available health organizational infrastructure. This is a report about the development of neonatal screening programs based on the disease prevalence and methodologies used in Latin American countries.

BACKGROUND

The neonatal research program began in Uruguay following an increase in the incidence of congenital hypothyroidism (HC) in 1990. Since 1994, screening for the condition is mandatory by examination of thyroid stimulating hormone (TSH) in cord blood. At that time, VDRL examination to detect congenital syphilis was compulsory as per the national Decree 183/94. In 2007, due to comprehensive reforms of the integrated national health system (SNIS) as dictated by the national decree 416/07, screening for phenylketonuria (PKU), by testing for phenylalanine, and congenital adrenal hyperplasia (HSC) by testing for 17-OH-Progesterone, was made compulsory. Subsequently, in 2009, following successful lobbying by the Society of parents of children with Cystic Fibrosis (CF), the national Decrees 677/09 made research on CF and screening for hearing defects mandatory. In 2011, following another national decree, acetyl CoA of medium-chain (MCAD) deficiency was also examined as part of the mandatory screening. The Ministry of Public Health Laboratory of the Bank of Social Security (BPS) was then the reference laboratory and the only laboratory responsible for Neonatal screening in the country. In 2013, the study of Hemoglobinopathies began using high-resolution liquid chromatography technique (HPLC) and a large number of carriers of mutations of the globin chains and sick children were identified.[2,3,4,5,6,7]

In Costa Rica the newborn screening program started by examining congenital hypothyroidism and phenylketonuria (PKU) in 1991, the program now screens for 29 conditions. In 2007, 98% of newborns were screened, this improved slightly (98.3%) by 2014.

In Mexico, Dr. Antonio Velázquez after completing his training in PKU with Dr. Guthrie in 1974, initiated the implementation of a national program. But it took till 1988 for the mandatory screening of HC to become a national standard.[8,9,10,11]

In Brazil, Dr. Benjamin Smith initiated research on PKU and HC in 1976 in the private sector. The initiative was supported by different philanthropic organizations and those dealing with disabled children. Eventually a federal law in 2001 gave the legal background for setting up the newborn screening program on a national level.

The Dominican Republic commenced its national neonatal screening program as recently as 2014.

MATERIAL AND METHODS

In Uruguay, the blood sample for the newborn screening program is collected from the heel. The sampling is done 40 hours after birth and the samples are analyzed at a centralized laboratory. The number of newborns in Uruguay is less than 50 000 annually, and more that 90% of the newborns are discharged within 48 hours. Sampling as such is done prior to discharge and this ensure a nearly 100% coverage. Sampling and documentation is the task of the staff at the maternity ward. The blood sample is allowed to dry for 4 hours before being mailed by certified post.

The following are techniques most used in the programs of Neonatal screening in Uruguay, Costa Rica, Mexico, Brazil and the Dominican Republic.

In Uruguay, the most common methods used are:

- Stimulating Thyroid Hormone (TSH): Currently serum samples are processed using a Roche Elecsys; blood samples are taken in a filter paper using the Bio Rad technique, but in the near future this will be done with Autodelfia.
- Phenylalanine: Since 2008, mass spectrometry is used for the test of phenylalanine and tyrosine.

- 17-OH-Progesterone: It is quantified by the Bio Rad ELISA method.
- Trypsin Immunoreactive: Currently, the Neonatal AutoDelfia Fluoroimmunoassay of Perkin Elmer is being used. Results that are above the cutoff point of 65 ng/mL are thought to be due to pancreatitis (PAP). For a confirmatory method the sweat test is done by the method of Clark Collip.
- Acetyl CoA Dehydrogenase chain Media (MCADD) deficiency: Acylcarnitine C8/C2, C8, C10, C6 is measured by spectrometry of atomic absorption by a 3000 API; confirmation is done by dosing the organic acids in urine by GC mass with an instrument from Agilent.

In Costa Rica, the methods mostly used are: mass-spectrometry, HPLC, immunofluorometry, fluorimetry. For confirmatory studies they use GC mass spectrometry and genome sequencing. The health care system supports and has established protocols for treatment of detected cases.

In Mexico, the methodologies employed primarily are: ELISA, absorption spectra and isoelectrofocusing.

In Brazil, these same methodologies are used, and in some states they are complemented by mass spectrometry.

Table 1 Number of positive cases of neonatal screening in Uruguay from 2009 to 2013

Diagnosis	Number of cases
Congenital Hypothyroidism [CH]	78
Congenital Adrenal Hyperplasia (CAH)	29
Cystic Fibrosis (CF)	29
Classic phenylketonuria (PKU)	13
Hyperphenylalaninaemia (HPA)	12
Medium-chain acyl-CoA dehydrogenase deficiency (MCAD)	5
Methylmalonic acidemia (methylmalonyl-CoA mutase, MUT)	2
Hemoglobinopathies (Hbp)	5
BH4 deficiency	1
Citrullinemia, type I (CIT)	1
3-Methylcrotonyl-CoA carboxylase deficiency (3-MCC)	1
Maternal B12 deficiency	4
Cobalamine Deficiency (CblCD)	1
Carnitine Deficiency (CUD)	1

RESULTS

Results from Uruguay between 2009 and 2013 are shown in Table 1.

The techniques used to investigate and confirm the diagnoses by the Neonatal screening laboratory testing in Uruguay are shown in Table 2, while those used in Costa Rica are illustrated in Table 3.

In Mexico, Marcela Vela-Amieva´s[10] group reported great variability of diseases that are included in the screening programs, it ranges from 1 up to 60 diseases. Additionally they also report a great variation in the methodology. This is due to the difference in protocols used by each health care institution, e.g., the Secretariat of health and assistance (SSA) only studies HC; HC and PKU are studied at the Mexican Social Security Institute (IMSS) and in some other clinics HSC is also diagnosed in addition; HC and PKU is done in some clinics at the Institute of security and social services for State employees (ISSSTE); in others, such as Petróleos Mexicanos (PEMEX) tests are conducted mainly to diagnose HC, Cystic Fibrosis, PKU, hemoglobinopathies, defects in the beta oxidation, galactosemia, the organic acidemias, and toxoplasmosis.

In Brazil, the following diseases are diagnosed in children: HC, PKU, Hb (SCD), CF, HSC, and biotinidase deficiency. These programs also comprise of detection and treatment of children who are carriers of the disease. It is important to note

Table 2 Neonatal screening panel in Uruguay

Disease	Metabolite	Screening technique	Verification	Verification technique
CH	TSH	Fluoroimmunometric in whole blood in paper, ECLIA in serum	T4	ECLIA
PKU	Phenylalanine	Mass	Tyrosine, F/T	Mass
CAH	17-OHP	Fluoroimmunometric	Na, K, cortisol, aldosterone	Ion selective electrode. Other derivated hormones
CF	TIR, PAP	Fluoroimmunometric ELISA	Sweat test	Clark Collin Method, dosifying Cl
Hemoglobinopathies	Hb A, A2, F, C, S	HPLC	BC and IEF	Isoelectrofocusing
Aminoacidopathies	AmAc	Mass	Aminoacids	HPLC
Organic acidemias	Related Carnitines	Mass	Organic acids	MGC
BH4 deficiency disorders	Related Carnitines	Mass	Organic acids	MGC

Table 3 Screening panel in Costa Rica

Endocrinopathies	CH, CAH
Aminoacidopathies	Galactosemia, PKU, MSUD, Citrullinemia, Argininemia, Homocystinuria, Tyrosinemia
Beta oxidation disorders	short chain SCAD, MCAD medium, long VLAD, Multiple dehydrogenase deficiency (GTAII), Carnitine, palmitoyl transferase deficiency (GPTII)
Organic acidemias	Isovaleric acidemia (IVA), Propionic Acid (PA), Methylmalonic Acid (AM), Hydroxymethylglutaryl CoA lyase deficiency (HMG), Methylcrotonyl CoA carboxylase deficiency (MCC), Glutaric acidemia type I (GAI), Beta ketothiolase deficiency (BKT), Multiple carboxylase deficiency (MCD)
Cystic Fibrosis	alpha-thalassemias, ßeta-thalassemia (Hb S/ßTh), Hb C, Hb S, Hb E, Hb D

that in 2013, the total number of children studied were 2.463.518, i.e, 84.9% of the newborns.

In the Dominican Republic, research programs will begin this year, and they include the following diagnoses HC, GAL, PKU, Hbs, G6DPD.

CONCLUSIONS

Several countries have implemented public child protection policies and promote programs for prevention of childhood diseases; Uruguay is a good example, being a country of low birth rate (about 48 000 births/year) where every child is a valuable capital for the country's future. All Latin American countries have followed suit and have introduced newborn screening programs. The utility of the program can be measured in terms of disease prevention and treatment, and the reduction in the burden to the social and health care system.

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