RESEARCH PAPER

An Optimal Capillary Screen Cut-off of Thyroid Stimulating Hormone for Diagnosing Congenital Hypothyroidism: Data from a Pilot Newborn Screening Program in Delhi

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Objective: To determine an appropriate cut-off of capillary Thyroid stimulating hormone (TSH) for congenital hypothyroidism.

Study design: Cross-sectional.

Participants: 174,000 neonates born in different hospitals of Delhi, India, from November 2014 to October 2016.

Main outcome measures: Correlation between initial and repeat capillary TSH level and subsequent venous free thyroxine (fT4) level.

Results: 102 newborns with initial/ repeat capillary TSH level of ≥20 mIU/L (*n*=174) were confirmed to have congenital hypothyroidism at mean (SD) age of 5 (4) days. A good correlation between capillary TSH level and confirmatory venous fT4 level and postnatal age of sampling was obtained (r -0.6, -0.4). The area under the ROC curve (AUC) was 0.81 (95%CI 0.75 to 0.88), indicating referral capillary TSH level of 20 mIU/L to be a good predictor of subsequent high venous TSH level.

Conclusion: A cut off of ≥20 mIU/L for capillary TSH screening beyond 24 hours of life is optimal in the Indian setting for deciding further recall and workup, keeping a balance between sensitivity and recall rate.

Keywords: *Dried blood sample, Evaluation, Free thyroxine.*

Neonatal screening programs allow for early detection and treatment of congenital hypothyroidism (CH), which is the most common preventable cause of mental subnormality [1,2]. Reported prevalence of CH has increased over the years due to factors like detection of milder forms, increasing maternal age, and lower capillary thyroid stimulating hormone (TSH) cut-offs [3-5]. CH can be either permanent or transient. Transient CH is a transient abnormality of thyroid function which reverts later to normal and may or may not require replacement therapy lifelong. Incidence of transient hypothyroidism is variable across different regions depending on whether the condition is defined on the basis of abnormal screen results or abnormal follow-up confirmatory results at three years of age [6]. Most centres evaluate the status of children diagnosed as CH in the neonatal period at 3 years of age for likely withdrawal of therapy. In recent years, transient neonatal hyperthyrotropinemia, a term applied to those neonates with abnormal initial transient elevation of neonatal TSH with normal serum thyroxine (T4) values which reverts to normal at re-examination within/after two weeks, has gained significance due to its identification as a risk factor for persistent childhood hyperthyrotropinemia [7].

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In India, where screening is likely to be initiated across the country with Rashtriya Baal Swasthya Karyakram having CH as a target disorder; it is important to define specific cut-offs, which can yield the best sensitivity and specificity and assist in initiation of empiric therapy. The purpose of this study was to evaluate the predictive value of various TSH levels. In resource-constrained settings, it is highly unlikely that one would obtain the results of venous sample on the same day or early enough on subsequent days.

METHODS

A total of 174,000 neonates (94.5% of the total births) were enrolled between November 2014 and October 2016 from

over 20 participating hospitals with a birth cohort of 184,000 births. All intramural neonates irrespective of gestation, birthweight and admission to NICU were included in the study. Only those neonates who died within 24 hours of birth, received blood transfusion within 24 hours of birth, or shifted to another participating hospital were excluded. In preterm neonates, a second mandated sample was taken at discharge or at two weeks of postnatal age, whichever was later. The values at repeat sample in preterm and sick neonates were considered confirmatory after evaluation of the corresponding venous profile.

Heel prick samples were collected after 24 hours of birth or at discharge, whichever was later, not later than 36 completed weeks of gestation in preterm or 14 days of life, whichever was later. They were dried and transported to a central laboratory of a tertiary-care teaching hospital located in Delhi. A high TSH with low free thyroxine (fT4) levels on subsequent venous sample was considered positive for congenital hypothyroidism. The Institutional Ethics Committees of all the participating hospitals approved the research protocol. All the parents or guardians of the infants signed an individual informed consent.

TSH testing was performed on dried blood samples (DBS) on 903 S & S GE, Whatmann filter paper using GSP model 2021 (Genetic Screening Processor, Perkin Elmer, Turku, Finland). When the TSH value on the filter paper was below 10 mIU/L, it was considered negative and no further action was pursued. Results between 10 and 19.9 mIU/L were considered borderline, and a new DBS was requested usually on the second or the third day, depending upon whether the patient was in the hospital or discharged. When the new DBS values were lower than 10 mIU/L, it was considered negative. When the result was still between 10-19.9 mIU/L, venous blood was collected for estimation of fT4 and TSH. TSH values of 20 mU/L and above on initial filter paper were considered positive for CH and the newborn was taken up for biochemical and clinical evaluation immediately. When venous TSH concentration was >10 mU/L and fT4 concentration was <12 pmol/L, the neonate was treated with L-thyroxine in the dose of 10-15 µg/kg/day and was categorized as presumptive CH. Newborns with elevated venous TSH and a normal fT4 were started on L-thyroxine after confirmation of hypothyroidism on thyroid scan or ultrasound finding of an ectopic or absent gland. Ultrasonography of the thyroid gland was performed during the first month of life usually at the time of confirmatory recall. Scintigraphy of the thyroid was performed prior to initiation of therapy or within 5 days of initiation of therapy.

Statistical analyses: Analyses were performed using STATA 11 software. Sensitivity, specificity and positive predictive value was calculated for different neonatal TSH ranges. An ROC curve was obtained for sensitivity and specificity at different capillary TSH cut-off levels. Spearman rank correlation was used to determine correlation between initial screening capillary TSH and venous fT4 and postnatal age sampling. Mean, median, percentile (0.5-99.5) capillary TSH values and false positive rates were calculated for different groups.

RESULTS

A total of 1,74,000 neonates were screened between November 2014 to October 2016. TSH levels ≥20 mIU/L was found in 174 neonates, either on initial or repeat capillary screen, out of which 168 were recalled and evaluated with estimation of fT4, free tri-iodothyronine (fT3), TSH, thyroid scan and ultrasonography. On follow up, 102 newborns were confirmed as positive for CH at a mean (SD) age of 5 (4) days, thus providing an overall prevalence rate of 1 in 1706 screened (***Fig.* 1**). A total of 2400 neonates had values between 10-19.9 mIU/L on initial capillary screen. Repeat capillary screen of these neonates identified five with TSH values ≥20 mIU/L, and amongst them, three newborns were confirmed to be positive for CH.

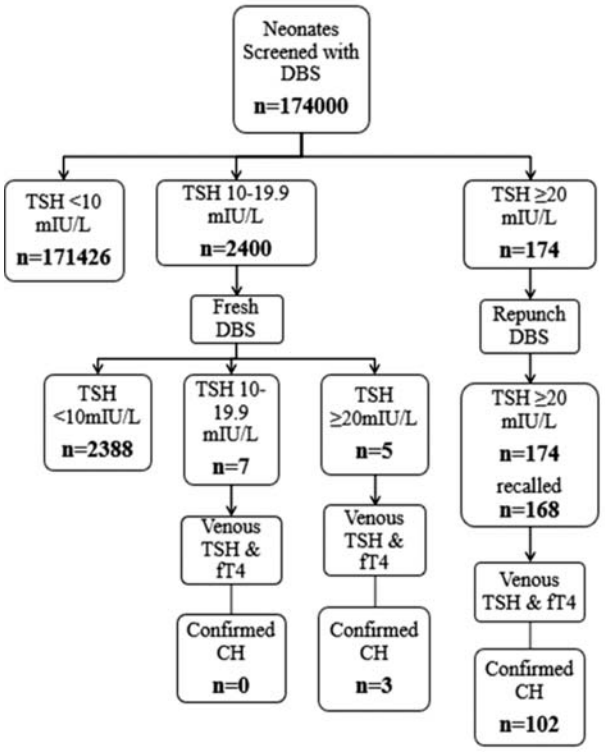

FIG. 1 *Flow diagram of the study.*

TABLE I DIAGNOSTIC PERFORMANCE OF NEONATAL CAPILLARY TSH VALUES

TSH value (mIU/L)	*Sensitivity (%)*	*Specificity (%)*	*PPV (%)*
≥20 (*n*=168)	97	99.6	61.4
≥30 (*n*=93)	76.4	99.9	83.8
≥40 (*n*=77)	66.7	99.9	88.3
≥50 (*n*=71)	62.7	100	90.1

TSH: Thyroid stimulating hormone, PPV:Positive predictive value.

Of the 102 confirmed cases, 20% (5 of 24) neonates with capillary TSH in range of 20-29.9 mIU/L had low fT4 levels (<12 pmol/L), while this was 65% (51 of 78) for neonates with capillary TSH above ≥30 mIU/L. ***Table* I** presents diagnostic performance at different TSH cut-off values. An ROC curve was obtained for sensitivity and specificity at different capillary TSH cut-off levels. Area under curve (AUC) was 0.81 (95% CI 0.75 to 0.88) (***Fig.* 2**) for a capillary TSH ≥20 mlU/L, indicating referral capillary TSH level to be good predictor of subsequent low venous TSH level.

Thyroid scan results were available for 48% of neonates. Three of the 11 newborns diagnosed as CH with capillary TSH in the range of 20-29.9 mIU/L had evidence of thyroid dysgenesis on thyroid scan indicating significant prevalence of hypothyroidism among newborns with mild elevation in capillary TSH level.

A negative correlation was seen between initial screening capillary TSH level and venous fT4 level (r= –0.6, *P*<0.001) and postnatal age of sampling (r= –0.4, *P*<0.001).

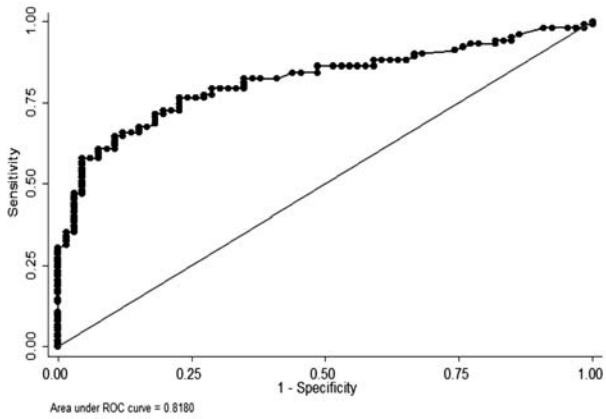

FIG. 2 *Receiver-operating characteristic (ROC) curve for capillary TSH value of ≥20 mIU/L to predict congenital hypothyroidism.*

Capillary TSH values and false positive rates were higher for newborns with samples collected within 24-<48 hours of birth (***Table* II**). The 99.5th percentile value for each group was well below the screening level of 20 mIU/L. Based on this analysis, data was retrospectively analysed with two sets of age at sampling specific cut-offs. Cut-off 1 was based on 99.9th percentile capillary TSH values for each group in postnatal age of sampling category as depicted in ***Table* III**. Cut-off 2 was adapted from published studies with capillary TSH cut off of 34 mIU/L for infants with 24-<48 hours of age at sampling and value of 28 mIU/L for newborns with age at sampling of 48 hours [9] .

TABLE II DESCRIPTIVE DATA OF NEONATES BASED ON THE POSTNATAL AGE

	Postnatal age of sampling			
	24-<48 hours	*48-<72 hours*	*72 hours -<7 days*	*≥7 days*
Number of neonates (%)	117116 (67.3)	45202 (25.9)	9781 (5.62)	1901 (1.1)
Median capillary TSH value	3.15	1.80	0.96	1.32
Percentiles (0.5-99.5)	0.37-14.75	0.17-10.56	0.08-9.7	0.11-9.22
False positive rate (%)	50	33.3	28.6	25

TABLE III SCREENING PERFORMANCE OF INDIVIDUAL CUT-OFFS FOR POSTNATAL AGE OF SAMPLING

Specific cut-off for postnatal age of sampling			*False positive rate (%)*	*Recall rate (%)*	*Sensitivity (%)*
Cut-off 1	24-<48 hours	29 mIU/L	31.1	0.046	67.4
	>48 hours	24 mIU/L	15.6	0.050	88.9
Cut-off 2	24-<48 hours	34 mIU/L	18.9	0.028	65.2
	> 48 hours	28 mIU/L	10.3	0.047	89.6

DBS: dried blood spot, Confirmed CH: TSH >20 mIU/L and fT4 <12 pmol/L.

WHAT IS ALREADY KNOWN?

- There is high prevalence of hypothyroidism in newborns with mild elevation of capillary thyroid stimulating hormone (TSH) in newborn screening.

WHAT THIS STUDY ADDS?

- A cut-off of ≥20 mIU/L for capillary TSH screening beyond 24 hours of life is optimal in the Indian setting.
- Repeat sampling is advised in newborns with initial TSH value between 10-20 mIU/L.

DISCUSSION

We report data collected from a large collaborative study group in Delhi, India,where the prevalance of CH was found to be 1 in 1706. Thyroid dysgenesis was present in significant number of neonates with confirmed CH. The referral capillary TSH level appears to be a good predictor of subsequent low venous TSH level. Significant negative correlation was obtained between initial screening capillary TSH level and venous fT4 and postnatal age of sampling. Three neonates with confirmed CH had initial screening capillary TSH of 10-20 mIU/L.

The prevalence in our study is comparable to reports from various newborn screening programs around the world [1,2]. Recent studies, including a multi-centric study by Indian Council for Medical Research, have also reported a higher prevalence of CH in India [10,11].

As seen in this study, an increase in a cut-off of capillary TSH from 20 mIU/L to 29 mIU/L will lead to an increase in sensitivity, but many (21 in our study) confirmed cases will be missed. This underscores the importance of maintaining lower TSH cut-offs to detect a significant proportion of CH cases. However, further lowering the cut-off to 10 will lead to an increase in recall rate from 0.1 % to 2%, reducing the efficacy of the program due to low positive predictive value. As all three confirmed cases with initial TSH between 10-19.9 mIU/L had a repeat capillary TSH value of ≥ 20 mIU/L, repeat sampling and recall is advisable in only those neonates whose repeat capillary screen levels of TSH is 10-19.9 mIU/L.

Similar to our findings on significant influence of postnatal age of sampling on TSH values, other studies have also suggested the use of age of sampling data in conjunction with absolute screening capillary TSH values to capture true positive cases [9,12]. Adjustment in cut-off based on postnatal age of sampling in this study also led to decrease in false positive cases and recall rates; however, there was a significant decrease in sensitivity with possibility of more than 15 confirmed cases being missed with both set of cut-offs. Thus, mild to moderate elevation of capillary TSH above screening level in newborns with postnatal age of sampling of >48 hours need to be evaluated more urgently due to lower false positive rates amongst these groups. Although, delaying postnatal age of sampling will benefit in terms of decreased recall rate but will lead to increase in overall cost of program due to increased days of hospital stay.

Across the world, except for some centres in USA and the Netherlands, most of the newborn screening programs use capillary TSH levels measured on DBS for screening CH. Cut-off for elevated TSH is different across various programs with region like Wales (Australia) using a cut-off as low as 6 mIU/L [13] to a high of 30 mIU/L [14] in Turkey. Such variations have been attributed to differences in age at sample collection and the specific type of assay used to measure TSH.

In a report from the Ontario newborn screening program, 24% newborns were confirmed to have CH with capillary TSH in the range of 17-29.9 mIU/L [12]. The combined data from 17 Italian screening centres with low TSH screening cut offs indicated that approximately 22% of neonates with permanent hypothyroidism were those who had been identified as a result of the lowered TSH cut off [15]. Studies from China, Iran and Sri Lanka consider capillary TSH of ≥20 mIU/L as a strong indicator for CH with screened newborns being referred immediately for biochemical and clinical evaluation [16-18]. In a study at a referral center in Lucknow, initial capillary TSH value between 20-40 mIU/L was utilized to recall newborns for repeat DBS at 10 days, and later on age at sampling based cut-off values of >34 mIU/L for 24-48 hours screen and >20 mIU/L for beyond 48 hours screen were used [10]. According to the recent recommendations by Indian Society for Pediatric and Adolescent Endocrinology (ISPAE), CH was confirmed and treatment was initiated when venous confirmatory TSH was >20 mIU/L before the age of 2 weeks and >10 mIU/L after the age of 2 weeks, with low T4 (<10 µg/dL) [19].

Very low TSH cut-offs (>8 mIU/L), as used by some

newborn screening programs, lead to higher sensitivity but there is added cost of fairly large number of false positive cases. Krude and Blankenstein have questioned the benefit of identification of these mild cases in terms of causing parental distress and higher recall rate [20]. In India, the public health sector is a highly resource-constrained system with limited provision for dealing with an additional number of false positive cases and high recall rate.

To conclude, optimal capillary TSH cut off is the most important determinant for success of any newborn screening program. Keeping a higher TSH cut-off increases the overall specificity but leads to significant number of missed cases and very low cut-offs leads to higher recall rate and cost of program. Thus capillary TSH cut-off of 20 mIU/L as used by our newborn screening program and other newborn screening programs across different regions can represent a way forward in this direction. Repeat capillary TSH spot is advised in newborns with initial TSH value between 10-19.9 mIU/L.

Contributors: PV, SK and BKT: conceived the idea of research paper; MK: performed the statistical analysis; PV: helped with study design; SK, SY, PV, SERB-NBS group: managed and followed up the newborns with congenital hypothyroidism. SK, BKT: conceived the primary newborn screening project and generated funding support; SERB-NBS group was involved in data collection and review of manuscript; VJ, SY, BKT and SK: critically reviewed the manuscript.

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REFERENCES

1. Grosse SD, Van Vliet G. Prevention of intellectual disability through screening for congenital hypothyroidism: how much and at what level? Arch Dis Child. 2011;96:374-9.
2. Olney RS, Grosse SD, Vogt RF Jr. Prevalence of congenital hypothyroidism–current trends and future directions: Workshop summary. Pediatrics. 2010;125:S31-6.
3. Rastogi MV, LaFranchi SH. Congenital hypothyroidism. Orphanet J Rare Dis. 2010;5:17.
4. Harris KB, Pass KA. Increase in congenital hypothyroidism in New York State and in the United States. Mol Genet Metab. 2007;91:268-77.
5. Deladoëy J, Ruel J, Giguère Y, Van Vliet G. Is the incidence of congenital hypothyroidism really increasing? A 20-year retrospective population-based study in Québec. J Clin Endocrinol Metab. 2011;96:2422-9.
6. Bhavani N. Transient congenital hypothyroidism. Indian J Endocrinol Metabol. 2011;15:S117-20.
7. Cuestas E, Gaido MI, Capra RH. Transient neonatal hyperthyrotropinemia is a risk factor for developing persistent hyperthyrotropinemia in childhood with repercussion on developmental status. Eur J Endocrinol. 2015;172:483-90.
8. Pokrovska T, Jones J, Shaikh MG, Smith S, Donaldson MD. How well does the capillary thyroid-stimulating hormone test for newborn thyroid screening predict the venous free thyroxine level? Arch Dis Child. 2016;101: 539-45.
9. Lott JA, Sardovia-Iyer M, Speakman KS, Lee KK. Age-dependent cutoff values in screening newborns for hypothyroidism. Clin Biochem. 2004;37:791-7.
10. Gopalakrishnan V, Joshi K, Phadke S, Dabadghao P, Agarwal M, Das V, *et al*. Newborn screening for congenital hypothyroidism, galactosemia and biotinidase deficiency in Uttar Pradesh, India. Indian Pediatr. 2014;51:701-5.
11. Christopher R, Rama Devi AR, Kabra M, Kapoor S, Mathur R, Muranjan M, *et al.* Newborn screening for congenital hypothyroidism and congenital adrenal hyperplasia. Indian J Pediatr. 2018;85:935-40.
12. Saleh DS, Lawrence S, Geraghty MT, Gallego PH, McAssey K, Wherrett DK, *et al*. Prediction of congenital hypothyroidism based on initial screening thyroid-stimulating-hormone. BMC Pediatr. 2016;16:24.
13. Pryce RA, Gregory JW, Warner JT, John R, Bradley D, Evans C. Is the current threshold level for screening for congenital hypothyroidism too high? An audit of the clinical evaluation, confirmatory diagnostic tests and treatment of infants with increased blood spot thyroid-stimulating hormone concentrations identified on newborn blood spot screening in Wales. Arch Dis Child. 2007;92:1048.
14. Büyükgebiz A. Newborn screening for congenital hypothyroidism. J Pediatr Endocrinol Metab. 2006;19: 1291-8.
15. Olivieri A, Corbetta C, Weber G, Vigone MC, Fazzini C, Medda E; Italian Study Group for Congenital Hypothyroidism. Congenital hypothyroidism due to defects of thyroid development and mild increase of TSH at screening: Data from the Italian National Registry of infants with congenital hypothyroidism. J Clin Endocrinol Metab. 2013;98:1403-8.
16. Zhao DH, Shen Y, Gong JM, Meng Y, Su L, Zhang X. Newborn screening for congenital hypothyroidism in Henan province, China. Clin Chim Acta. 2016;452:58-60.
17. Dorreh F, Chaijan PY, Javaheri J, Zeinalzadeh AH. Epidemiology of congenital hypothyroidism in Markazi Province, Iran. J Clin Res Pediatr Endocrinol. 2014;6: 105-10.
18. Lucas G. Guidelines on Management of Congenital Hypothyroidism in Sri Lanka. Sri Lanka J Child Health. 2015;44:75-6.
19. Desai MP, Sharma R, Riaz I, Sudhanshu S, Parikh R, Bhatia V. Newborn screening guidelines for congenital hypothyroidism in India: Recommendations of the Indian Society for Pediatric and Adolescent Endocrinology (ISPAE) - Part I: Screening and Confirmation of Diagnosis. Indian J Pediatr. 2018;85:440-7.
20. Krude H, Blankenstein O. Treating patients not numbers: The benefit and burden of lowering TSH newborn screening cut-offs. Arch Dis Child. 2011;96:121-2.

ANNEXURE 1: SERB-NBS INITIATIVE GROUP MEMBERS

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