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Original Article

Biochemistry Section

Neonatal Screening for Congenital Hypothyroidism through Dried Blood Spots: A Cross-sectional Study

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ABSTRACT

Introduction: Congenital Hypothyroidism (CH) is defined as the partial or complete loss of thyroid gland function present at birth. During the first 2-3 years of life, thyroid hormone plays a crucial role in brain development. If a baby is born with a deficiency of thyroid hormone (CH) and is not diagnosed and treated appropriately, it can lead to intellectual disability and growth retardation in the affected child.

Aim: To screen newborns for CH using Dried Blood Spot (DBS) sampling. Additionally, the study aimed to compare mean TSH values between two comparison groups based on gender, birth weight, gestational age, and type of delivery.

Materials and Methods: This cross-sectional study was conducted at Institute of Medical Sciences, Banaras Hindu University, Varanasi, Uttar Pradesh, India, from November 2021 to October 2022. A total of 250 live-birth newborns delivered either by Spontaneous Vaginal Delivery (SVD) or Lower Segment Caesarean Section (LSCS) were included in the study after obtaining written informed consent from their parents. Blood samples were obtained on DBS cards through heel prick in all newborns. TSH levels were assessed using a neonatal TSH sandwich ELISA kit. The cut-off value of TSH to label as screen positive was set at >20 mIU/L. A two-tailed Independent t-test was performed to compare mean TSH values between the two comparison groups based on gender, birth weight, gestational age, and type of delivery.

Results: Out of the 250 babies, 137 (54.8%) were male and 113 (45.2%) were female. The gestational age ranged from a minimum of 29 weeks to a maximum of 41 weeks in both male and female babies. TSH levels in male babies ranged from 0.16 mIU/L to 10.27 mIU/L, with a mean value of 3.98±2.16 mIU/L. TSH levels were below 20 mIU/L in all 250 newborns, indicating negative screening results for CH in all the neonates.

Conclusion: The results concluded that the serum levels of TSH obtained through heel prick were not statistically significant in both term and preterm newborns, as well as in Normal Birth Weight (NBW) and Low Birth Weight (LBW) newborns. Furthermore, there was no significant difference based on the type of delivery (SVD/LSCS) or the gender of the newborn (male or female).

Keywords: Growth retardation, Newborn screening, Thyroid, Thyroid stimulating hormone

INTRODUCTION

Hypothyroidism is defined as a deficiency in thyroid hormone production or secretion, resulting in a variety of clinical signs and symptoms of hypometabolism. This leads to insufficient metabolic and neurological effects at the cellular level [1]. The thyroid gland, a butterfly-shaped organ situated at the front of the neck, produces thyroid hormone and calcitonin. It plays an important role in the regulation of metabolism, growth, and serum concentrations of electrolytes such as calcium [2,3]. The term CH is used when thyroid hormone deficiency is present at birth. CH is the most common congenital endocrine disorder in childhood and is also the leading cause of intellectual disability, which can be prevented through early diagnosis and prompt treatment [4].

CH most commonly occurs when the thyroid gland does not develop properly, a condition known as thyroid dysgenesis. This can be due to agenesis (missing thyroid gland), hypoplasia (underdeveloped thyroid), or ectopia (thyroid located in the wrong part of the neck) [5]. In some cases, the gland is formed properly but does not produce hormones correctly, a condition known as dyshormonogenesis [6]. The prevalence of CH varies worldwide, with lower rates observed in Western countries (1 in 3000-4000) compared to higher rates in Asian populations (1 in 1200-2000) [7-9]. In India, the reported prevalence of CH differs across regions, such as Chandigarh (1 in 3400), Hyderabad (1 in 1700), Lucknow (1 in 1221), and Chennai (1.6 in 1000) [9-12]. This regional variation may be attributed to differences in screening strategies or the use of different cut-offs to identify screen-positive cases [9,10,13,14].

During the first trimester and early phase of the second trimester, the foetus relies on the transplacental transfer of maternal thyroid hormone since it is not yet able to produce thyroid hormone. The hypothalamic-pituitary-thyroid axis in the foetus begins functioning at 16-20 weeks of gestation and becomes fully mature at term. Inadequate secretion of thyroid hormones during this period can greatly impair the growth and maturation of the brain, both before and after birth, leading to intellectual deficits and neurological impairments [6]. Due to the absence of prominent clinical features at birth, many newborns with CH go undiagnosed and later develop mental retardation. Wilson JMG and Jungner F outlined the selection criteria for including a thyroid disorder in a Newborn Screening (NBS) program [15].

CH fulfills all the criteria given by Wilson JMG and Jungner F, as it is easy to screen, can be assessed before symptoms manifest through definitive diagnostic tests, is inexpensive, and affected children have excellent outcomes when treated in a timely manner. The European Society of Paediatric Endocrinology (ESPE) and the Indian Society of Paediatric and Adult Endocrinology (ISPAE) have suggested that the aim of neonatal screening programs should be to detect all forms of primary hypothyroidism. Primary TSH screening is more sensitive and specific than T4 screening for the diagnosis of primary CH. It should be noted that central CH (with an incidence of 1 in 13,000) cannot be detected through primary TSH screening, but the detection of this rare disorder is not the target of NBS [7].

One advantage of using DBS testing compared to conventional venepuncture is that only a small quantity of blood is required for DBS testing (approximately 50 µL, equivalent to one drop of capillary

blood). This minimal blood volume requirement is particularly important in paediatric diagnostics. DBS cards can be preserved for long periods with almost no degradation of the analytes [16-18].

Several state-level NBS programs have been initiated, including the Chandigarh Program initiated in 2007 [19], the Kerala State NBS program, and the Goa State NBS Program [20]. However, a national NBS program is yet to be initiated in India. Early diagnosis of CH and prompt treatment within a few weeks (preferably within one week) can prevent irreversible brain damage and result in normal neurodevelopmental outcomes for babies born with CH. Additionally, early diagnosis and treatment can reduce the number of cases of cretinism, a condition characterised by severe physical and mental growth subnormality due to untreated CH.

Hence, the present study was conducted to screen newborns for CH using DBS sampling.

MATERIALS AND METHODS

This cross-sectional study was conducted in the Department of Biochemistry, Institute of Medical Sciences, Banaras Hindu University, Varanasi, Uttar Pradesh, India, over a period of one year from November 2021 to October 2022. Ethical approval for the study was obtained from the Institutional Ethics Committee (IEC), IMS BHU Varanasi, in Ethics Committee (vide letter No 2947 dated 29.10.2021).

Inclusion criteria: All live birth newborns delivered either by SVD or LSCS after 72 hours of birth were evaluated and enrolled in the study after obtaining written informed consent from their parents.

Exclusion criteria: Parents of newborn who refused consent and cases of foetal demise were excluded from the study.

Sample size: All neonates delivered within the study duration and meeting the inclusion criteria were enrolled in the study.

Sample collection: Blood samples were collected on DBS cards through heel prick. The samples were collected from the Paediatrics Department and processed in the Department of Biochemistry, IMS BHU. The NBS Card has five circles, and this portion of the card is used for collection blood from the enrolled neonates. Blood samples were obtained by heel-prick on Whatman filter paper 903. Atleast three (preferably five) blood spots were collected from each neonate. The blood sample was collected in such a way that the circle was completely filled and the blood was evenly distributed on the filter paper. The filter paper was properly dried either by air drying at room temperature or in an incubator at 37°C.

Drying was done for atleast four hours. If the testing was not conducted on the same day, the DBS cards were stored in a refrigerator at 4°C. For longer durations, they were stored at -20°C. FDA-approved surgical lancets with a maximum tip length of 2.4 mm, marketed by Perkin Elmer, were used for this study.

To avoid calcaneal puncture and the risk of osteochondritis, heel puncture was performed on the most medial or lateral portion of the plantar surface. This also reduces the risk of injury to blood vessels and nerves.

Estimation of TSH: A neonatal TSH screening ELISA kit from Zentech Belgium was used for the quantitative estimation of thyroid stimulating hormone in dried blood samples collected on filter paper. The ROBONIK Read well Touch Elisa Plate Analyser, which has four filters, was used for plate reading. The recommended cut-off value for TSH was >20 mIU/L to classify as screen positive [12].

Principle of the assay: The neonatal TSH screening ELISA is an enzyme immunoassay used for the quantitative estimation of TSH in dried blood samples. It is a type of sandwich ELISA. Strips are coated with an anti-TSH antibody that captures TSH present in the sample. After incubation and a washing step to remove unbound material, a monoclonal antibody anti-TSH conjugated to Horseradish Peroxidase (HRP) is added and allowed to bind to TSH. After a second incubation and washing, the immunocomplex is detected by the reduction of Tetramethylbenzidine (TMB) by HRP. The development of the blue colour is directly proportional to the amount of antigen in the sample or standard. The enzymatic reaction was stopped by the addition of 0.5 M sulfuric acid, and absorbance was read at 450 nm using the ROBONIK Read well Touch, which consists of an ELISA reader with four filters.

Four comparison groups were created based on gender, birth weight, gestational age, and type of delivery:

- Gender of child: Male (M) or Female (F)
- Gestational age: Preterm (gestational age <37 weeks) or Term (gestational age ≥37 weeks)
- Birth weight: Low birth weight (LBW, birth weight <2.5 kg) and Normal birth weight (NBW, birth weight ≥2.5 kg)
- Type of delivery: SVD or LSCS

STATISTICAL ANALYSIS

The statistical analysis was performed using the GraphPad Prism statistical software. A two-tailed independent t-test was conducted to compare the mean TSH values between two groups. If the p-value <0.05, the difference between the two means was considered statistically significant.

RESULTS

A total of 250 samples were collected on DBS cards using a heel prick for the quantitative estimation of TSH values. None of the babies screened positive for CH as their TSH levels were <20 mIU/L. Among the 250 babies, 137 (54.8%) were male and 113 (45.2%) were female [Table/Fig-1].

Parameters	Mean SD	Range
Gestational age (weeks)	Pre term: 34.7±1.73	29-36
	Term: 38.3±0.97	37-41
Birth weight (Kg)	Low BW: 2.1±0.27	1.13-2.48
	Normal BW: 2.89±0.29	2.50-4.16
Maternal age (years)	26.58±3.72	20-38
TSH value (mIU/L)	3.98±2.16	0.07-11.79
Parameters	n (%)	
Mode of delivery	LSCS	158 (63.2)
	SVD	92 (36.8)
Gender	Female	113 (45.2)
	Male	137 (54.8)

[Table/Fig-1]: Demographic data of neonates.

The values of TSH in female babies ranged from 0.07 mIU/L to 11.79 mIU/L, while in male babies it ranged from 0.16 mIU/L to 10.27 mIU/L. The mean value of TSH in male children was 3.98, and in female children, it was 3.95.

Approximately 99% of the neonates had TSH values below 10 mIU/L, with the maximum values of TSH ranging between 2-4 mIU/L [Table/Fig-2].

Among the 250 neonates, 156 (62.4%) had NBW, and 94 (37.6%) were LBW babies. The mean value of TSH in LBW babies was

Level of TSH (mIU/L)	n (%)
0.00-2.00	44 (17.6)
2.01-4.00	100 (40.0)
4.01-6.00	65 (26.0)
6.01-8.00	30 (12.0)
8.00-10.00	9 (3.6)
10.01-12.00	2 (0.8)

[Table/Fig-2]: Distribution of heel prick TSH level.

4.18, and in NBW babies, it was 3.84. The percentage of newborns delivered at term was 175 (70%). Out of them, 82 were females and 93 were males, with TSH values of 3.76±2.21 mIU/L and 4.07±2.27 mIU/L, respectively.

The mean value of TSH was found to be higher in preterm babies (4.06) than in term babies (3.93), but this difference was not statistically significant. Out of the 250 babies, 158 were born by LSCS (63.2%), and the remaining 92 were born by SVD (36.8%). The mean value of TSH in babies born by LSCS was 3.84, and in babies born by SVD, it was 4.19. There was no statistical significance between the two groups in terms of their TSH values, as the p-value was 0.2061. In categories such as low birth weight babies and preterm babies, the mean value of TSH was higher than in normal weight and term babies. However, when comparing both groups, the difference was not found to be statistically significant, as the p-value was >0.05 [Table/Fig-3].

Comparison based on		N (%)	Mean±Std. Dev	SEM	p-value
Gender	TSH_F	113	3.95±2.17	0.20	0.9135
	TSH_M	137	3.98±2.16	0.18	
Gestational age	Preterm	75	4.06±1.95	0.23	0.6573
	Full-term	175	3.93±2.25	0.17	
Birthweight	Low_BW	94	4.18±2.33	0.24	0.2395
	Normal_BW	156	3.84±2.05	0.16	
Type of delivery	LSCS	158	3.84±2.08	0.17	0.2061
	SVD	92	4.19±2.28	0.24	

[Table/Fig-3]: Statistical comparison among TSH levels (mIU/L) measured according to Gender, gestational age, birth weight and type of delivery.
Unpaired t-test, level of significant p-value <0.05

DISCUSSION

Before the initiation of the NBS program, the diagnosis of CH relied on clinical presentation, including symptoms and signs of CH. Generally, symptoms in babies born with CH do not appear early due to the transplacental transfer of maternal thyroid hormone thyroxin (T4). This can result in the child going undiagnosed, leading to a delay in treatment initiation, which can cause irreversible brain injury. It has been observed that patients diagnosed through NBS have better outcomes compared to those diagnosed clinically based on signs and symptoms [21-24]. In India, the first-time screening for CH was initiated at BJ Wadia Hospital in Mumbai in 1982, using cord blood samples taken immediately after the baby's delivery [25,26].

The most important goal of CH screening programs is early diagnosis and prompt treatment initiation, ideally within the first month. After birth, there is a temporary elevation in TSH levels due to physiological neonatal surge, which remains elevated for the first 2-3 days of life. If sampling is done during this period, falsely elevated TSH values may be obtained. Therefore, the ideal time for sampling through a heel prick is after 72 hours of life [12]. In present study, samples were collected from 250 babies (≥72 hours after birth) on DBS cards using a heel prick, and CH screening was performed by estimating TSH levels. None of the babies had a TSH value higher than 20 mIU/L, indicating that all babies were screened negative for CH.

The data in present study shows similar values in both males and females, which was consistent with a study by Adele BC et al., that also demonstrated that no sex-based cut-off is required for CH screening in newborns [27]. When TSH levels were examined according to gestational age and birth weight, our study found higher mean TSH values in preterm and LBW babies. However, this result contradicts previous study done by Arasar T et al., [28] which showed higher mean TSH level in normal weight babies and study done by Adele BC et al., [27] which showed higher mean TSH level in full term babies. Nevertheless, in both studies, the differences were not statistically significant, suggesting that birth weight and gestational age may not warrant a change in the cut-off for CH screening.

In present study, the mean TSH level was lower in babies delivered by LSCS compared to those born by SVD which contradicts the findings of Adele BC et al., who reported higher mean TSH values in babies born by LSCS [28]. Transient TSH elevation, known as atypical hypothyroidism, can occur in some cases, particularly in preterm and low birth weight babies. It is commonly observed between 2-6 weeks of age and often resolves by 6-10 weeks of age. Cases like delayed TSH surge might have been missed in present study as preterm and LBW babies should undergo a second screening test two weeks after the first screening or at the age of two weeks, as there is a risk of delayed TSH rise in these babies [29]. A comparison of the findings in present study with contrasting studies is shown in [Table/Fig-4] [27,28,30,31].

Author name year of publication	Place of study	Size of study population and parameter measured	Conclusion
Arasar T et al., 2016 [28	Stanley Medical College, Chennai, Tamil Nadu, India	1,695 newborns DBS samples for TSH level estimation	Statistical significance between preterm and term babies with respect to their TSH values.
Adele BC et al., 2018 [27]	YGOPH, Cameroon, Nigeria	180 newborns DBS samples for TSH level estimation	Babies born by LSCS had higher mean TSH value than those delivered vaginally (SVD) but difference was not significant statistically.
Gopalakrishnan V et al., 2014 [30]	Lucknow, Uttar Pradesh, India	13,426 newborns DBS samples for TSH level estimation	Neonates with male sex, low birth weight and those born vaginally (SVD) had a significant higher TSH values.
Verma NR et al., 2021 [31]	Raipur, Chhattisgarh, India	1,216 newborns DBS samples for TSH level estimation	Significant negative correlation was observed between birth weight and TSH level.
Present study	Varanasi, Uttar Pradesh, India	250 neonates	No significant association was observed between birth weight, gender, gestational age and mode of delivery and TSH level.

[Table/Fig-4]: Comparison with previous studies [27,28,30,31].

During pregnancy, maternal total or bound thyroid hormone levels increase due to the serum concentration of thyroid-binding globulin, and TSH levels decrease in early pregnancy (first 12 weeks of gestation) due to weak stimulation of TSH receptors caused by human Chorionic Gonadotropin (hCG). This stimulates thyroid hormone secretion, leading to increased serum free thyroxine (T4) levels, which in turn suppress hypothalamic thyrotropin-releasing hormone and limit pituitary TSH secretion. In the later stages of pregnancy, TSH levels return to normal and progressively increase in the third trimester due to placental growth and production of placental deiodinase. These physiological changes are responsible for the decrease in TSH levels in newborns and the improvement in gestation and growth parameters later in life [31].

Limitation(s)

A limitation of present study was the small sample size, which could affect the generalisability of the results. Additionally, important factors such as the maternal history of thyroid illness, presence of any chronic illness, and history of drug intake by the mother that could potentially alter thyroid function in babies were not included in the study.

CONCLUSION(S)

Heel prick samples demonstrate good consistency, and mean TSH values vary among different comparison groups based on gender, gestational age, birth weight, and mode of delivery. However,

these differences were not statistically significant in present study. Therefore, it is feasible to use the same cut-off for screening babies in these comparison groups. Nevertheless, further extensive studies with larger sample sizes are needed, and it is important to include maternal factors such as the presence of thyroid or chronic illness and the history of maternal drug intake that could potentially affect the thyroid status of newborns. In the case of premature babies, a second screening should also be conducted, as cases of delayed TSH surge can be missed by the first screening alone, as may have been the case in present study.

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