The Diagnostic Challenges of Congenital Adrenal Hyperplasia in Preterm Neonates at a Tertiary Neonatal Intensive Care Unit in Trinidad and Tobago

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ABSTRACT

Congenital adrenal hyperplasia (CAH) is an autosomal recessive disorder that results from the deficiency of one of several steroidogenic pathways in cortisol synthesis. The worldwide incidence of classic CAH (21 hydroxylase deficiency) is between 1:15 000 and 1:16 000 in Europe and North America and 1:19 000 in Japan, with the non-classic type at 1:1000 (1). Classic CAH accounts for 90% of cases due to mutations or deletions in the CYP21A gene (1). In managing any neonate with ambiguous genitalia, CAH is a very important differential diagnosis as it has significant implications for future development, even gender assignment. The challenge of diagnosing CAH is compounded by prematurity associated factors that can lead to misinterpretation of traditional target levels for 17 hydroxyprogesterone (17-OHP), stress induced alterations in 17-OHP values and lack of population-based reference values especially at lower gestational ages.

Keywords: Ambiguous genitalia, congenital adrenal hyperplasia, newborn screening, false positive 17 OHP in preterm neonates

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INTRODUCTION

Universal screening for metabolic conditions such as congenital adrenal hyperplasia (CAH) is not widespread throughout the Caribbean. This is a surprising, given that The Bahamas has the third highest incidence of CAH worldwide at 1:5000 (2). If not properly diagnosed, CAH can cause death by shock, hyponatraemia and hyperkalaemia (3). In patients with classic CAH, 75% can develop severe salt-wasting (SW) and 25% may have the simple virilising (SV) form, which is less severe, with affected females having varying degrees of genital virilisation (4).

Classic CAH is biochemically diagnosed with elevated 17 hydroxyprogesterone (17 OHP) levels. One of the diagnostic challenges in CAH is the higher false positive rates in preterm infants. Such a high false positive rate is usually countered by the adjustment of cut-off levels for 17 hydroxyprogesterone (17 OHP) values in keeping with the birthweight. With no universal screening for neonates in Trinidad and Tobago, such cases remain a diagnostic challenge. The following two cases presented demonstrate the diagnostic difficulty with preterm neonates and the need for data to guide the implementation of a screening programme for rare conditions in our setting.

CASE REPORTS

CASE 1

A 710-gram neonate was delivered at 26 weeks gestation *via* spontaneous vaginal delivery with APGAR scores of nine in one and five minutes respectively to a 26-year-old, Afro-Caribbean female with a history of preterm, pre-labour rupture of membranes (PPROM) five days prior to

delivery. The mother, on presentation to the hospital, was started on erythromycin and received dexamethasone to accelerate fetal lung maturity, as per obstetric protocol. On admission to the neonatal intensive care unit (NICU), the infant was started on respiratory support, septic screened, and commenced on ampicillin, gentamicin and fluconazole, as per unit protocol. The neonate was noted early during admission to have a high sodium requirement of up to 7 meq/kg/day, but by day 14 of life, concerns were noted regarding the genitalia. On day 22 of life, the genitalia demonstrated a phallic clitoris 1 cm (Fig. 1) with a partially fused labia.

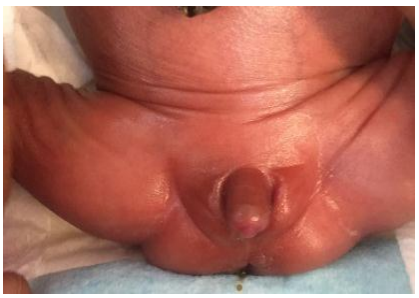

Fig. 1: Neonate demonstrating clitoromegaly and partially fused labia.

Karyotype studies were unavailable, however, serum hormone assay revealed the following: cortisol 9.43 µg/dL (normal > 14 µg/dL), testosterone 631 ng/dL (normal prepubertal range 10–20 ng/dL), 17 OHP 2260 ng/dL [normal 11–170 ng/dL] (4). Pelvic ultrasound showed an anteverted uterus with endometrial lining but was unable to identify ovaries or testes. The infant was referred to paediatric endocrinology with recommendations made to start fludrocortisone 0.1 mg orally once daily and hydrocortisone 15 mg/m^2 eight hourly. Due to the unavailability of the latter, prednisolone 0.4 mg orally once daily was initiated. The treatment plan however, was discontinued after four days due to the sudden onset of lethargy and abnormal electrolyte values. The medication was not restarted prior to discharge. Follow-up continued as an outpatient with infant's genital examination demonstrating normal female appearance. The 17 OHP was repeated

at a corrected gestational age 36+2 weeks (day of life 81) and found to be 57 ng/dL (Normal range 11–170 ng/dL).

CASE 2

A preterm neonate was born to a 40-year-old, Afro-Caribbean female at 28 weeks gestation with a birthweight of 620 grams. Due to a history of multiple first trimester losses, the mother was started on progesterone post conception. This pregnancy was complicated by pre-eclampsia, oligohydramnios and severe intrauterine growth restriction with fetal growth below the 5th centile, however, no physical abnormalities were detected antenatally on anomaly scans. The mother received dexamethasone three days prior to infant's delivery *via* emergency Caesarean section, secondary to impending eclampsia. Her other medications included prenatal vitamins and alpha-methyldopa. On delivery, the infant received APGAR scores of five in one minute and seven in five minutes and was admitted to NICU for respiratory support and management.

The infant was noted to have ambiguous genitalia from birth, with a phallus 1 cm in length, unfused urethral fold at the base and an unfused glans penis, but no obvious vaginal orifice (Fig. 2).

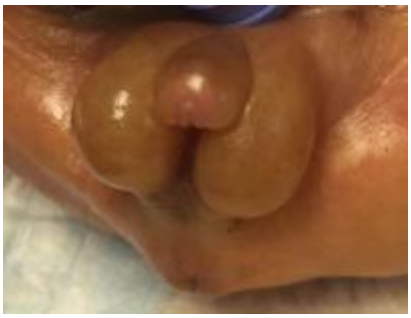

Fig. 2: Neonate with unfused labioscrotal folds and glans penis.

Labioscrotal folds appeared saccular with bilateral inguinal masses palpable, giving the appearance of an under-virilized, premature male infant. The infant otherwise appeared normal with no obvious dysmorphic features. Serum hormone studies: cortisol 19.92 µg/dL, (normal > 14 ug/dL), testosterone 461 ng/dL, (normal 10–20 ng/dL) and 17 OHP 2797 ng/dL [27.97 ng/mL], [normal 11–170ng/dL] (4). Unfortunately, it was not possible to continue investigations in this case since the infant died due to complications of extreme prematurity on day 16 of life.

DISCUSSION

The cases presented demonstrate the challenges of diagnosing CAH in preterm infants. At the extreme range of prematurity, physical findings can appear to evolve as growth progresses due to the stage of development at the time of birth. Such variation in the appearance of the external genitalia can prompt investigations for ambiguous genitalia and CAH. In low resource settings, the cost and logistics of the multi-tiered testing algorithm pose a significant barrier to the implementation of screening for CAH. Notwithstanding the higher cost due to second tier tests to reduce the false positive rates, screening is recommended by the European Society for Paediatric Endocrinology and the Lawson Wilkins Paediatric Endocrine Society to reduce mortality, incorrect gender assignment and initiate early treatment (1).

Screening for CAH, however, is plagued by a high false positive rate due to cross reactivity with other steroids and stress induced 17 OHP in premature babies. In the Caribbean region, newborn screening is not implemented in many countries including Trinidad and Tobago, Jamaica or The Bahamas, despite the latter recording the third highest incidence of CAH globally (2). The difficulties in establishing newborn screening in other countries for conditions such as CAH may also hold true for Trinidad and Tobago.

The policy for screening in two ethnically diverse regions in Brazil and New York State (NYS) in the United States were reviewed. In NYS, two million neonates were tested *via* dried blood spot (DBS) at 24–48 hours of life (5). Emergency cut-off values were established for immediate referral (Table 1) to specialty paediatric endocrinology centres using birthweight, prior to repeat confirmatory testing (5). Moreover, others were referred once the average of the tests was above the tiered cut-off level for weight [Table 2] (5). A similar tiered approach was used by the Brazilian National CAH screening programme [Table 3] (6), with serum studies and clinical evaluations done prior to starting treatment (6).

Table 1: New York screening emergency cut-off values for weight less than or equal to 1750 g or more than or equal to 1751 g. Value 17 OHP considered screen negative or repeat needed M Pearce *et al* 2016

Weight (g)	≤ 1750	≥1751	Screen Negative	Retest & Repeat
17 OHP (ng/ml)	≥150	≥110	≤ 22.4	≥ 22.4

Table 2: Current NYS screening algorithm for CAH. M. Pearce *et al* Elsevier 2016

Weight (g)	< 1000	1001-1750	1751-2250	> 2250
17 OHP (ng/ml)≥	149	117	79	64

Table 3: Brazilian National CAH Screening program, pilot study state Sao Paulo. Kopacek *et al* BMC Paediatrics 2017

Weight (g)	< 1500	1501-2000	2001-2500	> 2500
17 OHP (ng/ml)≥	110.4	43	28.2	15.1

Ideally, DBS specimens are taken between day three and seven of life, however, up to day 40 postnatal life can be acceptable (6). When compared to NYS and Brazil, the assay in the cases presented was performed in the accepted range of time, however, both reference ranges for 17 OHP in NYS and Brazil differ, making interpretation of the results somewhat challenging. The difference in values may simply be population-based demonstrating the need to have population-based references.

In the first case presented, the infant became lethargic with abnormal blood glucose and sodium values once started on the fludrocortisone and prednisolone, forcing the abandonment of the treatment prescribed by endocrinology. Having genetic analysis and second tier testing may have been pivotal in diagnosing or excluding CAH prior to initiating treatment in this case.

Genetic analysis provides reassuring information at an early stage and it is advisable to avoid statements about gender assignment prior to genetic assessment. In managing CAH, a multidisciplinary team approach is needed with an endocrinologist, geneticist, psychologist, surgeon and neonatologist. Karyotype studies are unavailable in public hospitals in Trinidad and Tobago but are available at private laboratories, with considerable delay and at a significant cost.

Using the incidence of CAH in Europe and North America and a birth rate of 14.6 per 1000 women (UN Data 2015) in Trinidad and Tobago, roughly one case per year can be anticipated (7). However, using the incidence in The Bahamas, an estimate of three to four cases may occur annually. Given the ethnic diversity of Trinidad and Tobago and a wider gene pool, the cost of picking up three to four cases per year *versus* the cost of screening must be analysed given the high-costs of implementation. The challenges to implementation of a newborn screening programme in Trinidad and Tobago are being overcome by outsourcing testing for a two-year period and evaluating the data obtained. After the collated data is analysed the cost-effectiveness

and overall benefit to having resources allocated to the public setting to assist in the diagnosis and management of CAH and other rare conditions, can be determined.

CONCLUSION

At the extreme range of prematurity, physical findings can appear to evolve as growth progresses. As screening for CAH in premature babies is plagued by a high false positive rate due to cross reactivity with other steroids and stress induced 17 OHP, the initial and repeat 17 OHP of neonates screened should be compared to the most current population-based reference tables, if available. Establishing cut-off values and repeat testing values is imperative for timely diagnosis and avoiding unnecessary treatment in extremely preterm neonates. Until robust data is available locally or regionally to guide the implementation of treatment, a thorough physical examination with close observation periods, selective testing and appropriate referral continues as the standard of care for suspected CAH cases in preterm neonates.

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