

Neonatal Screening Program for G6PD Deficiency in India: Need and Feasibility

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Glucose-6-phosphate dehydrogenase (G6PD) deficiency is a common genetic disorder affecting approximately 400 million people worldwide. In India, 390,000 children are born annually with this disorder causing significant morbidity and mortality in childhood. A National Neonatal Screening program for presumptive screening of all neonates using modified Formazan ring test method could be introduced. The test requires blood sample obtained using simple heel prick in the first 48 hours of life, and can be carried out using basic laboratory equipment and reagents. The screening program could be introduced in all institutional deliveries at tertiary hospitals in the major metropolitan cities and then gradually scaled up to cover institutional deliveries over the entire country. After field trials, the program can be expanded to cover home deliveries as well. Increased funding for the health sector under the National Rural Health Mission can provide the required financial support to the program.

Key words: Glucose-6-phosphate dehydrogenase (G6PD) deficiency, India, Modified Formazan ring test, Neonate, Screening program.

India is currently undergoing an epidemiological transition and congenital malformations and genetic disorders are gradually replacing sepsis as the major cause of perinatal and neonatal mortality. Presently, they constitute the fourth commonest cause (9.2%) of neonatal mortality in urban areas(1). This is because consanguineous marriages are still fairly common in many parts of India and these disorders which were hitherto masked by infections and malnutrition are being increasingly identified. Glucose-6-phosphate dehydrogenase (G6PD) deficiency, a hereditary predisposition to hemolysis, is the most common of all clinically significant enzyme defects in the whole of human biology. It is estimated to affect approximately 400 million people worldwide(2) with the highest prevalence rates in tropical Africa, the Middle East, tropical and subtropical Asia, some parts of the Mediterranean and in Papua New Guinea. Several countries in Europe, South East Asia, the Middle East and the United States of

America have successfully established a neonatal screening programme for this disorder. Taiwan has even established the first international program for quality assurance of G6PD screening(3). With almost 24 million children born annually in India, it is estimated that at least 390,000 children suffering from this disorder are born in the country every year(4). Several agents have been identified as triggers for hemolysis, viral and bacterial infections being the most common. Certain drugs (*e.g.* aspirin, chloramphenicol, chloroquine, primaquine, sulphamylamide etc.) and chemicals (naphthalene and henna) have been implicated as hemolytic triggers(5-7). Hemolysis may also be triggered by ingestion of fava beans (*Vicia faba*) or even inhalation of its pollen(8). Early detection and prevention of hemolytic episodes (by avoiding the triggers) is the only cure for this disorder. Hence, a neonatal screening program for G6PD deficiency is warranted with the increased availability of funds for the health sector under the National Rural Health Mission (NRHM).

THE CONDITION

G6PD deficiency is by far the most common genetic disorder in India(4). Though the exact incidence in India is not known, various studies have reported an incidence ranging from 2% to 27.9% in different communities(9). Four hundred different variants and ninety different mutations of this disease are known globally. In India the most common mutation is the G6PD Mediterranean (563 C->T) seen in the Vatalia Prajapatis of North India and the Parsis(10). The other two mutations commonly found in India are the G6PD Kerala-Kalyan mutation (949 G->A) reported from Maharashtra, Kerala, Andhra Pradesh, Tamil Nadu and Punjab; and the G6PD Orissa (131 C->G) found in the tribals of central, eastern and southern India. G6PD Mediterranean is the most severe variety. Children with G6PD deficiency usually present with prolonged neonatal jaundice or later in life with acute hemolytic crises. The disease as such causes significant morbidity and mortality in childhood. There are no primary prevention interventions available for this disease and the only way to avoid the adverse outcomes is to recognise such children early on in life and prevent exposure to agents which can trigger hemolysis.

THE SCREENING PROGRAM

Screening programs help us to identify those individuals who cannot be identified by routine observation and physical examination. Indeed, in India, only 32% of the G6PD deficient neonates had hyperbilirubinemia(10) and 55% of the G6PD deficient newborns did not require phototherapy(11). This implies that without a newborn screening program to identify the G6PD deficient newborns, these infants run a greater risk of unexpected hemolytic anaemia if exposed to triggers. Apart from the United States of America, many countries of South East Asia (*e.g.* Malaysia, Singapore, Taiwan, Hong Kong and the Philippines) the Middle East and Europe (where the incidence of G6PD deficiency is high) have been successfully running a neonatal screening program for this disorder. Singapore was one of the first countries to introduce this mass screening program in 1965. Data from Singapore reveal that with the preventative measures, the incidence of kernicterus has dropped

dramatically, and in the last 20 years there has been only one reported case of kernicterus in newborns(12). Greece, where such a program is in operation since 1977, has reported a fourfold reduction in the hospital admission of patients for the treatment of hemolytic crisis(13).

The need for a mass screening program for G6PD deficiency has long been perceived by the pediatric and public health experts in the country. The newborn screening program covering a number of genetic diseases including G6PD deficiency which was piloted in Bangalore and Hyderabad has been well received by the health professionals and the public(14). Introduction of the screening programme will substantially decrease hospital admissions due to acute haemolysis, thereby reducing number of blood transfusions and dialysis needed. The cost of introducing the screening program can be justified by the savings in the medical care as a whole for these patients.

THE TEST

Several screening tests for G6PD deficiency are now available. The International Committee for Standardization in Hematology has recommended the Beutler Fluorescent spot test for screening of G6PD deficiency(15). However, this test requires an UV light source which poses a serious limitation to its use in mass screening programs in resource poor settings. Recently, two new screening tests have been developed which are cheaper, easier to perform, do not require an UV light source and have a high sensitivity and specificity. One of them is the color reduction test involving reduction of a blue dye, dichlorophenol indophenol, to a colourless state and is produced by Sigma Diagnostics, USA(16). The other test is the modified Formazan ring test method(17) which uses the principle of the MTT-Linked Spot test recommended by the World Health Organization Scientific Group(8), with a minor modification. In individuals with normal G6PD levels, MTT, a soluble tetrazolium compound, is reduced to a purple insoluble formazan derivative with the diameter of the discoloration ranging from under 6 mm to over 7.5 mm.

For the screening program in India, it may be prudent to adopt the modified Formazan ring test

method(17) as it uses the same filter paper (Schleicher and Schuell #903C Duren, Germany) which is used in routine newborn screening for metabolic disorders allowing easy integration with other screening program for other inborn errors of metabolism. The test is rapid with results available in less than 24 hours, requires only basic laboratory equipments and does not require extensive training of personnel. Additionally, the cost of reagents for this method is approximately \$0.64 (less than Rs 30) per subject which is affordable. A positive screen is defined as a disc with absence of bluish discoloration or a bluish discoloration less than 5.5 mm. This cut-off value has been recommended as it gives the best sensitivity (96%) and specificity (90%). It is anticipated that since the test involves a simple heel prick within the first 48 hours of life, it should be acceptable to the population, this being not against any of the prevailing social values.

Those neonates who will be identified as positive for G6PD deficiency on the screening test will need to undergo a definitive, quantitative test using a commercial kit (Sigma Diagnostics, USA)(16). An enzyme level less than 100U/ trillion RBCs has been defined as the cut off for classifying the neonate as G6PD deficient(10). Since the kit is expensive and requires the use of a spectrophotometer, these tests can be made available only at apex laboratories. However, it must be noted that the screening test can only identify patients with enzyme deficiency and cannot identify G6PD variants with normal enzyme activity(17) or the female heterozygote(16).

THE PLAN

The program could be initially piloted in selected tertiary level Government Hospitals in the four metros (*i.e.* Delhi, Kolkata, Mumbai and Chennai). The pilot will also provide valuable data regarding prevalence of G6PD deficiency in the community. WHO recommends gradual scaling up of such programs in resource poor settings(18). Subsequently, the program can be extended to cover the entire country over a period of three to four years.

In consonance with the NRHM agenda to encourage and incentivise institutional deliveries(19), the screening program may first be implemented in all institutional deliveries both in

the government and the private sector. The blood sample can be collected any time in the first 48 hours of life and the babies should only be discharged from the hospital after receipt of the report. In case the discharge has to be made earlier, adequate contact information should be available with the hospital and feedback provided by the quickest possible means. Subsequent to successful introduction in institutional deliveries, this program may be extended to home-based deliveries. In this case, the sample collection may be entrusted to the Auxiliary Nurse Midwife (ANM) or the Accredited Social Health Activist (ASHA), who can collect the sample during their routine home visit in the first seven days of life. The ANM/ASHA can provide the feedback and arrange for blood collection for the definitive test in case of infants found positive for G6PD deficiency. However, field based trials will need to be conducted prior to this extension to verify that the cut-off value of 5.5 mm for the Formazan ring indeed holds good beyond the 48 hour period. The existing laboratories at the primary health centres have sufficient infrastructure for undertaking this screening program. To handle the enhanced laboratory workload generated by this program, the 9795 presently vacant positions out of a total of 26415 required positions of laboratory technicians at the PHCs and CHCs will need to be filled up(20). The Quality Council of India (QCI) can be entrusted to develop the quality assurance standards. They can also independently monitor the quality aspects of the programme through the National Accreditation Board for Hospitals and Healthcare Providers (NABH) and the National Accreditation Board for Testing and Calibration Laboratories (NABL).

Though many would argue that it would not be easy to find funds for implementing the screening program, there is scope within NRHM budget which is committed to increase to INR 4743.9 billion by 2012(19) from the present INR 1205 billion for 2008-09(21). Additionally, funds for the pilot project can also be sought from the Norway India Partnership Initiative (NIPI) which has committed to provide 500 million NOK (INR 36 billion) over 5 years *i.e.* till 2011 for strengthening neonatal health programs in India(22).

KEY MESSAGES

- G6PD deficiency is an important public health problem in India and warrants introduction of a neonatal screening program.
- Modified Formazan ring test involving a simple heel prick in the first 48 hours of life can be used in neonates.
- A positive screen is defined as a disc with absence of bluish discoloration or a bluish discoloration less than 5.5 mm.
- The screening program can be introduced initially in institutional deliveries in major metros and then gradually scaled up.

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