Indian J Pediatr (2010) 77:969–973
DOI 10.1007/s12098-010-0150-x

ORIGINAL ARTICLE

Preliminary Report on Neonatal Screening for Congenital Hypothyroidism, Congenital Adrenal Hyperplasia and Glucose-6-Phosphate Dehydrogenase Deficiency: A Chandigarh Experience

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Received: 3 December 2009 / Accepted: 22 April 2010 / Published online: 27 August 2010

Abstract
Objective To establish newborn screening in Indian scenario that could lay a framework for future such initiatives. Three disorders namely, congenital hypothyroidism (CH), congenital adrenal hyperplasia (CAH) and glucose-6-phosphate dehydrogenase deficiency (G-6-PDD) were selected for a preliminary study for newborn screening.
Methods Heel-prick blood samples were collected from live-born neonates at 24–48 h of birth as a part of a screening program after prior written consent from the parents. Blood levels of glucose-6-phosphate-dehydrogenase enzyme (G-6-PD), thyroid-stimulating hormone (TSH) and 17-α-OH progesterone (17-OHP) were measured using DELFIA time resolved fluoroimmunoassay.
Results Six thousand eight hundred and thirteen (6,813) neonates (86.3%), out of a total of 7,893 live births in our institute during the period May'2007 through July'2009, were screened for CAH, CH and G6PD deficiency. Major reason for missing samples was early discharge of the neonates and admission to the neonatal intensive care unit. G-6-PD deficiency was confirmed in 61 cases, congenital hypothyroidism (CH) in 2 cases and congenital adrenal hyperplasia (CAH) in 1 neonate, accounting for an incidence of 1/112 for G-6-PDD, 1/ 3400 for CH and 1/6813 for CAH.
Conclusions Preliminary data on prevalence of various genetic disorders viz. G-6-PDD, CH and CAH in the population of this region revealed that G-6-PDD is most prevalent disorder followed by CH and CAH. More efforts need to be undertaken to create awareness and emphasis on significance of preventive testing to make screening a successful program in India.

Keywords Neonatal screening in India · Neonatal screening · Genetic disorders in India

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Introduction

Congenital malformations and hereditary genetic diseases being the third most common cause of mortality in newborns in India constitute a significant health burden [1]. Factors contributing to their high prevalence include consanguineous marriages, high birth rate, improved diagnostic facilities and a lack of expertise in genetic counseling. In India, 1–4% of the population is mentally retarded and 5–15% of newborn sick babies have metabolic disorders [2, 3]. However, screening, prevention and management of genetic disorders has not yet been incorporated into the mainstream of health care system.

Approximately one-third of pediatric mental retardation stems from inability to detect metabolic disorders in early childhood [4]. Disorders like congenital adrenal hyperplasia (CAH), congenital hypothyroidism (CH), G-6-PD deficiency (G-6-PDD), galactosemia, and phenylketonuria manifest with serious complications in early neonatal life. Neonate may be normal in the initial few days of life and clinical manifestation may occur later depending on the dietary intake of proteins, carbohydrates, etc. [5].

In absence of a national policy regarding neonatal screening in India, there is an urgent need to introduce pilot studies in different parts of the country to assess the feasibility of a national screening program [6]. The first newborn screening (NBS) as a pilot project was carried out in 1980s at Bangalore for amino acids disorders in 12,500 newborns [7]. Homocysteinemia, hyperglycinemia, maple syrup urine disease, phenylketonuria, and hypothyroidism were found to be the common causes of mental retardation. Newborn screening in Andhra Pradesh revealed a high frequency of inborn errors of metabolism (IEMs) (1/1000) [6]. In the study conducted in 18,300 neonates, it was reported that CH is the most common disorder (1 in 1,700) followed by CAH (1 in 2,575). In Indian population, G-6-PDD frequency has been reported to be highly variable among the various populations depending on caste, tribe and ethnicity [8].

The present study was designed with the objective to confirm the incidence of CH, CAH and G-6-PD deficiency. Early detection of neonates with preventable genetic disorders through screening at birth will facilitate prevention of disability and mortality by early intervention, treatment and counseling.

Materials and Methods

Approximately 7,893 babies were born at Government Medical College and Hospital, (GMCH) Chandigarh during May, 2007 through July, 2009. Of these 6,813 neonates were screened for CAH, CH and G-6-PDD. Ethical clearance was obtained from ethical committee of the institution.

Heel prick blood sample were collected on Whatman 903 filter paper between 24 to 48 h after birth. Parents were counseled on necessity and benefits of screening and written informed consent was obtained prior to sample collection. Wallac Victor2D of PerkinElmer and Multicalc software were employed for estimation of 17 α-OH-Progesterone, neonatal TSH and G-6-PD enzyme. One month run-off period was used to standardize the sampling and laboratory techniques.

CAH: 17-OH-Progesterone estimation was carried out on dried blood spots for CAH using DELFIA (Dissociation-Enhanced Lanthanide Fluorescent Immunoassay) Neonatal 17 α-OH-Progesterone time resolved fluoroimmunoassay kit of PerkinElmer. Cut-off values were adjusted with respect to day of sampling, gestational age and birth weight of baby as described by Olgemoller et al. [9].

G-6-PDD: G-6-PD enzyme was measured on dried blood spots using neonatal G-6-PD Kit of PerkinElmer. Neonates with values less than 2.2 units/gm Hb were considered as positive for G-6-PDD.

CH: Quantitative determination of neonatal thyroid stimulating hormone (TSH) was carried out on dried blood spots by DELFIA Neonatal hTSH time resolved fluoroimmunoassay kit of PerkinElmer. TSH value up to 9 μU/ml was taken as normal, borderline-risk between 9 and 18 μU/ml, and high risk for CH above 18 μU/ml. All newborns with elevated TSH levels were called for diagnostic serum thyroid function test to evaluate T3, T4, and TSH for confirmation of CH.

Quality Control: Controls provided with kits were run with each assay of samples and results were validated only when control values attributed within the range specified by kit manufacturer. Genetic Centre is also a part of external quality assessment scheme of CDC Newborn Screening Quality Assurance Program, National Centre for Environment Health, Centre for Disease Control and Prevention, Atlanta, USA.

Results

A total of 6,813 neonates (86.3%) were screened for CAH, CH and G-6-PDD during May, 2007 through July, 2009. Early discharge and admission to intensive care unit comprised two major reasons of failure to collect samples (13.1%); while, refusal to participate in newborn screening program accounted for 0.3%.

Screening for CH in 6,813 babies identified 25 (0.37%) neonates with initial elevated TSH, of which 20 were false-positives on repeat testing. All borderline-risk category neonates were also called for serum thyroid function tests. Of 104 neonates who reported in borderline-risk, 44 (42.31%) did not respond inspite of repeated intimations. Of those who reported for repeat testing, 2 neonates reported abnormal thyroid profile (Table 1). The neonates were put on levothyroxine replacement therapy and responded to normalization without any clinical manifestation of hypothyroidism.

While screening for CAH, abnormal values were obtained in 22 cases out of which one was found to be true positive while 13 turned out normal on repeat testing

Table 1 Summary of the report on the prevalence of various genetic disorders

Metabolic disorders	Total neonates screened	Total number of high risk neonates	True positive	False positive	Others[a]	Prevalence
G6PD Deficiency	6,813	93	61	14	18	1/112
CH (High Risk)	6,813	25	–	21	4	1/3400
CH (Borderline)	6,813	104	02	56	46	
CAH	6,813	22	1	13	8	1/6813

Others[a] : Lost to follow-up; Refused for further evaluation; and Death within neonatal period

(Table 1). In 7 neonates repeat testing could not be performed. At early stages of screening program, uniform criterion for interpretation of 17-OHP values resulted in large number of high-risk cases (false-positives). It has been reported that 17-OHP values are influenced by gestational age, birth weight and day of sampling [9]. Variations due to these factors were incorporated into defining values and this markedly reduced the rate of false-positives for 17-OHP [9].

Screening 6,813 babies for G-6-PDD revealed 93 neonates had initial decreased G-6-PD levels. On repeat testing, 61 were found to be G-6-PD deficient. These included 50 males (81.97%) and 11 females (18.03%); while 14 turned out as false positives (Table 1).

Discussion

Neonatal Screening at GMCH was conducted as a pilot study to gain first-hand experience required for initiating such programs at a larger scale and to examine the feasibility and success of such an effort in India. There is no comprehensive data on prevalence of genetic disorders in India, although, meta-analysis has placed a very high prevalence of G-6-PDD and CH [3, 6, 8]. Earlier criteria of 'direct benefit to newborn' for selection of disorders for NBS, laid down by Wilson and Jungner, has recently been replaced by a 'broader conception of benefit' which includes benefit to family, society and indirect benefits to child to justify screening for poorly understood conditions [10, 11]. In view of limited information on prevalence of genetic disorders for Indian population and in particular the North-India, three most common inborn errors of metabolism namely, G-6-PDD, CH, and CAH were selected for this study.

There are many controversies over the selection of cord blood or heel-prick sample for screening. In this study, heel-prick method was adopted for sample collection from newborns. It is noteworthy that metabolism of newborn is initiated only after the cord is cut and hence, cord blood will not reflect metabolic status of neonate in true sense. Cord blood is useful for screening of G-6-PDD and CH; however, it is not adequate for CAH and PKU or other disorders with metabolite accumulation after birth [12]. Heel-prick method, besides being minimally invasive and easily accessible gives added advantage of testing many other disorders simultaneously through same blood spot. Long term objective of our centre is gradual expansion of NBS program to include more IEMs.

Ideal sample collection time recommended for testing of IEMs is between 24 h to 1 week [13]. In view of thyrotropin surge after birth, American Academy of Pediatrics (AAP) and American Congress of Obstetricians and Gynecologists state that it is highly desirable that CH testing is done between 2 to 4 days of age, when TSH surge has subsided; but there are situations in which this gets virtually impossible because of early discharge policies worldwide [14, 15]. In case of coupled screening with CAH, screening is recommended before day 3 of life [16]. AAP recommends ensuring total participation of all newborns and obtaining blood specimen from every neonate before baby is discharged or transferred from nursery, regardless of nature or status of infant's feeding or age; and that timing of specimen collection should be as close to time of discharge for full-term neonate [12]. For CH and CAH, specimens collected during first 24 h gives high false positive rate (FPR) which gets compensated when weighed against the risk of failure to obtain a sample or missing a true positive case. In this study, a uniform time of sample collection was employed for testing of CH, CAH and G-6-PD deficiency and samples were collected between 24 to 48 h for full-term neonates.

Blood samples by heel-prick were collected in 84% of total live born neonates. Attempts were made to minimize factors leading to loss of samples such as early discharge, parental reluctance and fostering greater cooperation and coordination between associated departments and staff. Parental counseling preceding sample collection resulted in remarkably low refusal rates (0.3%), reflecting the willingness of the parents to participate in the screening programs for the benefit of their child.

Recall rate for CH, CAH and G-6-PDD in this study was 2.06% (excluding borderline-risk neonates for CH); out of which 80.14% responded and 43.97% were true positives for

at least one of the IEMs. Recall rate increased to 3.59% when borderline TSH was included and response rate changed to 70.61%. Recall rates and FPR were found to be high in this study leading to additional work load of follow-up and repeat testing and greater parental anxiety. High FPR constitutes an unfortunate but unavoidable side effect of screening every neonate [11]. The experience on recall rate in our study is in concurrence with that reported earlier as response to recall is generally poor among the Asian population [17].

The biggest challenge in our study was ensuring follow-up visit by parents and substantial loss of patients due to early discharge. A follow-up program facilitates timely diagnostic testing and management that is crucial to avoid mortality, morbidity, and disabilities [18]. The reluctance to follow-up in our study could be due to lack of awareness, illiteracy, lesser confidence in preventive testing and poverty as majority of patients at GMCH comprise migrant and rural population from villages around Chandigarh. Frequent changes in mobile numbers and also addresses in some cases made it extremely challenging to contact high-risk cases either telephonically or through post. Response to recall rates has been reported to be poor even in highly literate regions like Hong Kong [19].

In our study, recall rate for CH (1.8%) was high as compared to 0.36% reported earlier [6]. Despite high recall rates, especially in borderline-risk group, cut-off values were not modified to a higher value as two neonates identified with CH had TSH level within this range. Attempt was also made to contact all neonates who reported low-risk in the initial screening and document false negative cases, if any. However, accessibility impediments (change of phone numbers and addresses) could not provide any significant data, and hence it has not been reported in this study. Several modifications were carried out to improve and streamline the program and reduce the rate of false-positives without affecting the sensitivity of test.

Initially, 17-OHP values were classified into three groups namely, low-risk (<30 nmol/l), borderline-risk (30–90 nmol/l) and high-risk (>90 nmol/l). This provided an unacceptably high number of false-positives for CAH creating confusion at testing laboratory and follow-up section and also contributing to significant parental anxiety. Correction of CAH defining values by incorporating variations due to gestational age, age of sampling and birth weight markedly reduced rate of false-positives for 17-OHP [9].

Screening for CH also resulted in high number of borderline-risk cases. Cut-off values for CH were taken from the AAP Guidelines for newborn screening of congenital hypothyroidism [20]. Screening larger number of neonates is required to obtain ideal cut-off values for CH. It has been reported that higher values for samples taken at <24 h are unreliable and cutoff value must be set to a higher value to prevent excessive number of false-positive results; however, this increases the chance of missing a truly hypothyroid baby [21].

Incidence of G-6-PDD, CH and CAH in this study must be considered in light of the fact that this was only an initial attempt by the authors to develop and provide NBS facility and, gain a firsthand experience of problems encountered to lay a stronger framework for such studies in future. More newborns must be screened before a true representation of incidence of these IEMs in this region is obtained.

In this study, incidence of CH is 0.29/1000 (1 in 3,400) which is close to worldwide incidence of 1 in 4,000 [22]. However, another study from South-Indian population reported incidence of CH as 1 in 1,700 [6]. The neonate identified with CAH in our study was male with salt wasting CAH and was normal at birth without any clinical signs of the metabolic disorder. The family was counseled and prenatal testing was advised for subsequent pregnancies. In the present study the prevalence of CAH is 1 in 6,813, although, as reported in another study from India the incidence of CAH was as high as 1 in 2,575 [6].

G-6-PDD is most common enzyme deficiency affecting 400 million people worldwide. In India, exact incidence of G-6-PDD is not known. However, it has been reported to vary with ethnicity from less than 1–28% [8, 23]. The incidence of G-6-PD deficiency in this study was found to be 1 in 112 with a higher prevalence in males than females.

NBS programs have enormous public health benefits. There are many important issues surrounding the debate on universal screening, including financial resources, level of screening, continuity of care and informed consent. Depending on available technology, the benefit may or may not be balanced against financial and other costs which also include setting up of infrastructure and laboratory techniques including cost of creating awareness [24]. NBS deals with rare disorders and benefits cannot be easily shown without very large studies and when the test is investigational or being developed [25].

Through this study attempt was made to establish NBS facility and develop a framework for future such programs. Difficulties encountered particularly with respect to ensuring complete coverage and maintaining total follow up need further consideration. Since, this program was instituted on research basis to find the feasibility of such program, to obtain firsthand information on prevalence of certain disorders, and to develop a facility; a cost-benefit analysis for this study was not undertaken. The program was funded entirely by government and all tests were offered free of cost. Since approximately half of the births in the world occur in Asia Pacific Region, it is important to implement and expand efforts for screening at birth so that children in developing countries can attain same health status as children in more developed parts of the world [26].

As delayed diagnosis of IEMs affects mental and physical development, every effort is essential to over-

come parental non-compliance and ensure efficient recall, shorter time for reporting, early follow-up and treatment. There is also a need to redefine cut-off values according to time of sampling, birth weight, gestational age, etc. to improve efficacy of screening procedures. Greater awareness through mass media is crucial to prevent IEMs from exacerbating to physical and mental handicap. Furthermore, more coordination between pediatricians, geneticists, health workers and parents is vital to establish an efficient neonatal screening program which could be used as a model in other parts of developing countries like India.

Acknowledgement The authors would like to acknowledge the complete financial assistance extended by Chandigarh Administration for this study. All neonates were screened free of cost.

Contributions Kaur G: Design and plan of the study.
Srivastav J: Data Analysis, Calculation of prevalence and Preparation of manuscript.
Jain S: Neonatologist responsible for follow-up and management of high risk neonates.
Chawla D: Neonatologist responsible for follow-up and management of high risk neonates.
Chavan B.S: Design and plan of the study
Atwal R: Biochemical analysis of 17α-OH Progesterone, neonatal TSH and G6PD.
Randhawa G: Pre-test counseling to all parents signifying the importance of screening and consent taking in prescribed Performa.
Kaur A: Pre test counseling to all parents signifying the importance of screening and consent taking in prescribed Performa.
Prasad R: Evaluation of data and preparation of final manuscript.

Conflict of interest None

Role of funding source To establish neonatal screening program in a government organization to identify metabolic disorders in asymptomatic phase and prevent associated physical and mental handicap.

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