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Original Article

Evaluating the Value of Quadruple Thyroid Screening in the Diagnosis of Hypothyroidism among Premature Neonates in Tehran in 2019-2020

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ABSTRACT

Background: Neonate screening is a preventative measure that can contribute to early diagnosis of treatable diseases. Hypothyroidism in neonates is among the common preventable factors leading to intellectual disability. This study aims to describe the prevalence and experience with four-step screening to diagnose congenital hypothyroidism.

Methods: The statistical population of this cross-sectional study included 392 preterm neonates (gestational ages less than 36 weeks and 6 days) in 2018-2019 from the health centers covered by Shahid Beheshti University of Medical Sciences (Tehran, Iran) who had been referred for screening. Neonates were screened in the first 3-6 days after birth and then on days 14, 42, and 72. In neonates with TSH serum levels of 10 mU/L, TSH-T4 was measured. Next, the physician decided on the medication based on the results.

Results: At the primary screening of the neonates average TSH level was 2.08 ± 2.4 mU/L. Thirty-three neonates (8.4%) indicated disrupted screening results. The secondary screening of 388 neonates (98.97%) revealed an average TSH serum level of 1.9 ± 1.96 mU/L, and 19 (5.9%) indicated TSH serum levels higher than 5 (mU/L). The third screening test was performed on 382 (97.44%) of the neonates. The average TSH level was 1.3 ± 2.4 mU/L, 340 (85.96%) indicated normal levels, and 42 (11.47%) had unfavorable results. Finally, the fourth screening test was performed on 373 (95.15%) neonates. The mean TSH level was 2.21 ± 1.9 mU/L, which revealed 24 (6.12%) unfavorable test results. The percentage of hypothyroidism in neonates was 5.6% (23 cases). In this study, the prevalence of hypothyroidism was 4.33% (17 cases) in girls and 1.53% (6 cases) in boys.

Conclusion: The results of this study revealed the high incidence rate of congenital hypothyroidism among preterm neonates. Therefore, preventing congenital hypothyroidism misdiagnosis requires a series of screening tests. Also, findings indicated that the results of screening tests for preterm neonates with prolonged GAs and higher birth weights are more accurate and reliable.

Keywords: Hypothyroidism, Neonate, Screening test

Introduction

Neonate screening is a preventative measure that can lead to early diagnosis of treatable diseases, including endocrine, metabolic, genetic, hematologic, and infectious diseases. These programs have been a significant healthcare objective among most developed countries over the past two decades (1) .

Hypothyroidism in neonates is among the

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common preventable factors contributing to intellectual disability (2). According to the clinical manifestations, the diagnostic rates of neonates with hypothyroidism in 1 month, 3 months, 1 year, and 3-4 years after their birthdate are 10, 35, 70, and 100%, respectively. Without screening, early diagnosis and proper treatment for neonates are not possible. Therefore, a reduction in intelligence quotient (IQ), incidence of mental disabilities, and growth and development disorders among neonates would be inevitable (3).

About 4-8% of the neonate population is preterm neonates (4). According to recent studies in Iran, 10% of neonates are preterm. These neonates are the largest group of neonates admitted to the NICU (5). Due to the undeveloped hypothalamic-pituitary-thyroid system, presence of respiratory disorders, hypoxia, inadequate nutrition, dysfunction of the cardiovascular and digestive systems, sepsis, and nervous system issues, they are susceptible to higher risks for thyroid disorders (6, 7).

According to the reports, before the screening programs for neonates with CH diseases, the incidence rate was 1 in 7,000-10,000 cases (8). Recent scientific reports indicate that the incidence of CH diseases in Iran is increasing. This finding can be attributed to factors including the improvement of the quality of laboratory tests and more accurate results, switching the primary screening test from T4 to TSH, enhancement of detection range for TSH screening tests using the filter paper soaked in blood from the neonate's heel, and higher survival chance of the neonates (8). The incidence of delayed hypothyroidism in full-term neonates was 1 in every 100,000 live births, although it was significantly higher in preterm neonates. According to reports, it was as high as 1 in every 300 live births (9).

About 5-10% of low birth weight (LBW) neonates with hypothyroidism may not be identified in the first screening stage. For this reason, in many studies, "re-screening" is necessary. Screening neonates benefit patients and their families and prevent a significant loss of financial and human resources. It also contributes to the nations' development by maintaining the IQ of the patients (10). According to screening programs, the CH rate in preterm and low birth weight neonates is higher than in term neonates due to the immaturity of the hypothalamus-pituitary axis. However, one-third of these neonates can be identified with a screening program. Accordingly, some studies suggest repeating screening in low birth weight and premature neonates (11).

Accordingly, this study aims to investigate the accuracy of repetitive screening tests to diagnose hypothyroidism in preterm neonates screened by Shahid Beheshti University of Medical Sciences (Tehran, Iran) health centers.

Methods

The method employed in the present study is cross-sectional. Out of 392 preterm neonates in this study, 23 cases had hypothyroidism. This study was conducted in 2018-2019 in the health centers covered by Shahid Beheshti University of Medical Sciences (Tehran, Iran). At these health centers, the neonates (with gestational ages less than 36 weeks and 6 days) had been subjected to screening tests for thyroid diseases. Samples were selected based on targeted sampling using available methods. In this way, all cases were examined until reaching the required volume of the study in the period of 2019-2020. Determining the sample size and its determination method: According to the conducted studies, the minimum number of samples required with 95% confidence and 80% power will be equal to:

$$\frac{Z^2 p(1-P)}{d^2} = \frac{1.96^2 \times 0.8 \times 0.2}{(0.5)^2} = 245.8 \sim 246$$

Therefore, 246 patients are needed in this study. However, to increase the accuracy and validity of the findings, 392 patients were included (12).

The first screening of the neonates was performed at Iranian health centers after a minimum of 72 hours from birth. Screenings were according to the national protocols of features of the filter paper, method of sampling, storage, and sample submitting. According to the national protocols, the screening of preterm neonates was repeated 14, 42, and 72 days after birth. In case of unfavorable results at each level, the families were informed and recommended to repeat the screening. In neonates with TSH serum levels of 10 mU/L, TSH-T4 was measured. Eventually, the physician decided on the medication according to the results.

The vice-chancellor of health affairs of the Shahid Beheshti University of Medical Silences provided us with the results of thyroid screening tests, and information on neonates.

Statistical analysis

We analyzed the acquired data using Statistical Package for the Social Sciences (SPSS), version 26 (IBM, Armonk, NY). For continuous variables, we

used mean and standard deviation (mean ± SD) to show the amount of central tendency or dispersion, respectively. Also, the categorical variables, such as gender, were analyzed using the frequency. Furthermore, the chi-square test, t-test, and Pearson correlation coefficient were employed for categorical continuous variables to find associations and calculate the association between variables, respectively. It is noteworthy that the marginal significance level for the tests (p-value) was considered 0.05.

Ethical approval

The patient's parents signed a written informed consent for participating in the study. The ethical approval code for this study was IR.SBMU.MSP.REC.1398.1003.

Results

The statistical population included 392 preterm neonates (with gestational ages less than 36 weeks and 6 days) in 2018-2019 who had been referred for screening. Also, of 392 studied neonates, 180 (46%) were female, and 212 (54%) were male. The average gestational age (GA) was 35.5 ± 1.6 weeks. Most neonates (47.7%) had GAs higher than 36 weeks. The average birth weight of the neonates was 2368 gr (± 379.2), their average height at birth was 46.49 (± 2.4) cm, and their average head circumference at birth was 33.89 (± 1.5) cm.

Among the mothers, 22 (5.6%) had a hypothyroidism history, and 21 (%95.45) were on levothyroxine medication. None of the mothers had hyperthyroidism.

The primary screening of the neonates was 3-6 days (average of 4.8 days) after the birth, and the average TSH level was 2.08 ± 2.4 mU/L. A total of 359 (91.5%) of the neonates had acceptable levels (TSH < 5 mU/L).

Thirty-three neonates (8.4%) indicated disrupted screening results. Six (1.53%) had TSH levels higher than 10 (mU/L), which were referred for TSH-T4 serum level evaluation. Two (0.51%) neonates indicated TSH serum levels higher than 20 (mU/L) and started levothyroxine treatment. According to the TSH serum level, four neonates started levothyroxine medication.

The secondary screening of 388 neonates (98.97%) on an average of 8.3 days after birth revealed an average TSH serum level of 1.9 ± 1.96 mU/L. A total of 369 neonates (94.1%) had normal levels, and 19 (5.9%) indicated TSH serum levels higher than 5 (mU/L). Seven (1.7%) neonates had TSH serum levels higher than 10 (mU/L).

Among the 19 neonates, 12 (3.6%) had unfavorable primary screening results, and 7 (1.78%) indicated TSH serum levels higher than 5 (mU/L) for the first time. Eventually, 6 neonates started medication.

The third screening test was performed on 382 (97.44%) neonates in the sixth week with an average of 43.6 days. The average TSH level was 1.3 ± 2.4 mU/L, 340 (85.96%) indicated normal levels, and 42 (11.47%) had unfavorable results.

Of the 42 (11.47%) neonates with TSH serum levels higher than 5 (mU/l) in the third screening test, 6 (1.78%) neonates indicated unfavorable screening results at the primary and secondary tests, and 9 (2.29%) indicated adverse test results only at the second test.

Based on the obtained results, 10 (2.55%) neonates indicated adverse results at the primary and third screening tests, while their second test results were within the normal range. In addition, 29 (7.9%) neonates had TSH levels higher than 5 (mU/L) for the first time. In this stage, nine (2.29%) neonates started medication.

The fourth screening test of 373 (95.15%) neonates were performed on the tenth week with an average age of 74.5 days. The mean TSH level was 2.21 ± 1.9 mU/L, which revealed 24 (6.12%) unfavorable test results. Only one of the neonates that showed unfavorable results on the fourth screening test had TSH levels higher than 5 (mU/L) at three previous tests. Two neonates had TSH levels higher than 5 (mU/L) in the secondary and third tests. Moreover, 11 neonates indicated TSH levels higher than 5 (mU/L) in the third test, and 12 showed TSH levels higher than 5 (mU/L) in the fourth test for the first time. Finally, 7 neonates started medication in this stage.

The results of quadruple screening tests of the neonates are shown in Table 1.

The significant statistical correlation between the validity of the quadruple screening tests and the GA of the neonates ($p = 0.01$ and $r = 0.3$; $p = 0.01$ and $r = 0.25$; $p = 0.01$; and $r = 0.21$, $p = 0.01$ and $r = 0.6$) reveals that the validity of thyroid screening enhances with increasing the neonates GA.

There was a statistically significant relationship between the birth weight of the neonates and the validity of the screening tests ($p = 0.00$ and $r = 0.1$; $p = 0.31$ and $r = 0.28$; $p = 0.04$ and $r = 0.31$; and $p = 0.01$ and $r = 0.6$). This data indicates that with the increasing birth weight of the neonate, the probability of the correspondence of all four screening tests with a hypothyroidism-positive diagnosis increases.

There was a statistically significant

Table 1. The results of quadruple screening tests of the neonates

Screening stage	Gender	Mean ±SD (mu/L)	Min	Max	TSH>5mu/L	TSH>10mu/L	TSH>5 mu/L*	TSH>10 mu/L*	TSH>5 mu/L**	TSH>5mu/L***	TSH>5mu/L****	TSH>10mu/L*****
Stage 1	F (180)	1.98±2.2	0.1	19	12±3.6	2±0.05						
	M (212)	2.24±2.59	0.1	20	21±5.35	4±1.02						
	T (392)	2.07±2.3	0.1	20	33±8.41	6±1.05						
Stage 2	F (179)	1.91±2.8	0.1	16	7±2.04	3±1.02	5±1.53	1±0.02				
	M (209)	2.24±2.59	0.1	12.5	12±3.06	4±1.02	7±1.78	0				
	T (388)	1.96±1.9	0.1	16	19±5.1	7±2.06	12±1.2	1±0.2				
Stage 3	F (176)	2.5±2.5	0.1	12	20±5.86	5±1.78			2±1.53	4±1.02		
	M (206)	2.32 ±2.39	0.1	14	22±5.61	6±1.52			4±1.78	6±1.53		
	T (382)	2.42±2.4	0.1	14	42±11.47	11±2.81			6±1.78	10±2.55		
Stage 4	F (170)	1.91±2.59	0.1	16	10±2.55	4±1.02					0	0
	M(202)	2.24±2.59	0.1	12	14±3.58	3±0.76					1±0.02	2±0.5
	T (373)	2.21±1.9	0.1	11	2.4±5.1	7±2.8					1±0.2	2±0.5

*; First stage screening
**; First and second stage screening
***; First and third stage screening
****; First, second,third and fourth stage screening
*****; Second, third and fourth stage screening
F: Female, M: Male; T: Total

relationship between the head circumference of the neonates and the validity of the screening tests of all four-stages (p = 0.00 and r = 0.52; p = 0.03 and r =0.63; p = 0.01 and r = 0.2; p = 0.01 and r = 0.26). It appears that by increasing the head circumference, the result of the screening tests would be more accurate.

There was no significant correlation between the gender of the neonates, the mother's hypothyroidism history, and medication at any of the screening phases. Factors affecting quadruple screening tests are shown in Table 2.

Table 2. Factors affecting quadruple screening tests

Variable		First screening Mean (standard deviation) number (percentage)	P- value	Second screening Mean (standard deviation) number (percentage)	P- value	Third screening Mean (standard deviation) number (percentage)	P- value	Fourth screening Mean (standard deviation) number (percentage)	P- value
Gestational age		35.1 w (±1.4)	0.025	35.1 w (±1.4)	0.001<	35.1 w (±1.4)	0.001<	35.2 w (±1.3)	0.001<
Gender of the neonate	Girl Boy	180(46%) 212(54%)	0.432	179(45%) 209(53%)	0.66	176(44%) 206(52%)	0.172	170(43%) 203(51%)	0.821
Neonate's weight		2368 gr (±37.4)	0.008	2373 gr (±37.6)	0.005	2370 gr (±37.3)	0.175	2386 gr (±36.3)	0.403
head circumference		32.8 cm (±1.5)	0.001<	32.9 cm (±1.4)	0.003	32.9 cm (±1.4)	0.001<	32.9 cm (±1.4)	0.044
height neonate		46.49 cm (±2.4)	0.001<	46.5 cm (±2.4)	0.001<	46.49 cm (±2.4)	0.001<	46.5 cm (±2.3)	0.662
Drug consumption in mother	Yes No	0.574				18(4.59%) 3(0.76%)			
Maternal hypothyroidism	Yes No	0.792				22(5.61%) 370(94.38%)			

Discussion

The present research aimed to investigate the accuracy of successive screening tests to diagnose hypothyroidism in premature neonates. Accordingly, we can prevent the decrease in IQ of neonates with effective treatment by predicting the influencing factors.

The results showed that the rate of congenital hypothyroidism in preterm neonates was 5.6%, indicating a high percentage of congenital hypothyroidism in preterm neonates. The findings were inconsistent with those of other researchers (3, 6).

Ordoukhani et al., in a study between 1997 and 2001 in Tehran, reported the incidence rate of congenital hypothyroidism at 1.91% (6). Also, in a study conducted in Shiraz by Keramizadeh and Amir Hakimi, the prevalence of this disease was reported as 1.41% (3). This difference can be attributed to the difference in the gestational age of the examined neonates, the overall prevalence of hypothyroidism in each province, the amount of iodine deficiency in each region, and the health and treatment facilities during the pregnancy.

Tfayli et al. reported a 5-times higher hypothyroidism ratio of preterm neonates to full-term neonates compared to our study (12). This difference might be due to higher GA and weight of the neonates in our study. In this study, most of the neonates were VLBW , but in our study, this group of neonates was relatively low in number.
On the other hand, despite the high rates of congenital hypothyroidism among Iranian neonates, the risk factors are not mentioned in any studies. Furthermore, we could not interpret the high rates of hypothyroidism due to the lack of studies on the insufficiency of iodine among pregnant and nursing mothers in Iran.

Overall, the high rates of hypothyroidism do not seem unreasonable considering the high risks of preterm hospitalization and low birth weight neonates, the use of disinfectants containing iodine in these centers and high chances of exposure to the disinfectant, and the tendency of mothers to cesarean.

In this study, the average gestational age (GA) of the neonates was 35.5 weeks, and most neonates (47.7%) had a gestational age of more than 36 weeks. According to the average gestational age in our study (35.5 weeks), the corrected age of most neonates is around the time of the third screening. Regarding the acceptable sensitivity and specificity of this stage, screening at the corrected gestational age of about 40 weeks may increase the screening accuracy and sensitivity. The findings were consistent with those of Vigone et al. In the study of Vigone et al., the best time for screening is the corrected age of the neonate up to 36 weeks (5).

Various studies recommended multiple stages of screening for premature and low-weight neonates. Researchers, including Zdraveska et al. and Belfort et al., advised that the TSH threshold for these neonates should match the GA. This recommendation seems infeasible because various studies reported different values, and these values are not the same throughout the world (13, 14).

Kaluarachchi et al. recommended evaluating the TSH and T4 levels to prevent the misdiagnosis of hypothyroidism in preterm neonates (15).

Moreover, Srinivasan et al. indicated that by lowering the TSH level to 6 mU.L, no CH case would remain undiagnosed, and only one screening test at birth is sufficient (16).

Our findings indicate that performing a minimum of 3 screening tests is required for early diagnosis of the accurate number of neonates with hypothyroidism. Besides, Rastogi et al. studied 263 neonates with GAs between 26 to 41 weeks and reported that two weeks after birth is the best time for screening (17).

The incidence rate of delayed hypothyroidism in our study was 15.3% (n = 59). These neonates showed normal TSH levels at the first screening test; however, they indicated an increase in TSH levels at the next screening tests. Other studies also have reported a delayed increase of TSH after 2-3 weeks, corresponding to our findings.

Research limitations

Considering the severe prematurity in the target population of the current study (neonates referring to health centers in the north of Tehran), many preterm neonates hospitalized in various hospitals were not included. Also, due to the lack of follow-up or preference of the families to perform the tests at the private centers, a large portion of the target population was not accessible. Moreover, since the screening tests were performed at different centers with different qualities, there is a lack of data on the timing and method of sample delivery. Hence, prospective studies with extended follow-up periods and evaluating the background risk factors to determine the prevalence of congenital hypothyroidism among the full-term and preterm neonates of Iran are necessary.

Conclusion

The results of this study revealed the rate of congenital hypothyroidism in preterm neonates is significantly high. Therefore, preventing congenital hypothyroidism misdiagnosis requires a series of screening tests. The results also showed the importance of four-stage screening. When normal TSH levels appear in the primary screening, it indicates a disruption in the next stage of screening. The hypothyroidism percentage of neonates was 5.6% (23 cases). In this study, the hypothyroidism prevalence was 4.33% (17 cases) in girls and 1.53% (6 cases) in boys.

Also, findings indicated that the results of screening tests for preterm neonates with prolonged GAs and higher birth weights are more accurate and reliable.

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Conflicts of interest

The authors have no conflict of interest.

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