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Biochemistry

SCREENING FOR CONGENITAL HYPOTHYROIDISM IN NEONATES WITH UNCONJUGATED HYPERBILIRUBINEMIA

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ABSTRACT Congenital hypothyroidism (CH) is a condition seen in infants, resulting from a partial or complete deficiency in thyroid function. This occurs when the thyroid gland is absent, abnormally located, or significantly underdeveloped, which accounts for 80–85% of cases. If not diagnosed and treated early, CH can lead to abnormal physical development and intellectual disabilities. Unconjugated hyperbilirubinemia is often linked to delayed development of the liver enzyme uridine diphosphate glucuronyl transferase (UDPG-T), which is responsible for converting indirect (unconjugated) bilirubin into its direct (conjugated) form. Thyroid hormones enhance the expression of UDPG-T, facilitating this conversion. When thyroid hormone levels are low, thyroid stimulating hormone(TSH) levels rises, and UDPG-T activity decreases, increasing the risk of unconjugated hyperbilirubinemia. Newborn screening, particularly TSH-based programs that have been implemented globally for over five decades, plays a crucial role in reducing the morbidity and mortality associated with CH through early detection and intervention.

KEYWORDS : Congenital Hypothyroidism(CH), Thyroid Stimulating Hormone(TSH), Unconjugated Bilirubin.

INTRODUCTION

Hypothyroidism is a state of deficiency in thyroid hormone secretion and action. It is a widespread disorder that affects 2% to 15% of people in mild and severe forms (Burtis et al., 2015). Congenital hypothyroidism (CH) is a condition that affects infants and is caused by a partial or complete lack of thyroid activity. It happens when the thyroid gland malfunctions or fails to produce thyroid hormone. The thyroid gland is either missing, positioned incorrectly, or much smaller in 80 to 85% of instances. The thyroid gland is either enlarged or of normal size in the remaining cases, but thyroid hormone synthesis is either missing or reduced. Congenital hypothyroidism can cause aberrant growth and intellectual impairment if left untreated (Sharma et al., 2019)

The worldwide incidence of CH varies by region and ethnicity but generally falls within the range of 1 in 2,000 to 1 in 4,000 live births (Danner et al.,2023; Braga et al., 2025). The true incidence in India is unknown due to lack in screening programs, although prior research has shown that it ranges from 1:500 to 1:3400 in Indian infants, which is significantly greater than the frequency described in Western literature (Choudhury et al., 2018).

The anterior pituitary release thyroid-stimulating hormone (TSH), which controls the release of the two thyroid hormones thyroxine(T4) and triiodothyronine(T3). Thyrotropin-releasing hormone (TRH), which is produced by the hypothalamus, controls the production of TSH (Boron & Boulpaep, 2012).

Neonatal screening is a significant preventative public health initiative. TSH-based newborn screening programs have been in place for more than 50 years in numerous nations. Neonatal screening aims to lower the newborn's morbidity and mortality rate. The newborn must clearly benefit from screening, and it must be more affordable than the cost of postponing treatment (Kumar et al., 2014).

Indian society of paediatrics and adolescent endocrinology recommended that newborns should be screened for CH after 48–72 hours, since there is physiological TSH surge during first 1-3 days of life. Thus estimating TSH levels during first 2-3 days may give false high TSH levels. TSH value if more than 20 mIU/L, requires follow-up. TSH more than 40 mIU/L needs immediate confirmatory venous T4/FT4 and TSH testing. For mild TSH elevation, a repeat test at 7 to 10 days is advised. Preterm, low birth weight, and sick infants should be screened within the first week. CH diagnosis is confirmed if venous TSH >20 mIU/L before 2 weeks or >10 mIU/L after 2 weeks, with low T4/FT4 (Desai el al., 2018).

Neonatal hyperbilirubinemia is a common issue, which is characterised as a total blood bilirubin level greater than 5 mg/dl. Clinical jaundice occurs in the first week of life in as many as 60% of full-term babies. Clinical estimation of the total blood bilirubin level can be based on the degree of caudal extension: 5 mg/dl for the face, 10 mg/dl for the upper chest, 12 mg/dl for the abdomen, and more than 15 mg/dl for the palms and soles (Porter & Dennis, 2002).

There have been reports of several forms of bilirubinemia in newborns, including pathological jaundice, physiological jaundice, jaundice due to breastfeeding or breast milk, and haemolytic jaundice, which includes three subtypes caused by ABO blood group incompatibility, Rh factor incompatibility, and jaundice linked to a deficiency in glucose-6-phosphate dehydrogenase (Ullah et al., 2016)

Jaundice resulting from physiological immaturity often manifests between 24 and 72 hours of age, peaking between 4 to 5 days in term and 7 days in preterm neonates and disappearing by 10 to 14 days of life (Dennery et al., 2001). The most common type of bilirubin is unconjugated bilirubin, which typically has a blood level of less than 15 mg/dl (Maisels & Gifford, 1983). A number of illnesses, including congenital hypothyroidism, haemolytic diseases, nursing jaundice, urinary tract infections, and genetic abnormalities such Gilbert syndromes or Crigler-Najjar syndromes, can also cause prolonged unconjugated hyperbilirubinemia (Alizah et al., 2024).

Thyroid hormones have a significant indirect impact on bilirubin metabolism, mostly through altering liver function and enzyme activity. Hepatic uridine diphosphate glucoronyl transferase (UDPG-T) enzyme activity appears to be delayed in maturation, which is linked to congenital hypothyroidism, a well-known cause of prolonged unconjugated hyperbilirubinemia. The liver's UDPG-Transferase enzyme is responsible for conjugating bilirubin. The conversion of indirect (unconjugated) bilirubin to direct (conjugated) bilirubin is improved by thyroid hormone's upregulation of UDPGT expression. Reduced UDPG Transferase activity and increased TSH synthesis are caused by low thyroid hormone, which raises the risk of unconjugated hyperbilirubinemia. Investigating any cause of jaundice in infants must take into account the likelihood that it could be the initial symptom of congenital hypothyroidism (Jain & Agarwal, 2019).

AIM

Screening for congenital hypothyroidism in neonates with unconjugated hyperbilirubinemia.

OBJECTIVE

1) Estimation of neonatal TSH from dried blood spot sample.
2) Correlation of neonatal TSH with unconjugated bilirubin levels.

MATERIALS & METHODOLOGY

This institutional based cross sectional observational study was

conducted after obtaining institutional ethics committee clearance, at department of Biochemistry, Gauhati Medical College & Hospital, a tertiary care hospital in Assam from April 2024 to October 2024.

Study Population
120 Newborn babies born at Gauhati Medical College & Hospital.

Inclusion Criteria
1) Newborn babies of age between the thirth and fifth day of life.
2) Neonatal unconjugated bilirubin levels >5 mg/dl

Exclusion Criteria
1) Blood transfusion.
2) Parents history of hypothyroidism.
3) Major congenital malformation.
4) Deceased newborn.
5) Unconjugated bilirubin level < 5mg/dl.
6) ABO and Rh incompatibility.
7) Parents did not give consent.

Sample Collection
All the subjects who were enrolled in this study, their parents were interviewed using standard interview schedule. After their verbal and written consent in accordance with the institutional ethical guidelines. Particulars like age, birth weight, gestational age at time of birth, mode of delivery of the subjects, and bilirubin levels were recorded in a proforma and these were compiled and analyzed at the end of the study. Dried Blood Spot(DBS) sample collected from the neonates with bilirubin levels more than 5 mg/dl. Samples were collected between the third and the fifth day of life (49 to 120 hours after birth) from the neonate's heel only using the medial or lateral portion of the plantar surface.

Estimation of TSH: The neonatal TSH levels from collected dried blood spot sample were estimated using ELISA. The Neonatal TSH Screening ELISA kit from Zentech is an enzyme immunoassay (Sandwich ELISA) for the quantitative estimation of TSH in dried blood samples on 903 filter paper. The 2 levels of control were processed with the collected samples at each run of the samples. The results were obtained by using the SKAN IT program. The neonatal TSH value of more than 20 mIU/L is considered as cut-off to term as screened positive.

Statistical Analysis
The data was compiled in Microsoft Excel 2016 spreadsheet and statistical analysis was done using Statistical Package for Social Science(SPSS). Descriptive variables were expressed in percentage and continuous variables were expressed in mean and standard deviation. The study population was divided into three groups on the basis of levels of unconjugated bilirubin. One way ANOVA test was applied to analyse the mean TSH levels and unconjugated bilirubin among the study groups. Association with p-value of <0.05 were considered to be statistically significant.

RESULTS
A total of 120 neonates of age between 3-5 day who fulfilled the criteria were included in the study. Out of 120 neonates 51(42.5%) were female babies and 69(57.5%) were male babies. Among the neonates 62.5% were delivered by lower segment caesarean section(LSCS) and 37.5% were delivered by vaginal delivery.

Table 1: Demographic Profile of Study Population.

		Frequency	Percent
Gender	Female	51	42.5%
	Male	69	57.5%
Mode of delivery	AVD	1	0.8%
	LSCS	75	62.5%
	NVD	44	36.7%
		Mean	Standard Deviation
Birth Weight (Kg)		2.5	0.6
Gestational Age (Weeks)		36.4	3.1
TSH value mIU/L		3.7	6.0
Unconjugated bilirubin level (mg/dl)		8.6	2.4

Table 1 shows the mean birth weight of the study population was 2.5±0.6 Kg and the mean gestational age was 37.4±3.1 weeks. The mean TSH value was 3.7±6.0 mIU/L and the mean unconjugated bilirubin was 8.6±2.4 mg/dl in the study population.

Table 2 Distribution of Unconjugated Bilirubin Levels In The Study Population.

Unconjugated bilirubin levels (mg/dl)	Frequency	Percent
5 to 10	98	81.7%
10.1 to 15	18	15.0%
> 15	4	3.3%
Total	120	100.0%

Out of 120 neonates, 81.7% had unconjugated bilirubin values between 5 to 10 mg/dl, 15% had unconjugated bilirubin values between 10.1 to 15 mg/dl and on 3.3% neonates had unconjugated bilirubin value more than 15 mg/dl.

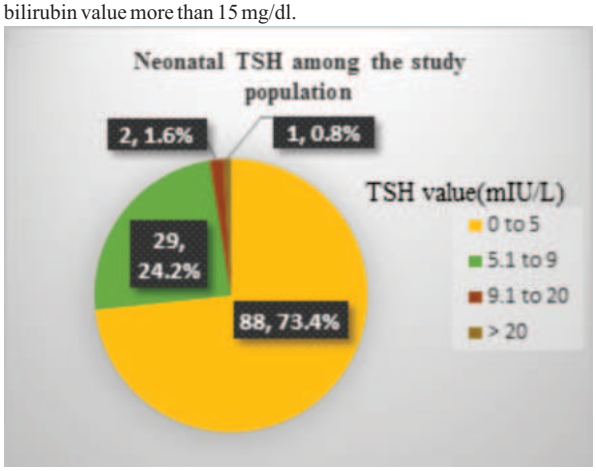

Figure 1 Distribution of Neonatal TSH Values in the Study Population.31

Figure 1 shows, among the study population, 88(73.4%) of neonates had TSH values between 0 to 5 mIU/L, 29(24.2%) neonates had TSH values between 5.1 to 9 mIU/L and 2(1.6%) neonates had borderline TSH value between 9.1 to 20 mIU/L. Only 1(0.8%) neonate had TSH value 64.57mIU/L which is > 20 mIU/L which was labelled as screen positive case.

Table 3 Mean TSH Values with Respect to the Unconjugated Bilirubin Levels.

Unconjugated bilirubin levels (mg/dl)	Mean TSH values (mIU/L)	Standard Deviation	P value
5 to 10	3.1	2.1	P <0.001
10.1 to 15	3.4	2.8	
> 15	20.0	29.8	

*** One way ANOVA Test. Statistical significance if p value <0.05.

The mean TSH among the neonates having unconjugated bilirubin value 5 to 10 mg/dl, 10.1 to 15 mg/dl and more than 15 mg/dl were 3.1±2.1 mIU/L, 3.4±2.8 mIU/L and 20.0±29.8 mIU/L respectively. The p value among the study group with respect to mean TSH was <0.001, which is statistically significant.

DISCUSSION
The present study screened 120 neonates of age 3-5 days with unconjugated hyperbilirubinemia for congenital hypothyroidism and also aiming to assess the relationship between TSH and unconjugated bilirubin. Neonatal hyperbilirubinemia can stem from multiple factors including congenital hypothyroidism, though this condition lack specific symptoms. While looking into the issue of neonatal jaundice, it is important to keep in mind that it could be the initial indication of congenital hypothyroidism. Thus, identifying any correlation between TSH and bilirubin levels can be essential for timely intervention.

In this study, only one neonate (0.8%) was screened positive with a TSH value of 64.76 mIU/L, and two neonates (1.6%) have borderline TSH value, while rest have normal TSH levels(<9 mIU/L). The mean TSH value in the study population was 3.6±6.0 mIU/L. Kumar et al. (2023) conducted their study among 250 neonates using heel prick dried blood spot sample in Banaras, Uttar Pradesh, the mean TSH value was 3.98 ± 2.16 standard deviation(SD) in their study, which is very consistent with our study.

Our study shown the higher levels of unconjugated bilirubin among the neonates having high and borderline TSH values. The serum

unconjugated bilirubin of the screened positive neonate having TSH value 64.67 mIU/L is 15.20 mg/dl. Labrune et al. (1992) reported a case following a comprehensive investigation, one instance with a blood bilirubin level of 18 mg/dl was diagnosed as congenital hypothyroidism. This is consistent with the finding that elevated TSH levels in newborns are associated with increased serum bilirubin values.

TSH and unconjugated bilirubin levels were shown to be significantly correlated in this study. Because there is no feedback inhibition to TSH synthesis, a decrease in thyroid hormone would raise TSH levels and may result in prolonged indirect hyperbilirubinemia, which is correlated with the UDPG-T enzyme's delayed maturation activity. In cases of congenital hypothyroidism, the lack of thyroid hormone may result in a substantial drop in hepatic UDP-G T levels or activity (Dwijayanti et al., 2020).

In line with our findings, a study conducted by Kayiran and Gurakan (2010) examined the association between TSH and thyroxine and third-day blood bilirubin levels found by neonatal screening revealed a noticeably weak correlation. However, this study did not analysed thyroxine or triiodothyronine levels among the study population, since only the TSH assay was used for the screening of congenital hypothyroidism.

CONCLUSION

The study highlights the relationship between the TSH levels and unconjugated hyperbilirubinemia. The TSH assay is one the most important tool of screening for congenital hypothyroidism. The study shows increased TSH levels is significantly higher in the neonates having unconjugated hyperbilirubinemia. Since, the hyper-bilirubinemia may be the one of the earliest sign of congenital hypothyroidism, all the neonates having hyperbilirubinemia should be screened for congenital hypothyroidism. Early diagnosis of congenital hypothyroidism will prevent the delay in treatment and thus prevent the complication due to congenital hypothyroidism. Overly delayed diagnosis often results in irreversible changes that have long-term effects, particularly in the central nervous system, which causes cerebral palsy, intractable seizures, growth retardation, mental retardation, and many other problems. This emphasises how important early screening and prompt action are in preventing complications from the condition.

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Author Declare no conflict of interest.

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