

Newborn Screening using Dried Blood Spot for Seven Metabolic Disorders- A Retrospective Study from a Tertiary Care Hospital in Southern India

KARTHIKEYAN KADIRVEL¹, VINOD BABU SUGUMARAN²,
SUMATHI SRI RAMACHANDRAN³, SOUNDARARAJAN PALANISAMY⁴



ABSTRACT

Introduction: Newborn Screening (NBS) is an important public health measure in many developed countries. In developing countries like India, the benefits of NBS have been acknowledged and that screening is slowly gaining attention.

Aim: To estimate the proportion for seven conditions screened in a tertiary care hospital in Southern India namely Congenital Hypothyroidism (CH), Congenital Adrenal Hyperplasia (CAH), Glucose-6-Phosphate Dehydrogenase (G6PD) deficiency, Biotinidase Deficiency (BD), Galactosemia, Phenylketonuria (PKU) and Cystic Fibrosis (CF).

Materials and Methods: The present descriptive study was conducted at a tertiary care teaching hospital in Southern

India during a three year period between January 2018 to December 2020. A retrospective analysis of the results of NBS by dried blood spots was done. There were 3152 live births during this period out of which 1649 babies were screened (52% coverage). Heel prick samples after 48 hour of life and prior to discharge were analysed by quantitative assessment. Neonates having positive screening results were recalled by telephonic call for confirmatory tests.

Results: The CH, BD and G6PD deficiency were the most common disorders with a proportion of 1:824, 1:1649 and 1:1649, respectively. Galactosemia, CF and PKU were not found in study population.

Conclusion: These results need to be corroborated with larger studies from the same geographical area.

Keywords: Biotinidase deficiency, Congenital hypothyroidism, Glucose-6-phosphate dehydrogenase deficiency

INTRODUCTION

The NBS is screening of newborns with the goal to identify conditions that are treatable, but not clinically evident during early life. On tracking the history, NBS was initiated in 1961 by Guthrie R which is considered as one of the greatest public health achievements [1]. The recommended time for heel prick sampling is after 72 hours and within seven days of life. Early suspect and confirmation will facilitate the provision of appropriate interventions that will alter the clinical course of the disease and prevent or ameliorate the clinical manifestations. The disorders that qualify for screening must have the following critical aspects: simple and easy sample collection, a specific and cost-effective method of testing, and specific treatment is available. It is important to estimate the incidence of disorders like CH, CAH, G6PD deficiency, Galactosemia, CF, BD and PKU as there is paucity of data from India studies conducted on large sample size [2].

Screening for the above listed seven conditions has been initiated in the study in January 2018. Retrospective review of the data regarding the test reports and instigation of therapy for infants confirmed with the above disorders would be important to improve the screening programs within the available resources and local context. Information derived out of the present analysis could have a relook at the scenario and tend to revise the NBS protocols. This three-year retrospective cohort study aimed to evaluate the status of the enlisted disorders among the screened population.

MATERIALS AND METHODS

This descriptive retrospective cohort study was conducted at a tertiary care centre in Southern India between January 2018 to December 2020. Institution Human Ethics Committee approval was obtained before the collection of data (MGMCRI/IRC/04/2020/31/IHEC/199). Waiver for obtaining consent was

applied and approval obtained from the committee for the same. The identity of the neonates was kept confidential.

Inclusion and exclusion criteria: Study included all neonates who have undergone NBS. The screening included seven conditions namely CH, CAH, G6PD deficiency, Galactosemia, CF, BD and PKU. Data which was incomplete either in the clinical details or the test reports were excluded for analysis.

NBS in the Study Site

All intramural neonates, irrespective of clinical status, were offered NBS and those newborns whose parents were willing were screened between 48 hours of life and prior to discharge. Infants admitted to intensive care were screened after initial stabilisation and initiation of feed. Pretest counselling was done and samples were collected by residents of Department of Paediatrics after informed verbal consent. Three drops of capillary blood were collected on 903 S and S filter paper through a heel prick. The dried blood samples were routed through the hospital's central laboratory to the NBS laboratory. The kits used for quantitative determination are manufactured by PerkinElmer, Greenville, SC 29611. The quantitative analysis was done by enzyme assay/enzyme immunoassay [Table/Fig-1]. Reports were sent to the separate mail address created for this purpose, which was accessible only by the principal investigator. The parents of the screened neonates were informed of the reports over phone. The neonates who had an initial borderline or abnormal screening result are recalled by telephonic call for confirmatory tests. Confirmation was done by quantitative analysis of enzymes/abnormal metabolites using venous whole blood sampling and other laboratory studies as indicated. Confirmation of CF was done by Cystic Fibrosis Transmembrane conductance Regulator (CFTR) gene sequencing (Exon 11 and Exon 12).

Disorder	Assay	Cut-off value
G6PD deficiency	G6PD	>2.0 Units/gm of Hb
Congenital adrenal hyperplasia	17-hydroxyprogesterone (17-OHP)	<30 ng/mL
Cystic fibrosis	Immunoreactive Trypsinogen (IRT)	<70 ng/mL
Hyperphenylalanemia (PKU or non-PKU)	Phenylalanine	<2.5 mg/dL
Galactosemia	Total Galactose (TGAL)	<10 mg/dL
Congenital Hypothyroidism	Thyroid Stimulating Hormone (TSH)	>10 mIU/L
Biotinidase deficiency	Biotinidase enzyme activity	>50 U

[Table/Fig-1]: Disorder and the corresponding screening test with cut-off values.

Data Collection

The data regarding the demographic details was obtained from the case records. The NBS results were obtained from the e-mail

to which the results were sent. Details regarding the recall were obtained from the NBS register available to the department. Details of treatment and follow-up were procured from the case records.

STATISTICAL ANALYSIS

The data was collected and entered in Microsoft Excel. Analysis was done using JASP 0.8.4v. Data were analysed using descriptive statistics such as frequencies and proportions.

RESULTS

The NBS was carried out for 1649 neonates during January 2018 through May 2020 (The enrollment of retrospective data was between January 2018 to May 2020. Data entry and analysis was done till December 2020). The total number of live births during the above period was 3152. Around 52% of delivered babies could be successfully sampled. Logistic reason (42%) was the prime reason for refusal to participate. Fear and cultural reasons accounted for 30%, which comprised two major reasons of failure to collect samples; while, admission to the intensive care unit (15%) was the third cause. Other reasons contribute to the remaining 13%. The mean reporting time of the results was 8±1.2 days.

[Table/Fig-2] depicts the total infants screened and confirmed for the disorders. Total 11 infants were called for confirmatory tests for CH using a fixed cut-off of Thyroid Stimulating Hormone (TSH) >10 mIU/L. One child was lost for follow-up. Of the remaining 10, two were diagnosed to have transient CH. They could be taken off therapy at six and nine months of age, respectively, due to very low TSH values on therapeutic doses of thyroxine (12.5 µg/kg). They have remained euthyroid on follow-up until 12 months of age. Proportion of CH in the study population was 1:824. Only four neonates had elevated levels of 17-OHP. Three were preterm and second sampling obtained at the corrected age of 40 weeks was normal. One neonate expired with severe metabolic acidosis with a provisional diagnosis of CAH as per the hospital records. Proportion of CAH appeared to be low in the study population however more number of neonates needs to be screened to state that this geographical population has a low incidence of CAH. All 16 neonates screened positive for galactosemia had negative results by the confirmatory tests (GALT assay). Out of 17 children who had biotinidase enzyme in the range of partial deficiency in the initial screening sample, one baby was found to be affected with BD on confirmatory test. This baby was started on biotin tablet 10 mg per day and on regular follow-up. Screening for G6PDD revealed nine neonates had initially decreased G6PD levels. One infant was found to be G6PD deficient. Proportion of BD and G6PD deficiency were 1:1649 in the present population. One infant had elevated IRT (immunoreactive trypsinogen) and had no pathogenic mutation identified in gene sequencing. No samples were screened positive for PKU. Hence, Galactosemia, CF and PKU were not found in present population.

Disorder	Screening positive	Diagnosis confirmed	Proportion
CH (n=1649)	11	2 (Transient)	1:824
CAH (n=1649)	4	-	-
BD (n=1649)	17	1	1:1649
G6PDdef (n=1649)	9	1	1:1649
Galactosemia (n=1649)	16	0	-
CF (n=1649)	1	0	-
PKU (n=1649)	0	0	-

[Table/Fig-2]: Screened positive and confirmed cases in the study population.

CH: Congenital hypothyroidism; CAH: Congenital adrenal hyperplasia; BD: Biotinidase deficiency; G6PDdef-G6PD deficiency; CF: Cystic fibrosis; PKU: Phenylketonuria

DISCUSSION

This retrospective study suggests that CH, BD and G6PD deficiency are common Inherited Metabolic Disorders (IMDs) and are to be included as mandatory disorders to be screened in this region. High incidence of CH has been documented from limited published data from India. Screening of over 22,000 newborns from different parts of the country with and without iodine deficiency revealed the incidence of CH varied from 0.6-13% among normal and deficient iodine population respectively [3,4]. Desai MP et al., screened 12407 newborns for CH and the incidence extrapolated was 1:2481 [5]. The observations from published data recommend a universal screening program should include a minimum two to four disorders of which CH has top priority [6,7]. CH is a disorder that is well understood, easy to detect, and inexpensive to treat. From a financial point of view, the return on investment is very high [8].

The incidence of CAH was found to be 1:2575 in a survey conducted in Southern India [9]. Another such survey observed 38% of children with ambiguous genitalia had CAH [10]. In the same study, it was additionally observed the difficulty of missing the diagnosis among the subset of affected boys with salt losing syndrome. With appropriate counselling of the parents about the need for screening, likely outcome of treatment, and easy access to therapy along with implementation of universal screening being implemented, the scenario is bound to change. More studies involving all neonates in the study site are required for detecting CAH without phenotypic markers and thus can be recommended for screening at the national level.

Data from Western countries show that PKU is the commonest genetic disorder leading to mental retardation. In India, this disorder is relatively uncommon. Kaur G et al., screened 4451 cases for inborn errors of metabolism in Delhi and detected PKU in 4 (0.08%) cases [7]. A higher incidence of PKU has been reported in Southern India [11,12]. Appaji Rao N screened 172,369 newborns in Bangalore, detected six cases of PKU (1 in 28728 screened) [13]. PKU induced mental retardation

can be prevented by a phenylalanine restricted diet, the special diet is difficult to obtain in India and is expensive.

Wolf B conducted a worldwide survey for BD and estimated the incidence to be 1:61067 population although severe or profound disease is much rare (1: 137401 population) [14]. Only two reports of BD could be traced in Indian literature, of which one was detected on routine neonatal screening [9,15,16].

In a study done by Bisoi S et al., the prevalence of G6PD was 14.7% on screening 32,903 babies [17]. Kumta NB projected about 1.5% of the babies born in India every year has G6PD deficiency [18]. Results from Goa 2.0 program showed an incidence of 0.36% G6PDD among 10,000 neonates screened [19]. High prevalence of this disorder has been observed among tribal populations with sickle cell anaemia in India [20]. Screening G6PD deficiency should also be considered for universal screening in India.

The CF is considered to be very rare in the Indian subcontinent. Screening done among immigrants from Indian subcontinent to UK and USA revealed a prevalence of CF estimated to be between 1/10,000 and 1/40,000 [21]. One study estimated the carrier frequency of F508del mutation among neonates using cord blood samples to reflect prevalence of CF in the study population [22]. More studies are required before it can be recommended for its inclusion in the nation wide screening program.

The NBS for common metabolic and genetic disorders should be an integral part of neonatal care as early detection and treatment can help prevent intellectual and physical defects and life-threatening illnesses [23]. Present study results may contribute to the existing data on the IMDs. Local incidence and outcome data are used to convince officials to include screening as priority health program. Observations from various studies recommend that a universal screening program should include two to four disorders [6,7,24].

This study establishes the need for education of parents prior to the birth of the child about NBS as only 52% of the babies could be screened and the main reason was refusal to participate, the authors feel that education of parents may improve the status. Education about these metabolic disorders also is needed for primary care physicians and other healthcare professionals. These measures can facilitate implementation of universal NBS.

Newborn care among developing countries has flourished over the last few decades as reflected by decline in infant mortality rates. In this scenario the morbidity among survivors will be next priority for which NBS plays a vital role in detecting the treatable conditions that leads to Neurodevelopmental Disorders (NDD). Many developing countries do not yet have a policy that mandates universal NBS. Priority should be given to the disorders like CH, CAH, G6PD deficiency and BD. Population-based NBS should be carried out in predominantly rural and inner-city populations.

Limitation(s)

Present work has some potential limitations. Universal screening was not possible due to the enlisted reasons; hence the prevalence of these disorders could not be calculated. The study population was from a teaching institution serving the rural and urban parts of the study site, which may not reflect all characteristics of the population in this region. Hence, calculation of true prevalence was not possible. Further evaluation among neonates with transient CH could not be done due to limitations in estimating maternal TSH levels, maternal anti-TSH receptor antibody, and urinary iodine levels.

CONCLUSION(S)

The neonates who underwent NBS contribute to 52% of the total deliveries during the study period and the major reason for not participating was logistic reasons. CH, BD and G6PD deficiency were the most common disorders detected. Galactosemia, CF and PKU were not found in present population. The authors recommend larger studies from the geographical area to determine the prevalence of IMDs for mandating universal NBS.

REFERENCES

- [1] Guthrie R. Screening for phenylketonuria. *Triangle*. 1969;9(3):104-09.
- [2] Kapoor S, Kabra M. Newborn screening in India: Current perspectives. *Indian Pediatr*. 2010;47:219-24.
- [3] Kochupillai N, Karmarkar MG, Weightman D, Hall R, Deo MG, McKendrick M, et al. Pituitary-thyroid axis in Himalayan endemic goitre. *Lancet*. 1973;1(7811):1021-24.
- [4] Kochupillai N. Neonatal hypothyroidism in India. *Mt Sinai J Med*. 1992;59:111-15.
- [5] Desai MP, Colaco MP, Ajaonkar AR, Mahadik CV, Vas FE, Rege C, et al. Neonatal screening for congenital hypothyroidism in a developing country: Problems and strategies. *Indian J Pediatr*. 1987;54(4):571-81.
- [6] ICMR Task Force on Inherited Metabolic Disorders. Newborn screening for congenital hypothyroidism and congenital adrenal hyperplasia. *Indian J Pediatr*. 2018;85:935-40.
- [7] Kaur G, Thakur K, Kataria S, Singh TR, Chavan BS, Kaur G, et al. Current and future perspective of newborn screening: An Indian scenario. *J Pediatr Endocrinol Metab*. 2016;29:05-13.
- [8] Therrell BL. Newborn screening for congenital hypothyroidism in India: Let's Just Do It! *Indian Pediatr*. 2019;56:277-78.
- [9] Devi AR, Naushad SM. Newborn screening in India. *Indian J Pediatr*. 2004;71:157-60.
- [10] Menon PS, Virmani A, Sethi AK, Verma IC, Rohatgi M, Gupta DK, et al. Congenital adrenal hyperplasia: Experience at intersex clinic, AIIMS. *Indian J Pediatr*. 1992;59(4):531-35.
- [11] Sridhar Rama Rao BS, Narayanan HS, Narayana Reddy GN. Biochemical investigations in five patients with phenylketonuria. *Indian J Med Res*. 1970;58:1753-57.
- [12] Verma IC. Genetic disorders in India. In: Verma IC, editor. *Medical genetics in India*. Pondicherry: Auroma Enterprises; 1978;1:05-16.
- [13] Appaji Rao N. Genetic consequences of inbreeding in a large human population. *Proc Indian Natl Sci Acad*. 1991;57:361-68.
- [14] Wolf B. Worldwide survey of neonatal screening for biotinidase deficiency. *J Inher Metab Dis* 1991; 14:923-927.
- [15] Apte BN. Private practices in genetics: Future scope in Mumbai. *Bombay Hosp J*. 2006;48:93-99.
- [16] Gulati S, Vaswani M, Kalra V, Kabra M, Kaur M. An approach to neurometabolic disorders by a simple metabolic screen. *Indian Pediatr*. 2000;37:63-69.
- [17] Bisoi S, Chakraborty S, Chattopadhyay D, Biswas B, Ray S. Glucose-6-phosphate dehydrogenase screening of babies born in a tertiary care hospital in West Bengal. *Indian J Public Health*. 2012;56(2):146-48.
- [18] Kumta NB. Inborn errors of metabolism—An Indian perspective. *Indian J Pediatr*. 2005;72:325-32.
- [19] Mookken T. Universal implementation of newborn screening in India. *Int J Neonatal Screen*. 2020;6(2):24.
- [20] Colah RB, Mukherjee MB, Martin S, Ghosh K. Sickle cell disease in tribal populations in India. *Indian J Med Res*. 2015;141:509-15.
- [21] Ahuja AS, Kabra SK. Cystic fibrosis: Indian experience. *Indian Pediatr*. 2002;39:813-18.
- [22] Kapoor V, Shastri SS, Kabra M, Kabra SK, Ramachandran V, Balakrishnan P, et al. Carrier frequency of F508del mutation of cystic fibrosis in Indian population. *J Cyst Fibros*. 2006;5(1):43-46.
- [23] Miller F. The complex promise of newborn screening. *Indian J Med Ethics*. 2009;6:142-49.
- [24] Shah H. Newborn Screening. In *Proceedings of the Consultative and Policy Meet, New Delhi, India, 22 September 2015*. Available online: <https://www.researchgate.net/publication/323534983> (accessed on 26 July 2019).

PARTICULARS OF CONTRIBUTORS:

1. Associate Professor, Department of Paediatrics, Mahatma Gandhi Medical College and Research Institute, Puducherry, India.
2. Associate Professor, Department of Biochemistry, Mahatma Gandhi Medical College and Research Institute, Puducherry, India.
3. Assistant Professor, Department of Paediatrics, Mahatma Gandhi Medical College and Research Institute, Puducherry, India.
4. Professor, Department of Paediatrics, Mahatma Gandhi Medical College and Research Institute, Puducherry, India.

NAME, ADDRESS, E-MAIL ID OF THE CORRESPONDING AUTHOR:

Dr. Karthikeyan Kadirvel,
1a, Type 1 Staff Quarters, Mahatma Gandhi Medical College, Pillayar Kuppan
Pondiche 2, Chekkadi Street, Perambalur, Tamil Nadu, Puducherry, India.
E-mail: drkk3179@gmail.com

PLAGIARISM CHECKING METHODS: ^[Lain H et al.]

- Plagiarism X-checker: Apr 16, 2021
- Manual Googling: Aug 28, 2021
- iThenticate Software: Sep 15, 2021 (14%)

ETYMOLOGY: Author Origin

AUTHOR DECLARATION:

- Financial or Other Competing Interests: None
- Was Ethics Committee Approval obtained for this study? Yes
- Was informed consent obtained from the subjects involved in the study? No
- For any images presented appropriate consent has been obtained from the subjects. NA

Date of Submission: **Apr 15, 2021**
Date of Peer Review: **Jul 22, 2021**
Date of Acceptance: **Aug 28, 2021**
Date of Publishing: **Sep 30, 2021**