Original Article

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Paediatrics Section

Comparison of Diagnostic Accuracy of Cord Blood TSH and 3rd Day Venous Blood TSH in Screening Congenital Hypothyroidism: A Cross-sectional Study

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ABSTRACT

Introduction: Neonatal screening for Congenital Hypothyroidism (CH) is necessary, as it is one of the most common disorders related to mental impairment and growth retardation in newborns. Screening for CH can be conducted through either cord blood testing or venous blood testing. The accuracy of these tests in screening for CH is important for clinicians.

Aim: To compare the diagnostic accuracy of cord blood Thyroid Stimulating Hormone (TSH) with day 3 venous blood sample TSH estimation in screening for CH and to estimate the incidence of CH in full-term neonates.

Materials and Methods: The present cross-sectional study conducted in the Department of Paediatrics, Dr. D.Y. Patil Medical College, Navi Mumbai, Maharashtra, India, from April 2021 to October 2022. Term newborns born to euthyroid mothers were screened for CH at birth to analyse the cord blood TSH levels, and a repeat TSH estimation was performed on the 3rd postnatal day for those with abnormal values. Neonates with day 3 TSH levels greater than 10 mIU/L were subjected to a repeat thyroid function test at two weeks of life; persistence of elevated TSH levels was considered indicative of CH. The values were statistically analysed. Sensitivity, specificity, Positive Predictive Value (PPV), Negative Predictive Value (NPV) and diagnostic accuracy were calculated.

Results: A total of 1,066 term newborns were screened for CH. The mean±Standard Deviation (SD) maternal age was 27.34±4.52 years. The male-to-female ratio among neonates was 1.5:1. Out of these 1,066 newborns, 100 had cord blood TSH levels greater than 10 mIU/L. Among these 100 newborns, 19 had day 3 TSH levels greater than 10 mIU/L. Of these 19 newborns, three were diagnosed with CH upon further follow-up at two weeks. The incidence of CH was three cases in 1,066 newborns. The diagnostic accuracy of cord blood TSH with cut-offs of greater than 10 mIU/L and greater than 20 mIU/L was 18% and 75%, respectively. In contrast, the diagnostic accuracy of day 3 TSH with the same cut-offs was 85% and 98%, respectively.

Conclusion: Venous blood TSH has a higher accuracy in screening for CH than cord blood TSH; however, it requires an invasive prick and a three-day hospital stay.

Keywords: Euthyroid mothers, Predictive value of tests, Incidence, Neonatal screening, Thyroid stimulating hormone

INTRODUCTION

Congenital Hypothyroidism (CH) is the most common preventable cause of intellectual disability [1]. It is caused by thyroid hormone deficiency at birth [2]. The hormones produced in the thyroid gland are essential for the growth and maturation of many target tissues, such as the brain, skeleton and gonads in children [3,4]. The thyroid gland controls the rate at which the body uses energy and regulates the body's sensitivity to other hormones. These hormones also regulate the growth and function of many other systems in the body. Thyroid hormones stimulate the oxygen consumption of most cells and help regulate lipid and carbohydrate metabolism, which are necessary for normal growth and maturation. The absence of thyroid hormones can lead to mental and physical slowing, poor resistance to cold, intellectual disability and dwarfism in children [5].

The incidence of CH worldwide is between 1 in 500 to 1 in 3,000 live births, depending on ethnicity [6]. In India, the incidence is approximately 1 in 1,000 [7]. CH can be classified as primary or secondary. Primary hypothyroidism occurs due to either thyroid dysgenesis or thyroid dyshormonogenesis. Secondary or central hypothyroidism at birth results from a deficiency of TSH; some cases may be familial, usually caused by inborn errors of thyroid hormone synthesis, and may be associated with goitre [8]. Thyroid hormones play a significant role in multiple organ systems, especially the brain. Most infants with CH appear normal at birth and show no signs of the condition. This can be attributed to the transplacental passage of maternal thyroid hormones or to moderately functioning thyroid tissue [9]. The main objective of screening for CH is early detection and treatment to prevent or minimise neuropsychological damage, which can become irreversible if treatment is delayed beyond the first few weeks of life [10].

Neonatal screening methods measure thyroid function tests in either cord blood or samples taken on the 3rd or 5th day of life [10]. Studies conducted by Al Juraibah F et al., and Alameer S et al., showed the superiority of cord blood testing, while a study by Vani KT and Prakash SS, demonstrated the superiority of venous blood testing [11-13]. All these studies discuss the sensitivity, specificity, and diagnostic accuracy of both cord blood testing and venous blood testing. This study was conducted to examine the variations in diagnostic parameters at different cut-offs of TSH.

With this background, the present study was conducted to compare the diagnostic accuracy of cord blood TSH with TSH estimation from

day 3 venous blood samples in screening for CH and to estimate the incidence of CH in full-term neonates.

MATERIALS AND METHODS

This cross-sectional study was conducted in the Department of Paediatrics, Dr. D.Y. Patil Medical College, Navi Mumbai, Maharashtra, India, from April 2021 to October 2022. The study was approved by the Institutional Ethical Committee (IEC) (IEC Ref No: DYP/IECBH/2021/059).

Inclusion criteria: Term neonates with a birth weight of more than 2.5 kg, born to euthyroid mothers who delivered at the study Institute were included in the study. A thyroid profile test for mothers was conducted at the first visit during the antenatal period.

Exclusion criteria: Neonates admitted to the NICU, preterm and low birth weight neonates, neonates discharged before three days of life, and outborn babies were excluded from the study.

Sample size: A total of 1,066 newborns, following the inclusion and exclusion criteria, were screened during the study period.

Study Procedure

Samples were collected in a sterile container from cord blood at birth and from venous blood on day 3 for TSH analysis. TSH was measured using the chemiluminescent immunoassay technique on Simon's machine. Differences between cord blood testing and venous blood testing are explained in [Table/Fig-1] [11].

Cord blood testing	Venous blood testing
Sample collected is cord blood	Sample collected is venous blood
Easy to collect	Difficult to collect
Non-invasive	Invasive
Sample collected at birth	Sample collected on 3rd or 5th day of life
Less chances of infection to baby	More chances of infection to baby
Results are available before mother is discharged	Results are late as they are sent after day 3
TSH surge due to stress of delivery at birth leading to false positive results	TSH levels normalise by day 3, so less false positive results

[Table/Fig-1]: Comparison of cord blood testing vs venous blood testing [11].

Maternal and neonatal details were documented, including maternal age, parity, maternal illness, baby gender, nature of delivery and birth weight of the neonates. All the enrolled babies were observed until discharge. A level of >10 mIU/L was considered the cut-off value for high TSH in cord blood, which was classified as positive for cord blood TSH [14]. Neonates with day 3 TSH >10 mIU/L were subjected to a repeat thyroid function test at two weeks of life, and clinical manifestations were noted. Persistence of elevated TSH in the second week of life was considered CH [15].

STATISTICAL ANALYSIS

All the data was collected, and appropriate graphs and tables were created using the software Statistical Package for Social Sciences (SPSS) 22.0. The appropriate tests were applied, including the Chi-square test, Fisher's exact test, and calculations for sensitivity, specificity, PPV, NPV and diagnostic accuracy were performed.

RESULTS

The 1,066 newborns were screened during the study period, out of which 100 newborns had high TSH levels in their cord blood. Among these neonates, the mean±SD maternal age was 27.34±4.52 years. The majority of them were multigravida (62%). The male-to-female ratio was 1.5:1. Most of the neonates were born via normal vaginal delivery (53%), while 46% were delivered by Lower Segment Caesarean Section (LSCS), and one was delivered via vacuum-assisted delivery. The mean±SD birth weight was 2,889.81±356.54 g. Mothers of 18 (18.0%) neonates had maternal illnesses, such as Pregnancy-induced Hypertension (PIH) (10%) and Gestational Diabetes Mellitus (GDM) (8%), while 82 (82.0%) mothers did not have any maternal illnesses. The mean cord blood TSH was 20.02±18.09 mIU/L. The mean TSH on day 3 was 7.48±10.66 mIU/L [Table/Fig-2].

Characteristics	Mean±SD/ n (%) N=100
Maternal age (years)	27.34±4.52
Parity	
Primigravida	38 (38.0)
Multigravida	62 (62.0)
Maternal illness	18 (18.0)
PIH	10 (10)
GDM	8 (8)
Baby gender	
Male	60 (60.0)
Female	40 (40.0)
Nature of delivery	
Normal Vaginal Delivery (NVD)	53 (53.0)
LSCS	46 (46.0)
Vaccum	1 (1.0)
Birth weight (gm)	2889.81±356.54
Cord blood TSH (mIU/L)	20.02±18.09
Venous blood TSH at day 3 (mIU/L)	7.48±10.66

[Table/Fig-2]: Distribution of baseline characteristics included in the study.

A total of 1,066 term newborns were born during the study period to euthyroid mothers who were screened for CH. Out of these, 100 neonates were found to have cord blood TSH levels greater than 10 mIU/L. Among them, 74 (74%) of the participants had cord blood TSH levels between 10 mIU/L and 20 mIU/L, while 26 (26.0%) had cord blood TSH levels greater than 20 mIU/L. The mean±SD of cord blood TSH was 20.02±18.09 mIU/L, and the cord blood TSH levels ranged from 10-150 mIU/L.

Out of these 100 neonates who had cord blood TSH levels higher than 10, 81 (81.0%) had venous blood TSH levels of ≤10 mIU/L. Fourteen (14.0%) of the participants had venous blood TSH levels between 10 mIU/L and 20 mIU/L, while 5 (5.0%) had venous blood TSH levels greater than 20 mIU/L [Table/Fig-3]. The mean±SD of venous blood TSH was 7.48±10.66 mIU/L, and the venous blood TSH levels ranged from 0.4-100 mIU/L.

The diagnostic performances of cord blood and venous blood testing at different TSH cut-offs are explained in [Table/Fig-4]. The diagnostic accuracy of venous blood TSH with cut-offs of >20 and >10 was 98% and 85%, respectively, whereas for cord blood TSH, the accuracies were 75% and 18% for the same cut-offs. A high significance (p-value< 0.001) was noted for venous blood testing.

Venous blood TSH at day 3	Cord blood TSH, n (%)			p-value
	10-20 mIU/L	>20 mIU/L	Total	
≤10 mIU/L	61 (82.4)	20 (76.9)	81 (81.0)	0.242
10-20 mIU/L	11 (14.9)	3 (11.5)	14 (14.0)	
>20 mIU/L	2 (2.7)	3 (11.5)	5 (5.0)	
Total	74 (100.0)	26 (100.0)	100 (100.0)	

[Table/Fig-3]: Association between 'cord blood TSH' and 'venous blood TSH'.

conducted by George RT et al., and Raj S et al., have reported higher incidence levels [Table/Fig-5] [10,17-20]. The recent increase in the incidence of CH may be attributed to improvements in screening techniques for CH.

Upon analysing demographic details such as birth weight, maternal age, parity, nature of delivery and maternal illness, the present study found no significant associations between CH and these variables. A study by Jacob AS et al., also shows a similar pattern [21].

Predictor	Sensitivity	Specificity	PPV	NPV	Diagnostic accuracy	p-value
Cord blood TSH (Cut-off: >10 mIU/L)	100.0%	15.5%	3.5%	100.0%	18.0%	0.460
Cord blood TSH (Cut-off: >20 mIU/L)	66.7%	75.3%	7.7%	98.6%	75.0%	0.103
Venous blood TSH (Cut-off: >10 mIU/L)	100.0%	84.5%	16.7%	100.0%	85.0%	<0.001*
Venous blood TSH (Cut-off: >20 mIU/L)	100.0%	97.9%	60.0%	100.0%	98.0%	<0.001*

[Table/Fig-4]: Comparison of the diagnostic performance of various predictors in predicting Congenital Hypothyroidism (CH).
*The p-value <0.05 was considered statistically significant

Out of the 1,066 neonates who underwent CH screening during the study period, 3 (0.28%) were found to develop CH by the end of the second week of life on repeat thyroid function tests.

DISCUSSION

Congenital Hypothyroidism (CH) is a preventable cause of intellectual disability and developmental delay [16]. Universal screening of all neonates for TSH levels has long been recognised as the most effective method to prevent severe developmental and physical abnormalities associated with CH [17]. The ideal sample for newborn thyroid screening remains controversial. Therefore, the present study was conducted to compare cord blood TSH and venous blood testing, as well as to estimate the incidence of CH.

The incidence of CH in India is 1 in 1,000 live births [7]. However, the incidence of CH was three in 1,066 neonates in this study. Manglik AK et al., Rasul CH et al., Sanghvi U and Diwakar KK, have reported incidences similar to those in this study [18-20]. In contrast, studies

In the present study, when considering a cord blood TSH level of 10 mIU/L as the cut-off, 100 (9.3%) newborns had high cord blood TSH levels. Conversely, when considering a cord blood TSH level of 20 mIU/L as the cut-off, 26 (2.4%) newborns had high cord blood TSH levels. Statistically, cord blood TSH with a cut-off >10 mIU/L has a sensitivity of 100%, but a specificity of 15.5% and a diagnostic accuracy of 18%. In contrast, when using a cut-off >20 mIU/L, sensitivity decreased to 66.7%, while specificity and diagnostic accuracy increased to 75.3% and 75%, respectively. The present study is comparable to that of Vani KT and Prakash SS, who observed that 131 out of 1,550 newborns had high cord blood TSH (8.4%) when using a cut-off of 20 mIU/L [13]. The diagnostic parameters reported by Vani KT and Prakash SS, are similar to those of the present study [Table/Fig-6] [11-13]. In the present study, the diagnostic parameters of cord blood TSH were not statistically significant (p-value=0.103).

Studies conducted by Al Juraibah F et al., and Alameer S et al., who established a cord blood TSH cut-off of 30 mIU/L, demonstrated

Name of the author	Place/Year of the study	Sample size	Method of estimation Cord blood/venous blood	Incidence of CH
Present study	Navi Mumbai/ 2022	1066	Both cord blood and venous blood	3 in 1066
Manglik AK et al., [18]	Kolkata/ 2004	1200	Both cord blood and venous blood	2 in 1200
Rasul CH et al., [19]	Dhaka, Bangladesh/ 2010	1000	Cord blood	1.5 in 1000
Sanghvi U and Diwakar KK [20]	Kochi/ 2006	2872	Venous blood	2.1 in 1000
George RT et al., [10]	Ernakulam/ 2017	272	Both cord blood and venous blood	4 in 272
Raj S et al., [17]	Trivendrum/ 2010	430	Both cord blood and venous blood	3 in 430

[Table/Fig-5]: Comparison of incidence of CH in different studies [10,17-20].

Name of the author	Place/Year of the study	Sample size	Cut-off	Sensitivity	Specificity	Diagnostic accuracy
Present study	Navi Mumbai, Maharashtra, India/2022	1066	Cord blood TSH >10	100%	15.5%	18%
			Venous blood TSH >10	100%	84.5%	85%
			Cord blood TSH >20	66.7%	75.3%	75%
			Venous blood TSH >20	100%	97.9%	98%
Vani KT and Prakash SS, [13]	Davangere, Karnataka, India/2023	1550	Cord blood TSH >20	100.0%	91.7%	91%
			Venous blood TSH >20	100%	91.7%	91%
Al Juraibah F et al., [11]	Riyadh, Saudi Arabia/ 2013	17729	Cord blood TSH > 30	100%	99.6%	99.6%
			Venous blood TSH >20	100%	98.3%	98.3%
Alameer S et al., [12]	Jeddah, Saudi Arabia/ 2019	21012	Cord blood TSH >30	75%	99.9%	99.9%
			Venous blood TSH >20	100%	98.3%	98.3%

[Table/Fig-6]: Comparison of diagnostic accuracy of cord blood TSH of the present study with previous studies [11-13].

higher diagnostic accuracy levels when compared to the present study and Vani KT and Prakash SS, [Table/Fig-6] [11-13]. This suggests that as the cut-off value increases, the sensitivity of the test decreases, while specificity, PPV and diagnostic accuracy increase. Thus, cord blood TSH in predicting CH shows fair diagnostic performance. The requirement for a high cut-off for cord blood TSH is primarily due to the TSH surge that occurs as a result of the stress of delivery [10].

Out of the 100 newborns with high cord blood TSH, 19 (19%) had venous blood TSH levels greater than 10 mIU/L, and 5 (5%) had venous blood TSH levels greater than 20 mIU/L on day 3. The present study is comparable to the work of Raj S et al., where venous blood TSH estimation performed on day 3 in 125 babies with high cord blood TSH levels revealed that only 5 (3.94%) babies had abnormal TSH levels [17]. Similarly, a study conducted by Vani KT and Prakash SS, found that 3 (2.3%) out of 131 babies with high cord blood TSH had elevated venous blood TSH levels on day 3 [13]. In George RT et al.,'s study, 17 newborns (6.25%) out of 272 had high cord TSH values, and 11 newborns (4.05%) had elevated venous blood TSH levels on day 3, demonstrating the superiority of venous blood TSH testing [10].

In the present study, venous blood TSH with a cut-off of greater than 10 mIU/L exhibited a sensitivity of 100%, with specificity and diagnostic accuracy of 85%. Conversely, venous blood TSH with a cut-off of greater than 20 mIU/L achieved a sensitivity of 100%, with specificity of 97.9% and diagnostic accuracy of 98%. The diagnostic parameters for venous blood TSH were statistically significant and reliable (p-value<0.001).

The present study found that specificity and diagnostic accuracy were higher in venous blood testing compared to cord blood testing. The study conducted by Vani KT and Prakash SS, reported the same sensitivity, specificity, and diagnostic accuracy for both cord TSH and venous blood TSH [13]. In contrast, studies by Al Juraibah F et al., and Alameer S et al., noted a decrease in specificity and diagnostic accuracy for day 3 TSH testing when compared to cord blood testing, which may be attributed to the use of a high cord blood TSH cut-off [Table/Fig-6] [11,12].

A postnatal increase in serum catecholamine concentration occurs at the time of parturition, and the thyroid gland is adrenergically innervated. Stimulation of thyroid adrenergic receptors may enhance TSH-induced changes in the T3 to T4 ratio. The decrease in TSH that follows during 72-96 hours after birth results from feedback inhibition by T3 [11]. Therefore, day 3 TSH testing in predicting CH has excellent diagnostic accuracy. Hence, a small prick on day 3 can mitigate the burden of severe complications, such as mental retardation.

Limitation(s)

The sample size is small; a larger sample size is required to estimate the incidence of CH and to compare the different testing methods.

CONCLUSION(S)

The present study concludes that, with a high cut-off for cord blood TSH, diagnostic accuracy increases, but sensitivity decreases. Venous blood TSH on day 3 is more accurate in screening for CH. However, venous blood testing requires an invasive prick and a three-day hospital stay. Screening for CH is recommended on or after day 3 of life, as it has better accuracy and reliability than cord blood TSH. Cord blood TSH can be used as a screening tool in settings where early discharge is practiced.

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