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Hafsa Majid

Lena Jafri

Sibtain Ahmed

Khadija Humayun

Salman Kirmani

See next page for additional authors

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Authors

Hafsa Majid, Lena Jafri, Sibtain Ahmed, Khadija Humayun, Salman Kirmani, Natasha Bahadur Ali, Bushra Moiz, Aysha Habib, and Bushra Afroze

Perspective on newborn screening (NBS): Evidence sharing on conditions to be included in NBS in Pakistan

Hafsa Majid¹, Lena Jafri², Sibtain Ahmed³, Khadija Humayun⁴, Salman Kirmani⁵, Natasha Ali⁶, Bushra Moiz⁷, Aysha Habib Khan⁸, Bushra Afroze⁹

Abstract

Newborn screening aims at detecting treatable disorders early so that the treatment can be initiated to prevent mortality and morbidity. Such programmes are well established in most developed countries, and all newborns are screened for selected metabolic, endocrine and other disorders based on disease epidemiology, testing and treatment availability, efficiency and cost-effectiveness. Even in developing countries, such screening programmes are initiated using heel prick capillary blood collected on filter paper. The current narrative review was planned to provide a perspective with evidence in favour of starting newborn screening for different disorders. The programme project should be initiated nationwide, taking one disorder, congenital hypothyroidism, as the prototype and a newborn screening panel can then be extended to include other disorders. A task force should be set up to recommend disorders to be included in the panel, develop the national plan policies, and define procedures to strengthen the testing.

Keywords: Newborn screening, Pakistan, Congenital hypothyroidism, Congenital adrenal hyperplasia, Biotinidase deficiency, Galactosemia, Sickle cell disease, Hemoglobinopathies.

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Introduction

Newborn screening (NBS) for inherited diseases is a secondary prevention strategy that involves evaluating neonates within the first few days of life before the clinical manifestation of the diseases screened.¹ The aim is to provide early diagnosis and to prevent or ameliorate long-term consequences from a treatable disorder.

NBS programmes have been an important public health strategy for more than 50 years and have evolved tremendously over the years in the developed world.² It

^{1-3,6-8}Department of Pathology and Laboratory Medicine, Aga Khan University, Karachi, Pakistan; ^{4,5}Department of Pediatrics and Child Health, Aga Khan University, Karachi, Pakistan; ⁹Department of Pediatrics, Child Health, Pathology and Laboratory Medicine, Aga Khan University, Karachi, Pakistan.

Correspondence: Aysha Habib Khan. Email: aysha.habib@aku.edu

started from simple blood or urine screening test for phenylketonuria (PKU) in 1963 by bacterial inhibition assay; now, with the advent of 'omics', it is a more comprehensive and complex screening system capable of detecting over 50 different disorders.¹ In 2006, the American College of Medical Genetics (ACMG) recommended a screening panel comprising 44 inherited metabolic disorders (IMDs) (aminoacidopathies, fatty acid defects and organic acidemias), two endocrinopathies, three haemoglobinopathies, hearing impairment and cystic fibrosis.³ The majority of the conditions in the core panel and in secondary targets require tandem mass spectrometry (MS) as the main methodology, and others require immunoassay and fluorometry.⁴

There has been increasing pressure to add more conditions to NBS programmes, advancing omics. However, establishing and expanding screening programmes is challenging as many of these conditions are still not well understood or treatable and have the potential to shift the implications and uses of NBS. So, while establishing a NBS programme, the first and foremost is to have a consensus on conditions to be included in the programme.⁵

The W&J criterion developed in 1968 remains the gold standard to select screening conditions, and several adaptations have since emerged.⁶ The World Health Organisation (WHO) in 2008 proposed some adaptations of classic criteria, emphasising more on adequate governance and regulatory framework, programme effectiveness, integrating education, testing clinical services, programme management and evaluation from the outset.⁵ The WHO also addresses quality assurance issues by defining mechanisms for the target population to minimise potential risks, ensuring informed choice, confidentiality, respect for autonomy, promoting equity and access to screening. The overall benefits of screening should outweigh the harm.⁶ The conditions included in NBS must be carefully evaluated based on medical and scientific evidence surrounding the natural history of the condition and the local ability to decrease morbidity and mortality through screening.

The current narrative review was planned to present

evidence for including congenital hypothyroidism (CH), congenital adrenal hyperplasia (CAH), glucose 6 phosphatase dehydrogenase (G6PD) deficiency, sickle cell disease (SCD), biotinidase deficiency (BTD) and galactosemia for NBS in Pakistan. The data presented on incidence is mostly from hospital-based studies.

Congenital hypothyroidism: CH is a serious disorder afflicting neonates resulting in intellectual disability (ID) if left undiagnosed and untreated. The worldwide incidence of CH is around 1:2000-1:4000, while the estimated CH incidence in Pakistan ranges from 1:1000 to 1:1600.^{7,8} Local endemic iodine deficiency has been reported as a cause of the higher incidence of CH in Pakistan.⁹

In the United States, every state has a different core NBS panel, but all the states screen for CH. In addition, CH is included in the mandatory core NBS panel of all the developed countries, while in many developing countries, it is emerging as a priority. Some countries are performing nationwide NBS for CH and certain other congenital and metabolic disorders, whereas other countries offer screening to high-risk groups only. Most of these programmes are supported by the government of the respective countries.^{3,4}

There is a need for a nationwide NBS programme for CH in Pakistan. Primary CH screening is done by measuring thyroid-stimulating hormone (TSH) on blood spot at 48-72 hours of age.¹⁰ Babies identified with high TSH on screening should be seen within the next 24 to 48 hours for clinical assessment and advised follow-up testing to confirm CH. If TSH is ≥ 20 mU/L and / or free thyroxin (FT4) is below normal for age, treatment is initiated as early as possible.^{10,11}

Congenital adrenal hyperplasia: CAH is an autosomal recessive (AR) inherited deficiency of enzymes involved in adrenal steroidogenesis. The most common cause of CAH

is 21-hydroxylase enzyme deficiency (21-OHD), with an overall incidence of 1:14000 live births, being equally prevalent among both genders with variable phenotype.¹² Globally, morbidity and mortality from CAH has significantly reduced by the introduction of NBS for 21-OHD. Added advantages of screening include decreasing the time of gender assignment for babies born with ambiguous genitalia, planning for puberty, avoiding the development of precocious puberty and short stature in the simple virilising forms. Furthermore, carrier detection and genetic counselling for families having an affected child minimise the long-term complications for the families.¹³

From a Pakistani perspective, the incidence reported by scattered hospital-based studies is almost similar to global studies, but has a relatively higher prevalence with late diagnosis than the countries with an established NBS programme.¹⁴ Moreover, alarmingly high numbers of CAH cases, i.e. 53% and 82%, were reported from tertiary care centres from Lahore and Karachi while evaluating children with ambiguous genitalia and precocious puberty, respectively.^{14,15} Additionally, our healthcare system lacks clear policies on complex issues commonly seen in CAH, such as ethical concerns and religious obligations in legislation.¹⁶

Global experience of NBS with dried blood spot (DBS) demonstrated reliability and efficiency for CAH testing. Considering the availability of economical, effective treatment and the high number of cases of CAH reported from Pakistan, now is the time for Pakistan to start NBS for 21-OHD. There is a dire need for advocacy and national policy to cater to a suitably designed and organised infrastructure for CAH NBS alongside genetic counselling for affected patients and families. Screening and confirmatory tests available for NBS are shown in table below.

Table: Incidences of disorders which can be included in a newborn screening (NBS) panel for Pakistan.

S #	Disorder	Screening Test	Diagnostic Test	Worldwide Incidence	Estimated Burden in Pakistan
1	Congenital Hypothyroidism	TSH on dried blood spot	Serum TSH and FT4	1:2000 to 4000 live births	1:1000-1600
2	Congenital Adrenal Hyperplasia	17 OHP on dried blood spot	Serum 17OHP, Synacthen stimulation test and Molecular testing	1:14000 live births	1:5000
3	Biotinidase deficiency	Biotinidase enzyme activity on dried blood spot	Plasma Biotinidase enzyme activity	1: 60000 to 109,921 live births	1:115 in patients suspected with IMDs (unpublished data)
4	Galactossemia	Total Galactose or GALT enzyme activity on dried blood spot	Plasma GALT enzyme activity	1:40,000 to 60,000 live births	Not known
5	Glucose 6 phosphate dehydrogenase deficiency	G6PD enzyme activity on dried blood spot	Plasma G6PD activity Quantitative analysis and molecular testing	1:350 to 700 live births	4-14% of jaundiced infants
6	Sickle cell disease	Haemoglobin isoelectric focusing of an eluate from the dried blood spots	Haemoglobin HPLC (high performance liquid chromatography)	1: 500 to 16,300 live births	4% of the patients evaluated for Anaemia or other blood related disorders

Glucose-6-phosphate dehydrogenase deficiency: G6PD deficiency is an X-linked disorder affecting about 400 million individuals around the globe.¹⁷ Neonates with G6PD deficiency are susceptible to hyperbilirubinaemia and acute bilirubin encephalopathy, kernicterus and even death if untreated. The haemolytic crisis may occur later in life when exposed to certain drugs and food. Neonatal screening of jaundiced infants identified G6PD deficiency in 4-14% of babies in two large hospitals in Pakistan.¹⁸⁻²⁰ Unfortunately, up to 22% of them developed kernicterus and 4% died. This makes a strong case for mass screening of G6PD deficiency through biochemical testing. The investigation is anticipated to identify all G6PD-deficient males and homozygous females who may require extended hospitalisation and monitoring. An additional advantage would be an opportunity for parental education for seeking medical care when required.

Sickle cell disease: SCD is the most common AR genetic haematological disorder. The common genotypes include sickle cell trait (HbAS) and sickle cell anaemia (HbSS).²¹ In the US and Europe, all newborns are screened for SCD, which helps identify carriers, subsequently leading to the screening of females with positive family history, planning for pregnancy.^{21,22}

Shabbir et al. have reported a frequency of approximately 4% and 2% for SCD and HbAS, respectively, in Pakistan.²³ In 2018, recommendations from a pan-European consensus conference suggested NBS for SCD in all the participating countries.²² Early detection and appropriate management lead to improved quality of life and helps in counselling to avoid risk factors that trigger painful crisis and administration of prophylactic penicillin and pneumococcal vaccine. NBS for SCD can be done by targeted screening, restricted to "at risk" ethnic groups or universal screening when the whole newborn population is screened. The screening and diagnostic tests include complete blood picture with peripheral smear, haemoglobin (Hb) electrophoresis or high-performance liquid chromatography (HPLC) and deoxyribonucleic acid (DNA)-based tests to identify globin gene mutations.²⁴

Biotinidase deficiency: BTM is an AR inherited disorder involving an enzymatic defect in the biotin cycle.²⁵ Biotin is a cofactor for four carboxylases involved in gluconeogenesis, fatty acid synthesis and catabolism of several branched-chain amino acids. Patients with BTM may present with seizures, hypotonia, breathing problems, hearing and vision loss, ataxia, skin rash and alopecia. If left untreated, children with BTM will develop irreversible ID, hearing impairment and optic atrophy.²⁵

A review of literature identifies BTM as one of the

commonest IMDs in Pakistan.²⁶ Till to date, more than 150 patients suspected of BTM have been identified from the biochemical genetics laboratory at the Aga Khan University (AKU) on urine organic acid chromatography from across Pakistan (unpublished data). Over ten years, 33 BTM cases were confirmed using biotinidase assay and BTM gene analysis.²⁷ The BTM is an easily treatable IMD by a simple and cheap oral supplementation of biotin, preventing neurological deficit, hearing impairment and optic atrophy and preventing life-threatening complications and death. BTM is a strong candidate to be included in NBS in Pakistan as sensitive and specific screening testing on DBS and confirmatory BTM enzyme activity in plasma can be developed.^{27,28}

Galactosemia: Galactosemia is an AR inherited disorder that results from a deficiency of any one of the three enzymes catalysing the conversion of galactose to glucose: galactose-1-phosphate uridylyltransferase (GALT), galactokinase (GALK), and uridine diphosphate galactose-4-epimerase (GALE). The incidence of galactosemia reported in the literature is about 1:53,554.²⁹ The disorder is not uncommon in Pakistan. A study from Pakistan reported 22 patients with galactosemia out of the 239 patients suspected of IMDs.³⁰ An accepted low-cost treatment for patients with recognised galactosemia and facilities for diagnosis and treatment can be made available in Pakistan. Clear algorithms for diagnosis and management of screening tests for galactosemia include total galactose or GALT enzyme activity in DBS, whereas enzyme activity testing and mutation analysis are the confirmatory tests.³¹

Why Pakistan Needs NBS? Pakistan has the highest neonatal mortality rate with 44 per 1000 live births, and is declared the most dangerous country for a baby to be born. The causes of neonatal mortality reported in Pakistan are sepsis, stillbirth, birth asphyxia, pneumonia and seizures. The early neonatal presentation of IMDs are often non-specific, symptoms mimicking sepsis-like illness or birth asphyxia. Due to efforts and intervention to reduce birth asphyxia and sepsis by WHO and the government of Pakistan, neonatal mortality has declined to 44.2 per 1000 live births from 51 per 1000 live births.³² In the absence of NBS in Pakistan, these babies are not investigated; thus, there is no data to provide information on how many babies diagnosed with sepsis or birth asphyxia had an underlying IMD. It has been demonstrated that the leading cause of neonatal mortality in developed countries is non-communicable diseases, including genetic disorders. It is high time that NBS is implemented in Pakistan for selected disorders.

NBS is a system; not a test: NBS is a complete system

involving five essential components that are crucial for a successful NBS system.³³ They are as follows:

- Raising awareness and educating the public about the importance and benefits of NBS. Acceptance of NBS by the public is likely to be suboptimal without this essential component.
- Targeting the expected new parents by imparting education during the prenatal period through the healthcare team involved in pregnancy and baby delivery.
- Appropriate method of screening selected for the particular group of disorders, e.g. heel prick for IMD, haemoglobinopathies or endocrinopathies.
- A robust system to retrieve all babies with NBS-positive results to confirm all NBS-positive results through a confirmatory diagnostic test. Timely intervention providing immediate and long-term evidence-based quality treatment is essential for a complete NBS programme.
- Outcome assessment of all NBS-positive babies and all patients with true-positive results. Both the institute and province/state are responsible for ensuring that regular audits, lost to follow-up rates, developmental, physical, and mental outcomes among the affected children, presence of ongoing evaluation of the effectiveness of various treatment protocols/regimens, evidence of use of national standards to collect data and link systems are implemented.

Recommendation for Pakistan: In the light of the discussion above, we recommend including CH, CAH, BT, galactosaemia, G6PD and SCD in the screening panel for Pakistan. The conditions included should not only fulfill the W&J criterion, but must be carefully evaluated based on medical and scientific evidence surrounding the natural history of the condition and the local ability to decrease morbidity and mortality through screening.¹

Expanded NBS is performed in many developed countries for the screening of amino acidopathies, including PKU, maple syrup syndrome, fatty acid defects, like medium-chain acyl Co-enzyme A (CoA) dehydrogenase deficiency, and organic acidemias, like methylmalonic academia. However, introducing NBS for these disorders in Pakistan at this stage without concrete evidence on prevalence is not feasible due to the inequitable and limited availability of screening and diagnostic testing, special milk formulae or foods for special medical purpose, and lack of trained personnel, including metabolic specialists. It is imperative that a pilot NBS should be performed prior to

recommending any of the IMDs for NBS. In addition, as per the W&J criterion, only disorders that have appropriate, cost-effective treatments, sensitive, accurate screening and diagnostic tests available should be included in a NBS panel.¹ Screening for another disorder that is prevalent in Pakistan, but not recommended in this paper is thalassemia. However, the best strategy for preventing thalassemia major cases is a premarital screening of the couple for thalassemia trait, rather than employing NBS.

Establishing NBS services requires collaboration between laboratories, pathologists, nurses, obstetricians, paediatricians, physicians, parents/families and integration of the systems for robust delivery of services. We recommend establishing the NBS programme taking CH as the prototype because of high incidence and cost-effective tests/treatments. Once the infrastructure is in place, the programme can be expanded further to include other disorders.

Care must be taken in introducing new diseases/programmes not to make the same mistakes made by others. Carefully planned pilot-testing should always include a thorough analysis of public health impact and cost-effectiveness with an eye on the future. Developing programmes must continually take advantage of progress already made by others. Conditions and inherited disorders are nominated and vetted to be included or excluded from the NBS programme based on the burden, severity of the condition, the availability of sensitive and specific screening tests, including second-tier tests, and the availability and efficacy of treatment modalities.

Systemic approach to initiating NBS in Pakistan: We recommend careful planning of NBS. This needs to start with forming an NBS advisory committee, which should include stakeholders from the government, including the Ministry of Health, paediatricians, pathologists, obstetricians, metabolic physicians representing both private- and public-sector hospitals and involvement of lady health workers in the community. Standard operating procedures for each of the five critical components of the NBS system described earlier will need to be developed. Careful selection of disease to be included in the national NBS panel will need to be decided. Access to treatment and long-term follow-up become even more important issues when NBS is expanded. It is important to avoid conditions under which children who have undergone mandatory screening are left with a diagnosis, but no resources and means to treat their condition.^{33,34}

Conclusion

The central idea of early disease detection and treatment is essentially simple. However, the path to its achievement

(on the one hand, bringing to treatment those with the previously undetected disease, and, on the other, avoiding harm to those persons not in need of treatment) is far from simple though sometimes it may appear deceptively easy. It is important to highlight that screening does not cover all inherited diseases and that diagnosis is presumptive, as screening may yield false-negative or false-positive results. A firm diagnosis can only be reached after confirmation. Confirmatory testing can be done using the same technology or different approach on blood spot, urine and plasma. All positive NBS results either need to be confirmed or excluded after confirmatory testing.

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