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NEWBORN SCREENING PROGRAM FOR CONGENITAL HYPOTHYROIDISM IN MONTENEGRO

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Objective – The aim of this study was to assess the prevalence of congenital hypothyroidism (CH) over the last 5 years and analyze and summarize the status of newborn screening in Montenegro. **Materials and methods** – This is a population-based retrospective study. Blood samples were collected from the heels of newborns 48 – 96 hours after birth and thyroid-stimulating hormone (TSH) was determined. The cut off value in our laboratory was 10 mIU/l in whole blood. Dissociation-enhanced lanthanide fluorescent immunoassay (DELFIA) was used for detection. **Results** – Over the period January 2008 – December 2012, a total of 40,758 newborns were screened and 17 cases were confirmed as CH - both transient and permanent. The program covered all live born infants (100%). Recall rate was 0.97 – 1.36%. Mean age of starting treatment was 21.4±6.4 days. **Conclusion** – During the period of analysis in our study, 17 cases of CH were detected. The overall prevalence of CH was 1 in 2397 live births. This is the first report on newborn screening for congenital hypothyroidism in Montenegro.

Key words: Congenital hypothyroidism ▪ Neonatal screening ▪ Montenegro.

Introduction

Screening for congenital metabolic diseases allows early detection of a disease before the symptoms appear, as well as the early introduction of therapy for prevention and relieving symptoms of the disease. Neonatal screening has been introduced in many countries, and in the last several years it has been improved by the use of tandem mass spectrometry, which allows the identification of over 50 hereditary metabolic diseases from several drops of blood (1).

Montenegro is a Southeast European country with a population of 625,266. Neonatal screening for congenital hypothyroidism (CH) was launched in March 2007 in the maternity clinics of the municipal hospitals of Podgorica, Kotor, Bijelo Polje and Berane. After two months, the screening program also covered the maternity clinics of the 9 remaining towns: Pljevlja, Mojkovac, Rožaje, Plav, Nikšić, Cetinje, Meljine, Bar and Ulcinj, as well as newborns from the Neonatology Center of the Institute for Sick Children of the Clinical Center of Montenegro.

The introduction of screening was selflessly supported by the knowledge and experience of many colleagues from the region, namely: Prof Dragan Katanić and Prof Jovan Vlaški from Novi Sad, Prof Husref Tahirović from Tuzla, Tatjana Milenković MD, PhD, Prof Slobodan Radmanović and Dr Tašić Svetlana from Belgrade, Prof Mirjana Kočova from Skopje and Prof Ciril Kržišnik from Ljubljana.

The goal of this study was the assessment of the status of neonatal screening in Montenegro.

Method

Sampling

Blood is taken by heel prick between 48-96 hours of age and applied to special filter cards. The cards are dried horizontally and kept at a temperature of 2 - 8°C until they are transported to the Clinical Center of Montenegro within 1-7 days after sampling. The cards contain information on birth date, sampling date, sex, possible therapy of a newborn or mother, address and contact phone number. In the Center for Clinical-Laboratory Diagnostics, the samples are kept at 2-8°C until the thyroid-stimulating hormone (TSH) testing, 1 to 4 days after the arrival (2).

Laboratory screening methods

Detection of blood TSH is used as the primary screening test in Montenegro. TSH concentrations are measured on a dried blood spot specimen using a commercially available fluoroimmunoassay DELFIA Neonatal hTSH kit (Wallac Oy, Turku). The inter-assay coefficient of variation for the evaluated period is between 3.78% and 4.38%. The cut off value for neonatal TSH is ≥10 mIU/l in whole blood. Samples with TSH values ≥10 mIU/l are assayed in triplicates, otherwise assays are performed once.

Clinical management of infants with elevated TSH includes the following: a complete history, including prenatal thyroid status (maternal drugs and medications) and physical examination. Confirmatory tests are based on serum TSH and free thyroxine (FT4) determination by chemiluminescent immunoassay (Architect, Abbott, USA). If there is a positive maternal history of autoimmune thyroid disease, we investigate maternal and baby TSH receptor antibodies, or antithyroglobulin and antimicrosomal antibodies if the former are not available. We use ultrasound imaging to determine the presence of a thyroid gland. Diagnostic criterion for congenital hypothyroidism is serum TSH value ≥10 mIU/l with low or normal FT4 value. For neonates with serum TSH value between 5 and 9 mIU/l (grey zone) serum TSH and FT4 are followed up every 15 days. L-thyroxine is prescribed for hypothyroid neonates at a dose 10-15 mg/kg/day as soon as the diagnosis is confirmed.

For preterm infants and multiple pregnancy neonates, the first heel prick is performed, as in other cases, at between 48-96 hours of age, and they are then retested again two weeks later to avoid false negative results due to the typically delayed rise of TSH levels (3, 4). The goal of the treatment is to keep T4 in the upper half of normal range and the TSH around 1 mIU/l. When no permanent thyroid disease is established (absent thyroid tissue confirmed by ultrasound examination), l-thyroxin administration is discontinued for 30 days when the child turns three years of age. At that time, serum is obtained for measurement of thyroxine (T4) and TSH levels. If T4 is low and the TSH level is elevated, permanent hypothyroidism is confirmed and therapy is reinstituted.

Quality control

Since January 2008, the Program has been included in the international quality con-

trol in the Deutche Variente Gesellschaft für Klinische Chemie und Laboratoriumsmedzim e.V., Referenzinstitut für Bioanalytic, Bonn, Germany, and it constantly gives satisfactory results.

Organization of screening

The central laboratory is located in the Clinical Center of Montenegro where the samples are collected from 13 maternity clinics and the Neonatal Center of the Institute for Sick Children of the Clinical Center of Montenegro. Local hospitals send samples to the central laboratory in a hospital vehicle. After detection of TSH ≥10 mIU/l in whole blood, babies are immediately invited to the Screening Center of the Institute for Sick Children where the serum levels of TSH, triiodothyronine (T3), T4, FT4 and thyroglobulin are retested. The Center is responsible for diagnosing, inviting patients for retesting, treatment and monitoring of children diagnosed with CH. Results are entered into protocols which contain data on the condition upon birth, screening results, results of monitoring and therapies. The results are also kept as hardcopy documents in duplicate, in the Screening Center of the Institute for Sick Children and the Center for Clinical Laboratory Diagnostics of the Clinical Center of Montenegro (5).

Monitoring of patients with congenital hypothyroidism

Parents need to bring their babies to control examinations 2 and 4 weeks after the introduction of therapy, as well as every two months during the first two years of life, and then every 3-4 months until the completion of growth and development.

Ethical aspect

This study has been approved by the Ethical Committee of the Clinical Center of Montenegro.

Statistical analysis

The data are presented as absolute and relative numbers, mean, and standard deviation of the mean.

Results

From 2008 to 2012 the neonatal screening test was performed in 40,758 babies with coverage of 100%. Table 1 shows the results of screening: number of babies with TSH ≥10 mIU/l in whole blood, number of babies with confirmed diagnosis of CH, and the percentage of babies called for retesting.

During the five year period there were 17 confirmed cases of CH (both transient and

Table 1 Babies with CH in Montenegro in the five year period (2008-2012)

Parameters	Years					Total
	2008	2009	2010	2011	2012	
Screened newborns (n)	8120	8311	8084	8087	8156	40758
TSH ≥10 mIU/l (n)	79	113	86	139	98	515
CH confirmed and therapy started (n)	2	8	3	3	1	17
Recall rate (%)	0.97	1.36	1.06	1.71	1.20	1.26
Specificity (%)	99.1	98.7	99.0	98.3	98.8	98.8
Prevalence	1:4060	1:1038	1:2695	1:2695	1:8156	1:2397

CH=Congenital hypothyroidism, TSH=thyroid-stimulating hormone.

permanent). Before treatment, 53%, 5.8%, 17.6%, 17.6% and 5.8% of studied newborns had serum TSH level >200 mIU/l, 100-200 mIU/l, 40-100 mIU/l, 20-40 mIU/l and 10-20 mIU/l, respectively. The overall prevalence of CH was 1 in 2397 live births. There was also one case which concerned secondary hypothyroidism in a baby with pan-hypopituitarism which was diagnosed in the first month of life. Including this case, the prevalence of CH in Montenegro was 1:2264. The mean age of starting treatment was 21.4±6.4 days (11 to 30 days).

Discussion

Montenegro was the last country in the region to introduce neonatal screening for CH. After the first few months, the coverage of screening was 100%. This was accomplished thanks to the great enthusiasm with which we entered the program, as well as the responsibility and support of all participants in the program, simple communication between the maternity clinics and parents, small distances from places of residence to the local hospitals and screening centers. The prevalence of CH was 1:2397 live births - both transient and permanent. CH prevalence in Montenegro before 2007 was 1:6450 live births.

The prevalence of congenital hypothyroidism varies. In Europe (1985-1990) it was 1:3801(6), in Italy 1: 2700(7), in USA from 1:4098 to 1:2370, (8) in Japan circa 1:3856 (6), in some Chinese provinces 1:1678 (9). The USA notes an increased trend of CH incidence, which demands the reconsideration of the established practice of confirming diagnoses (10).

According to the available data from the neighboring countries, the prevalence of CH in Croatia was 1:4127 (11), in Slovenia 1:3100 (12), in Serbia 1:3000 (13), in Macedonia 1:2804 (14), and in Bosnia and Herzegovina 1:3957 (15). The screening strategy and method (Delfia) were the same as in Montenegro, but the cut-off value varied: from 8 (Slovenia) to 20 (Bosnia-Herzegovina).

Results of some studies indicate that lower TSH cut off and retesting enable better diagnosis in newborns with functional defects of the thyroid gland, children with transient CH, prematurely born children, children with low birth weight, children conceived via vitro fertilization and children from multiple pregnancies (3, 7). With lower TSH cutoffs, additional cases are detected and treated, but there is no evidence of benefit of this intervention regarding intellectual outcome (16, 17). We should also note that permanent hypothyroidism still has not been confirmed in all babies with CH, since not enough time has passed for safe termination of therapy.

The number of metabolic disorders included in national screening programs varies from one disease (Finland, Montenegro, Malta, and Macedonia) to 29 diseases (Austria) (1).The average annual number of samples in our laboratory is 8151. In Europe, the average number of samples processed in one laboratory varies between 2050 (Malta) and 121,852 (Greece). It is not easy to determine the limit of optimum annual number of samples for one laboratory, but it is estimated that this would be approximately 30 000-50 000 (1).

During the five year period, the number of children called for retesting varied between 0.97 and 1.36%. The relatively large number of retested children could be explained by the low limit TSH value, but in Ireland the TSH cut-off is also 10 mIU/l with a recall rate of 0.8, in Norway the cut-off is 8 mIU/l with a recall rate of 1.45, in Slovenia the cut-off is 8 mIU/l with a recall rate 0.75 (13).

In our study, the mean age of treatment was 21.4±6.4 days. However, in most countries treatment is now started within the first 2 weeks of life (2). In order to be able to start treatment as soon as possible, we should have campaigns directed at healthcare professionals on the importance of collecting blood

samples properly, and quickly sending them to the reference laboratory.

Conclusion

These are the first results of the neonatal screening on CH, which was launched in Montenegro 6 years ago. We are planning to extend screening to phenylketonuria and some other disorders such as cystic fibrosis and congenital adrenal hyperplasia, when the economic circumstances in the country allow such projects.

Authors' contributions: Conception and design: MS and NG-B; Acquisition, analysis and interpretation of data: NG-B ; Drafting the article NP and MP-S; Revising it critically for important intellectual content: MS.

Conflict of interest: The author declares that she has no conflict of interest.

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