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Positive Predictive Value of Dried Blood Sampling of TSH in Diagnosing Congenital Hypothyroidism in Neonates Born at a Tertiary Care Hospital

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ABSTRACT

Congenital hypothyroidism (CH) is one of the most common preventable causes of intellectual disability in children. Early detection through newborn screening is essential for timely intervention. However, the predictive accuracy of DBS-TSH varies across populations, and limited local data are available from Pakistan. **Objectives:** To determine the positive predictive value (PPV) of dried blood samples of thyroid-stimulating hormone (DBS-TSH) in diagnosing congenital hypothyroidism (CH) in neonates. **Methods:** This retrospective cohort study using screening registry data was conducted at the newborn screening program, Aga Khan University Hospital, Karachi, from March 2023 to March 2024. Records of neonates screened from April 2019 to December 2022 were reviewed. Neonates with DBS-TSH >10 mIU/L were labeled screen-positive and underwent confirmatory testing with serum TSH and free T4 within two weeks. Neonates with Serum TSH (>20 mIU/L) were considered true positive for congenital hypothyroidism. **Results:** Of 30,402 neonates screened, 538 (1.76%) were screen positive. Mean DBS-TSH was 16.1 ± 12.6 mIU/L. Among them, 478 (88.8%) had 10–20 mIU/L (Group 1), and 60 (11.2%) had >20 mIU/L (Group 2). Confirmatory testing was available for 385 infants, of whom 33 were true cases of CH, yielding a PPV of 8.57% (95% CI: 5.9%–11.7%). Higher PPV was observed when sampling occurred at >48 hours. A DBS-TSH cutoff ≥35 mIU/L showed PPV of 95% (95% CI: 76.18 – 99.88), which may justify early treatment consideration. **Conclusions:** DBS-TSH screening at a cutoff of >10 mIU/L yields modest PPV for CH. Higher DBS-TSH values and appropriate sampling time significantly enhance predictive accuracy.

INTRODUCTION

Congenital hypothyroidism (CH) is one of the most common treatable endocrine disorders in neonates and a leading preventable cause of intellectual disability [1]. If untreated, thyroid hormone deficiency in early life leads to permanent cognitive impairment, motor dysfunction, and growth abnormalities [2, 3]. Recent global estimates suggest a rising prevalence, with reported rates ranging from 1 in 2,000 – 4,000 live births [4, 5]. The thyroid hormone plays a crucial role in brain maturation, particularly during the neonatal period [6]. Delays in detection and initiation of levothyroxine therapy are associated with irreversible neurological sequelae, whereas early treatment prevents long-term complications [2, 7]. This highlights the importance of effective and timely newborn screening programs. Dried blood spot thyroid-stimulating hormone (DBS-TSH) measurement has become the cornerstone of CH screening worldwide. It is inexpensive, minimally invasive, and well-suited for large-scale implementation, even in resource-limited settings [8, 9]. International studies demonstrate its predictive accuracy, though performance depends on the timing of sampling and cut-off thresholds. A large Chinese cohort study involving more

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than 50,000 newborns showed that optimal DBS-TSH cut-offs (9–11 mIU/L) improved both sensitivity and specificity [10]. Similarly, lowering TSH thresholds increased detection of mild CH cases but also raised false-positive rates [11]. Evidence also suggests that sampling after 48–72 hours reduce false positives compared to earlier collection [12]. Despite these global insights, data from Pakistan remains limited. Local studies have reported increasing trends of CH and highlighted gaps in newborn screening coverage [13, 14].

However, the positive predictive value (PPV) of DBS-TSH–based newborn screening has not been comprehensively evaluated in the Pakistani population, limiting evidence for optimizing national screening strategies. This study aimed to determine the PPV of DBS-TSH in diagnosing congenital hypothyroidism among neonates born at a tertiary care hospital, to inform refinement of national screening protocols.

METHODS

This retrospective cohort study using screening registry data was conducted at the Newborn Screening Program, Department of Pediatrics, Aga Khan University Hospital, Karachi, from March 2023 to March 2024. After obtaining approval from the Institutional Review Board (2023-8367-23754), data were retrieved for all neonates screened for congenital hypothyroidism (CH) between April 2019 and December 2022. As this study involved a retrospective review of existing medical records without direct patient contact, informed consent was not required. Patient confidentiality and anonymity were strictly maintained throughout the study. A DBS-TSH concentration greater than 10 mIU/L was considered screen-positive according to our hospital's newborn screening protocol. A non-probability consecutive sampling technique was used, whereby all screened positive neonates, with gestational age ≥33 weeks, during this period were included. Infants with incomplete records or missing follow-up data were excluded. Screen-positive infants were stratified into two groups: Group 1 (DBS-TSH 10–20 mIU/L) and Group 2 (DBS-TSH >20 mIU/L). Furthermore, screen-positive infants were also stratified based on age at the time of sampling. The sample size was calculated using the formula for estimating a single proportion, with the positive predictive value (PPV) of DBS-TSH in diagnosing congenital hypothyroidism as the primary outcome. Based on a previous regional study [6], which reported a PPV of 8.6%, and using a 95% confidence level (Z = 1.96) and a margin of error of 2.5%, the minimum required sample size was 478 screen-positive neonates. Accounting for an anticipated 20% loss to follow-up, the target sample size was increased to 600. The study included 538 screen-positive infants, meeting this calculated requirement. A non-probability consecutive sampling technique was used, whereby all screened positive neonates with gestational age ≥33 weeks during the study period were included. Data collection included demographic information like gestational age, birth weight, sex, and maternal thyroid and pregnancy history. Moreover, the age of neonates and TSH value through DBS and later confirmatory serum analysis were recorded along with FT4 from electronic medical records of Aga Khan University Hospital. DBS was collected at the hospital from neonates using standardized heel prick procedures onto filter paper cards for newborn screening. TSH levels were subsequently measured from these samples using liquid chromatography-tandem mass spectrometry (LC-MS/MS) at the AKUH Clinical Laboratories, Karachi. All samples were processed following institutional standard operating procedures to ensure quality and accuracy. Neonates with TSH levels >10mIU/L on DBS are advised to get confirmatory serum tests done, involving serum TSH for group 1 and serum TSH and free thyroxine (FT4) for Group 2, to confirm the presence of congenital hypothyroidism. Follow-up is done for all screen-positive cases within 48 hours of initial DBS-TSH results and continued for two weeks via telephonic messages, emails, and letters to the mailing address (whichever is available), and details of confirmatory testing, including date and results, were documented. Neonates are labelled as true positives for congenital hypothyroidism based on plasma TSH >20Miu/L and FT4 testing (<0.97 ng/dl). After extraction of data from medical records and NBS program registry statistical analysis was performed using Stata version 17 (Stata Corp, College Station, TX, USA). Continuous variables were expressed as mean ± standard deviation, while categorical data were summarized as frequencies and percentages. Positive predictive value was calculated as the proportion of screen-positive neonates who were confirmed to have congenital hypothyroidism on serum thyroid-stimulating hormone and free thyroxine testing, using the formula: Positive Predictive Value = (True Positives) / (True Positives + False Positives). Exact 95% confidence intervals were calculated using the Clopper–Pearson method for binomial proportions. Newborn screening was performed in 30402 neonates during the period of study, of which 538 (1.76%) were found to be screen positives (Figure 1).

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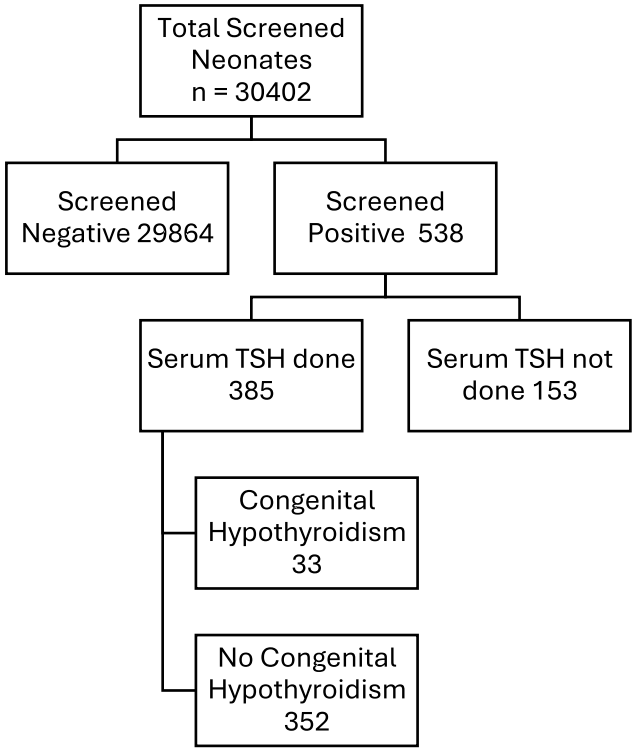

Figure 1: DBS-TSH screening and Serum TSH Confirmatory Testing in Neonates (n=30402)

RESULTS

The mean age of the neonates at the time of sampling was 38.3 ±18.8 hours. The majority of the DBS-TSH sampling was done within 24-48 hours of age, i.e., 315 (58.5%), while 37 (6.8%) was done before 24 hours of age, and 186 (34.5%) was sent after 48 hours of age. The cohort comprised 268 (49.8%) girls and 270 (50.2%) boys. The mean birth weight was 2853.8 ± 417.3 grams. Low birth weight (less than 2500 g) was observed in 86 (16%) of the neonates. The majority of the neonates were term infants, i.e., 466 (86.6%). The mean gestational age was 38.0 ±1.5 weeks. The mean DBS-TSH value was found to be 16.1 ±12.6 mIU/L. A total of 478 (88.8%) had DBS-TSH results between 10-20mIU/L (Group 1), whereas 60 (11.2%) had DBS-TSH results >20mIU/L (Group 2). Among the total cohort, 153 neonates (28.43%) were lost to follow-up, leaving 385 neonates (332 from group 1 and 53 from Group 2) (71.56%), who underwent confirmatory serum TSH testing. The mean serum TSH level among these neonates was 12.5 ± 28.7 mIU/L. Serum FT4 levels were measured in 90 neonates (16.7%), with a mean value of 1.5 ± 1.6 ng/dL. Of the 385 neonates who completed confirmatory TSH testing, congenital hypothyroidism was diagnosed in 33 cases, yielding a proportion of confirmed CH among screen-positive infants of 8.57% (Table 1).

Table 1: Descriptive Findings of Serum TSH, DBS-TSH, Final Diagnosis, and Serum FT4 (n=538)

Characteristics	Mean ± SD / Frequency (%)
DBS-TSH Results (mIU/L)	16.1 ± 12.6
Group 2 (DBS-TSH >20mIU/L)	60 (11.2%)
Group 1 (DBS-TSH 10-20mIU/L)	478 (88.8%)
Serum TSH done	385 (71.6%)
Serum TSH not done	153 (28.4%)
Contact established but not repeated by parents	117 (76.5%)
Contact not established	36 (23.5%)
Serum TSH Results (mIU/L), n=385	12.5 ± 28.7
Serum FT4 Levels, n=90	1.5 ± 1.6
Congenital Hypothyroidism	33 (8.57%)
No Congenital Hypothyroidism	352 (91.4%)

DBS: Dried blood sampling, PPV: Positive predicted value, SD: Standard deviation, TSH: Thyroid-stimulating hormone

The PPV of DBS-TSH in predicting congenital hypothyroidism was found to be 8.57% (95% CI: 5.9%–11.7%) in our cohort (Table 2).

Table 2: Positive Predictive Value of DBS-TSH in Diagnosing Congenital Hypothyroidism (n=385)

Final Diagnosis	DBS-TSH, Frequency (%)	PPV (%)	95% CI (%)
Congenital Hypothyroidism	33 (8.57%)	8.57%	5.97 – 11.38
No Congenital Hypothyroidism	352 (91.425)		
Total	385		

DBS: Dried blood sampling, PPV: Positive predicted value, TSH: Thyroid-stimulating hormone. The PPV confidence interval was calculated using the exact (Clopper–Pearson) binomial method to provide precise estimates of statistical uncertainty.

Stratification by predictor variables showed that the PPV of DBS-TSH increased with both sampling age and higher TSH concentrations. Neonates sampled after 48 hours of age had a PPV of 14.07% (95% CI: 8.69–21.10%) (Table 3).

Table 3: Stratification of Predictor Variables and Positive Predicted Value of DBS-TSH in Diagnosing Congenital Hypothyroidism (n=385)

Variables		Final Diagnosis, Frequency (%)		PPV (%)	95% CI (%)
		Congenital Hypothyroidism	No Congenital Hypothyroidism		
DBS-TSH Category					
Group 1 (10-20 mIU/L)	332	12 (3.6%)	320 (96.3%)	3.6	1.88 – 6.23
Group 2 (>20 mIU/L)	53	21 (39.6%)	32 (60.3%)	39.6	26.45 – 53.99
Age of Neonates at Time of Sampling					
<24 Hours	26	0 (0%)	26 (100%)	0	0 – 13.23
24-48 Hours	223	14 (6.27%)	209 (93.7%)	6.27	3.47 – 10.31
> 48 Hours	135	19 (14.07%)	116 (85.92%)	14.07	8.69 – 21.10

Further analysis of incremental DBS-TSH cutoff thresholds showed a progressive increase in PPV with rising TSH values. A DBS-TSH cutoff of ≥ 35 mIU/L yielded a PPV of

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95.2% (95% CI: 76.18–99.88%). Comparative PPV values for all evaluated cutoff points are summarized (Table 4).

Table 4: Comparison of Positive Predictive Values of Different DBS-TSH Cutoffs

DBS-TSH Cutoff Value (mIU/l)	No. of Screen Positives	No. of Screen Negatives	(Serum TSH >20 mIU/l) No. of True Positives	(Serum TSH >20 mIU/l)	(Serum TSH >20 mIU/l) No. of False Positives	(Serum TSH >20 mIU/l) No. of False Negatives	PPV (%)	95% CI (%)
≥ 10	385	0	33	0	352	0	8.57	5.97 – 11.83
≥ 11	326	59	33	59	293	0	10.1	7.07 – 13.93
≥ 12	252	133	31	131	221	2	12.3	8.51 – 17.00
≥ 13	197	188	28	183	169	5	14.2	9.66 – 19.88
≥ 14	142	243	26	236	116	7	18.3	12.32 – 25.67
≥ 15	110	275	24	266	86	9	21.8	14.51 – 30.70
≥ 16	96	289	23	279	73	10	23.9	15.83 – 33.75
≥ 17	77	308	22	297	55	11	28.5	18.88 – 40.00
≥ 18	64	321	21	309	43	12	32.8	21.59 – 45.69
≥ 19	59	326	21	314	38	12	35.5	23.55 – 49.13
≥ 20	54	331	21	319	33	12	38.8	25.92 – 53.12
≥ 25	38	347	21	335	17	12	55.2	38.30 – 71.38
≥ 30	28	357	21	345	7	12	75	55.13 – 89.31
≥ 35	21	364	20	351	1	13	95.2	76.18 – 99.88
≥ 40	18	367	17	351	1	16	94	72.71 – 99.86
≥ 50	16	369	16	351	0	17	100	79.41 – 100.0

DBS: Dried blood sampling, PPV: Positive predictive value, TSH: Thyroid-stimulating hormone, CI: Confidence Interval. PPV confidence intervals were calculated using the exact (Clopper–Pearson) binomial method to provide precise estimates of statistical uncertainty.

DISCUSSION

This study evaluated the positive predictive value (PPV) of DBS-TSH for neonatal screening of congenital hypothyroidism (CH) at a tertiary care center in Pakistan. The overall PPV was 8.57%, which is consistent with findings from other regions. Alameer *et al.* reported a PPV of 8.6% in Saudi Arabia [6]. while Liu *et al.* recently demonstrated wide variation in global prevalence and screening performance, reflecting differences in cutoffs and protocols across populations [3]. Current analysis showed that DBS-TSH ≥10 mIU/L identified 538 neonates, of whom 33 were confirmed to have CH, supporting this threshold as an effective screening cutoff. Importantly, a higher cutoff (≥35 mIU/L) yielded a PPV of 95%, suggesting that immediate treatment could be considered in such cases while awaiting confirmatory results. In addition, neonates sampled after 48 hours of life demonstrated higher PPVs, underscoring the importance of optimal timing for sample collection. These findings are consistent with recent European and international studies highlighting improved predictive value at higher TSH thresholds and later sampling [15-17]. These comparisons underscore the variability in PPV across different settings, influenced by factors such as screening protocols, cutoff values, and demographic characteristics of the neonates. Our study's findings contribute to the body of evidence supporting the effectiveness of DBS-TSH screening in identifying neonates at risk of congenital hypothyroidism, with implications for early detection and treatment to prevent adverse outcomes in our hospital and laboratory settings [18-20]. This study has several strengths. It is one of the few studies conducted in Pakistan that evaluates the PPV of DBS-TSH screening for congenital hypothyroidism in neonates. Current findings provide valuable insights into the performance of this screening method in a local context. Furthermore, the study included a relatively large sample size, enhancing the statistical power and reliability of our results. The identification of factors associated with higher PPV, such as age at the time of sampling and DBS-TSH levels, adds to the existing knowledge and can inform future screening strategies. Recent studies from Pakistan have reported that the true prevalence of congenital hypothyroidism in the Pakistani population is not available [10, 11]. Our study contributes to addressing the gap in knowledge regarding the true prevalence of congenital hypothyroidism in the Pakistani population by screening out cases with newborn screening, which would have been missed, but cannot provide prevalence estimates because nearly one-third of screen-positive infants did not undergo confirmatory testing. As a result, our findings are limited to the proportion of confirmed CH cases among those who completed follow-up rather than reflecting population-level prevalence. By evaluating the PPV of DBS-TSH for screening congenital hypothyroidism in neonates, our research provides valuable insights that can inform future epidemiological studies and screening strategies in Pakistan.

The study relied on DBS-TSH measurements without assessing full diagnostic parameters such as sensitivity, specificity, and negative predictive value. Additionally, incomplete follow-up limited the evaluation of long-term

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outcomes and may have led to underestimation of true disease burden. Future research should incorporate comprehensive screening performance measures, long-term neonatal follow-up, and evaluation of maternal and environmental factors affecting neonatal TSH levels.

CONCLUSIONS

Current study demonstrates that DBS-TSH screening in neonates has a PPV of 8.57% for diagnosing congenital hypothyroidism. Higher DBS-TSH levels and sampling after 48 hours were associated with increased predictive accuracy. These findings highlight the effectiveness of DBS-TSH as an initial screening tool for early detection of congenital hypothyroidism.

Authors' Contribution

Conceptualization: HM
Methodology: WAK, FN, KNH, IN
Formal analysis: SK
Writing and Drafting: WAK, FN, MA
Review and Editing: WAK, FN, MA, HM, IN, KHH

All authors approved the final manuscript and take responsibility for the integrity of the work.

Conflicts of Interest

All the authors declare no conflict of interest.

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