Neonatal Screening for Congenital Hypothyroidism in Nicaragua: Audit of a Cord-blood Thyrotropin-based Program (2005-2015)

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Abstract

The aim of this study is to evaluate the Nicaraguan screening program for congenital hypothyroidism in terms of coverage and effectiveness of detection and confirmation of cases with the condition throughout a decade. Thyrotropin was quantified in cord-blood samples by a validated ELISA and a cut-off of 20 mU/l was applied. Coverage, positive predictive value, recall rate and prevalence were retrospectively analysed. Babies with positive screening results were contacted for confirmation by means of determination of thyrotropin and thyroid profile in serum samples. 272,338 babies were screened during the period 2005-2015. The mean coverage reached by the program in the participating departments was 71%, with a positive predictive value of 83% and a recall rate of 0.055%. Eighty cases of congenital hypothyroidism were identified, representing an incidence of 1 in 3229 live births, most of them (81%) being severe. The performance of the Nicaraguan screening program is comparable to those in Latin America also using cord-blood samples. The incidence of congenital hypothyroidism is within the low range of other countries worldwide. Strategies are needed to expand the program to the whole country, improve recall rates and achieve earlier treatment of babies, with the condition.

Keywords

Congenital hypothyroidism, newborn, screening, cord-blood, TSH, Nicaragua.

Introduction

Congenital hypothyroidism (CH) is the most common cause of preventable mental retardation. With the introduction of CH neonatal screening programs, babies affected with this condition are detected before the clinical manifestations are evident and irreversible. CH screening enables timely replacement with thyroxin and efficiently reduces the risk for cognitive defects [1–3].

In Latin America the first pilot neonatal screening program for CH started in Argentina in 1985, while the first nation-wide established program for CH was implemented in Cuba in 1986. CH screening programs expanded from 1985 to 2005 and reached fourteen countries of Central and South America. However, mass screening programs in Guatemala, Haiti, Honduras, El Salvador and Belize have not yet been formally implemented.[4,5] Out of 14 countries studied, only four (Cuba[6], Costa Rica[7],

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Uruguay[8] and Chile[9]) reached a coverage close to 100%. The rest had widely varying coverage rates between 1-80%[4]. However, most countries have increased their coverages over 70% in the last decade.[5,10–14]

The reported incidence of CH in Latin America ranges from 1 in 1715 newborns (a local program in Guatemala) to 1 in 3616 (Cuban nation-wide program)[4]. These discrepancies may be influenced among others by specific characteristics of the different programs (local *versus* national), heterogeneity of coverages reached in the different countries, program strategy (cord-blood *versus* heel blood after 24 h), socioeconomic status of the region and the improved sensitivity of TSH immunoassays. This has allowed many programs around the world to reduce the TSH cut-offs, making possible the identification or cases that otherwise would have been missed, therefore increasing the incidence of congenital hypothyroidism detected by these programs.[15–20]

In 2005, a pilot screening program for CH started in the Department of León, Nicaragua, as result of an international collaborative initiative. In ten years, the program expanded to reach more than the 50% of the national territory.

Figure 1. Time of participation of the Nicaraguan Departments in the Newborn Screening Program for CH. The national map reflects in shades of light grey, dark grey and black the 10 provinces departments (out of 17 in the country) which participated in the program at any time between 2005 and 2015**.**

Objective

The aim of this study is to evaluate the Nicaraguan screening program for congenital hypothyroidism in terms of coverage and effectiveness of detection and confirmation of cases with the condition throughout a decade.

Patients and Methods

Screening Program Design

The CH screening program began in the department of León, within the frame of the Program of Cooperation between the University of Alcalá and the UNAN-León, with the subsequent incorporation of different institutions from Spain (see authors' affiliations). Although the program was initially implemented in the University Hospital (HEODRA), from 2005 to 2015 nine additional departments gradually joined the screening effort, involving a maximum of 12 hospitals (Fig. 1).

Samples were collected in hospitals and other healthcare institutions for onward shipment to the centralized laboratory. Personal and clinical data from the mothers and their babies were recorded in a printed form.

Hormone Determinations

Thyrotropin (TSH) in dried blood spots. Immediately after delivery blood samples from the umbilical cord were collected and placed on Whatman 903 (Schleicher & Schuell, USA) or TFN filter paper (Muntkell, Sweden) as three circular spots. An immune-enzymatic sandwich assay (ELISA) was used for the quantification of neonatal TSH. Basically, 96-well plates (Bio-one high binding, Greiner) were coated with 100 µl/well of a monoclonal anti-TSH antibody (3 µg/ml in Tris-buffered saline, TBS) and incubated for 60 minutes at 37°C. This antibody recognises an epitope of the TSH-β subunit. After washing five times with TBS-containing 0.05% Tween (TBST) using DELFIA WASHER Disk remove (Perkin Elmer), non-specific binding was blocked by adding to each well 200 µl of TBST containing 0.5% bovine serum albumin (Sigma Aldrich). Three-millimeter diameter circles of cards containing dry standards and controls (Auto DELFIA kit of neonatal human TSH dry blood, Perkin Elmer, USA) and cord-blood samples were punched with the aid of a DBS puncher, (DELFIA Dried Blood Spot Puncher, Perkin Elmer, USA) and two of these circles were used per well. Plates were incubated 60 minutes at 37°C in 200 µl TBST-BSA per well with mild agitation. After overnight incubation at 4°C and 60 minutes at 37ºC with mild agitation they were washed with TBST five times and 100 µl of a mixture 1/1 of two biotinylated antibodies directed respectively against the α- and β-subunits of the hormone (0.5 µg/ml) were added and incubated for an additional hour at 37°C.

All the antibodies were generated in the Department of Immunology and Oncology at the Centro Nacional de Biotecnología (CNB-CSIC, Madrid, Spain). They were obtained by immunization with the whole hormone-dimer and they are TSH-specific (mean 0.1% cross-reactivity with LH, HCG and FSH).[21]

After washing, 100 µl of Streptavidin-Peroxidase Polymer Ultrasensitive (Sigma-Aldrich) diluted 1 to 16,000 in TBST

were added and plates were incubated for 1 h at 37°C. Plates were washed thereafter and 100 µl o-phenylenediamine dihydrochloride (Sigma Fast OPD, Sigma-Aldrich) were then added, and incubated for 30 min. in the dark to develop colour. Reaction was stopped by the addition of 50 µl 3N sulfuric acid. Absorbance at 405 nm was measured in a Multiskan FC microplate reader (Thermo Fisher Scientific, USA). The TSH concentration in samples was calculated automatically with internal software by means of calibration curves built with the calibrators ran in every 96 well assay. The standard curve had a dynamic range for TSH measurement between 0.7-234 mU/l. Detection and quantification limits were 1.5 and 5 mU/l, respectively. Intra- and inter-assay variations were 12 and 13%, respectively.

Serum hormones – TSH, total thyroxine (T4) and triiodothyronine (T3) – were measured using ELISA commercial kits from Human Diagnostics, Germany.

Decision Flow-chart

A general flowchart for screening and confirmation of CH cases is shown in Fig. 2. A cut-off value of 20 mU/l TSH was chosen to consider a sample positive, a threshold frequently used in programs using cord-blood samples.[6,22–24] Since a range of cut-offs from 15 to 40 mU/l TSH have been used by different programs and countries[3,8,24,25], a trial was conducted from 2009 to 2013 to define the most efficient threshold for our conditions by lowering the cut-off to 10 mU/l.

Parameters used for the confirmation of CH were: serum TSH values above 20 or 10 mU/l as stated above and/or T4 lower than 6.9 µg/dl.[26–29]. Therefore, with these hormonal limits, hyperthyrotropinemia (HT) cases were initially considered as CHs, and when the serum results were within normal values children were considered as false positive (FP).

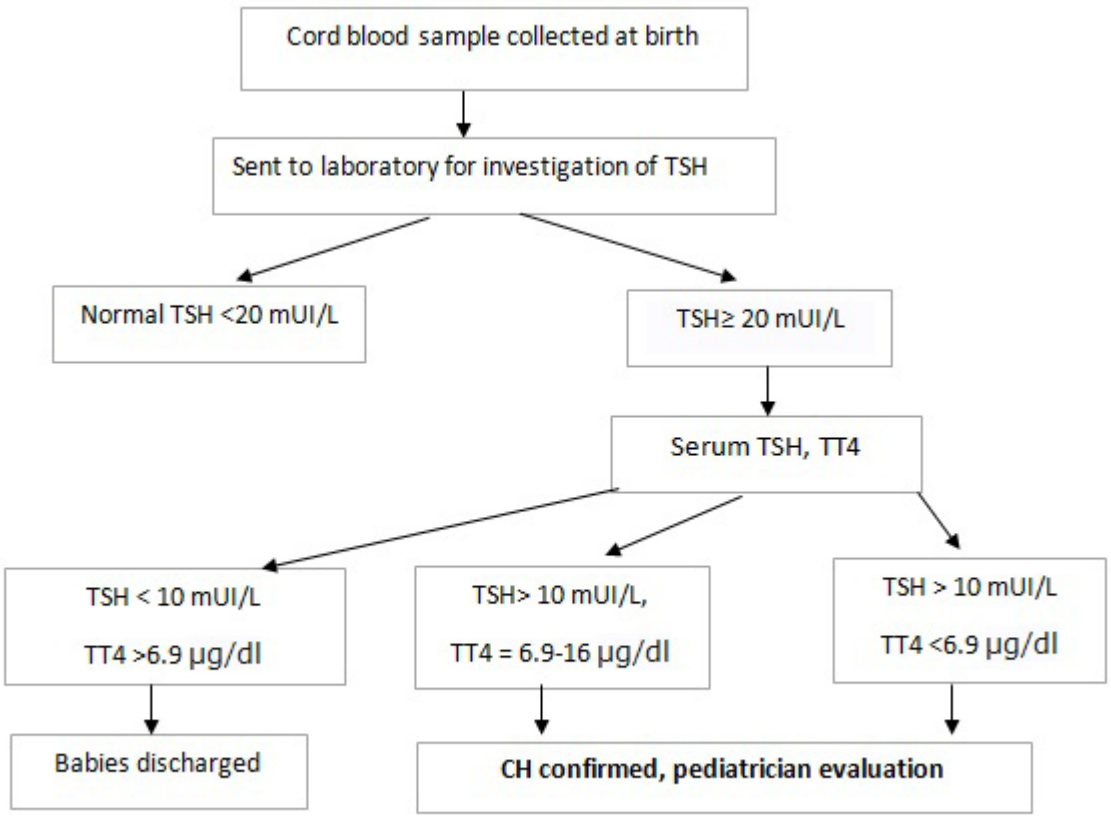

Figure 2. Flowchart for Screening of Congenital Hypothyroidism. The diagnostic of congenital hypothyroidism begins with either abnormal newborn screening test result, leading to serum thyroid function test (TSH and TT4) to confirm the diagnostic. Other diagnostic studies can be undertaken to determine the underlying etiology.

In each hospital, an appointed paediatrician in charge of CH patients started levothyroxine replacement and the clinical follow-up of the patients.

A database was created for registration of samples and screening results.

Classification of CH Severity

We followed the criteria of the European Society for Paediatric Endocrinology (ESPE)[30] based on serum T4 levels at confirmation lower than 40 nmol/l (3.13 µg/dl) to consider CH as severe.

Performance Analysis of the Nicaraguan CH Screening Program

The coverage of the program was determined by the ratio between the total number of screened babies and the total number of live births in each of the participating departments, for which national databases of the Nicaraguan Ministry of Health were consulted (www.minsa.gob.ni, www.inide.gob.ni). The incidence of CH was calculated as the ratio between the number of confirmed CH cases and the total number of newborns screened. The positive predictive value (PPV) for the program was calculated as the true positive (confirmed) CH patients divided by the total number of children retested for confirmation. Both the PPV and the false positive rate (FPR) evaluate the efficiency of mass screening program.

Statistical Analysis.

The data were processed using Microsoft Excel for Windows 10. Significance was defined at $p<0.05$.

Results

Coverage and positive predictive value of the program and incidence of CH in Nicaragua.

The demographic characteristics of the population covered by the Nicaraguan neonatal screening program for CH and the results of the program are summarized in Table 1. In its first year the program covered only the department of León. Subsequently, other departments, mainly on the Pacific Coast and Central regions, the most densely populated areas, joined the program (Fig 1).

272,338 infants were screened for CH in 10 years, representing an average coverage of 71% of life births in the participating departments (Table 1). The coverage in the participating departments gradually increased from the initial 55% to a maximum of 87% at its best performance. However, when the total number of children born in the country is considered, only 18% benefited from screening during 2005-2015.

In the study period, 153 children were positive (TSH in cord blood ≥ 20 mU/l), representing an average 0.06% of all samples analysed (samples received minus inadequate ones). Such percentage of positive children progressively decreased from 0.3% to 0.04% in those years in which more than 25,000 samples were analysed (Table 1).

Inadequately collected blood samples, unsuitable for TSH determination, accounted for a 5.9% in average. Experience gained in blood sampling lead to a decrease from the initial 12.5% to the 2.3% more recently (Table 1).

All the positive children at screening (153) were called for retesting representing an average rate of 0.055 %, a value in accord with international standards for TSH based screenings.[31] From these babies only 96 were retested for confirmation of the condition, giving a mean recall success rate of 68%, as defined by the percentage of children that underwent retesting versus those positive at screening.

Finally, 80 out of 96 children retested were confirmed for CH or HT, showing decreased total T4 and elevated TSH or isolated TSH elevation, respectively (Table 1). These data indicate an estimated incidence of CH in Nicaragua of 1 in 3229 live births, a figure in accordance with CH incidence in other Latin American countries, a PPV of 83% and a relatively low FP (16/96, 17%) (Table 1). This relatively low incidence of CH may be influenced by the low rate of children brought to the laboratory to be retested, since the estimation is based on the children who were actually retested.

Sensitivity of the screening program for moderate CH cases

In CH screening programs based on the analysis of cord-blood samples are obtained when TSH is physiologically increased.[3,29,32] This implies a limitation to establish the most efficient cut-off TSH level, balancing the highest CH identification rate possible without the cost of excessive FP.[6]

To investigate the sensitivity of the Nicaraguan program towards cases of moderate hypothyroidism, including HT, we categorized the positive children at screening in three groups: those ranging from 20 to 39 mU/l TSH, those with TSH between 40-100 mU/l and those exceeding 100 mU/l TSH (Table 2). Then, we retrospectively analysed the percentage of children confirmed with serum hormone tests as CH (elevated TSH, low T4), HT (elevated TSH, normal T4) or FP (normal TSH and T4) in each category (Table 2). From 96 children retested in serum, 71% had CH, 13% showed HT and 17% were FP (Table 2). Most babies retested (95%) had shown cord-blood TSH levels higher than 40 mU/l, while only 29% of those with screening TSH of 20-39 kept their appointments for serum retesting (Fig. 3), concentrating the lowest recall rate in the series.

Twelve babies with congenital HT were detected by the program (Table 2). Most HT cases (68%) were found from the group with cord-blood TSH levels between 20-39 mU/l, but not exclusively. Four cases with HT were detected with cord-blood TSHs levels above 40 mU/l. Remarkably, one case with very

Table 1. Characteristics and results of the neonatal screening for CH in Nicaragua over a period of 10 years (2005-2015).
[1]Number of Nicaraguan Departments participating in the screening program per year. [2]Inadequate samples for TSH determination due to faulty technique in cord-blood sample collection. [3]The percentage of children retested in serum for CH confirmation does not consider children who died before serum confirmation test. [4]Children deceased before recall for serum confirmation of CH.

Year	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015	Total (average)
DEMOGRAPHY Deparments[1]	1	3	5	9	10	10	9	6	6	6	6	6.5
Live births	3.761	7.876	21.287	43.310	48.311	44.383	49.844	44.093	38.990	40.713	40.085	382.653
Screened Children	2.081	4.761	14.822	29.455	39.305	38.791	33.939	30.833	25.931	26.495	25.925	272,338
Coverage (%)	(55)	(60)	(70)	(68)	(81)	(87)	(68)	(70)	(67)	(65)	(65)	(71)
SCREENING Positive children	4	13	8	13	21	17	12	12	21	21	11	153
Positive children (%)	(0.2)	(0.3)	(0.1)	(0.04)	(0.1)	(0.04)	(0.04)	(0.04)	(0.1)	(0.1)	(0.04)	(0.06)
Inadequate samples[2]	261	269	1312	2.076	2.357	2.078	937	1.367	795	620	1.925	13997
Inadequate samples (%)	12.5	5.7	8.8	7	5.9	5.4	2.8	4.4	3.1	2.3	7.4	(5.9)
CONFIRMATION Retested Children	3	7	6	8	9	12	6	5	15	16	9	96
Recall success rate (%)[3]	(75)	(54)	(86)	(62)	(45)	(75)	(75)	(45)	(83)	(76)	(90)	(68)
Deceased Children[4]	-	-	1	-	1	1	4	1	3	-	1	12
Children with HC	3	7	6	8	7	9	5	3	12	14	6	80
False positives	-	-	-	-	2	3	1	2	3	2	3	16
False positives (%)	-	-	-	-	22	25	16	40	20	12.5	33	(16.9)

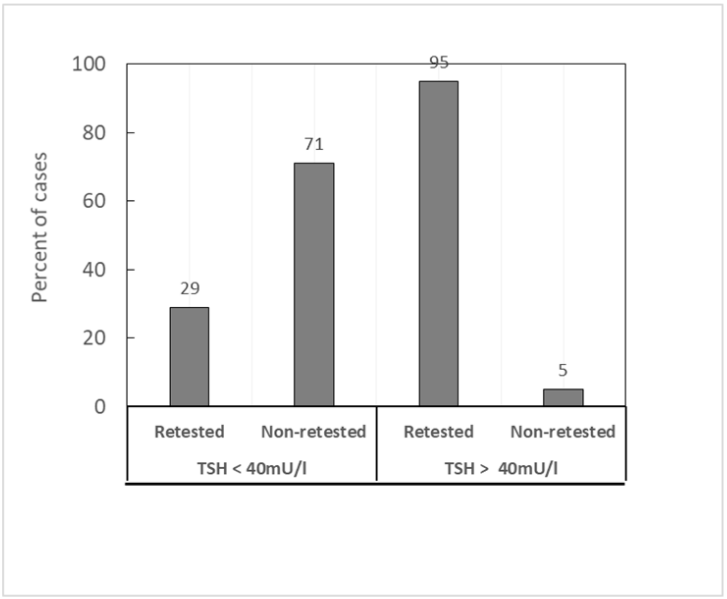

Figure 3. Percentages of retested and non-retested children who were positive at CH screening, considering their cord-blood TSH level (less than 40 mu/L or above 40 mU/l).

Table 2. Thyroid hormone profile of neonates positive at the CH screening, categorized in 3 groups depending on cord-blood TSH levels: 20-40, 40-100 and >100 mU/l, respectively. CH, congenital hypothyroidism; HT, hyperthyrotropinemia; FP, false positive; cbTSH, TSH from cord blood (eluate from filter-paper spots); sTSH: serum TSH at recall; T4, serum total thyroxine at recall; T3, serum total tri-iodothyronine at recall. M mean; SD, standard deviation; n, number of individuals. For reference ranges of serum TSH, T4 and T3 (see references 26-29). Reference ranges for sTSH: < 10 mU/l (Hubner et al 2002; Djemli et al., 2004; Kapelari et al., 2008, Rastogi & LaFranchi 2010), and <7 mU/l between 1-12 months of life (Hubner et al 2002; Djemli et al., 2004; Kapelari et al., 2008) for T4: 6.9-16 µg /dl (Rastogi & LaFranchi, 2010) and for T3: 0.9-2.4 ng/ml (Djemli et al 2004).

CH NEWBORN SCREENING (2005-2015) (cut-off 20 mU/l TSH)													
		TSH 20-39 mU/l				**TSH 40-100 mU/l**				**TSH > 100 mU/l**			**Total**
Positive samples		n= 75				n= 41				n= 37			153
Retested children		n= 22				n=39				n= 35			**96**
Recall rate		29 %				95 %				95 %			
		M	***SD***	***Range***	***n***	***M***	***SD***	***Range***	***n***	***M***	***SD***	***Range***	***n***	
CH	**cbTSH *mU/l***	28.5	3.5	25-34	5	67	19	40-98	30	158	44.0	102-239	33	**68** (70.8%)
	sTSH *mU/l*	354	374.4	4.9-750	3	535	482	1.5-1400	20	546.7	646.9	64-2775	21	
	T4 µg/dl	1.9	2.5	0.4-4.8	3	0.6	0.4	0.4-1.7	20	0.4	0.2	0.1-1	20	
	T3 *ng/ml*	0.6	0.6	0.2-1.3	3	0.5	0.5	0.17-1.8	20	0.4	0.5	0.2-0.8	20	
		n=5 (7.4 %)				**n=30 (44.1 %)**				**n=33 (48.5 %)**			
		M	***SD***	***Range***	***n***	***M***	***SD***	***Range***	***n***	***M***	***SD***	***Range***	***n***	
HT	**cbTSH *mU/l***	24.9	3.0	22-29	8	6.7	2.2	52-100	3	280	-	-	1	**12** (12,5%)
	sTSH *mU/l*	15.6	5.1	10-25	8	19.3	9.2	14-30	3	26	-	-	1	
	T4 µg/dl	10.2	2.1	8.3-13.6	6	9.8	3.2	7.5-12	2	9.3	-	-	1	
	T3 *ng/ml*	1.9	0.3	1.6-2.3	5	1.6	1.0	0.9-2.3	2	-	-	-	-	
		n=8 (66.7%)				***n=3 (25%)***				***n=1 (8.3%)***			
		M	***SD***	***Range***	***n***	***M***	***SD***	***Range***	***n***	***M***	***SD***	***Range***	***n***	
FP	**cbTSH *mU/l***	26.8	5.0	20-34	9	56	22.4	44-100	6	253	-	-	1	**16** (16.7%)
	sTSH *mU/l*	3.4	1.4	2.2-6.5	9	3.0	0.9	2.0-3.5	6	4.9	-	-	1	
	T4 µg/dl	10.9	1.8	8.6-13	6	10.1	2.5	8.6-13	3	13	-	-	1	
	T3 *ng/ml*	2.0	0.3	1.7-2.4	7	1.6	0.3	1.4-1.9	3	1.2	-	-	1	
		n=9 (56.25%)				**n=6 (37.5%)**				**n=1 (6.25%)**			

high cord-blood TSH (280 mU/l) evolved into mild euthyroid HT (TSH = 26 mU/l and T4 = 9.3 µg/*dl* in serum) (Table 2).

Most FP cases showed neonatal TSH lower than 40 mU/l (56%), but a substantial 37% had cord-blood TSH above 40 mU/l.

According to ESPE criteria for categorization of CH severity (see Methods), the vast majority of patients (81%) detected by the Nicaraguan screening program are severely hypothyroid. Indeed, the correlation study between the cord-blood or serum TSHs values and the serum T4 shows that, for a wide range of TSH levels, many positive children with CH had nearly undetectable T4 with a significantly higher correlation with serum than cord-blood TSH (Fig. 4 A, C). In the case of T3, almost undetectable values were also present, with a significant correlation similar with both serum and cord-blood TSH (Fig. 4 B, D), although the low values of T4 and T3 could also be influenced by the excessive time elapsed between positivity at screening and serum hormone retesting.

In an attempt to increase the detection rate for moderate/mild cases of hypothyroidism, a four-year trial (2009-2013) was implemented by lowering the cut-off TSH value to 10 mU/l. Table 3 shows the results of this trial, comparing the percentage of children with CH and HT and the FP identified within 3 different group of babies having, respectively, TSH cord-blood levels of 10-19, 20-40 and above 40 mU/l TSH at screening. Babies screened with neonatal TSH of 10-20 mU/l, were all FP cases (Table 3). Therefore, lowering the cut-off TSH from 20 to 10 mU/l TSH did not improve the detection rate of patients with neither CH nor HT, while worsening the PPV of the program from 83% to 62.5%. These results indicate that the initially used cut-off of 20 mU/l is the most convenient threshold for the cord-blood

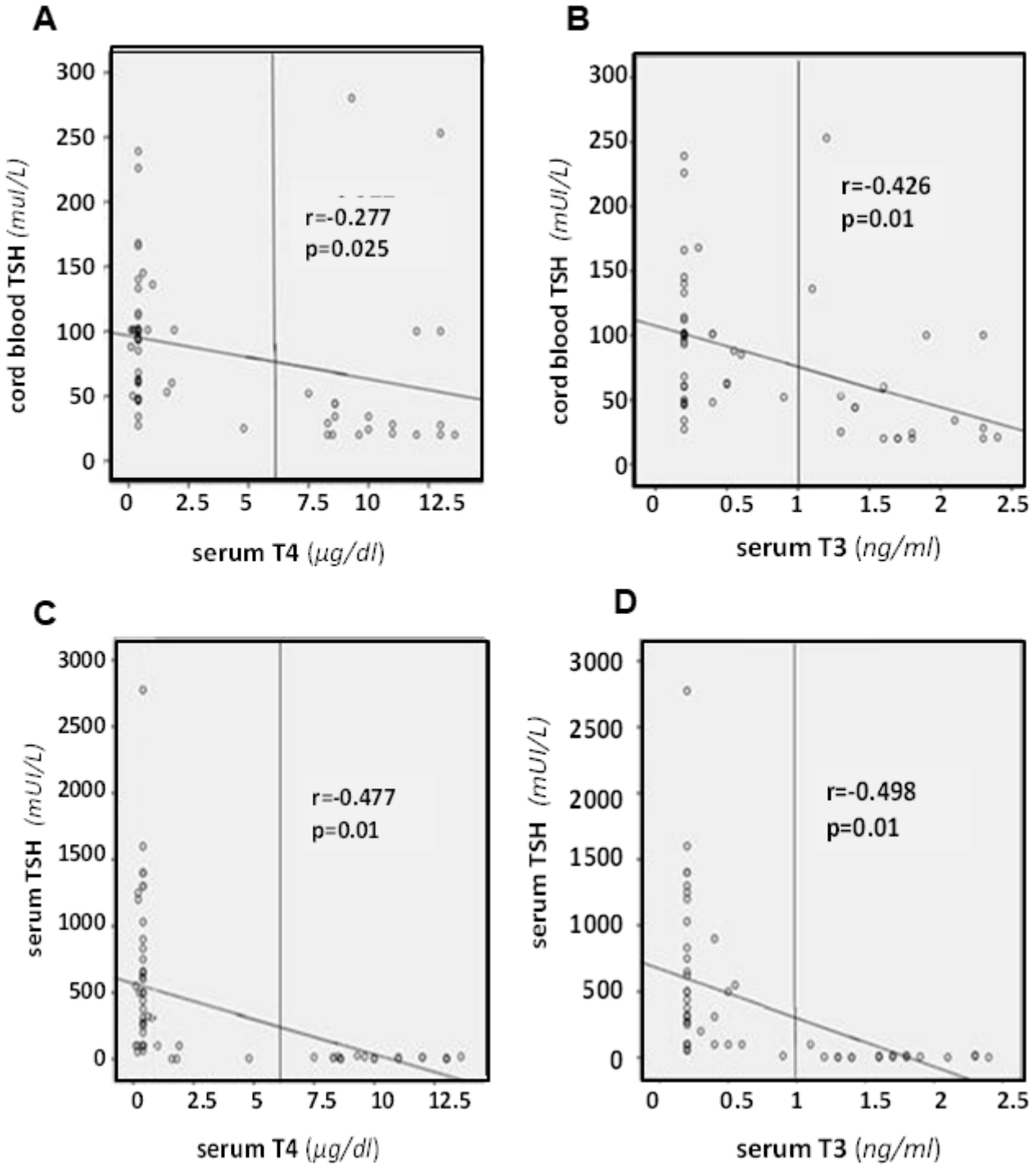

Figure 4. Correlations between cord blood TSH at CH screening, or serum TSH at CH confirmation and thyroid hormones, T4 or T3 at CH confirmation. **A**. Correlation between cord blood TSH and serum T4 (p= 0.025, r= -0.277) **B.** Correlation between cord blood TSH and serum T3 (p= 0.01, r= -0.426). **C.** Correlation between serum TSH and T4 (p= 0.01, r= -0.477). **D**. Correlation between serum TSH and T3 (p= 0.01, r= -0.498).

Table 3. Thyroid hormone profile of positive children at the Nicaraguan CH screening trial (2009-2013) categorized in 3 groups depending on cord blood TSH levels: 10-20, 20-40 and >40 mU/l, respectively. CH, congenital hypothyroidism; HT, hyperthyrotropinemia; FP, false positives. cbTSH, TSH from cord blood (eluate from filter-paper spots); sTSH: serum TSH at recall; T4, serum total thyroxine at recall; T3, serum total tri-iodothyronine at recall. M, mean; SD, standard deviation; n, number of individuals. For reference ranges of serum TSH, T4 and T3 (see references 26-29). Reference ranges for sTSH: < 10 mU/l (Hubner et al 2002; Djemli et al., 2004; Kapelari et al., 2008, Rastogi & LaFranchi 2010), and <7 mU/l between 1-12 months of life (Hubner et al 2002; Djemli et al., 2004; Kapelari et al., 2008) for T4: 6.9-16 µg /dl (Rastogi & LaFranchi , 2010) and for T3: 0.9-2.4 ng/ml (Djemli et al 2004).

CH NEWBORN SCRRENING TRIAL (2009-2013) (cut-off point 10 mU /l)													
		TSH 10-20 mU/l				**TSH 20-40 mU/l**				**TSH > 40 mU/l**			**Total**
Positive samples		115				38				40			193
Retested children		32				17				37			86
Recall rate		27.8 %				44.5 %				92.5 %			
		M	***SD***	***Range***	***n***	***M***	***SD***	***Range***	***n***	***M***	***SD***	***Range***	***n***	
CH	**cbTSH *mU/l***	-	-	-	0	29.4	3.4	26.5-34	4	118.4	58.5	46-239	32	**36 (42%)**
	sTSH *mU/l*	-	-	-	-	299.5	321	50-750	4	286.9	333.8	54-1400	32	
	T4 µg/*dl*	-	-	-	-	0.4	-	0.4	2	0.4	0.2	0.1-1.0	22	
	T3 *ng/ml*	-	-	-	-	0.2	-	0.2	2	0.3	0.1	0.1-0.6	21	
		n=0				**n=4 (11%)**				**n=32 (89%)**				
		M	***SD***	***Range***	***n***	***M***	***SD***	***Range***	***n***	***M***	***SD***	***Range***	***n***	
HT	**cbTSH *mU/l***	-	-	-	0	21.1	3.0	20-28	7	77	32.5	54-100	2	**9 (10.5%)**
	sTSH *mU/l*	-	-	-	-	16.4	4.1	10-22	7	22	11.3	14-30	2	
	T4 µg/*dl*	-	-	-	-	10.1	2.1	8.3-13.6	6	12		-	1	
	T3 *ng/ml*	-	-	-	-	1.9	0.3	1.7-2.3	4	2.3		-	1	
		n=0				**n=7 (78%)**				**n=2 (22%)**				
		M	***SD***	***Range***	***n***	***M***	***SD***	***Range***	***n***	***M***	***SD***	***Range***	***n***	
FP	**cbTSH *mU/l***	14	2.4	10-18	32	24.8	5.4	20-34	6	113.7	120.7	44-253	3	**41 (47.5%)**
	sTSH *mU/l*	4.0	1.6	1.4-8.3	32	2.2	1.9	2.2-6.8	6	3.5	2.1	2-4.9	3	
	T4 µg/*dl*	12.5	1.1	11-15	32	11. 9	2.2	8.6-14	5	10.8	13	8.6-13	2	
	T3 *ng/ml*	1.8	0.4	1.2-2.6	32	2.2	0.3	1.7-2.4	5	1.3	0.1	1.2-1.4	2	
		n=32 (78%)				**n=6 (14,6%)**				**n=3 (7.3%)**				

TSH-based CH screening in the Nicaraguan circumstances. This is in agreement with other efficiency trials performed in other countries using cord-blood for screening.[6,23].

Finally, the lapse between the birth date and the initiation of L-thyroxine treatment in CH positive children was lower than 15 days in 15% of cases, 15-30 days in 10% and above 30 days in 75% of children. The average start of treatment was 57±34 days from birth, ranging from 10 to 120 days.

Discussion

Nicaragua is a developing country with a population of 6 million inhabitants and with one of the lowest per capita gross domestic income in America.[33] A regional screening program for CH was launched in 2005, as result of an international collaboration. In our knowledge, such a non-profit volunteering international initiative was unique. The program expanded from one to ten departments of the country (from a total of 17) in its ten years of existence. The number of neonates screened for CH increased approximately 15-fold in this period. The program reached a mean coverage of 71% of babies born in the participating departments, evolving from the initial 55% to a maximum of 87%. This is remarkable for a non-governmental program not benefitting from the logistic and economic support of a national health system. So far, in Latin America only countries with long-standing screening programs of more than 20 years of experience (Cuba, Costa Rica, Uruguay and Chile) have succeeded to reach coverage rates over 95%.[5]

One hundred and fifty-three children were detected as suspicious for CH (total positive children at screening) in 10 years. Of them 96 were retested (recall rate 68%, this percentage of children retested in serum for CH confirmation does not consider children who died before serum confirmation test) and 80 were confirmed as CH positive while 16 were euthyroid. The rate of recall was low due to several factors: the lack of official institutionalization of the program, the fact that children who are slightly affected do not attend the reevaluation, probably due to the erroneous perception of absence of illness by their relatives. The family economic situation is also a factor that limits the affordability of the displacement for retesting.

The PPV of the program is 83% a figure close to those of the CH programs in Mexico (75%)[25], Paraguay (87%)[34] and Argentina (88%)[35]. The FP was 16.7%. We hypothesized that this efficient FP could be influenced by the relatively low recall rate achieved. Therefore, we analyzed whether the children that actually attended the call from the central laboratory to be retested could be biased towards the most severe cases, as judged by their high TSH levels in cord-blood. Indeed, we showed that the mean neonatal TSH of children who did not attend the call for retesting ("no-retested" babies) was significantly lower than the TSH of children retested (Fig. 3). This suggests that the relatives of the children with mild hypothyroidism did not observe any clinical sign and subsequently disregarded the recall.

The incidence of CH, including congenital HT, in Nicaragua is therefore estimated as 1 in 3,229 newborns. We must point out that this incidence does not take into account the samples identified as inadequate from the total of screened children (Table 1), this incidence being within the range of incidences reported in other Latin American countries, either using heel blood (1:3670 in Brazil[36]) or cord-blood screening (1:3616 in Cuba[4] to 1:2313 in Uruguay[37]) .

The incidence found may be influenced by two unavoidable factors: low return rate of babies for CH confirmation (incidence can only be calculated over confirmed cases, in serum) and the high rate of inadequate blood samples at initial years of the program. If we suppose that the incidence of CH is the same in the non-retested group than in the retested group, the total number of children with CH would have been 127, resulting in a theoretical incidence of 1 in 2023 neonates. The wide variation of incidences through the region, as for the rest of the world, can be influenced by technical issues, but also by ethnic (genetic) backgrounds as well as by environmental conditions like iodine deficiency[2] . Another fact emerging for the audit of the Nicaraguan program is that most of the babies identified were severely hypothyroid as judged by their T4 levels at confirmation, according to the categorization of CH severity by the ESPE guidelines[30]. Nevertheless, the low T4 values could be the consequence of the excessive elapsed time between delivery and hormone retesting in serum. Both the low CH incidence found, and the high proportion of severely hypothyroid babies identified led to the hypothesis that 20 mU/l TSH could be a relatively high cut-off for the detection of babies with moderate hypothyroidism. However, a 4-year trial lowering the TSH cut-off to 10 mU/l did not improve the detection rate of patients, neither with CH nor with HT, while it worsened the PPV from 83% to 62.5 %. Nevertheless, the fact that when using this cut-off only 28% of the children come to the hormone evaluation in plasma, does not allow to claim the inexistence of HC in the 72% lost for confirmation.

Taking into account that the PPV worsened, we suggest that the cut-off of 20 mU/l is the most efficient threshold for the cord-blood TSH-based CH screening Nicaraguan strategy. This is the most widely used cut-off in cord-blood based screening programs worldwide[6,23,24,32]. Our study indicates a possible limitation of using cord-blood (collected at birth) with respect to using heel-blood (collected after 24 h) when attempting to increase the detection of moderate and mild cases of congenital hypothyroidism, since heel-blood based programs truly achieved such goal by lowering cut-offs from 20 to 10-12 mU/l TSH. [2,36,38]

Finally, the Nicaraguan screening program has some obvious limitations in its development and faces clear challenges for the future. The mean sample rejection rate in the Nicaraguan program is still high (5.9%), reflecting the need of regular training of the personnel involved in sample collection[3]. Nevertheless, this challenge represents a straightforward opportunity to increase the coverage of the program in the future. The recall rate of positive children at screening is also low, although similar to those found in countries with poor socioeconomic status, deficient transport and health care infrastructures, and population living in rural and remote areas (43.1%).[5] The lapse between the birth date and the initiation of L-thyroxine treatment in confirmed CH children was high compared to other Latin America countries.[39,40].

Conclusions

Performance of the Nicaraguan CH screening program is comparable to those in Latin America also using cord-blood sampling in terms of coverage, detection effectiveness and incidence. The future screening efforts should concentrate in expanding the coverage of the screening to the whole country since only the 18.1% of babies benefited from screening along the 10-year program´s existence, increasing the recall rate of positive babies and reducing the lapse between diagnosis and treatment of CH.

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Ethics Approval and Consent to Participate

All procedures were in accordance with the ethical standards of the Ethical Committee for Biomedical Research of the Faculty of Medical Sciences of UNAN-León and with the Helsinki Declaration of 1975, as revised in 2004. Informed consent was obtained from mothers to take cord-blood to perform the early diagnosis screening.

Authors' Contributions

Fúnez A: Data collection analysis and interpretation, drafting the article, final approval of the version to be published.
Lara ME: Design of the work and data collection (responsible for diagnosis of congenital hypothyroidism).
Chévez AC: Data analysis (responsible for evaluation of the thyroid profile in serum).
Castellón EA: Design of study and data collection (coordinator of activities for the neonatal screening program for congenital hypothyroidism).
Perán S: Design of the study and critical revision of the article.
Toro MJ: Design of the study, critical revision of the article.
Montoya E: Design of the study, technical optimization of screening procedure and critical revision of the article and final approval of the version to be published.
Moreno JC: Design of the study, analysis of data, writing of the article and final approval of the version to be published.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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