Diagnostic Utility of Cord Blood Thyroid Stimulating Hormone in Congenital Hypothyroidism in the Era of Expanded Newborn Screening

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Abstract Even in the era of expanded newborn screening, utility of cord blood thyroid stimulating hormone (cTSH) for diagnosis of congenital hypothyroidism (CH) cannot be marginalised. This study was to assess the diagnostic utility of cTSH > 20 μ IU/L for screening CH. Generation of new cTSH value was the main outcome measure, to increase specificity. Designed as a cross-sectional analytic study in the neonatal unit of teaching hospital, 1200 term neonates with birth weight ≥ 2500 g, with no perinatal complications were included. Newborn cTSH assay was done by chemiluminescence. All screen positive were followed up on day five, 14 and 28 of life, to rule in or out CH (true or false positive). Positive predictive value and specificity were calculated. Receiver operating characteristic (ROC) was done to assess diagnostic accuracy of cTSH > 20 μ IU/L and to ascertain new cut-off to reduce false positivity. Of 1200 newborns screened, 69 (5.8%) were screen positive and followed up. In five, CH was confirmed (true positive); one in 240 newborns required thyroxine therapy. False positivity was noted in 59. Recall and dropouts were 6.25 and 7.2% respectively. Median cTSH of screen, true and false positives were 28.8, 43.5 and 27.2 μ IU/L respectively. Comparison of median values of cord blood (U = 59; $p = 0.017$) and day five serum TSH (U = 0.0; $p < 0.001$) among true and false positive subjects were statistically significant. Specificity calculated was 94.6% and positive predictive value 7.25%. ROC generated new permissible cTSH cut-off value of 30 μ IU/L. In conclusion,

an extended cTSH cut-off value of 30 μ IU/L improves specificity.

Keywords Mental retardation · Public health program · Transient hypothyroidism · TSH surge

Background

Expanded newborn screening (NBS) by heel-prick dried blood spot (DBS) has become an integral part of public health system in developed countries for pre-symptomatic detection of specific metabolic, endocrine and hematologic disorders. Wilson and Jungner in 1968 put forth criteria for inclusion of a disorder under population screening that was reviewed by Anderman in 2008 [1, 2]. Current recommendations for our country include a phased introduction of screening for congenital hypothyroidism (CH), congenital adrenal hyperplasia and glucose 6-phosphate dehydrogenase deficiency by DBS [3, 4]. A public health based population screening program needs to be comprehensive and coordinated. It includes education of parents and health providers, standardised screening techniques, interpretation of results, follow-up contact, confirmatory diagnosis by second tier testing, treatment initiation and program evaluation. Screening for CH satisfies all the criteria put forth but yet to be realised as a public health program.

CH is a preventable and treatable cause of mental retardation and delay in recognition and initiation of treatment are associated with long term effect on cognition. The worldwide incidence of CH ranges from 1:3000 to 1:4000 newborns. The recent Indian Council of Medical Research multi-centric study comprising of more than 100,000 newborns shows a higher incidence of 1:1172, and

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specifically, 1:727 in Southern India [5]. In the southern Indian state of Kerala, cord blood screening for CH was introduced in 1978 with the availability of immunoassays for serum thyroxine (T4) and thyroid stimulating hormone (TSH) [6]. Indication for cord blood TSH (cTSH) screening in the developed countries is currently limited to maternal thyroid disorders or family history of CH [7]. Single TSH measurement, in either cord blood or heel-prick DBS on the fifth day of life, is simple with a relative low false positive rate but will not detect central hypothyroidism. It may be false negative in preterm and low birth weight due to hypothalamic immaturity. The true positive rate for CH in NBS is 1 in 26 (3.9%) of screen positive.

There are 200,000 children born every month in our country, majority in public health centres. Early detection and treatment of CH during the first few weeks of life has been proven to be cost effective. Failure to screen for hypothyroidism results in a huge burden of children with mental disability. In the absence of NBS as a public health programme, it is extremely important that we have a screening and follow up protocol in place using cTSH for early diagnosis of CH for initiation of thyroxine therapy. Though a cTSH cut off of 20 $\mu\text{IU/L}$ has been used in several studies it is shown to be associated with high recall and dropout rate [8]. An effective social system whereby babies could be reached at home or re-called after discharge is practically non-existent. There are no national guidelines on follow-up of newborns who are screen positive and subsequently with intermediate results in confirmatory tests. Thus, this study was designed to assess the diagnostic utility of cTSH > 20 $\mu\text{IU/L}$ in NBS of CH.

Methods

Design, Subjects and Method

It was a cross-sectional analytical study of cTSH of 1200 neonates born over a period of 18 months in the affiliated hospital of our institution. Sample size was calculated for 3% (0.03) screen positivity and absolute precision of 0.01. Educational material on the need for NBS for CH in local languages were prominently displayed in the post-natal wards and parental counselling was done for neonates who required definitive testing and follow-up. Inclusion criteria included term neonates with gestational age >37 completed weeks by dates or sonogram and birth weight ≥ 2500 g. Neonates with birth asphyxia requiring positive pressure ventilation, tertiary care, major congenital malformation or syndromes and maternal hyperthyroidism were excluded. Umbilical cord blood sampling was done

after placental delivery. Cord was cleaned and the clamp briefly released and three millilitres of blood was collected into a sterile vacutainer. TSH assay was done by chemiluminescence method and was expressed as $\mu\text{IU/L}$. Maternal thyroid status, gender, and neonatal anthropometry were recorded.

Interpretation of Screening Results and Follow-Up of Screen Positive

Newborns with cTSH < 20 $\mu\text{IU/L}$ were considered screen and true negative and no further follow up was done. Newborns with values ≥ 20 $\mu\text{IU/L}$ were considered screen positive. Screen positive babies were followed up with serum TSH (sTSH) and free T4 (FT4) as a measure of unbound T4 on day 5 (D5) of life. Intermediate results on D5 were followed up on days 14 and 28 of life with thyroid function tests to rule in or out CH. Interpretation of the tests were based on pre-determined protocols as outlined in flow charts (Fig. 1a, b). Further evaluation for definite CH was done with radiograph of the knee and where feasible, technetium isotope scan.

Statistical Analyses

Statistical analysis was done using software package SPSS 15.0. For categorical data, proportions were calculated and for continuous data mean or median were used. Mean was expressed as $\pm$ standard deviation. Screen positive were divided into true and false positive based on follow-up values. Comparison of groups was done using Mann–Whitney U test as positive values were skewed. Positive predictive value was estimated using the formula: True positive/(True positive + False positive). Specificity was calculated by using the formula: True negative/(True negative + False positive). It was presumed that all screen negative was true negative based on literature [8]. Receiver operating characteristic (ROC) was done to assess the diagnostic accuracy of cTSH and to ascertain a new cut off to reduce false positive rate.

The study was approved by Institutional Ethics Committee and consent was obtained from either of the parent.

Results

A total of 1200 term newborns with birth weight more than 2500 g were screened for CH using cTSH of which 69 (5.8%) were screen positive with value ≥ 20 $\mu\text{IU/L}$. Five screen positive newborns were followed up only clinically and were not excluded from analysis.

Fig. 1 Flow chart showing interpretation and follow-up of **a** cord blood thyroid stimulating hormone screening, **b** intermediate thyroid function results in screen positive

Demography of Study Population

Of the newborns screened, 608 (50.7%) were male and 592 (49.3%) female. The mean gestational age was 38.6 ± 1.07 weeks. The mean birth weight, length and head circumference corresponded to 2941.27 ± 374.73 g, 49.45 ± 1.01 cm and 34.37 ± 0.86 cm respectively. Thirteen (1.1%) mothers were treated for hypothyroidism during pregnancy. The mean cTSH value was 10.28 ± 32.22 , median 7.30 and mode 5.0 μIU/L. Standard error of mean was 0.93. The 5th, 50th and 95th centiles of cTSH value corresponded to 3.36, 7.30 and 22.09 μIU/L respectively.

Demography of Screen Positive Subjects

Of the screen positive newborns, 39 were male and 30 female which accounted for 6.4 and 5.1% of the respective gender. No statistical significance was noted between screen positivity and gender (Chi square: 1.004; *p* value: 0.32). None of the babies born to mothers with hypothyroidism were screen positive. The mean cTSH was 48.24 ± 128.53 with median at 28.8 μIU/L. The minimum value was 20.3 and maximum 1095 μIU/L. The 5th, 50th and 95th centiles were 21.0, 28.8 and 71.6 μIU/L respectively. The mean TSH of the screen negative babies was 7.96 ± 3.78 μIU/L.

Follow-Up of Screen Positive

Of the screen positive, in five newborns the cTSH and FT4 values on D5 suggested definite CH with need for thyroxine therapy and were considered to be true positive (Table 1). Five (7.2%) did not agree for further evaluation and this included one newborn with intermediate result.

Thyroid function done on D5 was normal in 53 (76.8%), intermediate in six (8.6%) requiring repeat evaluation on day 14 which was subsequently normal. No newborn required day 28 follow up. Other newborns (*n* = 1131) who were screen negative were taken as true negative. The mean cTSH value of five true positive cases was 251.56 ± 471.57 with median 43.5 μIU/L. All these newborns had absent knee epiphyses suggesting an intra-uterine onset. The newborn with cTSH 1095 μIU/L was confirmed to have organification defect by isotope scan. Maternal carbamazepine therapy contributed to another. The mean and median values among false positive cases were 31.13 ± 11.66 and 27.2 μIU/L respectively. Comparison of cTSH among the true (*n* = 5; mean rank = 55.2) and false (*n* = 64; mean rank = 33.42) positive subjects was statistically significant (*U* = 59.0; *p* = 0.017). Similar comparison of serum TSH on D5 among the true (*n* = 5; mean rank = 63.0) and false (*n* = 60; mean rank = 30.5) positive subjects was also statistically significant (*U* = 0.0; *p* < 0.001).

Diagnostic Utility of cTSH ≥ 20μIU/L and a New Cut-Off

All true positive had cTSH > 30μIU/L. In 35 newborns who did not require thyroxine therapy, cTSH showed clustering of values between 20 and 30 μIU/L in the scatter plot (Fig. 2). The need for initiation of thyroxine therapy in this study was 1 in 240 newborns screened and 1 in 18 of screen positive. Specificity calculated was 94.6% and positive predictive value 7.25%. The highest sensitivity of 1.00 and the least (1—specificity) of 0.36 derived by ROC corresponded to cTSH value of 31.34 μIU/L (Fig. 3).

Hence, a value of 30 mIU/ml may be designated as cut off value, to increase the specificity.

Table 1 Serial thyroid function test values in children with intermediate results and definite congenital hypothyroidism (CH)

Sl. no.	Cord blood TSH ($\mu\text{IU/L}$)	Day 5		Day 14		Remarks
		TSH ($\mu\text{IU/L}$)	FT4 (ng/dl)	TSH ($\mu\text{IU/L}$)	FT4 (ng/dl)	
1.	34.7	26.5	2.1	–	–	Definite CH; absent epiphysis in knee x-ray
2.	31.4	44.5	1.2	–	–	Definite CH; maternal carbamazepine therapy
3.	53.1	21.1	2.1	–	–	Definite CH; absent epiphysis in knee x-ray
4.	43.5	49.3	1.5	–	–	Definite CH; absent epiphysis in knee x-ray
5.	1095.0	484	1.4	–	–	Definite CH; radioisotope scan showed organification defect
6.	78.8	9.0	0.7	–	–	Intermediate; did not consent for Day14 testing
7.	37.4	12.0	3.0	3.4	2.1	Intermediate
8.	37.0	11.0	2.5	3.0	1.5	Intermediate
9.	25.5	11.0	2.5	2.0	1.5	Intermediate
10.	27.2	11.7	3.6	2.0	1.5	Intermediate
11.	28.8	10.7	4.0	3.0	2.0	Intermediate

TSH thyroid stimulating hormone, FT4 free thyroxine

Fig. 2 Scatter diagram of cord blood and day five (D5) thyroid stimulating hormone (TSH) with arbitrary cut-offs shown at 30 and 20 $\mu\text{IU/L}$ in the two axes. An outlier value of 1095 $\mu\text{IU/L}$ is not shown

Discussion

Primary abnormalities in thyroid development, most commonly a dysgenesis with ectopia or dysmorphogenesis with goitre contribute to most causes of CH. These are usually permanent and would require imaging studies to confirm. The incidence of CH in India is higher than population based incidences reported in western countries [5, 9, 10]. Central CH resulting from a defective hypothalamo-pituitary axis is uncommon with an incidence of 1:25,000. Transient CH is seen with prematurity, endemic iodine deficiency, maternal medications and antibodies and may necessitate thyroxine therapy if thyroid function

Fig. 3 Receiver operating characteristic curve of cord blood thyroid stimulating hormone (TSH) for prediction of congenital hypothyroidism. The cut-off value for maximising area under curve was 31.34 $\mu\text{IU/L}$

abnormalities persist beyond 4 weeks [11]. Early clinical diagnosis is difficult at birth and the time of initiation of therapy is a critical determinant of outcome [12].

In 130 neonates Seth et al. [13] found no statistically significant difference in TSH sampled by cord blood and venous sample obtained on day 3, which suggest that cTSH assay is easier and ethically appropriate when screening is limited to CH. This also ensures better compliance and reduced dropout rate. Several authors report spurious high

or low cTSH values with perinatal factors such as prematurity, low birth weight, maternal thyroid diseases, birth asphyxia and forceps extraction [7, 12, 14]. Hence screening should be limited to term neonates with normal birth weight not requiring intensive care and the babies at risk should undergo definitive evaluation. Nair et al. [15] re-evaluated 36 children started on thyroxine based on NBS at 3 years of age. Only 50% of them were confirmed to have permanent hypothyroidism. Considering the consequences of deficient thyroxine during developmental period, it is imperative that newborns receive therapy even if it is likely to be a transient CH.

Manglik et al. studied 1200 cTSH in term babies with birth weight ≥ 2500 g. A complete thyroid profile was done at 7–10 days of age in all babies with cTSH > 20 $\mu\text{IU/L}$ [8]. Out of 22 babies who were screen positive, CH was confirmed in two (9%). The study concluded that a cut off value of cTSH > 20 $\mu\text{IU/L}$ is adequate for neonatal thyroid screening in Indian settings. In a recent study from a tertiary care hospital in Kerala using cTSH > 10 $\mu\text{IU/L}$ as the cut-off, 403 of 1950 babies that included preterm, required rescreening with a recall rate of 10.1% [9]. Intermediate results were followed up and the need for therapy was noted for 1 of 243. Desai et al. [16] screened 12,407 newborns for CH using cTSH measurement and anticipating follow-up difficulties, T4 was assayed in cord sera when TSH was > 30 $\mu\text{IU/L}$. About 2.81% (350) babies needed recall. Only 30% of 302 babies responded to recall letters and were all normal; 48 babies with cTSH > 90 $\mu\text{IU/L}$ had to be traced by home visits and CH was confirmed in 5 (10.4%). The prevalence of CH in their study was 1:2481. In our study 6.25% (75 visits) babies required recall and thyroxine requirement was seen in 1:240. The dropout rate in our study was 7.2% of screen positive. This study has been successful in reducing the recall and dropout rates due to selection criteria, pre-determined protocols and parent education. Adding cord T4 estimation to NBS adds complexity to interpretation and additional expenditure. As screening tests require confirmation with follow-up serum samples it may be limited to only cTSH estimation. Although previous studies have utilized 20 $\mu\text{IU/L}$, the cut-off value of 30 $\mu\text{IU/L}$ allows for greater specificity and decreased cost burden, as a public health programme. Limitation in this study is all screen negative were considered to be true negative.

In conclusion, one in 240 newborns required thyroxine therapy following screening. Pre-determined protocols and parental education would considerably improve the compliance for follow-up and confirmatory evaluations. An extended cTSH cut-off value of 30 $\mu\text{IU/L}$ is of diagnostic utility. Until expanded NBS is made a public health programme, cTSH screening will continue to be relevant. The expenditure incurred during implementation of NBS for

CH pales in comparison to the impending augmentation of the Quality-adjusted life year (QALY) index, marking it as being invaluable.

Compliance with Ethical Standards

Ethical Approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed Consent Informed consent was obtained from all individual participants included in the study.

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