RESEARCH PAPER

Newborn Screening for Congenital Hypothyroidism, Galactosemia and Biotinidase Deficiency in Uttar Pradesh, India

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Objective: To assess feasibility and recall rates for newborn screening for congenital hypothyroidism, galactosemia and biotinidase deficiency in a predominantly rural and inner city population in and around the city of Lucknow in Uttar Pradesh, India.

Design: Prospective observational study.

Setting: Two tertiary-care and 5 district hospitals in and around Lucknow.

Participants: All babies born in above hospitals during the study period.

Methods: Heel prick samples were collected after 24 hours of life. Dried blood spot TSH, total galactose and biotinidase were assayed by immunofluorometry. Age related cut-offs were applied for recall for TSH. For galactosemia and biotinidase deficiency, manufacturer-suggested recall cut-offs used initially were modified after analysis of initial data.

Main outcome measure: Recall rate for hypothyroidism, galactosemia and biotinidase deficiency.

Results: Screening was carried out for 13426 newborns, 73% of all deliveries. Eighty-five percent of those recalled for confirmatory sampling responded. Using fixed TSH cut off of 20 mIU/L yielded high recall rate of 1.39%, which decreased to 0.84% with use of age-related cut-offs. Mean TSH was higher in males, and in low birth weight and vaginally delivered babies. Eleven babies had congenital hypothyroidism. Recall rates with modified cut-offs for galactosemia and biotinidase deficiency were 0.32% and 0.16%, respectively.

Conclusion: An outreach program for newborn screening can be successfully carried out in similar socio-cultural settings in India. For hypothyroidism, the high recall rate due to early discharge was addressed by age-related cut-offs.

Keywords: *Feasibility, Recall rate, Metabolic disorders, Neonate.*

Population-based newborn screening (NBS) has been carried out in developed nations for the past 40 years [1]. Many factors have limited the establishment of such a service in less developed countries: the high birth rate, low number of institutional births, and poor awareness about preventive health, among others. Most published studies on NBS in India have been carried out in a single-hospital setting [2-8]. For a national NBS program to be initiated, its feasibility has to be assessed in the framework of a regional network, with one center providing laboratory as well as scientific knowhow, serving as the node for transport of samples from all the maternity centers of the region [9]. We carried out such an outreach program for NBS in a predominantly rural and inner city population in and around Lucknow, to assess feasibility and recall rates for congenital hypothyroidism (CH), galactosemia and biotinidase deficiency (BTD) screening.

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METHODS

The study was carried out from October 2011 to December 2012. The study was approved by the Institute Ethics Committee. Pretest counseling was offered to all families and included verbal counseling along with a printed information brochure. Samples were collected after parental informed written consent. Though sampling should occur after decline of the physiological postnatal TSH surge at 48-72 hours, blood samples were collected after 24 hours of birth so that the least number of babies were missed due to early discharge [10]. Caesarean births could be sampled up to 72-96 hours of life. Babies who were sick, had birth weight <1.5kg, were born at a gestational age <35 weeks or one of twins, were advised a second sampling at 2 weeks of life. The logistic constraints

of our program did not permit second sampling in babies with birth weight of 1.5 to <2.5 kg or gestation of 35 to <37 weeks. Caregivers of neonates discharged within 24 hours of birth were counseled to come for sampling to the hospital of birth, within one week. Heel prick samples were collected on Whatman 903 filter paper. These were stored at room temperature (or at 4ºC in summer) until transportation to the laboratory at SGPGIMS, Lucknow. Assays were performed by fluoroimmunoassay (Perkins Elmer, Victor 2D, Turku, Finland). The sensitivity of the TSH assay was 4.4 mIU/L serum units (or 2 mIU/L whole blood units).Total galactose (both galactose and galactose-1-phosphate) was measured by fluorescent galactose oxidase method (sensitivity 1.3 mg/dL) and biotinidase was measured by semiquantitative fluorometric assay (sensitivity 16U). Our laboratory successfully participated in the CDC (Centre for Disease Control, USA) proficiency test.

Recall Cut-offs and Procedure

Recall rate referred to the number of infants called back for confirmatory test after an abnormal screening result, and was expressed as a percentage of the total number of infants screened. Cut offs- refers to the analyte level at which recall is done.

CH: The initial protocol adopted in our program was as per the American Academy of Pediatrics recommen-dations [10]. Neonates with screen TSH 20-40 mIU/L serum units were recalled for repeat filter paper TSH at 10 days. Those with screen TSH >40 mIU/L were recalled for immediate venous serum TSH and T4 by chemiluminescence assay (Siemens, Llanberis, UK). After the first 6500 neonates, in view of high recall rates, age-related cut-offs were used: TSH >34 mIU/L during 24-48 hours of life and >20mIU/L after 48 hours for repeat filter paper TSH, and >40 mIU/L at any age for immediate venous TSH and T4. These cut-offs were adapted from previously published programs [11,12]. Infants with confirmed CH underwent technetium thyroid scan and thyroid ultrasonography. They were immediately started on levothyroxine at a dose of 10-15 µg/kg/day.

Galactosemia: Laboratory cut-off for recall was initially decided as per the manufacturer's recommendation: total galactose ≥6.5 mg/dL (presumptive normal <6.5 mg/dL, equivocal 6.6-9.9 mg/dL and presumptive positive >10 mg/dL). On analyzing mean, standard deviation and analytic variation of the data of the initial 1791 samples for galactosemia, the cut-off for recall was changed to ≥11.7 mg/dL for galactosemia (presumptive positive >13.9 mg/dL, equivocal result 11.7-13.9 mg/dL). For those with abnormal screening results, repeat heel prick sample for total galactose assay and galactose-1-phosphate uridyl-tranferase (GALT) assay was done by fluorometricassay (Synergy HT, Biotek Instruments, USA).

Biotinidase deficiency: Initial cut offs for recall were ≥77U (Equivocal 50-77 U, presumptive positive <50U). After interim analysis on data of initial 1680 babies, these cut-offs were changed to ≤45 U (presumptive positive < 36 U, partial BTD: 17-35.9 U/l, complete BTD <17 U, equivocal 36.1-45 U). For biotinidase deficiency confirmatory testing, repeat heel prick sample and direct enzyme assay in serum was performed by spectrophotometric assay (Synergy HT, Biotek instruments, USA).

Statistical analysis: All statistical analyses were performed using SPSS Version 16. Comparison of means was done by Mann Whitney test and correlation by Spearman test.

RESULTS

NBS was carried out for 13426 neonates. Seventy-three percent of delivered babies could be successfully sampled. The mean reporting time of results was 8.8 days of age.

***Table* I** shows the trend of mean TSH values; with increasing age of sampling, there was a decline in the mean values, as expected. Interim analysis showed our recall rate for CH to be 1.4% using a fixed cut off of TSH >20mIU/L. Using the prevalence of approximately 1:1100 from recent Indian reports [13,14], this recall rate meant that for every infant diagnosed to have CH, a large number (15) were recalled who were found to be normal during confirmatory test. In the interests of using our resources for those babies most likely to have permanent severe CH, after 6500 babies we used the age-related cut offs mentioned above. Using these, recall rate fell to 0.84%.

Neonates with male sex, low birth weight and those born by vaginal delivery had a significantly higher TSH. There was no effect of gestational age and season of sampling on TSH levels (***Table* II**). Birth weight was the only variable which had a significant (but weak) correlation with TSH (r= -0.031, P<0.001).

A total of 139 babies were recalled for confirmatory samples. There was 85.7% response to recall. Eleven children were diagnosed to have CH. Their characteristics

TABLE I: MEAN SCREEN TSH (SERUM UNITS) AT VARIOUS SAMPLING AGES

Age at sampling	*Percentage of babies sampled*	*Mean (SD) (mIU/L)*
24 - <48 hrs	39.5	7.2 (5.2)
48 - <72 hrs	27.1	6.1 (5.1)
≥72 hrs	33.4	5.6 (4.8)

TABLE II PARAMETERS AFFECTING TSH LEVELS ON SCREENING

Parameter	*No.*	*TSH (mIU/L)**	*P value*
Mode of delivery			
Vaginal *vs.* Caesarean	7294; 5738	6.8 (4.5); 5.6 (3.8)	<0.001
Birth weight			
< 2.5 kg *vs.* ≥2.5 kg	3061; 10365	6.5 (4.5); 6.3 (4.2)	0.017
Sex (Male *vs.* Female)	7519; 5907	6.4 (4.4); 6.2 (4.1)	0.007
Season# (Winter *vs.* Summer)	3567; 4588	6.3 (4.1); 6.5 (4.5)	0.67
Gestational age (Preterm *vs.* Term)	1190; 11846	6.3 (4.4); 6.3 (4.2)	0.92

** Values in Mean (SD); #Winter - December to February; Summer - April to June.*

are shown in ***Web Table* I**. The male to female ratio was 1:2.6. Mean (SD) age at start of therapy was 17.7 (4.6) days. One child was lost to follow up. Of the remaining 10, two were diagnosed to have transient CH. They could be taken off therapy at 5 and 9 months of age, respectively, due to high T4 and suppressed TSH values on minimal doses of thyroxine (12.5µg). They have remained euthyroid on follow-up till 18 months of age.

For the initial 1791 samples, recall rate for galactosemia was 4.1%. After revising cut-offs for recall (as described in methodology), it was 0.32% (43/13426). All neonates who were tested by confirmatory tests were negative for galactosemia after repeat galactose assay and/or GALT assay.

In the initial 1680 samples analyzed, recall rates for biotinidase deficiency was 8%. Based on calculation of our own data, cut-offs for recalling patients was revised. Using these newer cut offs, over all recall rate of biotinidase deficiency was 0.16%. All these 22 children had biotinidase enzyme in the range of partial deficiency in the initial screening sample. None of the babies was found to be affected with biotinidase deficiency on confirmatory test.

DISCUSSION

In a regional network catering to predominantly rural and low socioeconomic strata population, we were able to demonstrate more than 70% success in sampling, more than 80% success in recall and 90% success rate in follow-up of neonates diagnosed to have CH. None of the screened infants had galactosemia or biotinidase deficiency.

Early discharge resulted in an unacceptably high recall rate; this could be due to a residual neonatal TSH surge at the time of sampling. To combat this problem, we adopted age-related cut-offs being used by other NBS programs[11,12]. Though some neonates with CH may have been missed by these higher cut-offs, a high false positive rate causes unnecessary anxiety to parents and also burdens the system. The additional yield with lowered TSH cut-offs in various programs has been mainly (but not solely) in the category of subclinical mild or transient hypothyroidism [15]. Such considerations have led many developed countries to also adopt higher cut-offs [16]. A study from India also showed a high incidence of transient hypothyroidism in children who were being treated for CH, when they were re-evaluated at age of 3 years [17]. After applying age-related cut-offs, our recall rate reduced to 0.84%; however, this is still high in comparison to some other countries using similar cut-offs [18]. Similar high recall rates have also been described from other Asian countries [19]. The high recall rate in our study could be explained by earlier age of sampling (however, even the subset sampled after 72 hours of birth had a recall rate of 0.86%), iodine deficiency[20], and inherently higher TSH in Asian and Hispanic populations [21].

Our finding of significantly higher TSH in low birth weight infants, in male babies, and in those born by vaginal delivery is in consonance with other reports [21,22]. We did not find any significant correlation between gestational age and TSH [23]. It is possible our program had too few samples from preterm infants to bring out significant associations.

Our study had some potential limitations. The sample size was relatively small and may not be adequate for calculation of a true prevalence. We were unable to do maternal TSH levels, maternal anti-TSH receptor antibody and urinary iodine levels which would have been useful for determination of etiology in cases of transient CH. Further, we were able to do repeat sceening at two weeks for babies <35 week gestation and <1.5 kg weight as our program was not equipped to handle the numbers of second screen for all babies between 35-37 weeks gestation and 1.5-2.5 kg weight. We could not detect any patient of biotinidase deficiency or

WHAT IS ALREADY KNOWN?

- Newborn screening is a cost-effective preventive health measure.
- The prevalence of CH is higher in expatriate Indian populations than the local Caucasians of European descent.

WHAT THIS STUDY ADDS?

- Newborn screen is acceptable and successful as a regional network program.
- Locally developed and/or age-related cut-offs are relevant for newborn screening, to address the problem of high recall.

galactosemia to make any interpretation about these disorders.

To conclude, population-based newborn screening could be successfully carried out in our predominantly rural and inner-city population. Age-related cut-off for TSH is useful in dealing with the problem of a high recall rate due to early discharge. Irrespective of the age at sampling, TSH in our newborns appears higher than those in Caucasian populations in developed countries. These results need to be corroborated with larger studies from our country.

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REFERENCES

1. Léger J, Olivieri A, Donaldson M, Torresani T, Krude H, van Vliet G, *et al*. European Society for Pediatric Endocrinology consensus guidelines on screening, diagnosis, and management of congenital hypothyroidism. J Clin Endocrinol Metab. 2014; 99:363-84.
2. Desai MP, Colaco MP, Ajgaonkar 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;54:571-81.
3. Desai MP, Upadhye P, Colaco MP, Mehre M, Naik SP, Vaz FE, *et al*. Neonatal screening for congenital hypothyroidism using the filter paper thyroxine technique. Indian J Med Res. 1994;100:36-42.
4. Manglik AK, Chatterjee N, Ghosh G. Umbilical cord blood TSH levels in term neonates: a screening tool for congenital hypothyroidism. Indian Pediatr. 2005;42:1029-32.
5. Sanghvi U, Diwakar KK. Universal newborn screening for congenital hypothyroidism. Indian Pediatr. 2008;45:331-2.
6. Kaur G, Srivastav J, Jain S, Chawla D, Chavan BS, Atwal R, *et al*. Preliminary report on neonatal screening for congenital hypothyroidism, congenital adrenal hyperplasia and glucose-6-phosphate dehydrogenase deficiency: a Chandigarh experience. Indian J Pediatr. 2010;77:969-73.
7. Gupta A, Srivastava S, Bhatnagar A. Cord blood thyroid stimulating hormone level – Interpretation in light of perinatal factors. Indian Pediatr. 2014;51:32-36
8. Rashmi, Seth A, Sekhri T, Agarwal A. Effect of perinatal factors on cord blood thyroid stimulating hormone levels. J Pediatr Endocrinol Metab. 2007;20:59-64.
9. Rama Devi AR, Naushad SM. Newborn screening in India. Indian J Pediatr. 2004;71:157-60.
10. American Academy of Pediatrics, Rose SR; Section in Endocrinology and Committee on Genetics, American Thyroid Association, Brown RS; Public Health Committee; Lawson Wilkins Pediatric Endocrine Society, Foley T, Kaplowitz PB, Kaye CI, Sundarajan S, *et al.* Update of newborn screening and therapy for congenital hypothyroidism. Pediatrics. 2006;117:2290-303.
11. National Newborn Screening and Genetics Resource Centre (NNSGRC), 2009 National Newborn Screening Information System. Available from: *http://genes-r-us.uthscsa.edu*. Accessed February 2, 2014.
12. Lott JA, Sardovia-Iyer M, Speakman KS, Lee KK. Age-dependent cutoff values in screening newborns for hypothyroidism. Clin Biochem. 2004;37:791-7.
13. Rama Devi AR. Newborn screening in India, Experience From Pilot Initiative (ICMR Multicenter Project). Abstract presented at 8th Asia Pacific Regional Meeting

of the International Society for Neonatal Screening. New Delhi, September 2013.
14. Mathai S. Newborn screening for congenital hypothyroidism- experience from India. Abstract presented at 8th Asia Pacific Regional Meeting of the International Society for Neonatal Screening. New Delhi, September 2013.
15. Korada SM, Pearce M, Ward Platt MP, Avis E, Turner S, Wastell H, *et al*. Difficulties in selecting an appropriate neonatal thyroid stimulating hormone (TSH) screening threshold. Arch Dis Child. 2010;95:169-73.
16. Albert BB, Cutfield WS, Webster D, Carll J, Derraik JG, Jefferies C, *et al*. Etiology of increasing incidence of congenital hypothyroidism in New Zealand from 1993-2010. J Clin Endocrinol Metab.2012;97:3155-60.
17. Nair PS, Sobhakumar S, Kailas L. Diagnostic re-evaluation of children with congenital hypothyroidism. Indian Pediatr. 2010;47:757-60.
18. Loeber JG. Neonatal screening in Europe; the situation in 2004. J Inherit Metab Dis. 2007;30:430-8.
19. Valizadeh M, Mazlomzadeh S, Nicsirat A, Shajari Z. High incidence and recall rate of congenital hypothyroidism in Zanjan Province, a health problem or a study challenge? Int J Endocrinol Metab. 2011;9:338-42.
20. Yadav S, Gupta SK, Godbole MM, Jain M, Singh U, V Pavithran P, *et al*. Persistence of severe iodine-deficiency disorders despite universal salt iodization in an iodine-deficient area in northern India. Public Health Nutr. 2010;13:424-9.
21. Herbstman J, Apelberg BJ, Witter FR, Panny S, Goldman LR. Maternal, infant, and delivery factors associated with neonatal thyroid hormone status.Thyroid. 2008;18:67-76.
22. Avis E, Turner S, Cheetham T. TSH levels in relation to gestation, birth weight and sex. Horm Res. 2009;72:120-3.
23. Slaughter JL, Meinzen-Derr J, Rose SR, Leslie ND, Chandrasekar R, Linard SM, *et al*. The effects of gestational age and birth weight on false-positive newborn-screening rates. Pediatrics. 2010;126:910-6.