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Evaluating the Two-Step TSH Screening Protocol for Congenital Hypothyroidism: Prevalence and Diagnostic Accuracy in Ninh Binh, Vietnam

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Abstract

Objectives. This study aimed to determine the prevalence of congenital hypothyroidism (CH) among newborns in Ninh Binh Province, Vietnam, and to evaluate whether adding a second thyroid-stimulating hormone (TSH) screening reduces false positives and improves diagnostic accuracy compared with the traditional single-step screening commonly practiced in Vietnam.

Methodology. A retrospective cohort study was conducted on 11,306 newborns screened between January 2019 and December 2020. TSH levels were measured from dried blood spot samples, with a threshold of >9 mU/L indicating high risk. High-risk cases underwent a second screening, followed by confirmation with serum TSH and free thyroxine. Screening performance (sensitivity, specificity, positive predictive value [PPV], negative predictive value [NPV]) and risk factors for CH were analyzed.

Results. The prevalence of high-risk CH was 2.40% (271/11,306 newborns), with four confirmed cases (incidence: 1:2,826). The two-step screening program achieved a sensitivity of 75.00%, with one false-negative case later detected clinically. Specificity improved from 97.64% in the first screening to 99.81% in the second, while PPV increased more than tenfold (1.11% → 11.54%). Low birth weight infants (≤2,500 g) had a significantly higher CH risk (OR: 10.04, 95% CI: 1.053–95.820, $p = 0.004$).

Conclusions. This first provincial evaluation of two-step CH screening in Vietnam demonstrated that repeat testing significantly reduced false positives and improved diagnostic accuracy without compromising sensitivity. The findings highlight the value of implementing a two-step strategy to optimize newborn screening, reduce unnecessary referrals and save resources in developing countries.

Key words: congenital hypothyroidism, thyroid-stimulating hormone screening, newborn screening, prevalence, two-step TSH screening

INTRODUCTION

Congenital hypothyroidism (CH) is a significant endocrine disorder in newborns, marked by insufficient thyroid hormone production, potentially causing severe developmental delays and intellectual disability if not detected early.[1-3] Globally, CH incidence ranges from 1:2,000 to 1:4,000 live births, influenced by geographic regions and screening protocols.[1,2,4] In Vietnam, with approximately 1.4–1.5 million annual births, an estimated 400 CH cases are diagnosed yearly. Newborn screening began in 1999, and at the National Hospital of Pediatrics, the number of diagnosed cases has risen to 70–80 per year since 2017. However, many infants remain unscreened, particularly in rural areas like Ninh Binh Province, where iodine deficiency is a concern.[5,6]

Prior to 2019, the standard approach in Vietnam was a single-step TSH screening, in which newborns with elevated TSH levels in dried blood spots were directly referred to central hospitals for confirmatory testing. This practice often resulted in a high proportion of false positives due to transient neonatal TSH elevations, prematurity or maternal factors, leading to parental anxiety and unnecessary referrals that placed heavy burdens on families and healthcare resources.[2,3]

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To address these limitations, a two-step TSH screening protocol was introduced in Ninh Binh Province in 2019. In this approach, high-risk infants identified in the first screen undergo repeat testing locally before confirmatory diagnostics. This method has the potential to reduce false positives and improve positive predictive value (PPV) without increasing false negatives, while also optimizing resource use at the provincial level.

We therefore conducted a retrospective cohort study of 11,306 newborns in Ninh Binh Province between January 2019 and December 2020. The objectives were to determine the prevalence of CH, to evaluate the performance of two-step TSH screening compared with the traditional single-step approach and to examine risk factors such as low birth weight. This study represents the first provincial evaluation of the two-step screening strategy in Vietnam and highlights its potential to strengthen newborn screening programs in resource-limited settings.

METHODOLOGY

Research objects

This retrospective cohort study was conducted at the Ninh Binh Provincial Obstetric and Pediatric Hospital in Vietnam from January 1, 2019 to December 31, 2020. All consecutive live births within this period who underwent congenital hypothyroidism (CH) screening were included.

Study population and sampling

A total of 11,306 newborns were screened. Convenience sampling of all eligible consecutive live births during the study period was applied, ensuring the sample reflected the provincial birth cohort.

Sample collection and handling

Heel-prick blood samples were obtained from newborns aged 24–72 hours by trained healthcare personnel. Five dried blood spots (DBS) were prepared on Whatman Protein Saver Cards (USA), air-dried, stored appropriately and transported within 24 hours to the Chemedic Vietnam Joint Stock Company testing center. Missing or incomplete records (e.g., incomplete demographic or laboratory data, poor-quality DBS, or biologically implausible results) were excluded; these accounted for <0.5% of the dataset and no imputation was performed.

Instruments and reagents

The Victor-2D system (PerkinElmer, USA), Cobas 6000 Chemiluminescence Apparatus (Roche, Switzerland), and Panthera-Puncher™9 Puncher (3 mm column diameter, PerkinElmer) were used. Kits included the Thyroid-stimulating hormone assay kit (time-resolved fluorescence, PerkinElmer), TSH detection kit, and free thyroxine detection kit (electrochemiluminescence, Roche).

Screening procedure

TSH concentrations in dried blood spots were measured using a fluorescence immunoassay (Neonatal TSH ELISA kit, PerkinElmer) on the Victor-2D system, according to the Thyrotropin Assay Kit Manual and GSP Operating Procedure. High-risk CH cases were defined as TSH >9 mIU/L. All high-risk newborns identified in the first screening underwent a repeat heel-prick sample at 1–2 weeks for second-step screening.

Diagnostic confirmation

High-risk newborns after initial or second screening underwent confirmatory testing. Venous blood samples were analyzed for TSH and free thyroxine (FT4) levels using an electrochemiluminescence immunoassay on the Cobas 6000 analyzer (Roche, Switzerland). CH was confirmed by elevated TSH and reduced FT4 levels, based on age-specific reference ranges.

Sample size and power

As a retrospective cohort, no *a priori* sample size was calculated. However, a post-hoc power analysis indicated that with 11,306 newborns and an observed incidence of 1:2,826 (0.035%), the study achieved >80% power to detect a prevalence difference of 0.02% at $\alpha = 0.05$.

Statistical analysis

Analyses were conducted using SPSS version 20.0 (IBM Corp., USA).

- ***Descriptive statistics*** were used to summarize prevalence, demographics, and screening outcomes.
- ***Diagnostic accuracy*** (sensitivity, specificity, positive predictive value [PPV], negative predictive value [NPV]) was calculated for the first and second screenings.
- ***Chi-square tests*** assessed differences in the proportions of diagnosed cases across subgroups (gender, birth weight, geographical distribution).
- ***Multivariate logistic regression*** evaluated risk factors for CH (low birth weight, gender and topographical classification). Odds ratios (ORs) with 95% confidence intervals (CIs) were reported.

No adjustments for multiple testing were applied, given the exploratory nature and the small number of confirmed CH cases; results were interpreted with caution.

Ethical considerations

The Institutional Review Board of Ninh Binh Provincial Obstetric and Pediatric Hospital approved the study before commencement. Written informed consent was obtained from parents or legal guardians prior to participation.

Table 1. Prevalence of high-risk congenital hypothyroidism by gender and birth weight

Category	No. of newborns (N)	High-risk 1st screening	High-Risk 2nd screening	Confirmed CH (n)	Incidence (1:n)	*p*-value*
Gender						
Male	6,055	151	14	1	1/6,055	0.252
Female	5,251	120	12	3	1/1,750	
Birth weight						
≤2,500 g	1,025	17	5	2	1/512	**0.004**
>2,500 g	10,281	254	21	2	1/5,140	
Total	11,306	271	26	4	1/2,826	–

*Chi-square test comparing subgroups.
High-risk is defined as TSH >9 mIU/L.
Incidence was calculated as the total confirmed CH cases divided by the total number of newborns.

Table 2. Multivariate logistic regression analysis for the incidence of CH in neonates

Influencing Factors	β	SE	Wald χ^2	*p*-value	OR	95% CI
Sex	1.241	1.091	1.29	0.252	3.46	0.408–29.341
Birth weight	2.307	1.153	4.00	0.004	10.04	1.053–95.820
Topographical classification*	-0.192	1.000	0.04	0.841	0.83	0.116–5.870

*Transitional lowland and coastal delta region vs. hilly midland region

RESULTS

Prevalence of CH

A total of 11,306 newborns were screened for congenital hypothyroidism (CH) between 2019 and 2020. After the first screening, 271 newborns (2.40%) were identified as high risk, decreasing to 26 (0.23%) after the second screening. Four newborns were confirmed with CH, yielding an incidence of 1 in 2,826.

Table 1 shows the prevalence of high-risk CH cases by gender and birth weight. The incidence was higher in females (1/1,750) than in males (1/6,055), though the difference was not statistically significant ($p = 0.252$). Low birth weight infants (≤2,500 g) had a significantly greater risk of CH compared with normal weight infants (OR 10.04, 95% CI: 1.053–95.820, $p = 0.004$). Geographic and topographic subgroup analyses are presented in Supplementary Table S1, with no statistically significant differences observed.

Risk factors

In Table 2, multivariate logistic regression analysis was performed to evaluate factors associated with congenital hypothyroidism (CH) in 11,306 neonates, with results presented for sex, birth weight and topographical classification. For sex (female vs. male), the odds ratio (OR) was 3.46 (95% CI: 0.408–29.341, $p = 0.252$). For birth weight (Low Birth Weight vs. Normal Birth Weight), the OR was 10.04 (95% CI: 1.053–95.820, $p = 0.004$). Finally, for topographical classification (Transitional lowland and coastal delta region vs. hilly midland region), the OR was 0.83 (95% CI: 0.116–5.870, $p = 0.841$). Overall, low birth weight remained the only significant predictor of CH (OR 10.04, $p = 0.004$). Gender and topographical classification were not significantly associated with CH incidence.

Screening performance

The diagnostic accuracy of the screening program is summarized in Table 3 and illustrated in Figure 1. At first screening, sensitivity was 75.00%, specificity was 97.64%, PPV was 1.11% and NPV was 99.99%. After the second

Table 3. Performance of congenital hypothyroidism screening using TSH quantification

Parameter	Formula	Result (1st screening)	Result (2nd screening)
True positive	(a)	3	3
False positive	(b)	268	23
False negative	(c)	1	1
True negative	(d)	11,034	11,279
Sensitivity	a/(a + c) (%)	75.00	75.00
Specificity	d/(b + d) (%)	97.64	99.81
Positive predictive value	a/(a + b) (%)	1.11	11.54
Negative predictive value	d/(c + d) (%)	99.99	99.99

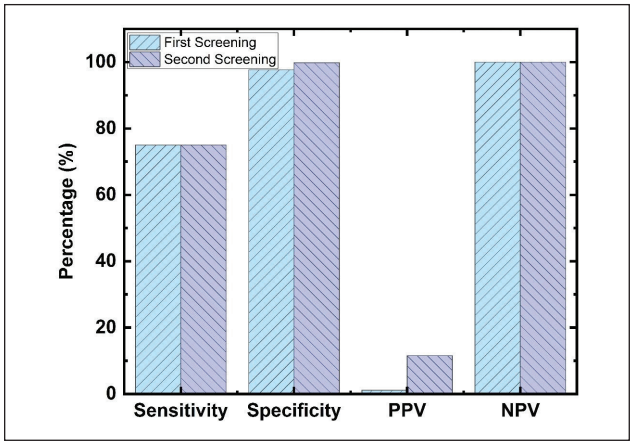

Figure 1. Comparison of screening performance between the first and second TSH-based screenings for congenital hypothyroidism (CH) in 11,306 newborns in Ninh Binh Province, Vietnam (2019–2020).

screening, sensitivity remained 75.00%, but specificity improved to 99.81% and PPV increased markedly to 11.54%, while NPV remained 99.99%. This demonstrates that the second screening substantially reduced false positives without increasing false negatives.

DISCUSSION

This study is the first evaluation of a two-step TSH-based screening program for congenital hypothyroidism (CH) in Vietnam. Conducted on 11,306 newborns in Ninh Binh Province during 2019–2020, it provides insights into prevalence, risk factors, and screening performance under real-world conditions in a resource-limited setting.

The incidence of confirmed CH was 1 in 2,826, which lies within the global range of 1:2,000–1:4,000 live births.[1,2,4,5] The relatively high proportion of initial high-risk results (2.40%) likely reflects transient TSH elevations and threshold effects, a phenomenon also noted in previous reports.[7,8] Low birth weight emerged as the only significant predictor, with an odds ratio of 10.04 (95% CI: 1.053–95.820, p = 0.004). Half of the confirmed CH cases were low birth weight infants, reinforcing findings from studies in Hainan and elsewhere that prematurity and reduced birth weight are associated with impaired thyroid function.[9] These results highlight the importance of tailored screening and follow-up for vulnerable subgroups.

A major contribution of this study is the demonstration that a repeat TSH test markedly improves diagnostic performance. Although sensitivity remained at 75.00%—with one false-negative case later diagnosed at ~3 months due to delayed growth, prolonged jaundice, constipation, and lethargy—specificity increased from 97.64% to 99.81%, and positive predictive value (PPV) rose more than tenfold (from 1.11% to 11.54%). This reduction in false positives (by >90%) is consistent with international recommendations that repeat testing minimizes unnecessary referrals and improves efficiency.[1] In the Vietnamese context, where abnormal first screens are usually referred directly to central hospitals, a second provincial-level screen can substantially reduce resource burdens while maintaining safety.

No statistically significant differences were observed across districts or topographic regions, either in univariate or multivariate analysis. These findings suggest that CH risk is not geographically clustered within Ninh Binh Province and support uniform province-wide screening policies.

The prevalence and screening performance observed here are broadly comparable to findings in other regions. Studies from China and Colombia have similarly highlighted the role of repeat or second-step testing in improving PPV.[9,10] Research in Kenya reported challenges with false positives and underdiagnosis in single-screen programs, underscoring the value of repeat testing.[11] Asia-Pacific regional reports also recommend two-step or tiered protocols to balance sensitivity with resource constraints (IAEA, 2005). Our findings add to this body of evidence and provide local data to guide newborn screening policies in Vietnam.

Several limitations should be acknowledged. First, confirmatory testing was restricted to high-risk newborns, raising the possibility of undetected CH cases and a true incidence higher than reported. Second, the small number of confirmed cases (n = 4) led to wide confidence intervals in logistic regression estimates, which should be interpreted with caution; future multicenter studies or extended follow-up periods are needed to improve statistical power. Third, maternal factors such as iodine deficiency and thyroid disease, which may influence neonatal TSH values, were not assessed. Finally, while the two-step method substantially reduced false positives, the persistence of false negatives indicates that clinical vigilance and follow-up remain essential.[10-12]

Overall, this study demonstrates that implementing a two-step TSH screening protocol is feasible and effective in reducing false positives in Vietnam. By reducing unnecessary referrals and focusing attention on true high-risk cases, this approach can optimize scarce healthcare resources while maintaining diagnostic accuracy. These findings provide a foundation for broader adoption of enhanced screening strategies in other provinces and similar resource-limited settings.

CONCLUSIONS

This study identified a high-risk prevalence of congenital hypothyroidism (CH) among newborns in Ninh Binh Province, Vietnam, of 2.40% (271/11,306), with four confirmed CH cases (incidence: 1:2,826), including one false-negative case diagnosed later through clinical manifestations. The two-step TSH-based screening program demonstrated high specificity (97.64% in the first screening, 99.81% in the second) and markedly improved positive predictive value (PPV) (11.54% vs. 1.11%), while maintaining sensitivity at 75.00%. Low birth weight infants had a significantly higher risk of CH (OR: 10.04, 95% CI: 1.053–95.820, p = 0.004), whereas sex, geography and topography showed no significant differences. These findings support the feasibility of two-step screening to reduce false positives, minimize unnecessary referrals, and optimize limited healthcare resources in Vietnam. However, the persistence of false negatives highlights the need for complementary clinical follow-up to ensure timely detection and intervention.

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Statement of Authorship
All authors certified fulfillment of ICMJE authorship criteria.

CRediT Author Statement
NHV: Conceptualization, Methodology, Writing – original draft, Writing – review and editing, Supervision; **TKH:** Conceptualization, Methodology, Writing – original draft, Writing – review and editing, Supervision; **NBN:** Formal analysis, Investigation, Data curation; **BTB:** Formal analysis, Investigation, Data curation; **NAN:** Formal analysis, Investigation, Data curation; **VTT:** Conceptualization, Methodology, Writing – original draft, Writing – review and editing, Supervision.

Data Availability Statement
Data are available upon reasonable request from the corresponding author.

Author Disclosure
The authors declared no conflict of interest.

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