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ORIGINAL ARTICLE

Newborn Screening for Congenital Adrenal Hyperplasia in India: What Do We Need to Watch Out for?

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About the Author

Dr. Kishore Kumar is a global Neonatologist, having qualified and worked as a Consultant in four continents—India, the UK, the USA, and Australia—with several years of experience. Having acquired his original qualification and working experience in India has given him immense experience of the global health vs Indian health system. He qualified as a Neonatologist in the UK—when there was no such official qualification to that effect in India. He has always worked in Teaching hospitals in the west and has had a university affiliation—which means he has always been in the teaching line—and he plans to "make a difference in the teaching faculty for the benefit of the Medical fraternity in our country." He is interested in "giving every Indian baby/child" the same opportunity as every "western Child".

Abstract

Background Congenital Adrenal Hyperplasia (CAH) is a disorder—an ideal candidate to deserve newborn screening. CAH accounts for a significant mortality and morbidity in India, and its awareness among obstetricians should be treated as highly important to prevent the problem.

Purpose of the Study It is very important for a country like India as the incidence of CAH is reasonably high justifying screening program. However, there are simple logistics that need to be followed, and the treating physicians need to be aware of, if one has to reduce the number of false positives and recalls.

Methods This article takes one through the steps involved in the analysis, interpretation, and reasons for false positives, why the false positives, so that unnecessary calls to parents for repeat sampling are minimized along with the emphasis and the need for the routine screening for CAH.

Results/Conclusion The results of samples can vary depending on the gestational age of the baby, weight of the baby, sampling time, and the knowledge of these data to the treating Obstetrician and Pediatrician is of paramount importance in preventing repeat samples and frustration for the family and the people involved.

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Introduction

Newborn screening is the most important preventive public health program of the twenty-first century [1]. It is implemented in majority of the developed countries [2]. Most of the developing countries are following suit. Once a country decides to implement screening—then what to screen for? [3] We do not have enough data to say that we need to screen for all possible disorders. Each country has to make its own choice of which disorders they need to screen [4–6]. Verma et al. in their recent article have explained that CAH is one disorder that needs to be screened in India [7]. Some of the European countries screen for anywhere between six and eight disorders [8]. Australia and the USA are on the top of the list with screening for nearly 50 disorders including tandem mass spectrometry (TMS) [9]. Philippines screens for four common disorders in their country—hypothyroidism, congenital adrenal hyperplasia (CAH), G6PD, and galactosemia [10]. China has joined the list recently in making it mandatory for all the Obstetricians and Pediatricians to provide the written information to all parents so that they can make informed choice. We present our data about screening for CAH which is quite important for a practicing Obstetrician or the Pediatrician to be aware as they are the first encounters to interact with the prospective parents, and their explanation to the parents goes a long way in ensuring the success or failure of such programs.

CAH is one of the most common causes of preventable neonatal mortality and morbidity, which can be picked up by newborn screening [11, 12]. The screening for CAH by 17-hydroxy progesterone (17-OHP) has fraught with repeated arguments about the values and timing of the screening; the sensitivity and specificity of picking up the babies with CAH which could be missed by just 17-OHP screening, as this could be normal in many non-17-OHP cases of CAH. In India, there are varied reports of CAH with the incidence ranging from 1:4000 to 1:12,500—but we report here our study—a prospective study, which has been done with confirmatory tests to confirm the screening diagnosis (Tables 1, 2, 3).

Materials and Methods

All babies born at the three centers of tertiary maternity group of Hospitals, Bangalore were screened for CAH during the period from January 2007 to October 2013—accounting for nearly 14,800 samples. Of these 14,800 neonates screened for CAH by 17-OHP, 15 were positive for CAH by the screening protocol.

Blood was collected from all newborn babies between 36 and 48 h along with other investigations, which were part of the hospital protocol. For example, in Cloudnine, all babies were checked for discharge bilirubin, blood group, and other screening disorders along with CAH. Parents were counseled on the need for and benefits of screening, and verbal consent was obtained prior to sample collection. For those who declined the test, despite the explanation about the importance of the screening, written consent was obtained as NON-CONSENT. Quantitative determination of 17-OHP was carried out on dried filter paper blood by DELFIA (FIA) kit of Perkin Elmer. 17 hydroxy-progesterone (17-OHP) values up to 30 nmol/L was taken as normal (as per international recommendations), and anything above 30 is taken as abnormal and considering worth repeating the test to confirm or deny the diagnosis of CAH.

Results

A total of 11,200 neonates were screened for CAH during the period from January 2007 to October 2013. Refusal to be screened accounted for less than 0.01 %, and failure to collect samples was none—as the early discharged parents came next day for the blood test. Follow-up of the babies were 100 %, and none was lost for follow-up. Screening identified 15 babies with initial elevated 17-OHP of which 11 babies were false positive on repeat testing. Repeat testing included checking serum 17-OPH along with a short Synacten test. Four were confirmed to have CAH.

Discussion

Newborn screening for CAH is known to Pediatricians for over 30 years and has been implemented only in some countries so far around the world. Recently, Sweden reported their 100 years of experience with CAH along with 28 years of their experience with newborn screening [12]. Other countries like Philippines and China too have commenced the screening for CAH [13, 14].

The traditional screening program for CAH is by measuring 17-OHP, although there are some debates on whether this will miss the rarer forms of enzyme defects leading to false sense of security [15–17].

However, there are multiple ways one can measure 17-OHP. Traditionally, it was being measured in the serum, but of late, it is measured in the blood, and the method used and the normal values for the method used along with gestation are very important in the interpretation of the results. In our hospital, although we have had 15 positive screening results, we

Table 1 99.9 centile for babies = 36 weeks' gestation

Day of sampling	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
GA (week) 36	46	38	32	30	31	32	33	34	34	33	32	30	29	29	28	28	27	26	26

Table 2 99.9 centile for babies > 36 weeks' gestation

Day of sampling	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
GA (week) >36	28	23	20	20	20	20	20	20	20	20	20	19	18	18	17	17	16	16	15

Table 3 99.5 centile for babies ≤36 weeks gestation

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
27	–	192	160	137	129	131	136	141	144	143	139	134	128	124	121	119	116	114	111	108
28	–	165	137	117	110	112	117	121	123	122	119	115	110	106	104	102	100	97	95	92
29	–	140	117	100	94	96	100	103	105	104	102	98	94	91	88	87	85	83	81	79
30	–	119	99	85	80	81	85	88	89	88	86	83	80	77	75	74	72	70	69	67
31	–	100	84	71	67	68	71	74	75	75	73	70	67	65	63	62	61	59	58	56
32	–	84	70	60	56	57	59	62	63	62	61	58	56	54	53	52	51	50	48	47
33	–	69	57	49	46	47	49	51	51	51	50	48	46	44	43	43	42	41	40	39
34	–	56	46	40	37	38	39	41	42	41	40	39	37	36	35	34	34	33	32	31
35	–	44	37	31	30	30	31	32	33	33	32	31	30	29	28	27	27	26	25	25
36	–	35	29	25	23	24	25	26	26	26	25	24	23	23	22	22	21	21	20	20

received 10 positive results in the first 2 years (2007–2009)—when we received the result as being marked ABNORMAL. Three assay techniques are utilized for initial screening: radioimmunoassay (USA), enzyme-linked immunosorbent assay (Japan), and time-resolved fluoro-immunoassay (Europe). Preterm newborns have higher 17-OHP concentrations in serum than babies born at term. Therefore, cutoff levels are based on gestational age (in Japan and Europe) or on birth weight (in the USA). There is a considerable variation in cutoff levels from one program to another. This is most likely due to the different antibodies and reagents used, varying thicknesses, and densities of filter paper used for sample collection and, most significantly, the characteristics of the reference population (in terms of birth weight and gestational age).

However, more than the method, the value depends on the gestational age of the baby and also the timing of the sample, which are crucial factors to be borne in mind, since the nomograms change for the respective times and ages. In a country like India where the incidence of LBW and late preterm babies are thought to be high, this is even more important if we want to reduce the false positives. ISNS recently issued the following data:

The day of birth is taken as = Day1.

The results are expressed as nmol/L blood.

There are no data for the first 24 h: these data have shown to be extremely variable and changing from hour to hour. ISNS (International Society of Newborn Screening) is working on producing a set of data stratified by hour after birth for the first 24–36 h.

Most of the false positives in our study were before 2009, when we did not have nomograms to refer to and 'interpreted' the laboratory results based on the results given by the lab. Most laboratories in India do not have nomograms when they issue the results; hence, the results depend on the clinician's interpretational knowledge which can lead to problems unless one is aware of the normal values for different gestations and ages. The screening process, however, is less reliable among LBW or preterm infants, and recent studies show that newly established normative reference levels based on birth weight or gestational age may minimize false-positive rates and improve the efficacy of newborn screening for CAH, particularly in LBW newborns.

More than 30 million newborns have been screened. The prevalence rates of CAH in the USA and Europe are approximately 1:15,000–16,000, and slightly lower in Japan (1:19,000) [17, 18]. In general, severe salt wasting can be prevented, but there is a remarkable variation in the number of false positives and false negatives among the various programs. Ongoing refinement of cutoff levels is needed to improve specificity and sensitivity. The effectiveness of early detection and treatment of CAH in Japan has been demonstrated by cost–benefit analyses. However, the false-positive rate of CAH screening in preterm infants remains too high compared with screening tests for term infants. To

improve the positive predictive value, they have employed 21-hydroxylase gene (CYP21A2) analysis on dried blood spots and high-performance liquid chromatography (HPLC) to measure 17-OHP, and currently use TMS (LC–MS/MS) as a screening technique. They suggest that LC–MS/MS should be used in the future to improve the accuracy of CAH screening in Japan.

CAH incidence rates have been reported to vary between 1:10,000 and 1:20,000 live births, in most parts of the world. In India, it is thought to be higher although the published reports have been sketchy, and many reports were published from a single center [19–22].

We have screened so far 14,800 babies and have found that we had four babies who were diagnosed with CAH, confirmed by subsequent tests and are currently being followed up by a Pediatric Endocrinologist. We had 11 false positive cases due to the lab error reporting as elevated 17-OHP results in view of "misinformation" with the baby being premature and the Guthrie card had no details mentioning the same, which lead to unnecessary anxiety and the need for confirmatory tests. In view of the high false positive rates, we had stopped screening for CAH routinely for sometime in mid 2009 and resumed again in early 2010, and we find with the new nomograms our false positives are lesser than the positive results, which is consistent with the philosophies of newborn screening.

Indian Council of Medical Research (ICMR) under the auspices of Government of India has done pilot projects, and the recommendations from the pilot projects are listed @ http://www.icmrmetbionetindia.org for all health professionals to follow, and even has leaflets for CAH screening in many languages to be downloaded for use.

Gurjit Kaur et al. from Chandigarh have done screening for three common disorders in India, i.e., congenital hypothyroidism, CAH and G6PD deficiency, and they have found it is worthwhile for these three disorders—but it appears to be from one regional center which can be containing skewed data.

In our study, we screened the babies at 36 h of age along with discharge bilirubin and TSH as per the hospital policy, and the 17-OHP was measured by RIA and any value over 30 nmol/L of whole blood was reported to be abnormal for follow-up. From our study, there is no doubt that CAH screening fits into the criteria of the diseases that need to be screened in India as the incidence rates in our study of less than 1:5000 are too high to ignore [23–25].

However, unlike the CAH and G6PD deficiency screening—the screening for CAH requires quite a number of issues to be addressed before it can be implemented across India, which include the following:

(1) Timing of the sample needs to be explained to all medical professionals. There is enough evidence that it should NOT be done from the cord blood as the 17-OHP values will be high.
(2) The values range with each day—better to be done after 36 or preferably 48 h.
(3) Gestational age is more important in determination of the normal values—which the local lab has to take into account before the results are issued.
(4) Method of 17-OHP assay should be standardized across the country and the values by different methods—we should have nomograms before mass screening is applied.
(5) Professional organizations like FOGSI, IAP, NNF, IMA—should have enough educational materials in their websites, journals, and in meetings so that the knowledge is disseminated widely to avoid unnecessary false positives before lives can be saved.
(6) Obstetricians and neonatologists should work together especially in identifying these babies who otherwise suffer silently before mortality or morbidity strikes the family.

Compliance with Ethical Requirements and Conflict of Interest All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2008 (5). Informed consent was obtained from all patients for being included in the study.

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