PERSPECTIVE

Newborn Screening in India: Current Perspectives

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Newborn screening aims at the earliest possible recognition of disorders to prevent the most serious consequences by timely intervention. Screening is not a confirmatory diagnosis and requires further investigations. Guidelines from some developed countries recommend newborn screening before discharge because of the high prevalence of certain endocrinopathies, metabolic errors and hearing loss which, if recognized later, contribute to significant morbidity(1). Although the exact list differs among, and sometimes within countries, testing for phenylketonuria (PKU) and hypothyroidism is universal in the developed world. However, neonates are not screened in India because the health policies have typically targeted mortality and infectious morbidities but not disabilities. These policies have been successful in lowering infant mortality rates, but the net effect of these gains has been somewhat offset by an increase in disability.

One of the basic requisites for a screening program is the availability of the epidemiological data regarding disease burden. The annual birth rate is 21.76 births/1,000 population and in Delhi alone nearly 900 births take place every day; considering this figure there would be one or two babies born in Delhi alone with a metabolic defect each day(2,3). However, the diagnosis is delayed due to lack of awareness among the professionals and of easily accessible technical expertise. Considering the large numbers born with these disorders, there is paucity of centers geared to deal with them. The need of the hour is to identify and equip regional centers to enable them to evaluate the metabolic disorders of wide spectrum and high complexity(4).

Blood collection after 72 hours and within 7 days of life on a filter paper is the standard method of screening newborns for hypothyroidism and metabolic disorders. In our country this practice has serious limitations due to high home delivery rate, early discharge from hospitals and cultural taboos related to newborns. Collection of cord blood may be a feasible alternative and has been used for newborn screening of congenital hypothyroidism (CH) in some countries e.g. Malaysia, but this is not a solution for other metabolic errors. Cord blood testing is of limited value in detecting inherited metabolic disease as the metabolites associated with most disorders are not elevated due to lack of dietary substrates i.e. milk, proteins or are at undetectable levels by currently used methods .

INDIAN DATA

There is paucity of published studies in the normal newborn population screening from India. A pilot newborn screening project was carried out on 125 thousand newborns(5). Homocysteneimia, hyperglycinemia, MSUD, PKU, hypothyroidism and G6PD deficiency were found to be the common errors. Another pilot program Expanded Newborn Screening was started in 2000 at Hyderabad to screen amino acid disorders, CH, congenital adrenal hyperplasia (CAH), G6PD deficiency, biotinidase deficiency, galactosemia and cystic fibrosis. Testing a total of eighteen thousand three hundred babies, the results revealed a high prevalence of CH (1 in 1700).

The next common disorder was congenital adrenal hyperplasia followed by G6PD deficiency. Aminoacidopathies as a group constituted the next most common disorder. Interestingly, a very high prevalence of inborn errors of metabolism to the extent of 1 in every thousand newborns was observed. The authors stressed the importance of screening in India, necessitating nation-wide large-scale screening(6).

FEASIBILITY AND IMPLEMENTATION

It may not be viable economically and ethically to screen for a complete range of disorders for which diagnostic modalities are available. Wilson and Jungner(7) have outlined specific criteria that serve as a template to decide what disorders to include in the screening at a national platform. These are: (*a*) biochemically well identified disorder; (*b*) known incidence in the population; (*c*) disorder associated with significant morbidity and mortality; (*d*) effective treatment available; (*e*) period before which intervention improves outcome; and (*f*) availability of an ethical, safe, simple and robust screening test. The developed countries have prioritized the diseases according to the incidence. For most developed countries, initial targets for screening were phenylketonuria and congenital hypothyroidism, but now include other genetic disorders like congenital adrenal hyperplasia (CAH), cystic fibrosis, galactosemia, G6PD deficiency, biotinidase deficiency, hemoglobinopathies *e.g.* Sickle Cell Disease (SCD), and nongenetic targets such as hearing and intrauterine infections, especially toxoplasmosis. Certain countries are using tandem mass spectrometry to screen for a wide range of disorders. The technique is expensive and available only at a handful of centers in India. In our opinion, the screening for various disorders should be phased out after judging feasibility and implementation at each level, and prioritized thereafter.

PRIORITIES AT THE NATIONAL FRONT: INCLUSION IN FIRST PHASE

Congenital Hypothyroidism

It has been included in newborn screening programs all over the world and serves as a template for both introduction, fulfillment of all criteria and cost effectiveness of the newborn screening. This is because of availability of simple therapeutic measures and the good response that follows early detection and treatment. Studies from India, though limited, show a high incidence of CH. The initial reports came from screening of over 22,000 newborns from different parts of the country with and without iodine deficiency to determine the incidence of CH(8,9). Cord blood thyroxine (T4) levels of <3 μU/mL and cord blood TSH levels of >50 mU/mL were used as cut offs. Their data showed that the incidence of CH was about a hundred-fold more in seriously iodine deficient endemic districts. However, newborn screening program was not a part of the evaluation exercise as this was a community survey and would have been useful for formulating guidelines.

Desai, *et al.*(10) screened 12407 newborns for CH using cord blood TSH measuments. 2.8% babies were called for retesting and the incidence extrapolated was 1: 2481(10). In 1994, the same group screened 25,244 neonates at 24-94 hours and measured filter paper T4(11). The babies recalled were 18.91%; however, this screening missed 3 out of 9 babies despite a high recall. The extrapolated incidence was 1:2804. Considering this high incidence of congenital hypothyroidism, availability of low cost therapy and a robust screening test like TSH, it is highly desirable to start a screening program nationwide to prevent the most preventable cause of mental subnormality. Both ELISA and time resolved fluroimmunoassay can be used in the screening phase and confirmatory tests can use either radioimmunoassay or chemiluminescence.

Deafness

There are no published studies on newborn screening for deafness from India. Scanty school surveys are available from both rural and urban setup and demonstrate prevalence of 6.3% of cases in the urban group and 32.8% in the rural group(12).

The importance for screening for deafness can clearly be understood from the fact that if hearing aid can be provided in the prelingual phase it can minimize the negative impact of sensorineural

hearing loss on speech and language acquisition. The recommendations can be the 1-3-6 guideline(13); i.e. (*a*) completed newborn hearing screening before 1 month of age, (*b*) diagnosis of hearing loss and hearing aid fitting before 3 months, and (*c*) enrollment in early intervention before 6 months. Techniques currently used in newborn hearing screening can discriminate peripheral (ie, cochlear) from central (ie, brainstem) auditory function. Two-phase screening using 2 different electrophysiologic measures, otoacoustic emissions (OAEs) and auditory brainstem response (ABR), allows detection of various failure patterns and provides more complete information about auditory function and should be followed in our country(14). Molecular studies as a part of newborn screening may be very useful but are extremely expensive at this time.

DISORDERS MERITING REGIONALIZED SCREENING

Hemoglobin Disorders

Hemoglobin disorders are considered to be a serious health problem by WHO. In India, the carrier frequency of beta thalassemia varies from 1-17% (mean 3.3%). It is estimated that about 10,000 babies affected with beta thalassemia are born every year(15). Sickle cell disease is predominantly found in tribal communities in India, which constitutes about 8% of total population of India(16). In a study by Balgir, *et al.*(17), it was seen that the most common hemoglobin disorders observed of 1015 cases were: sickle cell trait (29.8%), sickle cell disease (7.5%), sickle cell-beta-thalassemia (1.7%), betathalassemia trait (18.2%), thalassemia major (5.3%), thalassemia intermedia (0.9%), Hb E trait (0.9%), Hb E disease (0.3%), E-beta-thalassemia (0.7%), Hb D trait (0.2%) and SD disease (0.2%). Kar, *et al*.(18) also carried out a screening program involving 9,822 hospitalized patients which revealed the frequency of individuals with S gene to be 11.1 per cent. A population survey of 1000 randomized subjects from amongst about 70,000 people in one block of the area showed the frequency to be 15.1%. The gene was not confined to tribal people, but was prevalent throughout the society. Analysis of clinical data on the first 700 cases of sickle cell disease seen in the Sickle Cell Research Centre (ICMR) at Burla demonstrated that while most patients were SS and 8.1% were S-beta thalassaemia, cases of SD disease and SE disease were also encountered. A frequency of 0.32% of alpha thalassaemia gene was noted in SS patients against 0.28% in sickle cell trait and 0.12% in AA controls. The disease was found to manifest as early as 3 months or may remain asymptomatic till adult life(18). With the available Indian data, a sickle cell belt could be mapped out in the country.

Studies on prenatal diagnosis are also very few. Prenatal diagnosis in the years 1986 to 1997 was for 520 pregnancies but rose to 724 pregnancies in the period between 1998-2003(19). The trend which emerged was that nearly 32.9% couples reached the authors prospectively for a diagnosis before having an affected child. The same group had reported a low yield of 10% for prospective testing. A study done in UCLA on cord blood samples of newborns of African–American, Asian-Indian, Southeast Asian and Chinese population used multiplex PCR for common thalassemia mutations(20). Another study in Singapore on cord blood samples of multiracial Asian population reported carrier frequency of alpha thalassemia as 5.2% and beta thalassemia as 0.5% in Indians. Samples were screened for most common alpha and beta thalassemia mutations(21). We feel screening for this group of disorders may be regionalized depending upon the information obtained by gene frequency. Screening for thalassemia would indeed be beneficial but due to the lack of a single robust screening tool, is not feasible at present. Diagnosis of thalassemia using HPLC *en masse* is only possible after six months when switch to adult type of hemoglobin has occurred. Diagnosis using multiplex PCR is cumbersome and would miss a number of cases in whom mutation has not been tested. Screening for sickle cell disease using HPLC of hemoglobin variants should be undertaken in pockets of high incidence.

G6PD Deficiency

There are three recent studies on neonatal / community screening for G6PD deficiency from different regions of the country. In a retrospective hospital based study from Delhi, 2,479 male and female neonates consecutively born were screened

for G6PD levels(22). Incidence in males was 28.3% and in females was 1.05%. In another study from Surat (Gujarat),1644 random blood samples were collected from 404 families(23). Incidence of G6PD deficiency was found as 22%. Thirteen biochemically characterized variants have been reported from India. At the molecular level, G6PD Mediterranean is the most common deficient variant in the caste groups whereas, G6PD Orissa is more prevalent among the tribals of India. The third common variant seen in India is G6PD Kerala-Kalyan(24). However, since the belt in which these disorders are found in large frequency are different, we opine that G6PD screening should also be included in the first phase but in a regionalized manner. Both ELISA and flouroimmunoassay based tests can be used for screening.

DISORDERS FOR INCLUSION IN THE SECOND PHASE

Congenital Adrenal Hyperplasia

The incidence of CAH in India has been found to be 1: 2575 from a small sample survey(6). In a study from AIIMS, New Delhi, CAH was diagnosed in about 38% of children presenting with ambiguous genitalia. What was even more striking was that only one child out of the 53 cases studied was brought immediately after birth with 14 presenting after the age of one year(25). In a study from Kashmir, an incidence of 1.4% has been reported in females presenting with hirsutism. This group, however, studied 4,780 adult women and deduced the incidence of late onset CAH(26). A peculiar situation exists in India as far as the diagnosis of CAH is concerned. A separate group formulated by individuals with sexual ambiguity also colloquially known as the *Hijra* group electively and at times forcefully adopts all babies with sexual ambiguity. Therefore most female neonates with CAH are denied access to therapy. In a subset of affected boys with the salt losing wasting syndrome, the diagnosis is often missed. If screening is implemented and parents are explained the likely outcome of therapy and given access to therapy, the scenario is bound to change. More studies are required before the screening for CAH can be recommended at the national level.

Cystic Fibrosis

Cystic fibrosis (CF) is considered to be very rare in the Indian subcontinent. Based on reports of CF in migrants from Indian subcontinent to UK and USA, the prevalence of CF is estimated to be between 1/10,000 and 1/40,000 in this ethnic group(27). There is only one study which was done to estimate the carrier frequency of F508del mutation among neonates using cord blood samples to reflect the prevalence of CF in the study population(28). The prevalence of CF was estimated by using the proportion of F508del homozygous cases out of all CF patients, as reported in various studies (19-44%) from Indian subcontinent. The carrier frequency and gene frequency of F508del mutation in the Indian population was calculated to be 1/238 (0.42%) and 1/477 (0.21%), respectively. Frequency of CF patients homozygous for F508del mutation was 1/228,006. The estimated prevalence of CF was 1/43,321 to 1/100,323 in Indian population. More studies are required before it can be recommended to be included in a nationwide screening program.

OPTIMAL TIMING AND METHOD OF SAMPLING

The American Academy of Pediatrics has advocated the ideal time of sampling after 72 hours and within 7 days of life. However, this policy would be very difficult to adopt due to high birth rate, limited space in most hospitals and definite resistance, which we can anticipate from our Obstetric colleagues. A recent document suggests that the analytes can ideally be measured at 24-48 hours of life when enteral feeding has been established, renal function is improving and hepatic metabolism is in the process of becoming mature. Thus it may be ideal for our set up, to take the sample after first 24 hours of life.

Since dried blood spot remains stable for years, the mode of collection should be capillary blood from the heel, impregnation of drops of blood into filter paper, drying of these blood spots and transport of the specimens to a central screening laboratory.

STEPS FOR IMPLEMENTATION AND HURDLES

The Central Government has to take up this responsibility which may be shared by the State Governments in due course of time. Pilot studies

need to be initiated to assess the epidemiology of each disease, simultaneous with starting the program for CH and deafness. To begin with, the programs can be initiated in states with low infant mortality rates. The results of this assessment need to be then discussed in a common forum where expert professionals, policy makers, and media is involved and region-wise disorders could be added, depending upon prevalence in phase II. Government funding agencies should identify regional centers which can offer definitive diagnosis to high risk neonates and empower them with technical expertise to undertake this task. Such centers, being funded by the state governments, should be the reference center for diagnosis, therapy and prenatal diagnosis. Screening can only be initiated if confirmatory diagnostic and treatment facilities are available. The difficulties in initiation need to be tackled with creation of a Task force. Indian Council of Medical Research has taken the lead and constituted a task force, and has recently funded a multicenter project to assess the feasibility of newborn screening for CH and CAH.

A public private partnership is required to offer the program to run as a low cost model. Initially the big hospitals in the metropolitian cities should initiate the process. A reliable courier should be identified who is explained the time frame of implementation of the therapy and its consequent positive results. Later, the expertise should reach all state capitals with a move to teach collection to both aaganwadi workers and ANMs. This is the right target as they can collect samples in the ideal time frame.

Genetic centers identified should take up one disorder each, mutually exclusive of each other, to undertake the responsibility of molecular diagnosis, so that efforts are not duplicated. Mass education, media propagation and training centers are required for smooth take off of the program.

NGOs already stationed in the periphery can be roped in for better execution. The program should also address the therapy and follow up of the neonates detected. For a progressive country like India, what would be a better time to start? It is time to revive ourselves now, so as to rejoice later.

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