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NEWBORN SCREENING IN SLOVENIA

PRESEJANJE NOVOROJENCEV V SLOVENIJI

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ABSTRACT

Introduction. Newborn screening in whole Slovenia started in 1979 with screening for phenylketonuria (PKU). Congenital hypothyroidism (CH) was added into the programme in 1981. The aim of this study was to analyse the data of neonatal screening in Slovenia from 1993 to 2012 for PKU, and from 1991 to 2012 for CH.

Methods. Blood samples were collected from the heels of newborns between the third and the fifth day after birth. Fluorometric method was used for screening for PKU, CH screening was done by dissociation-enhanced lanthanide fluorescent immunoassay (DELFIA).

Results. From 1993 to 2012, from 385,831 newborns 57 were identified with PKU. 184 newborns out of 427,396 screened from 1991 to 2012, were confirmed for CH. Incidences of PKU and CH in the periods stated are 1:6769 and 1:2323, respectively.

Conclusions. Successful implementation of newborn screening for PKU and CH has helped in preventing serious disabilities of the affected children. Adding screening for new metabolic diseases in the future would be beneficial.

Keywords:
congenital hypothyroidism, phenylketonuria, mass screening, incidence, Slovenia

IZVLEČEK

***Uvod**. Presejanje novorojencev v Sloveniji se je začelo leta 1979 s presejanjem za fenilketonurijo (PKU). Leta 1981 je bil v program presejanja dodan še kongenitalni hipotireoidizem (CH). Cilj te raziskave je analiza podatkov presejanja novorojencev v Sloveniji v obdobju med letoma 1993 in 2012 za PKU ter med letoma 1991 in 2012 za CH.*

***Metode**. Vzorci krvi so bili odvzeti petim novorojencem med tretjim in petim dnem življenja. Pri presejanju za PKU se uporablja fluorometrična metoda, presejanje za CH pa poteka z metodo DELFIA.*

***Rezultati**. Od leta 1993 do leta 2012 je bil presejalni test za PKU izveden pri 358.831 novorojencih. Pri 57 otrocih je bil PKU potrjen. Pri 427.396 novorojencih med letoma 1991 in 2012 je bil izveden presejalni test za CH. Pri 184 otrocih je bil CH potrjen. V navedenih obdobjih je bila incidenca PKU 1:6769 in incidenca CH 1:2323.*

***Zaključki**. Uspešna implementacija presejanja novorojencev za PKU in CH je imela pomembno vlogo pri preprečevanju resnih zapletov pri obolelih otrocih. Smiselno bi bilo v program presejanja vključiti nove metabolne bolezni.*

Ključne besede:
kongenitalnihi potireoidizem, fenilketonurija, presejalni testi, incidenca, Slovenija

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1 INTRODUCTION

Newborn screening for metabolic diseases is an important public health programme, as an early identification of affected children can help in preventing disabilities and even death (1, 2). It is available in many developed countries and in all neighbouring countries of Slovenia (3-6). Newborn screening in Slovenia started in 1979 with screening for phenylketonuria (PKU) (7). Screening for congenital hypothyroidism (CH) started 2 years later in 1981 (8). PKU is an inborn error in amino acid metabolism, caused by mutations of phenylalanine hydroxylase gene. Phenylalanine hydroxylase converts phenylalanine to tyrosine, decreased activity of the enzyme leads to increased phenylalanine in the blood and the brain, which can have detrimental effects, such as intellectual impairment and other symptoms, like autism, seizures and motor deficits (9). CH, one of the most common preventable causes of mental retardation, is a thyroid hormone deficiency present at birth (10). Screening for PKU and CH in Slovenia helped to improve the outcome of most of the affected children. Screening for both diseases has been shown to be cost saving (11, 12).

2 METHODS

2.1 Organisation

Analyses were done at the University Medical Centre Ljubljana, Department of Nuclear Medicine. Only serum phenylalanine (Phe) was analysed at the University Medical Centre Ljubljana, University Children's Hospital, Unit for Special Laboratory Diagnostics. All children with elevated Phe and TSH values were followed-up at the University Medical Centre Ljubljana, University Children's Hospital, Department of Endocrinology, Diabetes and Metabolic Diseases.

2.2 Specimen Collection

Blood samples from newborns were collected between the third and the fifth day after birth from the heels of newborns. Samples were collected onto the filter paper Whatman 903 and dried. The dried blood samples were sent by mail (from nurseries not located in Ljubljana) or by a courier service (from the nursery in Ljubljana) to the Department of Nuclear Medicine.

2.3 Laboratory Methods

PKU was detected by quantitative determination of Phe in dried blood spot. From January 1979 to June 1992, screening for PKU was performed by the Guthrie method with the Phe cut off value of 0.12 mmol/L (7). Since July 1992, a fluorometric method was used (Neonatal Phenylalanine kit, PerkinElmer). For the fluorometric method, Phe values < 0.12 mmol/L were treated as normal after the responsible analyst approved them. Values of Phe between 0.12 mmol/L and 0.20 mmol/L, including 0.12 mmol/L and 0.20 mmol/L, were considered as increased threshold values. In this case, the analysis was repeated from a new dried blood spot which was acquired from the nursery. Increased values of Phe ≥ 0.20 mmol/L required confirmational analysis of Phe, which was quantified from a new serum blood sample. Serum blood sample was taken after contacting the nursery where the original sample was taken. It was quantified by the use of the ninhydrin and L-leucyl-L-alanine fluorometric test. PKU was confirmed by elevated serum Phe.

CH was detected by measuring the value of thyroid-stimulating hormone (TSH). From August 1981 to April 1989, radioimmunoassay (RIA) was used for this purpose (8). From then on, analysis was done by dissociation-enhanced lanthanide fluorescent immunoassay (DELFIA® Neonatal hTSH kit, PerkinElmer). Values for TSH were given as mU/L. The cut off value for RIA was 20 mU/L. The measured values with the current method were evaluated as follows. Values up to 8 mU/L were considered normal after the approval by the responsible analyst. In the case of TSH, values between 8 and 20 mU/L, including 8 and 20 mU/L, were considered as increased threshold values, analysis was repeated from a new dried blood spot acquired from the nursery. When TSH values were ≥ 20 mU/L, they were considered as increased values and confirmational analysis of TSH was done from a new serum blood sample taken after contacting the nursery where the original sample was taken. Analyses were done by ADVIA Centaur TSH-Ultra assay (Siemens). CH was confirmed by elevated serum TSH.

2.4 Quality Control

The dried blood spot controls were included in every analytical batch to monitor accuracy and precision within the system. The laboratory participated in UK NEQUAS scheme (http://www.ukneqas.org.uk/) for external international quality control for PKU and CH, and in RFB DGKL scheme (http://www.dgkl-rfb.de/) for external international quality control for CH.

3 RESULTS

3.1 Metabolic Phenotypes of PKU Patients

PKU was classified into different metabolic phenotypes, based on the measured Phe value in blood. Normal Phe value was between 0.05 mmol/L and 0.12 mmol/L. In mild hyperphenylalaninaemia, which does not require any dietary treatment to prevent neurological damage, Phe values were between 0.12 mmol/L and 0.60 mmol/L (9, 13). In mild PKU, Phe values were between 0.60 mmol/L and 0.90 mmol/L, in moderate PKU, Phe values were between 0.90 mmol/L and 1.20 mmol/L (some authors

do not use the term moderate PKU and consider mild PKU between 0.60 mmol/L and 1.20 mmol/L). Patients with classic phenylketonuria had Phe values higher 1.20 mmol/L (9, 13).

3.2 Incidence of PKU and CH

Results for PKU screening from 1993 to 2012, and for CH screening from 1991 to 2012, are given in Table 1 and Table 2. The numbers of positive results for both PKU and CH are given for the period between 2000 and 2012, older data was not available. Results of PKU screening from the implementation of screening until April 1993, and of CH screening until July 1991, were already published (7, 8). From 1993 to 2012, 57 cases were diagnosed positive for PKU, which is up to 6 positive cases of PKU per year. Between 1991 and 2012, there were from 3 to 16 positive cases of CH annually, which amounts to a total of 184 patients with CH. That gives average incidences of 1:6769 for PKU and 1:2323 for CH. Incidence of PKU in Europe is between 1:3000 and 1:30000, of CH between 1:1300 and 1:13000 (14).

Table 1. Neonatal screening for PKU in Slovenia from 1993 to 2012 (the number of live births annually was taken from the webpage of Statistical Office of the Republic of Slovenia, www.stat.si). N/A = data not available. / = Incidence can not be calculated as there were no confirmed cases in that year.

Year	No. of newborns	No. of positive results	No. of confirmed cases	No. of classic PKU	No. of moderate PKU	No. of mild PKU	Incidence
1993	19793	N/A	5	4	1	0	1 : 3959
1994	19463	N/A	3	2	0	1	1 : 6488
1995	18980	N/A	3	2	0	1	1 : 6327
1996	18788	N/A	2	2	0	0	1 : 9394
1997	18165	N/A	3	1	1	1	1 : 6055
1998	17856	N/A	5	4	1	0	1 : 3571
1999	17533	N/A	2	2	0	0	1 : 8767
2000	18180	265	3	1	0	2	1 : 6060
2001	17477	167	1	1	0	0	1 : 17477
2002	17501	368	0	0	0	0	/
2003	17321	476	3	1	0	2	1 : 5774
2004	17961	520	6	4	1	1	1 : 2994
2005	18157	556	4	3	0	1	1 : 4539
2006	18932	154	2	1	0	1	1 : 9466
2007	19823	112	1	1	0	0	1 : 19823
2008	21817	112	3	2	0	1	1 : 7272
2009	21856	196	6	5	0	1	1 : 3643
2010	22343	182	4	2	0	2	1 : 5586
2011	21947	154	1	0	0	1	1 : 21947
2012	21938	157	0	0	0	0	/

Table 2. Neonatal screening for CH in Slovenia from 1991 to 2012 (the number of live births annually was taken from the webpage of Statistical Office of the Republic of Slovenia, www.stat.si). N/A = data not available.

Year	No. of newborns	No. of positive results	No. of confirmed cases	Incidence
1991	21583	N/A	9	1 : 2398
1992	19982	N/A	7	1 : 2855
1993	19793	N/A	3	1 : 6598
1994	19463	N/A	8	1 : 2433
1995	18980	N/A	4	1 : 4745
1996	18788	N/A	6	1 : 3131
1997	18165	N/A	4	1 : 4541
1998	17856	N/A	9	1 : 1984
1999	17533	N/A	11	1 : 1594
2000	18180	127	14	1 : 1299
2001	17477	176	7	1 : 2497
2002	17501	201	8	1 : 2188
2003	17321	258	14	1 : 1237
2004	17961	238	10	1 : 1796
2005	18157	214	5	1 : 3631
2006	18932	238	8	1 : 2367
2007	19823	187	16	1 : 1239
2008	21817	185	12	1 : 1818
2009	21856	160	4	1 : 5464
2010	22343	195	8	1 : 2793
2011	21947	128	8	1 : 2743
2012	21938	207	9	1 : 2438

4 DISCUSSION

Worldwide newborn screening started in 1962, in Massachusetts, USA, with the introduction of bacterial inhibition assay for PKU (Guthrie test) (15). Since then, numerous countries have incorporated newborn screening in their public health programmes, and it is, nowadays, an established medical practice in developed countries (3-5). Screening has also expanded to include more diseases, beginning with CH and galactosemia, while many others were added later on (3, 16). More than 10 diseases are screened for in many European countries (17), while in the USA, more than 20 diseases are included in the newborn screening panels (18, 19).

With the technological advances came the introduction of tandem mass spectrometry (MS/MS), which allowed accurate measurements of acylcarnitines, amino acids and other metabolites important in diagnosing metabolic disorders (20-22). MS/MS is being increasingly used for newborn screening, as it provides important advantages over other techniques, such as the ability to screen for several diseases in a single run, short time of analysis, selectivity and sensitivity (23).

In the countries of southeastern Europe, including Slovenia, MS/MS has not yet been implemented into newborn screening programmes (24). In Slovenia, a pilot study is under way (25) and will be the first expanded newborn screening in Slovenia, which will make an important contribution in designing the optimal strategy for screening Slovene newborns for inborn errors of metabolism. It will also significantly contribute to the evaluation of the frequency of occurrence of each inborn error of metabolism in our population, as reports from countries that have already incorporated newborn screening showed that prevalences for some diseases are higher than expected (26-29). Slovene newborn screening is an area that should be expanded, and thus brought closer to other countries in the European Union (17, 18) and other parts of the developed world (3, 16, 30-33).

Early detection of PKU and CH can improve the outcomes of patients (31). One major downside of newborn screening using less sensitive methods is the high number of false positive results. An increase of stress has been shown in parents of infants with false positive screening results (34). With the use of MS/MS, there is a significantly lower number of false positive results (35).

Incidence of classic PKU in Slovenia is 1:10153, while the total incidence of all forms of PKU (classic, moderate and mild PKU) is 1:6769. Incidence of classic PKU in Slovenia between 2001 and 2010, was previously reported to be around 1:10000 (36), which is consistent with here reported data. Incidence of PKU in Europe in 2004, is between 1:3000 and 1:30000 (14). Incidence of CH in Slovenia, which is 1 : 2323, is on the lower end in Europe, where the incidence is from 1:1300 to 1:13000 (14). Our incidence (1:2323) is higher than the incidence reported in 2007 in Slovenia, which was 1:3100 (37).

Guidelines in several European countries recommend starting the treatment for PKU as early as possible for the best outcomes (38). Treatment for PKU is with a Phe restricted diet (39). According to the literature, treatment with levothyroxine for CH should start in newborns within 2 to 3 weeks of age (40). In Slovenia, treatment for both PKU and CH starts immediately after the confirmation of the disease, which is within two weeks after birth. This is in line with the previously stated limits of treatment, and shows that newborn screening has been successfully established for the best outcomes of the affected children.

5 CONCLUSIONS

The newborn screening programme in Slovenia has been successfully implemented in its public health programme, and has been beneficial for a significant number of affected newborns since the start of the programme. Based on positive experiences in screening for PKU and CH, and on the results from the expansion of screened conditions in developed countries, expansion of screened conditions should be the next step towards a better health programme in Slovenia.

CONFLICTS OF INTEREST

The authors declare that no conflicts of interest exist.

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ETHICAL APPROVAL

Not required.

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