Research Article

Preliminary Study of Newborn Screening for Congenital Hypothyroidism and Congenital Adrenal Hyperplasia in Indonesia

Aman B. Pulungan,[1*] Frida Soesanti,[1] Agustini Utari,[2] Niken Pritayati,[3] Madarina Julia,[4] Diadra Annisa,[5] Attika A. Andarie,[5] I Wayan Bikin[6]

[1]Department of Child Health, Faculty of Medicine Universitas Indonesia, dr. Cipto Mangunkusumo General Hospital, Jakarta, Indonesia
[2]Department of Pediatrics, Faculty of Medicine Diponegoro University, Semarang, Indonesia
[3] Cilegon Regional General Hospital, Banten, Indonesia
[4]Department of Child Health, Faculty of Medicine, Public Health and Nursing, Universitas Gadjah Mada, Yogyakarta, Indonesia
[5]Faculty of Medicine, Universitas Indonesia, Indonesia
[6]Department of Child Health, Faculty of Medicine, Universitas Udayana, Bali, Indonesia

*Corresponding Author: amanpulungan@mac.com
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Abstract

Newborn screening (NBS) is an effective public health policy to detect congenital disorders. In 2014, Indonesia marked its pivotal step by launching a national NBS program for congenital hypothyroidism (CH). The expansion of NBS program is expected. This study aimed to evaluate the feasibility and recall rates of NBS for CH and congenital adrenal hyperplasia (CAH). A preliminary study was conducted in 5 cities in Indonesia from October 2015 to January 2016. All newborns aged 2-5 days with gestational age ≥36 weeks were included in the study. Identity, gender, and age at the time of testing, and gestational age were recorded. The heel prick dried blood samples were taken and transported to the appointed standardized laboratories for TSH and 17-OHP testing. Samples were tested using immunoassay. A positive screen test prompted recalling for confirmatory testing and referral to pediatric endocrinologists. Out of 1226 patients, 1126 were screened for TSH while 1188 were tested for 17-OHP. The median age was 2 (2-5) days. The recall rate for CH was 1/1167 (0.09%). The patient was recalled and was found to be a true positive case. Out of 1188 patients who were screened for CAH, 8 had positive results (0.71%). After recalling, only 3 patients screened for CAH came for confirmatory testing, and 2 were found true positives. High rates of CH and CAH in Indonesia indicate the need of mandatory NBS program. CAH screening results in high false positive values; hence, second tier screening should be considered. Government support, good partnership with health services, and increased society awareness are of importance.
Keywords: *newborn screening, Indonesia, congenital hypothyroidism, congenital adrenal hyperplasia.*

Studi Preliminer Skrining Hipotiroid Kongenital dan Hiperplasia Adrenal Kongenital pada Bayi Baru Lahir di Indonesia

Abstrak

Skrining bayi baru lahir (newborn screening/NBS) adalah kebijakan kesehatan masyarakat yang efektif dalam mendeteksi penyakit kongenital. Pada tahun 2014, Indonesia meluncurkan program NBS nasional untuk mendeteksi hipotiroid kongenital (HK). Penelitian ini bertujuan untuk mengevaluasi kelayakan dan recall rate program NBS untuk HK dan hiperplasia adrenal kongenital (HAK). Penelitian ini dilakukan di 5 kota di Indonesia pada bulan Oktober 2015 hingga Januari 2016. Subjek penelitian adalah neonatus berusia 2-5 hari dengan usia kehamilan ≥36 minggu. Data identitas, jenis kelamin, usia saat skrining, dan usia kehamilan dikumpulkan. Sampel darah kering yang diambil dengan heel prick diuji TSH dan 17-OHP. menggunakan immunoassay. Jika hasil positif, maka subjek dipanggil kembali untuk uji konfirmasi dan rujukan ke spesialis endokrinologi anak. Dari 1226 subjek, 1126 diuji untuk TSH dan 1188 diuji 17-OHP. Median usia 2 (2-5) hari. Recall rate untuk HK 1/1167 (0,09%). Subjek dipanggil kembali dan memiliki hasil uji konfirmasi positif. Dari 1188 subjek yang diskrining untuk HAK, 8 memiliki positif (0,71%). Hanya 3 subjek yang datang untuk uji konfirmasi dan 2 subjek positif. Tingginya angka HK dan HAK di Indonesia mengindikasikan kebutuhan akan program NBS nasional. Skrining HAK memiliki nilai positif palsu yang tinggi, sehingga skrining lapis kedua perlu dipertimbangkan. Dukungan pemerintah, kerja sama yang baik dengan layanan kesehatan, dan peningkatan kesadaran masyarakat penting untuk mendukung suksesnya program ini.
Kata kunci: *skrining bayi baru lahir, Indonesia, hipotiroid kongenital, hiperplasia adrenal kongenital.*

Introduction

Newborn screening (NBS) is an effective policy to prevent negative health outcomes and death due to several congenital diseases. Diseases covered by NBS vary by country. Economic, geographic, and cultural differences are few of the challenges in developing a sustainable NBS program. According to the Indonesian Basic Health Research in 2016, the total live births in Indonesia reached 4.9 million per year, urging the need for NBS.

In the Asia Pacific, NBS was initiated since the 1960s using newborn dried bloodspot. Since then, NBS evolved from the detection of only one disease to an advanced system capable to detect more than 50 congenital diseases. By 2015, NBS coverage in The Asia Pacific reached more than 95% in developed countries, such as Australia, Japan, New Zealand, Palau, South Korea, Malaysia, Hong Kong, Singapore, and Taiwan. In contrast, its coverage was less than 5% in Bangladesh, Cambodia, India, Indonesia, Pakistan, and Vietnam.

Almost a decade ago, NBS was initiated in Indonesia for congenital hypothyroidism (CH) in two hospitals, dr. Cipto Mangunkusumo National Hospital (Jakarta) and Hasan Sadikin Hospital (Bandung). In 2014, Indonesian Ministry of Health released a decree for mandatory CH newborn screening. However, it is not yet integrated into national health programs nor funded by the national health insurance. As a consequence, current NBS coverage in Indonesia is still low, less than 2%.

CH and congenital adrenal hyperplasia (CAH) are the most common diseases with preventable mortality and morbidity which can be detected by NBS. Globally, the incidence of CH is around 1:3000. The prevalence of neonatal hypothyroidism is estimated to be higher in East Asia compared to Western Countries. In Japan and China, CH occurs in 6.8 and 4.8 per 10,000 infants, respectively. As for CAH, the worldwide incidence ranges from 1:14,000 to 1:18,000 births. In Asia Pacific, NBS is in the process of implementation, with coverage ranging from >90% in developed countries to <5% in lower-to-middle income countries such as Bangladesh, Cambodia, India, and Indonesia. No current data is presently available on the exact incidence of CH and CAH in Indonesia, as there are neither large multicenter studies nor data from the Ministry of Health. In 2009-2019, the Indonesian Pediatric Society registry recorded 644 pediatric patients with CH and 326 cases of CAH. We aimed to evaluate the feasibility and results of NBS for CH and CAH in Indonesia in order to guide its nation-wide implementation in the future.

Methods

A multicenter cross-sectional study was conducted in five cities in Indonesia namely Denpasar (6 hospitals), Banten (8 hospitals), Jakarta (14 hospitals), Semarang (1 hospital), and Yogyakarta (1 hospital) from October 2015 to January 2016. The study protocol was approved by the ethical committee of the Faculty of Medicine, Universitas Trisakti (no. 153/KER/FK/I/2020).

All newborns aged 2-5 days with gestational age ≥36 weeks in the participating hospitals were included in the study, unless parents refused consent. Identity, gender, and age at the time of testing, and gestational age were recorded. Heel prick dried blood spot samples were taken by nurses or laboratory technicians before hospital discharge at the age of two days, if possible. Premature and ill neonates were excluded. The blood specimens were then dried on filter papers. Blood samples that were taken from veins, tainted by anticoagulants, and received by the laboratory more than one week after extraction were excluded from the study.

Blood spot on filter paper needed at least 5-6 hours to dry. Dried blood spot samples were transported within 24 hours to the nearest standardized laboratories. Samples were tested for thyrotropin-stimulating hormone (TSH) and 17-hydroxyprogesterone (17-OHP) using fluoroimmunoassay in Victor™ 2D instrument. The analytical sensitivity for TSH and 17-OHP was 0.7 µU/mL and 4.4 nmol/L, respectively. TSH cutoff value for CH screening was 20 µU/mL, while 17-OHP cutoff value for CAH screening was 19.3 nmol/L (gestational age ≥36 weeks).

Parents whose child had positive screen results were contacted by hospital staff for a confirmation test on blood serum samples. CH was confirmed if serum TSH was increased (>20 mIU/L) and free T4 decreased, while CAH was confirmed if serum 17-OHP was below 19.3 nmol/L. All patients were tested under supervision of pediatric endocrinologists, patients with positive confirmation test results were referred to pediatric endocrinologists for treatment and follow up. Data were analyzed using SPSS version 20.

Results

A total of 1226 newborns had their heel prick blood samples taken. All newborns were tested for TSH while only 1188 (97%) were tested for 17-OHP.

Approximately 90% of blood spot samples were taken by the nurses, while the rest was taken by laboratory technicians. Retaking of blood samples that did not meet requirements were conducted by laboratory technicians. As some of the samples had inadequate amount of dry blood spot, not all patients could be tested for both parameters and TSH measurement was prioritized. In the group tested for TSH, 638 (52%) were male and 588 were female (58%), the male-to-female ratio was 1.09:1. The similar male-to-female ratio was observed in the group tested for 17-OHP (1.1:1), 621 (52.3%) male and 567 (47.7%) female. The median age at heel prick time was 2 (0-26) days. The majority of subjects (97%) had their heel prick blood taken at the recommended age (2-5 days), 34 (2.8%) were taken at age 1 day or younger, and 2 (0.2%) were taken at age 13 days or older. Subject characteristics and results of this study can be seen in Table 1.

Table 1. Baseline Characteristics of the Study Subjects

Characteristics	Screened for Congenital Hypothyroidism (TSH)	Screened for Congenital Adrenal Hyperplasia (17-OHP)
Number of patients tested	1226	1188
Gender, n (%)		
Boy	638 (52)	621 (52.3)
Girl	588 (48)	567 (47.7)
Age at heel prick, day	2 (0-26)	2 (0-26)
Recall rate	1/1226	10/1188
Response rate	1/1	3/10
Confirmatory positive	1	2
Positive predictive value	100%	20%

TSH and 17-OHP Screening

One newborn was found positive after TSH screening. This patient was successfully recalled and underwent confirmatory testing, the result was positive for CH. The patient was then referred to a pediatric endocrinologist for further treatment and follow-up.

Positive CAH screening results were found in 10 out of 1188 patients (0.84%). All patients were recalled but only three came for confirmatory testing. Out of these three patients, two resulted positive and were referred to the nearest pediatric endocrinologists. The positive predictive value for CAH screening using 17-OHP was 20%. CAH screening still resulted in high false positive; hence, it is still not as reliable as CH screening. Mean TSH and 17-OHP levels obtained from this study and the distribution of subjects who were screened positive can be seen in Table 2.

Table 2. Mean TSH and 17-OHP Levels and Number of Positive Subjects at Screening Based on Cities

Cities	Mean TSH Levels (µU/mL)	Mean 17-OHP Levels (nmol/L)	Number of Positive Subjects	
			CH	CAH
Jakarta	2.367	4.022	0	6
Cilegon	2.319	1.916	0	0
Semarang	2.825	3.531	1	1
Yogyakarta	3.040	3.194	0	2
Denpasar	2.751	3.681	0	1
Total	**2.592**	**3.667**	**1**	**10**

Discussion

Indonesia hosts 261.9 million populations with a total fertility rate of 2.33 children in 2017. In 2008-2017, demographic trends showed an inclining population growth and life expectancy rate, as well as a declining infant mortality rate.

Hence, the burden of non-communicable diseases is expected to rise, leading to a double burden of disease. CH and CAH) are the most frequent endocrine diseases affecting newborns detectable through NBS, although the incidence in Indonesia is currently unknown.

Congenital Hypothyroidism Screening

CH is considered the most common treatable and preventable cause of intellectual disability. Etiologies of CH are either thyroid gland dysgenesis or dyshormogenesis, with the former accounting for approximately 85% of CH cases.

The majority of newborns with CH do not exhibit clinical manifestations at birth due to the presence of maternal thyroid hormones. Signs and symptoms usually occur in patients with thyroid agenesis, the total absence of thyroid hormone, and maternal hypothyroidism. Clinical manifestations of CH include feeding problems, constipation, jaundice, macrosomia, large anterior fontanelle, edema, hypotonia, coarse facial features, and hypothermia.

Delayed diagnosis and treatment of CH cause impairment of intellectual functions and quality of life, as reported by Pulungan et al who studied several Indonesian children with CH. Other studies also reported lower health-related quality of life and higher cognitive function outcomes in patients promptly treated.

TSH testing is arguably the best and most sensitive test for CH screening. A second test at the age of two weeks or two weeks after the first screening may be required in preterm neonates, neonates with low and very low birth weight, ill neonates, and multiple births.

Several methods can be used to extract blood samples, such as heel skin prick, umbilical cord blood, venipuncture, or blood from a catheter - each with advantages and disadvantages. In this study, blood from heel skin puncture dried on a filter paper was chosen because blood collection and transport is quick and easier, requires a small volume of blood, and specimen collection can be done any time after birth.[5]

Congenital Adrenal Hyperplasia Screening

CAH is a group of autosomal recessive disorders that causes life-threatening adrenal crisis and difficulty in female sex determination due to impaired cortisol synthesis. CAH can cause medical emergencies due to adrenal crisis and a social emergency due to ambiguous genitalia. In over three-quarters of patients with severe enzyme deficiency, clinical features include salt wasting, failure to thrive, as well as hypovolemia and shock caused by aldosterone deficiency. The recommended first-tier newborn screening test for CAH uses 17-hydroxyprogesterone assays in dried blood spots on filter paper, which can detect the disease in affected but symptom-free neonates. The cutoff for 17-OHP is stratified based on gestational age. However, false-positive results can be seen due to high normal levels of 17-OHP at birth, lower 17-OHP in females compared to males, and higher levels in premature, sick, or stressed infants.[10]

Recall Rate

This study revealed that the recall rate for CH screening was 1/1,226. Mehran et al reported that the recall rate in various countries ranged from 0.01% to 13.3%. The factor which affected recall rates were different screening strategies, laboratory techniques, site of sample collection, iodine deficiency, recall rate criteria, human error, and incidence of CH. Shajira et al reported CH recall rate of 1.98% in Bahrain using cord blood TSH. Other studies in Asia reported various recall rates for heel-prick TSH test ranging from 0.37% in India, 0.69% in Sri Lanka, 2,37% in Laos, and 2,6% in Turkey. However, different cutoffs were used in these studies; hence, apple-to-apple comparison was difficult.

The TSH cutoff in this study followed the recommendation from IAEA (20 µU/mL).[5] Several studies across the world used lower cutoff points to detect less severe cases, but with an increased number of false positives. Other disadvantages of applying lower cutoffs were increased number of recalls, effort, cost, and psychological pressure of parents.[12]

For CAH, the recall rate was higher (10/1188). A study in Sweden found CAH recall rate of 0.06%. In contrast, a study in Switzerland found a very low recall rate of 0.0018%, but with high sensitivity (97%) and specificity (99.9%) for 17-OHP dried blood spot testing. In Asia, the recall rate for 17OHP dried blood spot test was 0.02% in Japan, and 2.37% in Laos[22]. However, these studies used different cutoffs.

Confirmatory Positive

The sample number did not reach the calculated sample size based on the previous prevalence of CH and CAH; hence, incidence of CH and CAH could not be determined. However, we found a relatively high occurrence of CH and CAH: one and

two cases of CH and CAH out of 1226 patients. The occurrence of CH may be comparable to previous reports in Bahrain (1:1489)[19], Sri Lanka (1:1682)[21], and India (1:1221). Additionally, several NBS research in Asia found a lower incidence of CH, ranging from 1:5681 in Laos[22], 1:6813 in India[20], and 1:7175 in Saudi Arabia.

As for CAH, precedent NBS studies in Asia reported that the incidence of CAH was 1:7908 in Saudi Arabia[28], 1:11,362 in Laos[22], and 1:19,859 in Japan[26]. The results of our study showed 1 in 594 babies screened was confirmed positive for CAH. This number, despite being obtained from a small sample size, shows a rough incidence rate that is notably higher than other countries. This study only conducted first tier screening of CAH. Due to the high false positive rate of 17-OHP screening, many countries conduct second tier screening and genetic testing to screen for CAH. The Endocrine Society recommend using a two-tier protocol, an initial immunoassay test followed by liquid chromatography or tandem mass spectrometry to increase sensitivity because only 1 in 100 neonates with positive initial test will have CAH. Guran et al reported the use of steroid profiling in DBS with a liquid chromatography-tandem mass spectrometry as a second-tier test in Turkey.

Our results described subjects that were positive in the screening process were scattered in different cities and due to the small sample size, we could not regard the numbers as representative for an entire province. Further studies are needed to explore if there are any provinces in Indonesia that have higher prevalence of CH and CAH and the factors that influence it.

Feasibility of Nationwide NBS in Indonesia

In this study, not all patients were successfully recalled. Out of 10 patients who had positive screening tests, only three came for confirmatory testing. The challenges to recall patients were a reluctance of parents to allow retesting and the low commitment of hospitals to contact and track newborns with positive screen results. This highlights the importance of increasing awareness in society on newborn screening and maintaining a good partnership with health services.

Methods to improve NBS coverage need to be developed. Although the procedure is relatively simple, training of human resources is required because this study found that not all nurses were confident to take the heel prick blood sample. Consent was an issue because some parents did not agree for their children to be taken repeated blood samples. Hence, community education of the importance of NBS should also be increased. Thus now, NBS is not included in national programs and not funded by the national health insurance. Hence, it is not viewed as a priority by the government and policymakers. Although newborn screening policy exists, community awareness is still low, reflected by the reluctance of patients' parents to undergo repeated testing. Government commitment and support for establishing a sustainable NBS program are crucial.

Conclusion

We found high recall rates for CH and CAH newborn screening compared to worldwide prevalence. Hence, newborn screening for CH and CAH should be routinely performed. Not all patients were successfully recalled due to difficulties in contacting parents and lack of awareness. Nationwide NBS was feasible with certain requirements. First, the Ministry of Health should recognize the importance of NBS through funding and integration of NBS in national health programs. Second, campaigns to increase awareness amongst parents should be done. Last, good partnership with healthcare providers and stakeholders is of urgency.

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