Case Report

Newborn Screening Pitfalls: A Missed Case of Salt-losing Type of Congenital Adrenal Hyperplasia

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Abstract   Congenital adrenal hyperplasia (CAH) is a rare group of inherited disorders of enzymes mediating adrenal steroidogenesis resulting in inadequate production of cortisol, aldosterone or both by the adrenal glands. Newborn screening for CAH using a dried blood spot sample to measure 17- hydroxyprogresterone (17-OHP) is employed for early detection of severe type of CAH. In this report, we describe a patient with classical CAH who had a normal newborn screening including second tier testing for CAH, but subsequently presented in salt-losing crisis at 1 month of age. Clinicians should not be falsely reassured by a negative newborn screening result, and they should proceed to diagnostic testing in clinical suspicious cases.

Key words   Congenital adrenal hyperplasia; Missed screening case; Newborn screening

Introduction

Congenital adrenal hyperplasia (CAH) is a rare group of inherited disorders of enzymes mediating adrenal steroidogenesis resulting in inadequate production of cortisol, aldosterone or both by the adrenal glands. Majority of the cases are due to 21-hydroxylase deficiency resulting from mutations or deletions in the CYP21A gene.[1,2] Salt-losing type of CAH accounts for two thirds of the classical CAH cases, the remaining being non-salt losing type or non-classical CAH. Salt-losing crisis are life threatening and newborns may present as hyponatraemia,

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hyperkalaemia, shock and even death. Hormonal replacement therapy can result in correction of the electrolyte imbalance. In view of the possibility of such a morbid presentation in the newborn period and treatment being readily available, CAH has been included in most western newborn screening programs. The Hong Kong newborn screening for inborn errors of metabolism (NBSIEM) program has been implemented in stages for all babies born in government hospitals. CAH screening is included in the screening program. Newborn screening for CAH relies on using a filter paper blood spot sample to measure 17-hydroxyprogresterone (17-OHP). Many factors affect the measurement of 17-OHP and thus second tier testing using 17-OHP and androstenedione/cortisol ratio is being used to increase the reliability of the test. Despite having second tier testing, screening tests have limitations. In this report, we describe a case of classical CAH who passed second tier testing for CAH, but subsequently presented in salt-losing crisis at 1 month of age.

Case

A female baby was born at 41 weeks of gestation with a birth weight of 3.07 kg at 25th percentile for age and sex. Antenatal and postnatal history was unremarkable.

NBSIEM was performed on day two of life, showing a minimal elevation in 17-OHP at 25.4 nmol/L (reference interval for full term babies: <25.0 nmol/L). Second tier testing was subsequently performed and showed 17-OHP + androstenedione/cortisol ratio was 0.37 ng/ml (reference interval: <2.0 ng/ml), which was considered normal.

On day five of life, she was admitted to a local hospital for excessive weight loss of 13.5% with hypernatraemic dehydration. Sodium on admission was 151 mmol/L (Reference range: 137-144 mmol/L) and potassium was 4.3 mmol/L (Reference range: 3.5-5.0 mmol/L) With rehydration therapy, sodium gradually normalised but potassium was found to be on the high side 5.6-6.4 mmol/L. This was attributed to difficult blood taking in a newborn. Physical examination was normal. She fed well and regained weight, and was subsequently discharged.

One month later, she was admitted again for fever to another hospital. She had no gastrointestinal nor respiratory symptoms. Feeding was reported to be satisfactory all along without any vomiting. However, body weight on admission was 3.56 kg which was at 3rd percentile for age and sex. Vitals were stable with a blood pressure of 99/51 mmHg and a pulse rate of 154 per minute but skin mottling was noted. Physical examination of respiratory, cardiovascular and abdominal systems were unremarkable. When saving urine sample, isolated cliteromegaly was noted. There was no associated skin hyperpigmentation. Initial investigation showed hyponatraemia with a sodium level of 126 mmol/L (Reference range: 139-146 mmol/L) and hyperkalaemia with a potassium level of 5.4 mmol/L (Reference range: 4.1-5.3 mmol/L). Serial plasma glucose ranged between 5-6 mmol/L. A salt-losing crisis was suspected despite a normal NBSIEM result. Further work up for CAH was performed. The 17-OHP was grossly elevated to 718 nmol/L (Reference range: <8 nmol/L). Testosterone was 2.13 nmol/L (Reference range: <2.15 nmol/L). Cortisol was 167 nmol/L (Reference range: of 101-536 nmol/L). Aldosterone was 578 pmol/L (Reference range: <488 pmol/L). Renin was 116.28 ng/ml-h (Reference range 0.08-3.84 ng/ml-h). Urine steroid profile was performed and showed significant elevation of metabolites if 17-hydroxyprogesterone, moderate elevation of ratio of 16-alpha-hydroxypregnenolone/16-alpha-hydroxy-dehydroepiandrosterone. This pattern was suggestive of 21-hydroxylase deficiency. Genetic test showed compound heterozygous mutation of CYP21A2 gene, with CYP21A2 c. 518T>A p. (Ile173Asn) and CYP21A2 c. 1069C>T p. (Arg357Trp) mutation, confirmed by parental analysis.

Empirical antibiotics were given after initial sepsis workup. Stress dose intravenous hydrocortisone was started on day 3 of admission, which was subsequently changed to oral hydrocortisone, fludrocortisone and sodium chloride supplement. She was stable and then discharged.

Upon follow-up visit, she had good drug compliance and was thriving well. Her body weight and body height were at 75th percentile and 50th percentile for age and sex now. She has also been referred to paediatric surgeon for assessment and reconstruction of external genitalia.

Discussion

CAH owing to 21 hydroxylase deficiency occurs with an incidence of 1:15000 in Canada and the United States combined.[1] The incidence in the Chinese population is even lower as reported by Lee et al.[1] Classical CAH fulfills the criteria for newborn screening because it has a fairly high incidence and tests are available for its diagnosis. Besides, effective treatment and timely treatment reduced mortality.[3,4]

Measuring 17-OHP concentration is the mainstay of newborn screening for CAH. However, 17-OHP can be affected by multiple factor including − sex, race, birth weight, gestational age and timing of newborn screening.[2] All these factors make false positive results a long-standing problem of newborn screening for CAH. Wisconsin state in United States of America has reported the sensitivity of CAH newborn screening to be 83% in male infants but only 60% in female infants.[2] Whereas New York state in United States of America reported a sensitivity of 95% and a specificity of 99.9% for the CAH screening program.[2] It also reports that the positive predictive value of the screening test was higher in full-term infants than in pre-term.[2] This has also been noted by Kopacek et al in Brazil who noted a higher number of false positive results among newborns with a birth weight <2000 g.[4]

Many strategies have been used in the past two decades to reduce the false positive rates. Adjustment of diagnostic levels of 17- OHP according to birth weight tiers was one proposed strategy. Birth weight data is still being used to determine 17-OHP cut-offs in many places including New York State. However, Van der Camp et al and Streigert et al both suggested that the efficacy of 17 OHP screening can be improved by adjusting cut-offs to gestational age rather than to birth weight.[5,6] Our newborn screening program, similar to the Netherlands and Switzerland, also adjusts cut-

offs to gestational age rather than birth weight.

Another strategy that our laboratory utilises to reduce the false positive rates is to perform a second tier test using liquid chromatography tandem mass spectrometry (LC-MS) to measure the levels of adrenal steroid in dried blood spots. Sarafoglou et al have reported that despite using the second tier testing, the positive predictive value for CAH testing remains relatively low.[7] In fact, the Minnesota program has the longest experience in using a single sample two-tier screening algorithm with steroid profiling by LC-MS/MS as the second tier in specimens that exceeded the first-tier 17-OHP cut-off. It showed that second tier testing can reduce false positive rate but the false negative rate are doubled[8] Our newborn screening program has only recently started and it is too early to draw a conclusion about the usefulness of second tier testing in CAH newborn screening.

With implementing all of the above strategies, we have managed to reduce the number of false positives but not the false negative cases. The aim of newborn screening for CAH is to detect the severe form of CAH, especially the salt-wasting form, in order to prevent life-threatening adrenal crisis. From literature, false negative cases are usually the non-salt losing type, also known as the simple virilising CAH or the non-classical CAH. The false negative cases are also usually female because they present with virilised external genitalia but such signs are not clear in males. A 2016 study from New York reviewed two million newborns screened by the New York State Newborn Screening Programme from 2007 to 2014 which diagnosed 105 babies with CAH. Three other confirmed CAH cases were reported with false negative newborn screening results. All 3 cases were female, and they all suffered from simple virilising CAH.[2] In another European study in 2005, researchers had analysed newborn screening cards of 110 patients with CAH in five middle European countries between 1988 and 2000 and compared with the newborn screening cards of 920 normal controls. It was found that 17-OHP level of all patients with salt-wasting CAH were largely above cutoff level, while 10 out of 33 patients with simple virilising CAH had 17-OHP values below cutoffs. They were likely to be missed if screening was done.[9] Both these studies show that false negatives cases in CAH newborn screening are usually simple virilising CAH.

Our patient has classical salt-losing CAH which was missed by newborn screening despite using gestational age adjusted cut-offs and using second tier testing. False negative cases of salt-losing CAH are uncommon but they have been reported in literature. In a study by the University of Wisconsin in 2015, screening data were collected from 2 one-screen states and 5 two-screen states in USA over 3 to 5 years. Four million babies were screened and 374 of them were diagnosed with CAH. Ten cases of salt-wasting CAH were not detected by newborn screening or had an initial specimen unsatisfactory for screening.[10] In another Minnesota study, 838241 babies were screened over 12 years from 1999 to 2010 and identified 52 cases with classical CAH. Fifteen cases were missed and five of them had salt-wasting type.[11] Table 1 shows the comparison of screening protocols, test essays and cutoff values of Hong Kong, New York and Minnesota centres.

One possible method to reduce the number of false negative cases is to adopt a two-screen test in which the first blood sampling is taken between 48-72 hours and the second between day 8 to day 14 of life. A delayed rise in 17-OHP level is seen in some newborns with CAH and this group of patients will be missed by single screening. In a 2015 Wisconsin study, by using the 2-screen method, 6.5% of classical salt-wasting CAH were detected in the second screen versus the first screen.[10]

Hong Kong employs a one-screen test based on measurement of 17-OHP level on from heel-prick blood sample collected on filter paper taken at 24 to 72 hours of life. Second tier test utilising tandem mass spectrometry and measurement of steroid ratios on the same blood sample is performed on cases with borderline results. Since two-screen testing is not available in Hong Kong, postponing the timing of first blood sampling to beyond 48 hours may be another option to improve sensitivity of the screening test. However, it may prolong hospital stay of the mother and baby. The default rate may increase if the screening is to be performed after discharge.

In conclusion, measurement of 17-OHP level is commonly used in newborn screening of CAH, and was thought to have good sensitivity for salt-losing CAH. However, false negative cases of salt-losing CAH were reported in literature and in local experience. Newborn screening has been implemented in Hong Kong recently and more experience is needed to assess whether second-tier testing is useful. More studies are required to establish whether second screening or postponing collection of blood sample to beyond 48 hours is applicable and cost-effective in Hong Kong. We would like to raise awareness amongst clinicians to suspect CAH in clinically suspicious patients, even they passed the newborn screening, and not to delay effective treatment which may be life-saving.

Table 1

	Hong Kong	**New York**	**Minnesota**	
Assay	First-tier test: DELFIA Neonatal 17α-OH-progesterone kit from PerkinElmer Second tier test: liquid chromatography tandem mass spectrometry (LC-MS)	AutoDELFIA Neonatal 17-OHP kit (Perkin Elmer, Turku, Finland) .	First-tier test: AutoDELFIA Neonatal 17-OHP kit, (PerkinElmer)	Second-tier test: Liquid chromatography-tandem mass spectrometry
Protocol	One-screen, two-tier test Heel-prick blood sample collected on filter paper at 24-72 hours of life	One-tier test Heel-prick blood sample collected on filter paper at 24-48 hours	One-screen, two- tier test Heel-prick blood sample collected on filter paper at 24-48 hours of life	
Cut off	Term: 17-OHP <25 nmol/L Preterm: We are adopting gestational age cutoff provided by the International Society for Neonatal Screening)	Any age: < 22.4 ng/ml (67.8 nmol/L)	Weight-based cutoff: <1500 g : <80 ng/ml, 242 nmol/L 1500 - 2499 g: <45 ng/ml, 136.2 nmol/L >2499 g : <24 ng/ml, 72.6 nmol/L	17-OHP (F): <4.0 ng/ml 17-OHP (M): <7.0 ng/ml 17-OHP+D4A Cortisol >2.5 11-deoxycortisol >10.0 ng/ml 21-deoxycortisol >1.6 ng/ml
Babies screened	50,649*	1,962,433	838241	
CAH picked up by NBS	1 (Classical)	105 SW-CAH: 90 SV-CAH: 8 NC-CAH 5 "Other enzyme": 2	52	
CAH missed by NBS	1 (Classical)	3 (SV)	15 (SW-CAH: 5, SV-CAH: 10)	

CAH: Congenital adrenal hyperplasia; 17-OHP: 17- hydroxyprogresterone; NBS: Newborn screening

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Conflict of Interest

The authors have no conflicts of interest to disclose.

References

1. Pang S, Shook MK. Current status of neonatal screening for congenital adrenal hyperplasia. Curr Opin Pediatr 1997;9:419-23.
2. Pearce M, Demartino L, McMahon R, et al. Newborn screening for congenital adrenal hyperplasia in New York State. Mol Genet Metab Rep 2016;7:1-7.
3. Lee HH, Kuo JM, Chao HT, et al. Carrier analysis and prenatal diagnosis of congenital adrenal hyperplasia caused by 21-hydroxylase deficiency in Chinese. J Clin Endocrinol Metab 2000; 85:597-600.
4. Kopacek C, de Castro SM, Padro MJ, da Silva CM, Beltrao LA, Spritzer PM. Neonatal screening for congenital adrenal hyperplasia in Southern Brazil: a population based study with 108,409 infants. BMC Pediatr 2017;17:22.
5. van der Kamp HJ, Oudshoorn CG, Elvers BH, et al. Cutoff levels of 17-alpha-hydroxyprogresterone in neonatal screening for congenital adrenal hyperplasia should be based on gestational age rather than on birth weight. J Clin Endocrinol Metab 2005:3904-7.
6. Steigert M, Schoenle EJ, Biason-Lauber A, Torresani T. High reliability of neonatal screening for congenital adrenal hyperplasia in Switzerland. J Clin Endocrinol Metab 2002:4106-10.
7. Sarafoglou K, Banks K, Gaviglio A, Hietala A, McCann M, Thomas W. Comparison of one-tier and two-tier newborn screening metrics for congenital adrenal hyperplasia. Pediatrics 2012:e12261-8.
8. Guran T, Tezel B, Gürbüz F, et al. Neonatal Screening for Congenital Adrenal Hyperplasia in Turkey: A Pilot Study with 38,935 Infants. J Clin Res Pediatr Endocrinol 2019;11:13-23.
9. Votava F, Török D, Kovács J, et al. Estimation of the false-negative rate in newborn screening for congenital adrenal hyperplasia. Eur J Endocrinol 2005;152:869-74.
10. Held PK, Shapira SK, Hinton CF, Jones E, Hannon WH, Ojodu J. Congenital adrenal hyperplasia cases identified by newborn screening in one- and two-screen states. Mol Genet Metab 2015; 116:133-8.
11. Sarafoglou K, Banks K, Kyllo J, Pittock S, Thomas W. Cases of congenital adrenal hyperplasia missed by newborn screening in Minnesota. JAMA 2012;307:2371-4.
12. Sarafoglou K, Gaviglio A, Hietala A, et al. Comparison of newborn screening protocols for congenital adrenal hyperplasia in preterm infants. J Pediatr 2014;164:1136-40.