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Neonatal screening for congenital adrenal hyperplasia: 17-hydroxyprogesterone cut-off values based on birth weight

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Abstract

Introduction: Congenital adrenal hyperplasia (CAH) is an adrenal disorder, most commonly caused by 21- hydroxylase enzyme deficiency. Newborn screening for CAH is done by the measurement of 17-hydroxyprogesterone (17-OHP).

Aim: To establish the reference limits of 17-OHP in newborns.

Materials and Methods: The study was conducted at Sri Ramachandra Medical College and Research Institute (SRMC& RI), Chennai. The clinical data of 360 neonates delivered over a period of two years from January 2015 to December 2016 were retrospectively analyzed. The newborns were grouped according to birth-weight: G1: < 1,250 g, G2: 1250-2249 g, G3: >2250 g. Based on birth weights 17-OHP cut-off values were determined for each group by rank number.

Results: The reference limits for the three group, < 1,250 g, 1250-2249 g, >2250 g are 2.23 - 59.3, 1.9 - 29.7 and 1.9 - 15.1 nmol/L respectively. The upper reference limits of all the groups are less than the values showed in the kit literature.

Conclusion: CAH can be diagnosed by measuring 17-OHP at birth as a part of neonatal screening. Population specific and birth weight specific cut off values of 17-OHP will reduce the false positives.

Keywords: Congenital adrenal hyperplasia (CAH), 17-Hydroxyprogesterone (17-OHP), Virilization, Dried blood spot (DBS), Salt-wasting.

Introduction

Congenital adrenal hyperplasia (CAH) is an adrenal disorder, caused due to mutation of genes coding for enzymes involved in cortisol synthesis. 90-95% of the cases are due to 21-hydroxylase enzyme deficiency, leading to over production of 17-hydroxyprogesterone (17-OHP) and its diversion to adrenal androgen synthesis. Deficiency of other enzymes of the cortisol synthesis constitutes only about 5 -10% of the cases.[1]

The incidence of classic form of 21-hydroxylase deficiency is 1 in 10,000 to 1 in 20,000 newborns, while the world wide prevalence rate of non-classic form is estimated to be 1 in 1,000.[2] From a study conducted in Hyderabad, India, CAH incidence was 1:2575. The prevalence is said to be 1:6813 from statistical data taken from a study in Chandigarh.[3]

A Task Force under the guidance of ICMR conducted newborn screening programs in 5 Centers in India and found the overall incidence to be 1 in 5,762 newborns, varying from 1 in 2,036 in Chennai to 1 in 9,983 in Mumbai.[4]

Clinical manifestations of classic (complete) form of CAH ranges from hypoadrenalism, electrolyte abnormalities and ambiguous genitalia in females; precocious pseudopuberty in males. Classic CAH is usually seen in infancy while the nonclassic (incomplete) form presents later in childhood and is a less severe form of this condition.[5]

The clinical symptoms of CAH are due to the deficiency of mineralocorticoid or glucocorticoid production or from the excess production of adrenal androgens. In cases of mineralocorticoid deficiency, there is severe dehydration due to renal salt-wasting, and glucocorticoid deficiency leads to the adrenal crisis. Depending on the presence or absence of mineralocorticoid deficiency, classical form of CAH is further subdivided into salt-wasting and simple virilizing forms.[6]

Salt-losing CAH appear normal after birth, later on it manifests as poor weight gain and the child develops vomiting, hyperkalemia, and hyponatremia within first few weeks of life. According to previous study, 75% of infants with classical CAH have the severe salt-wasting (SW) form of the disease with electrolyte abnormality and shock which leads to death.[7] Delayed diagnosis of babies with salt-wasting CAH still remains a major clinical problem. Affected infants should be followed up until there is a correct diagnosis or normalization of serum 17-OHP levels.

Serum 17-OHP estimation helps in identifying the CAH at birth. In the developed nations, it is included in the newborn screening for inherited metabolic disorders. The concentration of 17-OHP at birth varies with the gestational age and birth weight. Therefore cut-off values are established based on gestational age or birth weight. The Clinical Laboratory Standards Institute (CLSI) suggested a minimum of 120 reference

individuals to establish reference intervals for each group (or subgroup).[8]

Aim

To establish the reference limits of 17-OHP in newborns for South Indian population.

Materials and Methods

The study was conducted at Sri Ramachandra Medical College and Research Institute (SRMC & RI), Chennai. The clinical data of 360 neonates delivered over a period of two years from January 2015 to December 2016 were retrospectively analysed. The specimen used for newborn screening test was capillary blood samples taken directly from a heel prick after third day of birth, on special filter paper cards 'PerkinElmer 226'. The concentration of 17-OHP in dried blood spots was measured by Dissociation Enhanced Lanthanide Fluorescent Immunoassay (DELFIA). The newborns were grouped according to birth-weight: Group1 (G1): less than 1,250 grams, Group2 (G2): between 1250-2249 grams, Group3 (G3): more than 2250 grams. 120 samples of both male and female newborns in each group was included in this study and their 17-OHP value was included for statistical analysis.

Results

The newborns were categorised into three groups based on their birth weights. In group one (G1) newborns with less than 1,250 g, in group two (G2) 1250-2249 g and in group three (G3) newborns with birth weight more than 2250 g were included. Data analysis was performed using a non-parametric method, since the data distribution is not Gaussian. Based on rank numbers, the 2.5- and 97.5-percentiles values were observed.

Table 1: Reference Interval of 17-OHP for newborns with birth weight <1250 g based on rank numbers

17-OHP Value (nmol/L)	Lower Reference limit with 90% Confidence limits	Upper Reference limit with 90% Confidence limits
Kit manufacturer's value	Not available	77.6
SRMC& RI value	2.23 (1.52 to 2.84)	59.3 (47.6 to 68.6)

Table 2: Reference Interval of 17-OHP for newborns with birth weight 1250-2249 g based on rank numbers

17-OHP Value (nmol/L)	Lower Reference limit with 90% Confidence limits	Upper Reference limit with 90% Confidence limits
Kit manufacturer's value	Not available	45.9
SRMC& RI value	1.9 (1.9 - 2.05)	29.7 (23.4 – 40.2)

Table 3: Reference Interval of 17-OHP for newborns with birth weight > 2250 g based on rank numbers

17-OHP Value (nmol/L)	Lower Reference limit with 90% Confidence limits	Upper Reference limit with 90% Confidence limits
Kit manufacturer's value	Not available	19.6
SRMC& RI value	1.9 (1.9 - 2.01)	15.1 (12.6 - 17.4)

Discussion

Autosomal recessive disorder of congenital adrenal hyperplasia is due to common enzyme defect of 21 hydroxylase which leads to over production of 17-OHP and adrenal androgens. Early diagnosis of CAH is important to reduce the adrenal crisis and life-threatening complications. Swerdlow et al, showed that the cause of deaths in most of the CAH patients seemed to be due to adrenal crisis.[9]

Measuring 17-OHP levels in dried blood spots (DBS) of newborns is the screening test for CAH diagnosis. Diagnosis is obvious in females with ambiguous genitalia, but difficult to diagnose in partial enzyme defect in female and newborn males. Melissa et al, found that most of the CAH diagnosed female newborns presented with ambiguous genitalia.[10] Simple virilizing disease in males can be diagnosed only in later childhood due to rapid growth and accelerated skeletal maturation, which increases their mortality.[11]

The concentration of 17-OHP varies according to the gestational age at birth and birth weight. With increasing prematurity, there is a corresponding increase in the level of 17-OHP.[12] Therefore high values in preterm infants can be falsely classified as CAH.

According to recent study done by Rekha et al, 89% of girls and 60% of boys were diagnosed with salt-wasting crisis.[13] In various studies done by Sanches et al[14] and Muller et al,[15] 70 – 80% of males had salt-wasting disease. The difference in the incidences may be due to early death of male babies before the diagnosis of CAH and age group screened. To reduce the false positives, reference values are categorized based on birth weight and gestational age. Allen et al observed that there is a tenfold increase in positive predictive value of screening results when 17-OHP levels were categorized

according to birth weight[16]. To reduce false positive levels, Barra et al[17] and Hayashi et al,[7] used weight adjusted criteria in the newborn screening programs. In this study, decision values are fixed based on birth weights.

In this study, the concentration of 120 samples in each of the three categories based on weight does not follow the Gaussian distribution. The cutoff values for each category of birth weight is calculated by rank numbers.

The lower and upper reference limits for less than 1250 g group (Table 1) are 2.23 (1.52 to 2.84) and 59.3nmol/L (47.6 to 68.6) respectively in this study, which composed of South Indian population. The upper limit (90 percentile) given by the kit manufacturer (PerkinElmer, Finland) is 77.6 nmol/L in serum.

The lower and upper reference limits for 1250 - 2249g group (Table 2) are 1.9 (1.9 - 2.05) and 29.7 nmol/L (23.4 – 40.2) respectively in this study, which composed of South Indian population. The upper limit (90 percentile) given by the kit manufacturer (PerkinElmer) is 45.9nmol/L in serum.

The lower and upper reference limits for more than 2250g group (Table 3) are 1.9 (1.9 - 2.01) and 15.1 nmol/L (12.6 - 17.4) respectively in this study, which composed of South Indian population. The upper limit (90 percentile) given by the kit manufacturer (PerkinElmer) is 19.6 nmol/L in serum. The upper reference limit (90% Confidence limit) for all the three groups are lower in this study, which is based on South Indian population, when compared to the reference limits given by the kit manufacturer.

The difference in the limits obtained could be attributed to the various factors like sex distribution and ethnicity. This necessitates further confirmation using larger group and inclusion of sex of the newborns as a variable. During the study period of two years there was no positive case of CAH.

Conclusion

CAH can be diagnosed by measuring 17-OHP at birth as a part of neonatal screening. To overcome the false positive results, cut off values of 17-OHP based on birth weight and population specific can be helpful in the larger group of studies. The concentration of 17-OHP is more in newborns weighing less. Use of birth weight specific reference limits overcomes false positives in low birth newborns.

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