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Original Article

Prevalence of congenital hypothyroidism in North Macedonia: data from a newborn screening program conducted for twenty years

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ABSTRACT

Background. Congenital hypothyroidism (CH) is a common endocrine disorder that can be treated if timely detected by newborn screening, optimizing the developmental outcome in affected children. In the present study, we analyze the data of the national newborn thyroid screening program in North Macedonia collected over twenty years, including the CH prevalence as well as its geographical and ethnic variations.

Methods. The thyroid-stimulating hormone (TSH) was measured on a filter paper blood spot sample using the DELFIA fluoroimmunometric assay. A TSH value of 15 mIU/L whole blood was used as the cutoff point until 2010 and 10 mIU/L thereafter.

Results. Out of 377,508 screened live births, a total of 226 newborns with primary CH were detected, providing an overall prevalence of 6.0 per 10,000. Lowering the TSH cutoff led to an apparently increased prevalence of the transient CH, from 0.2 to 2.4 per 10,000 live births ($p<0.0001$) with an impact on the overall prevalence of primary CH (from 4.0 to 7.1 per 10,000, $p=0.0001$). Taking ethnicity into account, the significantly highest primary CH prevalence of 11.3 per 10,000 live births was observed among the Roma neonates, with a predominance of permanent CH (75.5%). There were also regional differences in the prevalence of primary CH. The highest primary CH prevalence of 11.7 per 10,000 live births was observed in the Vardar region, together with the highest regional prevalence of the transient CH (3.2 per 10,000). The highest prevalence of permanent CH was observed in the Pelagonia region (6.6 per 10,000) where the largest percentage of the Roma population lives.

Conclusions. The overall CH prevalence is high in North Macedonia, with substantial ethnic and geographical variations. Further analysis to elucidate the causes for the significant variations in the CH prevalence including environmental factors is warranted.

Key words: congenital hypothyroidism, ethnicity, prevalence, newborn screening, thyroid-stimulating hormone.

Congenital hypothyroidism (CH) is an endocrine disorder due to thyroid hormone deficiency present at birth, causing irreversible intellectual disability. It is classified as primary when the origin of the defect is in the gland itself, or secondary when the hypothalamic-pituitary axis is affected.[1-4] Primary CH can be further divided into permanent CH due to persistent thyroid hormone deficiency and transient CH that refers to a temporary deficiency of thyroid hormones.[1,5] Most neonates born with CH

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have a normal appearance without detectable physical signs, which can delay the diagnosis, leading to intellectual disability as the most severe outcome of the disease. Newborn thyroid screening allows early detection and treatment of CH so it is rightly considered one of the major achievements in preventive medicine that has changed the natural history of the disease and optimized the developmental outcome.[1,6] Additionally, the reported CH incidence of 1/7,000 to 1/10,000 prior to the onset of the newborn screening programs[7] was changed in the range between 1 in 2000-3000.[1,4] Different countries report different incidences of CH, and numerous studies are searching for the origin of these variations.[1-4]

The Republic of North Macedonia is a multiethnic country in the Balkan Peninsula with a population of approximately 2 million people.[8] It is divided into eight statistical regions (Eastern, Northeastern, Pelagonia, Polog, Skopje, Southeastern, Southwestern, and Vardar).[9] The newborn screening for congenital hypothyroidism in North Macedonia was introduced in April 2002 as a pilot study in five larger birth centers in the country. Since January 2007, it has become mandatory for all newborns in the country, as an integral part of the preventive programs for maternal and child health care of the Ministry of Health.

The aim of this retrospective study was to examine the national and regional CH prevalence in North Macedonia over the last two decades as well as the impact of the ethnic factor on the prevalence.

Material and Methods

Neonatal thyroid screening in North Macedonia is carried out by the centralized screening center located at the University Clinic for Pediatrics in Skopje, and covers a neonatal population of approximately 20,000 births per year. A total of 377,508 newborns delivered in all 27 public and private nurseries throughout the entire country were screened between April 2002 and April 2022. Whole blood was collected on filter paper Whatman 903 (Schleicher and Schuell Inc., Keene NH, USA) by neonatal heel prick, between 48 and 72 hours after birth. The whole-blood thyroid-stimulating hormone (TSH) was measured by the DELFIA time-resolved fluoroimmunometric assay based on the direct sandwich technique, in which two monoclonal antibodies (derived from mice) are directed against two separate antigenic determinants on the TSH molecule[10], using a neonatal TSH kit (DELFIA, Perkin-Elmer, Wallac Oy, Turku, Finland), and read by a 1420 VICTOR 2D Fluorometer (Wallac Oy, Turku, Finland). The analytical sensitivity of the DELFIA assays is typically better than 2 mIU/L blood. External controls from "Referenzinstitut fiir Bioanalytik," Bonn, Germany were included every 3 months. We have used a TSH cutoff value of 15 mIU/L whole blood until 2010 (period 1), and cutoff value of 10 mIU/L thereafter (period 2). The TSH values between 15 and 25 mIU/L in the period 1, and between 10 and 20 mIU/L in the period 2 were considered borderline and repeat analysis (new blood spot card) was requested usually 7 days after the previous test. The diagnosis of CH was made when the TSH level was higher than 10 mIU/L with low or normal thyroxine (T4) or free thyroxine (FT4) values on confirmatory serum measurements (IMMULITE 2000 chemiluminescent enzyme immunoassay system, Siemens Healthcare Diagnostics Inc., NY, USA). In all diagnosed children, treatment with L-T4 was immediately initiated.[11,12] The neonates with TSH serum levels between 5 and 9 mIU/L, and normal T4 level, were followed up every 15 days. Infants with repeatedly increased serum TSH levels (>5 mIU/L) up to 2-3 months of life also received substitution therapy with levothyroxine. The diagnosis of permanent vs. transient CH was made after a 4-weeks trial of treatment withdrawal in all children with "gland in situ" CH aged ≥3 years. The classification was made as follows: permanent CH (TSH>10mU/L, subnormal total or FT4), transient CH (normal thyroid tests after the trial off therapy and at least 6 months'follow-up period).[1,2] Based on this classification, only

CH cases obtained before 2020 (Apr 2002 – Dec 2019) were differentiated into transient and permanent forms of the disease, and used for assessing the prevalence of the transient and permanent CH while the remaining cases require more follow up, and those who were yet undetermined. This study was approved by the Ethical Committee of the University Clinic for Pediatrics, Faculty of Medicine in Skopje (Number: 12/2015 - 03-5515/13). The authors declare that all study procedures were in accordance with the Declaration of Helsinki and local laws and regulations. Formal consent was not required for the retrospective study.

Statistical analysis

Statistical descriptive analysis was performed using the Statistical Package for Social Sciences (version 20.0; SPSS Inc., Chicago, IL, USA). The comparison of two proportions was performed with Pearson χ^2 by MedCalc Software Ltd (Version 20.011, https://www.medcalc.org/calc/rate_comparison.php). Statistical significance was set at $p<0.05$.

Results

Over the twenty years of newborn thyroid screening, out of 388,940 live births, 377,508 have been screened. The nationwide coverage was 97.1%. It increased from 91.1% in 2002 to 98.8% in 2020. A total of 226 newborns with primary CH were detected with an overall prevalence of 1/1670 (6.0 per 10,000). Of them, 186 cases were differentiated into the permanent and transient forms of the disease. Thus, 135 (72.6%) of them had permanent CH and 51 (27.4%) were diagnosed with transient CH. In fact, a prevalence of 4 per 10,000 live births for permanent CH and 1.5 per 10,000 for transient cases ($p<0.0001$) was confirmed. Lowering the TSH cutoff from 15 to 10 mIU/L led to an apparently increased prevalence of the transient CH, from 0.2 to 2,4 per 10,000 live births ($p<0.0001$) while the prevalence of the permanent cases was insignificantly changed from 3.8 to 4.2 per 10,000 ($p=0.5864$). Moreover, the overall prevalence of primary CH was significantly increased (from 4.0 to 7.1 per 10,000 live births) after lowering the TSH cutoff value ($p=0.0001$).

Furthermore, we observed substantial regional variations of the primary CH prevalence (Table I). The highest CH prevalence of 11.7 per 10,000 live births was observed in the Vardar region, which was significantly different compared to the rates obtained in the Eastern region ($p<0.001$), Skopje ($p=0.003$), Southwestern ($p=0.005$) and Southeastern regions ($p=0.027$). In the Eastern region, the lowest CH prevalence in the country (2.1 per 10,000) was detected which was significantly lower than the rates observed in the Vardar region ($p<0.001$), Polog ($p=0.013$), Pelagonia ($p=0.002$), Skopje ($p=0.048$) and the Northeastern region ($p=0.027$). The evaluation of the prevalence of permanent CH in different regions showed the highest prevalence in the Pelagonia region (6.6 per 10,000) compared to the lowest prevalence of 1.8 per 10,000 live births detected in the Eastern region ($p=0.014$). On the other hand, the highest prevalence of the transient CH was detected in the Vardar region (3.2 per 10,000), while the lowest prevalence was observed in the Northeastern region (0.51 per 10,000), $p=0.070$.

We also found significant variations in the primary CH prevalence regarding the ethnicity. The highest primary CH prevalence of 11.3 per 10,000 live births was assessed among the Roma neonates with a predominance of the permanent CH (75.5%) in relation to the transient CH cases (23.5%), $p=0.031$. It was significantly higher in comparison with the primary CH rates obtained in all other ethnicities, Macedonian ($p=0.004$), Albanian ($p=0.012$) and Turkish ($p=0.001$), Table II. The lowest CH prevalence of 2.6 per 10,000 was detected among the Turkish neonates which was not statistically different from that in the Macedonian ($p=0.074$) and Albanian ($p=0.052$) neonates. Moreover, there was no statistically significant difference between detected the rates in the Macedonian and Albanian neonates, $p=0.633$ (Table II). The differentiation of the permanent CH cases from

Table I. Prevalence of primary CH in different regions of North Macedonia, during the period Apr 2002 - Apr 2022.

Region	Screened Newborns (n)	Newborns with Primary CH (n)	Incidence of Primary CH	CH Incidence per 10,000
Skopje	187,110	99	1/1890	5.3
Polog	42,989	30	1/1433	7.0
Pelagonia	43,376	37	1/1276	8.5
Eastern	18,812	4	1/4703	2.1
Northeastern	21,773	15	1/1452	6.9
Southwestern	24,910	10	1/2491	4.0
Southeastern	21,399	11	1/1945	5.1
Vardar	17,138	20	1/857	11.7
Total	377,508	226	1/1670	6.0

CH: congenital hypothyroidism, n: number of newborns with CH

Table II. Primary CH prevalence in North Macedonia by ethnicity, over twenty years (Apr 2002 - Apr 2022).

Ethnicity	Newborns with CH (n)	Incidence	Incidence per 10,000
Macedonians	113	1/1701	5.88
Albanians	82	1/1588	6.30
Roma	27	1/882	11.33
Turkish	4	1/3926	2.55

CH: congenital hypothyroidism

the transient ones in the Macedonian neonates did not show statistically significant differences (58.5% vs 41.5%, p=0.220) as well as in newborns of Albanian (48.8% vs 51.2%, p=0.878) and Turkish ethnicity (50% vs 50%).

Discussion

Newborn thyroid screening allows detection of CH shortly after birth in order to facilitate timely treatment and prevention of an irreversible neurodevelopmental delay optimizing its developmental outcome.[1,13,14] In 2007 after a five year pilot study it became mandatory in North Macedonia. The nationwide coverage over the two decades of the newborn thyroid screening program was 97.1%. An overall primary CH prevalence of 1/1670 live births was detected. It was higher than the last reported prevalence in the country (1/1976).[15] Lowering the TSH cutoff value from 15 to 10 mIU/L led to an almost two-fold increase in the primary CH prevalence (4 per 10,000 vs. 7.1 per 10,000). Similarly, a two-fold increase in the CH prevalence has been revealed by six newborn screening programs around the world, after lowering the TSH cutoff.[16] The increase in the prevalence of the primary CH is probably due to the 11-fold increase in the rates of transient CH cases detected after lowering the TSH cutoff value (p<0.0001), compared to the prevalence of the permanent CH (p=0.5864). The estimated prevalence of transient CH in the present study was 27% higher than previously reported in the country.[17] Lowering the TSH cutoff level contributed to the detection of transient CH cases in Central Serbia[18], Italy[19] as well as in Türkiye where an approximately 5-fold increase in transient CH prevalence (1/1154 vs. 1/6202) was reported.[20] In contrast, over a 37-year study period, prevalence of CH increased significantly in the Republic of Ireland despite a screening cutoff value that remained unchanged.[21] It is worth mentioning that lowering the neonatal screening TSH cutoff values, allows detection of newborns who would have been missed otherwise as

false negatives.[22] Furthermore, environmental, ethnic, and genetic factors should be considered in assessing the overall increase in CH prevalence.[23-25] For instance, some patients with the clinical expression of mutations in the *DUOX2*/*DUOXA2* genes required no treatment, and some of them had transient CH. On the other side, *DUOX* gene mutations can be associated with worsening of thyroid functions in the first weeks of life.[26] In a recent study that investigated genetic causes in Macedonian CH patients, *DUOX2* variants were identified in 5% of the "gland in situ" CH cohort (normal-sized, goitrous or hypoplastic thyroid glands). The heterozygous *DUOX2* variants were associated with transient hypothyroidism in all cases. However, monogenic *DUOX2* mutations are frequently associated with goitrous CH or TSH resistance.[27]

In the present study, we also revealed significant differences in the primary CH prevalence in neonates of different ethnic backgrounds. The primary CH prevalence in the Roma neonates (1/882) was almost two-fold higher than that among the Macedonians and Albanians, and 4-fold higher than that in the Turkish neonates. It was previously reported that high primary CH prevalence in one province of the Pelagonia region was associated with a higher percentage in the Roma population[28], as well as in the capital of the country[11] Similarly, the reported CH prevalence among Roma neonates (1/2192) in East Slovakia was statistically higher ($p<0.05$) than the one detected in white newborns (1/6284).[29] Genetic studies of the Roma population have shown high gene frequencies for private disease-causing founder mutations that often exceed the expected magnitudes for the global population. The limited gene flow points to the unique genetic heritage of the Roma, which makes this population genetically distinct from other European populations.[30] Furthermore, the predominance of permanent cases (75.5%) among Roma neonates with CH compared to other ethnicities could imply that genetic factors play a role in the ethnic variations of CH prevalence in the country.

In addition, we found the highest prevalence of permanent CH in the Pelagonia region (6.6 per 10,000), where the Roma population is the largest (3.4%), compared to the Southeastern region, which has only 0.4% Roma inhabitants ($p<0.0001$).[8,28] However, our results are in accordance with the reported data for the impact of the ethnic factor on the CH incidence.[24] Several US programs have obtained higher CH incidence in the Asian, Native American, and Hispanic populations and lower in the African American population.[2] Thus, race and ethnicity data revealed significantly higher CH incidence among Hispanic (6.1 per 10,000) and some Asian newborns (such as Asian Indians, 5.7 per 10,000) than among non-Hispanic white (3.6 per 10,000) and non-Hispanic black newborns (0.9 per 10,000) in California.[31] The processes of genetic drift and different levels and sources of admixture as well as the high fertility rate in the ethnic groups with a high risk for CH may have contributed to the rise of CH incidence.[32] Furthermore, the present study showed regional variations in the CH prevalence. The Vardar region had the highest primary CH prevalence which was 5-fold higher than the prevalence in the Eastern region, as a region with the lowest primary CH prevalence in the country. Taking into account that there is a similar ethnic composition in both regions with more than 80% of Macedonians, as a majority ethnic group[8,15], factors other than ethnicity are involved in different CH prevalence. Environmental factors such as exposure of chemical agents toxic to the thyroid gland may play a role in different regional variations in CH prevalence.[33] The natural and anthropogenic enrichment with heavy metals in the Vardar region, due to the location of two of the country's four smelter plants and the Allchar mine deposits on Kozhuf Mountain, may be linked to the highest prevalence of CH in this region.[15,34-36] However, more data are needed to elucidate this association. Moreover, we found the highest prevalence of the transient CH in the Vardar region (3.2 per 10,000), which was 6-fold higher in comparison with the lowest prevalence observed in the Northeastern

region (0.51 per 10,000), p=0.070. Given that iodine status is one reason for regional differences in CH prevalence, it is worth noting that the Republic of North Macedonia was designated an "iodine sufficient country" by the International Council for Control of Iodine Deficiency Disorders (ICCIDD) in 2018.[37,38] In Israel, however, there is a gradual decrease in CH incidence from the north to the south.[39] A recent study in China found that the prevalence of CH was significantly higher in coastal and inland areas than in remote areas, as a result of several suggested factors such as screening program practices, follow-up of screening positive cases, laboratory testing, and survival rates for preterm births across regions.[40] However, there is only one screening center in North Macedonia and all of the clinical centers that treat preterm births in the country are located in a single region (Skopje). Further analysis of the rate and proportion of the thyroid dysgenesis in different regions of the country is needed to assess the impact of the geographically delineated environmental exposure on the regional variations of the CH prevalence.

In conclusion, there has been a significant increase in the prevalence of primary CH in North Macedonia, probably due to an increased rate of transient CH cases after the lowering of the TSH cutoff value, as the most important factor. We found appreciable differences in the regional and ethnic-specific rates of CH over the two decades in North Macedonia. Further analysis to elucidate additional causes of the substantial variations in the CH prevalence is warranted since no complete data exist thus far.

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Ethical approval

The Local Ethics Committee and Human Research Ethics Committee of University Clinic for Pediatrics in Skopje, approved the study. (Number: 12/2015 - 03-5515/13).

Author contribution

The authors confirm contribution to the paper as follows: study conception and design: VA, MP, NZ, BZ, JMJ, MK; data collection: VA, MP, NZ; analysis and interpretation of results: VA, MP, NZ, MK; draft manuscript preparation: VA, BZ, JMJ. All authors reviewed the results and approved the final version of the manuscript.

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Conflict of interest

The authors declare that there is no conflict of interest.

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