ORIGINAL RESEARCH ARTICLE

Neonatal Screening for Congenital Hypothyroidism: A Study Conducted in a Tertiary Care Hospital of Gujarat

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Abstract

Background: Growth and differentiation of the fetal brain largely depend on the thyroidal biochemical cascade. The most common cause of congenital hypothyroidism (CH) is thyroid dysgenesis. Maternal iodine deficiency remains to be the predominant cause of underactive thyroid in neonates. Congenital hypothyroidism fulfils Wilson and Jungners criteria for newborn screening, in having a suitable test, and treatment and that the costs of screening, confirmation, and treatment are balanced against the overall costs of not screening.

Aims and objectives:

Aims: Assessment of possible methods for screening/early detection of CH in neonates.

Primary objective: Compare and correlate cord blood (CB) thyroid stimulating hormone (TSH) with heel prick TSH, as a marker of CH.

Materials and methods: In a prospective study approved by the Ethical Committee of GCS Medical College and Hospital, Ahmedabad, 100 neonates (50 males and 50 females) were assessed for thyroid status after excluding those born of known hypothyroid mothers or having a history of distress. Cord blood was collected at the time of delivery, and dried blood spots (DBS) from heel prick at 72 hours were collected and TSH was compared.

Results: The findings were comparable in both samples as mean TSH CB = 6.14 ± 2.82 mIU/L vs mean TSH DBS = 6.21 ± 3.03 mIU/L; *p*-value = 0.9268 at 95% confidence interval and *r*-value for TSH = 0.9783. This was true in normal delivery as well as LSCS births.

Keywords: Congenital hypothyroidism, Neonatal screening, Thyroid stimulating hormone.

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Introduction

Thyroid hormone deficit during the intrauterine period significantly impairs the growth of the fetus and compromises its adaptation to extrauterine life.[1] Growth and differentiation of fetal brain largely depend on the thyroidal biochemical cascade.[2] Thyroid hormone receptors are found in abundance in the fetal brain, and importantly, are present even before the time the fetus is able to synthesize the said hormones.[3]

As studies conducted by Lee and Petratos,[4] Porterfield and Hendrich[5] and Joseph Bravo et al.[6] Thyroid hormone levels, peaking during active myelination, finely regulate oligodendrocyte development and the terminal differentiation of oligodendrocyte precursor cells (OPCs) into myelinating oligodendrocytes by inducing rapid cell-cycle arrest and continuous transcription of pro-differentiation genes. The most common cause of congenital hypothyroidism (CH) is thyroid dysgenesis. It occurs by mutations in factors (e.g.: PAX8, TSHR) responsible for normal growth and development of the thyroid gland.[7,8] Dyshormonogenesis occurs when thyroid hormone production is affected-mutations in genes DUOX2, SLC5A5, TG, and TPO are implicated.[9,10] Maternal iodine deficiency remains the predominant cause of underactive thyroid in neonates.[11]

Congenital hypothyroidism fulfills Wilson and Jungners criteria for newborn screening, in having a suitable test, and treatment and that the costs of screening, confirmation, and treatment are balanced against the overall costs of not screening. There is a price to pay; both monetary and otherwise, for the lapse in detection and resulting permanent intellectual deficit. The American Office of Technology Assessment concludes a positive cost-to-benefit ratio (10:1) for thyroid screening.[12,13]

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There are three screening strategies for the detection of CH: (1) primary thyroid stimulating hormone (TSH) measurement with backup testing of thyroxine (T4) in infants with high TSH levels; (2) primary T4 measurement with backup TSH in infants with low T4 levels; and (3) simultaneous measurement of T4 and TSH levels.[14] Some guidelines have been developed by the Indian Society for Pediatric and Adolescent Endocrinology (ISPAE) for neonatal thyroid screening, according to which 20 mIU/L has been set as cut off, above which all neonates must be recalled for confirmatory testing with T4 or FT4.[14]

The specimens for performing newborn screening biochemical testing are urine, cord blood (CB), and skin puncture capillary blood from the heel as liquid or dried onto collection paper. Both CB and heel prick have been investigated for various parameters.[15]

Table 1: Mean values and statistical analysis of thyroid profile by both samples

Test	*CB*	*DBS*	*p-value at 95% confidence (students unpaired t-test)*
Mean TSH (mIU/L)	6.14 ± 2.82 mIU/L	6.21 ± 3.03 mIU/L	0.9268
Mean T4 (µg/mL)	13.76 ± 1.82 µg/mL	13.75 ± 1.31 mIU/L	0.969
Mean T3 (ng/mL)	59 ± 9.89 ng/mL	257.73 ± 76.79 mIU/L	<0.0001

The present study aims to compare the thyroid profiles, especially TSH in CB and heel prick samples.

Aims and Objectives

The aim of this study is:

- Assessment of possible methods for screening/early detection of CH in neonates.

The objectives are to:

- Compare and correlate CB TSH with heel prick TSH, as a marker of CH.
- Compare the levels of TSH, triiodothyronine (T3), and T4 in CB sample at birth vs levels of TSH, T3, and T4 from heel prick on 4th day.

Materials and Methods

In a prospective study approved by the Ethical Committee of GCS Medical College and Hospital, (IEC reference number: GCSMC/EC/Dissertation/APPROVE/2019/6654) Ahmedabad, 100 neonates were assessed for thyroid status. The study population consisted, by purposive sampling of 50 males, and 50 females excluding those born of known hypothyroid mothers, having a history of distress *in-utero*, sick babies, low birth weight, maternal death due to complications, and multiple/high-risk pregnancies. All neonates were born full-term either by normal vaginal delivery or elective cesarean section. Informed consent for thyroid testing was taken prior to delivery.

Two samples were collected from the 100 subjects: CB collected at the time of delivery and dried blood spots (DBS) from heel prick at 72 hours. In both samples, a thyroid profile consisting of three tests: TSH, T4, and T3 was performed. The cord samples were analyzed within 3 hours by the chemiluminescent immunoassay method (CLIA), analyzed on Roche Cobas e411. The DBS were isolated and allowed to stay in chilled phosphate buffered saline overnight, processed the next day to prepare the eluate by an appropriate method. Resultant eluates were assayed by CLIA. Data collected was processed in Microsoft Excel, and analyzed by unpaired *t*-test.

Results

The study conducted in 100 neonates, led to the following results:

Table 1 shows the findings of the present study which support the comparability of both methods-CB vs heel prick, as Mean TSH CB = 6.14 ± 2.82 mIU/L vs Mean TSH DBS = 6.21 ± 3.03 mIU/L; *p*-value = 0.9268 at 95% confidence interval and *r*-value for TSH = 0.9783.

Mean T4 CB 13.76 ± 1.82 µg/mL vs Mean T4 DBS = 13.75 ± 1.31 µg/mL; (*p*-value 0.969) *r*-value for T4 = 0.7576. Hence, there is a strong positive correlation between both methods for TSH and T4.

The Mean T3 CB = 59 ± 9.89 ng/mL, Mean T3 DBS = 257.73 ± 76.79 ng/mL, (*p*-value less than 0.0001; shows a significant difference between two methods; however, this can be attributed to T3 surge caused by perinatal factors.

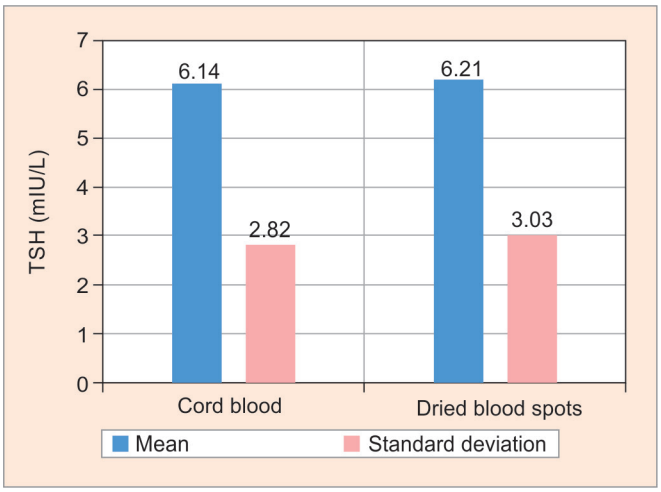

Fig. 1: TSH: Mean and standard deviation

Here, we will consider the results for TSH, as it is the most important and first-line test for screening program. The mean and standard deviations are summarized in Figure 1. The screening cut-off value was set at 20 mIU/L, in concordance with other programs. However, not a single neonate showed any signs of hypothyroidism or a grossly elevated TSH level.

Figure 1 shows, for 100 neonates, the Mean TSH CB = 6.14 mIU/L with a standard deviation of 2.82 whereas Mean TSH DBS = 6.21 mIU/L with a standard deviation of 3.03.

Figure 2 shows the results according to the mode of delivery. Out of the 100 neonates, 40 were delivered full term through the normal vaginal route whereas 60 opted for cesarean section. All mothers belonged to urban areas, poor socio-economic classes and were screened for thyroid status during the antenatal period. The mean TSH CB in LSCS was 4.94 mIU/L while in DBS was 5.07 mIU/L. In normal delivery patients, the Mean TSH by CB was 6.94 mIU/L while by DBS was 6.98 mIU/L respectively.

Figure 3 shows the equal distribution between male (50) and female (50) neonates. The results of mean TSH in CB and DBS in female babies were 5.92 mIU/L and 5.78 mIU/L respectively while for male babies it was 6.36 in CB and 6.64 mIU/L for DBS respectively.

Discussion

A 2024 European article by Costeira et al.[16] discusses the historical and current screening strategies for CH. The choice of sample for newborn biochemical screening is a complex mix of priorities and practicalities. For neonates, fluids utilized for biochemical screening are urine, CB, and whole blood from skin puncture in the heel.

Studies by Bhatia and Rajwaniya,[17] Alameer et al.,[18] Manglik et al.,[19] etc., preferred umbilical cord sampling because of availability

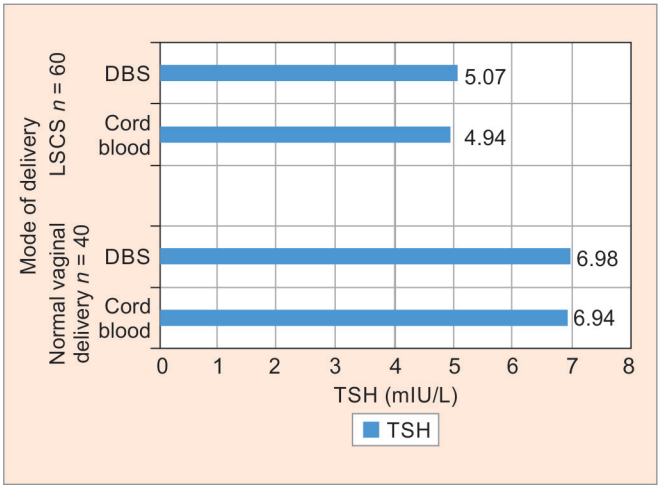

Fig. 2: Comparison of mean TSH according to mode of delivery

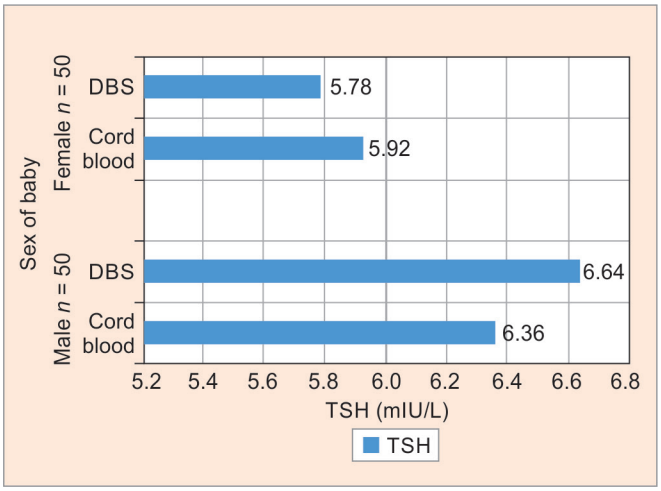

Fig. 3: Comparison of mean TSH according to sex of baby

in abundance, ethical appropriateness, and better compliance since it can be collected in all newborns in the hospital at the time of birth.

Studies carried out by Rashid et al.,[20] Kumari et al.,[21] Noble SE et al.,[22] etc., favor neonatal thyroid screening programs using the filter paper method. The preferential use of heel stick blood in most screening programs results from its ability to provide a satisfactory specimen for many other metabolic screening tests that are not possible from CB, which can facilitate later program expansion. Dried samples usually have the advantage of better analytic stability and ease of transport.

Multiple studies like Juraibah et al.,[15] Büyükgebiz[23] and Seth et al.[24] reported that both cord and heel-stick TSH testing detected all cases of CH and that the same cut-off value for recall can be used for CB and heel prick samples for screening of CH.

Studies by Ryckman et al.,[25] Herbstman et al.,[26] Trumpff et al.,[27] and others discuss the role of perinatal factors affecting neonatal thyroid status. Desai et al.[14] argue that although the fetal endocrine system functions largely independently of that of the mother, maternal endocrine disorders can adversely influence fetal development. The dilemmas in screening for CH can relate to maternal thyroid status, fetal factors like gestational age and maturity of the fetal Hypothalamo-Pituitary-Thyroid axis, intranatal factors (use of iodine application during delivery), and mode of delivery. Some factors like maternal ingestion of antithyroid drugs, goitrogens, or other factors from difficult deliveries can cause transient hyperthyrotropinemia in the neonate. Lao[28] and Miyamoto et al.[29] reported higher CB TSH concentrations in infants born vaginally compared to cesarean sections. Other studies such as Monen et al.[30] report higher TSH in operative deliveries. However, Miyamoto et al.[29] reported that this did not much interfere with CH screening.

In the present study, it was found that vaginal deliveries show higher TSH values. [Mean TSH CB-DBS for ND = (6.94–6.98) mIU/L vs mean TSH CB-DBS for LSCS = (4.94–5.07) mIU/L].

For T4, only slightly higher values are seen in CB in vaginal deliveries, but not in heel prick samples. (Mean T4 CB-DBS for ND = (13.83–13.62) µg/mL vs mean T4 CB-DBS for LSCS = (13.66–14.02) µg/mL. Triiodothyronine at birth is reflective of deiodinase 3 activity and is not very significant for screening, however, it is slightly high in cesarean section and following a surge, falls off to a somewhat higher degree thereafter. (Mean T3 CB-DBS for ND = (58.22–268.66) ng/mL vs mean T3 CB-DBS for LSCS = (60.58–241.33) ng/mL.

Taking into account the physiological rise in TSH and its persistence as a mechanism to fight cold stress, Gruñeiro-Papendieck L et al.[31] reported on the need to use samples collected from infants after 48 hours and highlighted concerns around the time of sampling and the lack of clear WHO guidelines for the same. The present study included heel prick samples only after 72 hours of life, drawing from Walfish.[32]

Hinton et al.,[33] Medda et al.,[34] Parks et al.,[35] and several other studies have indicated that CH is frequently found in the female child. Van Vliet et al.,[36] show that the preponderance of hypothyroidism in females is mostly associated with dysgenesis of the thyroid gland. A 2020 study by Dalmazi et al.,[37] showed slightly higher levels of thyroid hormones in the male neonate as compared to the female. No such conclusion can be drawn from the present study. Although the female-to-male ratio was 1:1 in the present study, there is no significant difference with regard to the sex of the baby. Mean TSH (mIU/L) in males 6.36 (CB) and 6.64 (DBS) is only slightly higher compared to the mean TSH (mIU/L) in females 5.92 (CB) and 5.78 (DBS). The mean T4 (µg/mL) in males 13.80 (CB) and 13.60 (DBS) is almost identical to the mean T4 in females (µg/mL) 13.72 (CB) and 13.96 (DBS). No hypothyroid neonate was found, male or female. For T3, sex differences are variable: Mean T3 (ng/mL) in males 57.8 (CB) and 278.93 (DBS) vs Mean T3 (ng/mL) in females 60.53 (CB) and 236.53 (DBS). However, all neonates in the present study were healthy, and no such correlation was obtained.

To minimize global disparities in NBS programs, health authorities must work towards developing uniform decision-making criteria and further harmonization efforts.[38]

Limitations

There were some limitations to this study. First, this is a cross-sectional prospective study. A larger-scale screening program can truly reflect the prevalence and help generate a problem statement. Secondly, CLIA is not widely available in India, owing to infrastructure and financial constraints. In its absence, the availability of an ELISA kit that works especially with DBS could be beneficial as it circumvents errors in extraction and elution. Third, all of the factors possibly affecting thyroid status cannot be evaluated, for example: maternal iodine levels, autoimmune disease, coinciding psychiatric factors such as depression, etc.

Conclusion

India faces a spectrum of challenges for newborn screening in totality, let alone for CH such as the lack of a national policy, high home delivery rate, especially in rural areas, early discharge from hospitals, lack of reliable laboratories with huge outreach, and non-availability of baseline data. Cord blood is a feasible sample for primary screening, for ease of collection, huge volume available, and owing to shorter hospital stays seen in the majority of patients. Dried blood spot by heel prick allows for non-invasive sampling, the much lesser volume required, and screening of multiple disorders, especially after the T3 surge has subsided considerably, and storage. It remains to be seen, how the most common preventable mental retardation in children can be picked up and dealt with in infancy itself in a growing country like ours.

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