Asian Biomedicine Vol. 5 No. 6 December 2011; 855-859 DOI: 10.5372/1905-7415.0506.112

Brief communication (Original)

Newborn screening for congenital adrenal hyperplasia in Srinagarind Hospital, Khon Kaen University, Thailand

Kittiphop Somboonnithiphol[a], Ouyporn Panamonta[a], Pakaphan Kiatchoosakun[a], Junya Jirapradittha[a], Manat Panamonta[a], Pagakrong Lumbiganon[a], Pichet Somsapt[b]
[a]*Department of Pediatrics,* [b]*RIA Laboratory unit, Srinagarind Hospital, Faculty of Medicine, Khon Kaen University, Khon Kaen 40002, Thailand*

Background: Salt wasting 21-hydroxylase deficiency congenital adrenal hyperplasia (CAH) is the most common cause of adrenal insufficiency during neonatal periods. Newborn screening for CAH will improve case early detection and decrease associated morbidity and mortality. The previous nationwide incidence of CAH in 1999 was 1:19,521. To date, CAH newborn screening has not been included in national newborn screening program.
Objective: We evaluated the incidence of CAH in newborns delivered at Srinagarind Hospital.
Methods: Between September 2005 and June 2008, the filter paper blood spot 17-hydroxyprogesterone (17-OHP) tests were determined in newborns delivered at Srinagarind Hospital. The tests were concurrently performed with TSH and phenylketonuria screening in national newborn screening program of the Ministry of Public Health of Thailand. Re-evaluation with completed physical examinations, repeated blood test for serum 17-OHP and serum electrolytes were performed in newborns who had 17-OHP levels higher than cut-off values. CAH was indicated in patients who had abnormal high serum 17-OHP concentration with or without hyperpigmentation and/or ambiguous genitalia in affected females and/or electrolyte imbalance.
Results: Five thousand seven hundred seventy one of 7,147 (80.74%) live births were screened for CAH. Fourteen infants (0.24%) were recalled for re-evaluation. Eight of fourteen (57.14% response rate) infants had the repeated blood tests. Abnormal elevated serum 17-OHP concentrations were found in two infants. Only one had clinical and laboratory findings indicative of CAH. The incidence of CAH was therefore 1:5,771.
Conclusion: The incidence of CAH from newborn screening in Srinagarind Hospital was obviously higher than national incidence of Thailand. The implement of CAH screening for all neonates should be reconsidered.

Keywords: Congenital adrenal hyperplasia, newborn screening, salt-wasting, 17-hydroxyprogesterone, 21-hydroxylase deficiency

Congenital adrenal hyperplasia (CAH) is, in more than 90% of patients, due to a 21- hydroxylation defect in the adrenal cortex. Biochemically, this results in low serum concentrations of aldosterone and cortisol and elevated 17 α-hydroxyprogesterone (17-OHP) and androstenedione. Clinically, early-onset classical CAH consisted of two types: the salt- wasting form (75%) and the non-salt wasting form (25%) [1]. Salt wasted individuals have salt loss, dehydration, which become life-threatening in the first few weeks of life. In non-salt wasted patients, no electrolyte imbalance is found. Prompt diagnosis and treatment of salt wasting CAH are essential to prevent potential mortality as well as physical and emotional morbidity [2].

The first microfilter method for 17-OHP radioimmunoassay screening for CAH was demonstrated in 1997 [3]. Thereafter, a pilot CAH newborn screening program was performed in Alaska. The results of this program confirmed that CAH screening by this method would improve case detection and decrease associated morbidity and mortality [4]. A number of screening program for CAH have subsequently been developed worldwide. The first CAH newborn screening project in Thailand was

Correspondence to: Professor Ouyporn Panamonta, MD, Pediatric Endocrinology Unit, Department of Pediatrics, Srinagarind Hospital, Faculty of Medicine, Khon Kaen University, Khon Kaen 40002, Thailand. E-mail: ouypan@kku.ac.th

performed in 1999 to determine the feasibility of including CAH screening into the national screening program, which previously screened for congenital hypothyroid and phenylketonuria. Fifty eight thousand five hundred sixty three newborns were screened for CAH and the incidence was 1:19,521. They concluded that this incidence was probably lower than the actual incidence because some babies might have died before the proper diagnosis could be made and some did not return for follow-up testing [5]. To date, CAH newborn screening has not been included in national newborn screening program.

There are many (five to eight cases each year) new cases of salt-wasting CAH in pediatric endocrine clinic at Srinagarind Hospital, the sole university hospital in northeast Thailand. For this reason, we cooperated with the Ministry of Public Health to do newborn CAH screening and reevaluate the incidence of CAH in infants born at Srinagarind Hospital, Khon Kaen University, Khon Kaen, Thailand.

Materials and methods

Between September 2005 and June 2008, heel-prick blood samples from infants aged 48 hours or more were collected on filter paper. The filter paper spot 17-OHP tests were concurrently performed with TSH and phenylketonuria in the newborn screening program of the Ministry of Public Health of Thailand. 17-OHP was measured by GSP neonatal 17 α-OH-progesterone kit (PerkinElmer, Inc., Waltham, MA). Birth weight related cut-off of 17-OHP levels as described in **Table 1** were used [6]. Newborns that had screening 17-OHP concentrations more than possibly abnormal cut off values, would be recalled for further evaluation. Re-evaluation consisted of completed physical examinations, serum electrolytes and repeated blood test for serum 17-OHP concentration. Quantitative serum 17-OHP concentration was measured by radioimmunoassay OHP-CT kit (Cisbio Bioassays, Bagnol/C□ze, France) at RIA laboratory unit, Srinagarind Hospital. ACTH test was performed and CAH was diagnosed if the patients had abnormal high serum 17-OHP concentration and low serum cortisol response with or without hyperpigmentation and/or ambiguous genitalia in affected females and/or electrolyte imbalance. Affected case was treated and followed up by pediatric endocrinologist. In borderline case, patient was followed up with serial blood tests for serum 17-OHP concentrations until the serum concentration turned to normal value.

Demographic data and 17-OHP concentrations of all patients in the study were collected. Clinical and laboratory findings of patients with abnormal screening tests were separately selected and showed. Descriptive statistics were used, percentages for the coverage, recall, and response rate while the incident rate was the number of cases per total number screened during the study period.

The Ethical Committee for Human Research of Faculty of Medicine, Khon Kaen University approved the protocol. Written informed consents were obtained from the parents.

Results

Between September 2005 and June 2008, 5,771 of 7,147 live births, 3,003 females and 2,768 males were screened for CAH. The screening coverage was 80.74%. There were newborns that did not include in the screening because some were dead from severe illness before screening time, most of them had inadequate or unqualified specimens (e.g., small amount of blood or clotted blood on filter paper). The age at screening time ranged from 2 to 20 days (95% of cases were screened at two to three days of life). Median gestational age was 38 (range 23-43) weeks. Median birth weight was 3,080 (range 640-4,910) grams. The 17-OHP concentrations ranged from 0.3 to > 245 ng/ml. The turnaround time for the result of the screening 17-OHP reported from the Ministry of Public Health was more than 20 days in all cases. Fourteen infants who had abnormal levels of 17-OHP

Table 1. Cut off levels of screening 17-OHP concentrations according to birth weight [6].

Weight (gm)	Possibly abnormal (ng/ml)	Definitely abnormal (ng/ml)
<1,300	≥135	
1,300-1,699	115-134	≥135
1,700-2,199	65-89	≥90
≥2,200	55-89	≥90

were recalled for re-evaluation establishing a recall rate of 0.24%. The clinical and laboratory findings of these infants were showed in **Table 2**. Eight of fourteen infants had the repeated blood tests for serum 17-OHP concentrations (57.14% response rate). Abnormal elevated serum 17-OHP concentrations from repeated blood tests were found in two infants (**Table 2**, infant 1&14). Only one infant had clinical and laboratory finding indicative of CAH. Another infant had no salt losing and was followed up. Other six patients were followed and monitored for serial serum 17-OHP concentrations. These serum levels turned to normal values without treatment. The incidence of CAH in our study was therefore 1:5,771.

The affected patient was a 41-week gestational age male infant (**Table 2**, infant 14). The parents brought him to our hospital at 26 days of age with history of poor feedings and poor weight gain since birth. The physical examinations revealed nine percent weight loss, hyperpigmented skin, and mucosa. The serum electrolytes showed hyponatremia, hyperkalemia, and metabolic acidosis. CAH was suspected in this patient. ACTH test was performed with low cortisol and very high 17-OHP response. Adrenal insufficiency was treated with hydrocortisone, fludrocortisone and rehydration and he responded well after treatment. We got the screening result of this patient one day after his admission and the level of 17-OHP concentration was 214 ng/ml. He was discharged 5 days after admission and had regular follow-up.

There was another female infant with high screening 17-OHP concentrations (**Table 2**, infant 3). She had mild hyperpigmentation and mild clitoromegaly, but no electrolyte imbalance. The serum 17-OHP concentration was serially performed; it was slowly declined but still high value. Unfortunately, ACTH test was not performed and she was diagnosed to be nonclassic CAH and treated with prednisolone. During follow-up periods, the serum 17-OHP concentration declined to normal level. Therefore, prednisolone was tapered off. After cessation of prednisolone, the serum 17-OHP concentration was still in normal range and she had no hyperpigmentation and clitoromegaly.

We did not perform the confirmed serum17-OHP in six infants with high screening 17-OHP tests. Three of them (**Table 2**, infant 6, 7, and 9) were preterm infants admitted at hospital with normal serum electrolytes and no signs and symptoms of adrenal insufficiency. The other three infants (**Table 2**, infant 5, 10, and 11) were discharged from hospital and the parents did not take them back for confirmation tests after the recalls. One infant was female with normal genitalia and no hyperpigmentation. Others were male infants who had history of perinatal stress.

Discussion

From the pilot study in Alaska, a number of screening program for CAH have subsequently been developed worldwide. The incidence of CAH ranged from a low of 1 in 21,270 (New Zealand) to a high of 1 in 5,000 (Saudi Arabia) live births. The highest prevalence of CAH has been found in two populations, the Yupik Eskimos of Western Alaska (1:282) and the French island of La Reunion in the Indian Ocean (1:6,071) [1]. At the present time, health organizations in most developed countries (such as the United States, New Zealand, Japan) screen for CAH in their routine newborn screening programs [1], but CAH screening is not currently performed in Thailand. They claimed that the majority of CAH patients will be identified clinically at birth and the results of the screening were not available before the development of an adrenal crisis.

The screening process, however, is less reliable among low birth weight or preterm newborns [7]. Therefore, the normative reference levels based on birth weight or gestational age were used to minimize false positive rates and improve the efficacy of newborn screening for CAH, particularly in low birth weight newborns [8-10]. In our study, we used birth weight related cut-off levels [6], even though a recent Netherlands study demonstrated that gestational age was a better predictor of 17-OHP in newborns and would result in greater specificity than birth weight [9]. In Thailand, early prenatal ultrasonography was not performed in most pregnancy and assessment of gestational age was less reliable. Thus, birth weight was chosen for cut off levels because it was highly accurate and reliable. Another factor that caused high 17-OHP concentration was perinatal stress [11, 12]. However a study from Sweden could not demonstrate any influence of perinatal stress factors on the screening levels of 17-OHP [13]. Despite the birth weight, related cut-off levels were used, there were thirteen false positive cases in our study. Most of them had factors that might have explained high screening false positive such as preterm delivery or history of perinatal stress. However, few cases had no

Table 2. Clinical and laboratory findings of fourteen infants with abnormal 17-OHP screening tests.

Clinical and laboratory findings	Infant 1	Infant 2	Infant 3	Infant 4	Infant 5	Infant 6	Infant 7	Infant 8	Infant 9	Infant 10	Infant 11	Infant 12	Infant 13	Infant 14
Sex	female	female	female	female	female	male	male	male	male	male	male	male	male	male
BW(grams)	670	835	1,330	2,790	3,170	700	990	1,920	1,940	2,400	3,350	3,360	3,370	3,850
GA(weeks)	24	24	30	40	38	26	28	32	32	33	37	38	38	41
Screening 17-OHP (ng/ml)	>245	218	216	>245	231	216	153.5	132	152	71.3	61.3	98.5	>245	241
Serum electrolytes	normal	normal	normal	not done	not done	normal	normal	normal	normal	not done	not done	normal	not done	hyponatremia, metabolic acidosis
History of perinatal stress*	+	+	+	-	-	+	+	+	+	+	+	+	-	-
First recall serum 17-OHP (ng/ml)	54.58	18.14	23.42	2.31	not done	not done	not done	13.39	not done	not done	not done	13.43	5.44	73.13
Latest serum 17-OHP (ng/ml)	16.48		0.32					3.76				2.85		0.62 (after treatment)
Ambiguous genitalia	-	-	+	-	-	-	-	-	-	-	-	-	-	-
Hyperpigmentation	-	-	+	-	-	-	-	-	-	-	-	-	-	+

BW = birth weight, GA = gestational age, 17-OHP = 17 □- hydroxyprogesterone, ng/ml = nanogram per milliliter

* perinatal stress: asphyxia, temperature instability, hypotension, sepsis

definite risk factors. For these false positive cases, follow-up with serial blood tests for serum 17-OHP concentrations were crucial.

The incidence of CAH among newborns screened in our hospital was 1:5,771, which was higher than the national data from previous study [5]. It might be from the inadequate power due to small sample size, limited by the budget from the Ministry of Public Health of Thailand. The low response rate in this study might be due to lack of information and awareness of CAH in health personnel and parents and/or inconvenient of transportation. Another problem was delayed recalled process, resulting in delayed confirmation due to lack of understanding and awareness of the severity of CAH among health personnel. To prevent neonatal adrenal crisis, it is necessary to shorten the time for recall to less than one week of age.

Conclusion

Our study showed that the incidence of CAH among newborns screened was higher than the previous national incidence of Thailand. The implement of CAH screening for every newborn should be considered. Moreover, improvement of screening system such as shortening of recall time, education of health personnel and parents are necessary.

Acknowledgement

The authors wish to thank all mothers and their children who participated in this project, the fifth year medical students who collected the blood samples and the Ministry of Public Health for providing 17-OHP tests. The authors have no conflict of interest to report.

References

1. Pang S, Shook MK. Current status of neonatal screening for congenital adrenal hyperplasia. Curr Opin Pediatr. 1997; 9:419-23.
2. White PC, Speiser PW. Congenital adrenal hyperplasia due to 21-hydroxylase deficiency. Endocr Rev. 2000; 21:245-91.
3. Pang S, Hotchkiss J, Drash AL, Levine LS, New MI. Microfilter paper method for 17 alpha-hydroxy-progesterone radioimmunoassay: its application for rapid screening for congenital adrenal hyperplasia. J Clin Endocrinol Metab. 1977; 45:1003-8.
4. Pang S, Murphey W, Levine LS, Spence DA, Leon A, LaFranchi S, et al. A pilot newborn screening for congenital adrenal hyperplasia in Alaska. J Clin Endocrinol Metab. 1982; 55:413-20.
5. Janejai N, Krasao P, Phansang J, Pankarnjanato R, Charoensiriwatana W. Congenital adrenal hyperplasia: should nationwide screening be implemented in Thailand? Southeast Asian J Trop Med Public Health. 2003; 34 Suppl 3:170-3.
6. Allen DB, Hoffman GL, Fitzpatrick P, Laessig R, Maby S, Slyper A. Improved precision of newborn screening for congenital adrenal hyperplasia using weight-adjusted criteria for 17-hydroxyprogesterone levels. J Pediatr. 1997; 130:128-33.
7. Hingre RV, Gross SJ, Hingre KS, Mayes DM, Richman RA. Adrenal steroidogenesis in very low birth weight preterm infants. J Clin Endocrinol Metab. 1994; 78: 266-70.
8. van der Kamp HJ, Oudshoorn CG, Elvers BH, van Baarle M, Otten BJ, Wit JM, et al. Cutoff levels of 17-alpha-hydroxyprogesterone in neonatal screening for congenital adrenal hyperplasia should be based on gestational age rather than on birth weight. J Clin Endocrinol Metab. 2005; 90:3904-7.
9. Torresani T, Gr□ters A, Scherz R, Burckhardt JJ, Harras A, Zachmanna M. Improving the efficacy of newborn screening for congenital adrenal hyperplasia by adjusting the cut-off level of 17α-hydroxyprogesterone to gestational age. Screening. 1994; 3:77-84.
10. Olgemoller B, Roscher AA, Liebl B, Fingerhut R. Screening for congenital adrenal hyperplasia: adjustment of 17-hydroxyprogesterone cut-off values to both age and birth weight markedly improves the predictive value. J Clin Endocrinol Metab. 2003; 88: 5790-4.
11. Ersch J, Beinder E, Stallmach T, Bucher HU, Torresani T. 17-Hydroxyprogesterone in premature infants as a marker of intrauterine stress. J Perinat Med. 2008; 36: 157-60.
12. Murphy JF, Joyce BG, Dyas J, Hughes IA. Plasma 17-hydroxyprogesterone concentrations in ill newborn infants. Arch Dis Child. 1983; 58:532-4.
13. Nordenstrom A, Wedell A, Hagenfeldt L, Marcus C, Larsson A. Neonatal screening for congenital adrenal hyperplasia: 17-hydroxyprogesterone levels and CYP21 genotypes in preterm infants. Pediatrics. 2001; 108:E68.