Original Paper

HORMONE RESEARCH

Horm Res 2005;64:287–292
DOI: 10.1159/000089488

Received: April 18, 2005
Accepted: September 15, 2005
Published online: November 1, 2005

Rate of Recalls in Congenital Hypothyroidism Based upon a Regional Survey in Isfahan, Iran, Using Serum T4 and TSH Analyses: Comparison of Two Different Recall Methods

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Key Words
Congenital hypothyroidism · Thyroxin · Thyroid-stimulating hormone

Abstract
Aims: To evaluate and compare the recall rate in congenital hypothyroidism screening project in Isfahan, first using an approach involving measures of both TSH and T4 and then using TSH alone. ***Methods:*** From June 2002 to January 2005, serum TSH and T4 level of referred neonates were measured at 3rd to 7th day of birth through venous sampling. If neonates' serum TSH was > 20 mIU/l or T4 was <6.5 μg/dl by the first protocol, or TSH was >20 mIU/l by the second protocol, they were recalled. TSH and T4 were measured using an immunoradiometric assay and radioimmunoassay, respectively. Neonates with TSH > 10 and T4 < 6.5 on their second measurement were considered as congenitally hypothyroid. ***Results:*** Serum T4 and TSH of 29,425 neonates by first and 57,235 neonates by second recall approach were measured. Recall rate was higher in the first protocol (2.2% vs. 0.6%, $p < 0.05$). Most of the recalled neonates in the first protocol were recalled for low T4 level ($p < 0.05$). The prevalence of CH was 1 in 350 livebirths. ***Conclusion:*** Although the recall rate was in the acceptable range by either approach, the TSH alone protocol seems to be a more sensitive and practical approach with the least recall burden and considering the high prevalence of CH in our region merit adaptation of widespread screening for CH using TSH measurements from heel stab blood spotted on filter paper.

Introduction

Congenital hypothyroidism (CH) is a preventable cause of mental retardation, which can be prevented in the case of early diagnosis and treatment. Clinical diagnosis of CH without neonatal screening is just impossible to do early enough in order to be effective [1], thus screening of CH in communities is essential. In this way, the screening program which is feasible, accessible and less costly for public must be implemented.

Proper recalls of neonates in CH screening projects have beneficial effects on parents' time, expense and mentality. The rate of recalls and recall criteria vary in different communities and studies. Not only can neonates' recalls based on proper criteria cover the community screening program properly but they also increase parent cooperation and organize project expenses [2, 3].

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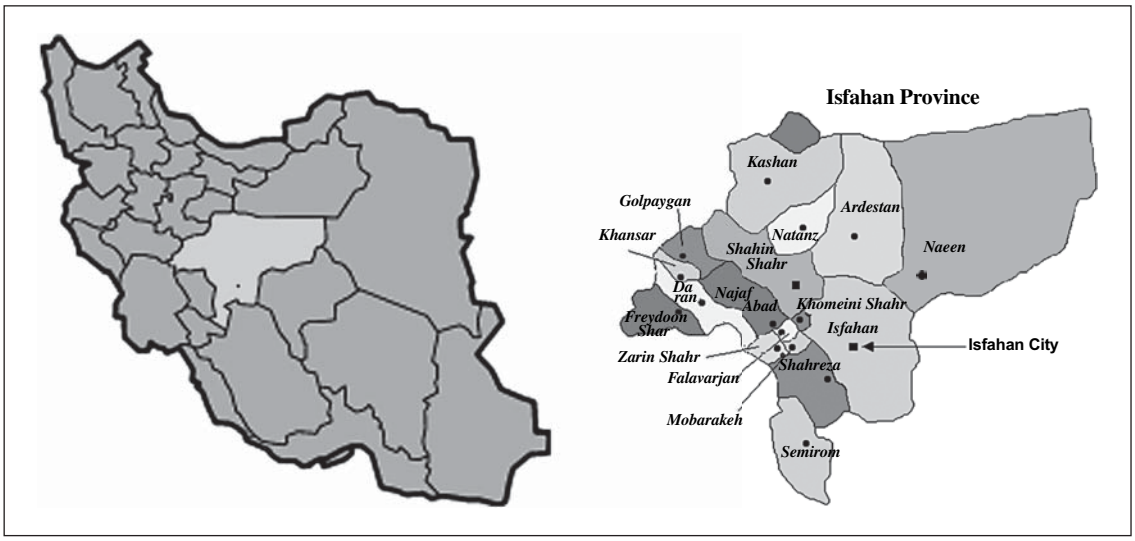

Fig. 1. The map of Iran and Isfahan Province.

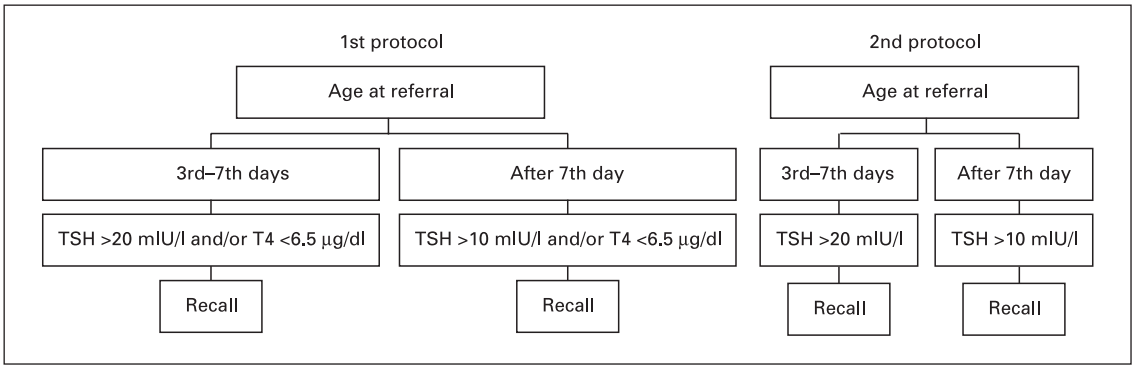

Fig. 2. First vs. second protocol used to recall neonates.

Considering the importance of prevention of the long-term consequences of CH, the current study evaluated the rate of recalls performed based on two different protocols in the CH screening project of Isfahan, a centrally located city of Iran (fig. 1), and compared them with other communities.

Material and Methods

This study was implemented from June 2002 to January 2005, and evaluated referred neonates from 17 maternity hospitals (private, public, educational, township) in Isfahan, Iran. The coverage percent of this project was achieved through calculating the ratio of referred neonates to total number of livebirths.

Serum T4 and TSH or only TSH concentrations of 86,660 neonates were measured on their 3rd to 7th day of birth through venous sampling (from the cubital vein), by trained nurses. A general information questionnaire, which recorded neonates' sex, length, weight, birth date and maternal age of pregnancy, joint with laboratory data, was sent to an endocrinologist and collaborating general practitioner. Therefore, they made a decision and determined which neonates must be recalled.

Two different protocols were used to recall the neonates (fig. 2). According to the first protocol, recall was implemented based on the level of T4 and TSH. In the case of TSH >20 mIU/l or T4 <6.5 μg/dl on the 3rd to 7th days after birth and TSH >10 mIU/l (for those referred after the 7th day of birth), the neonates were recalled. Immature neonates (GA <37 weeks), with low levels of T4 for their weight or high TSH levels for their age were recalled. The levels of both serum T4 and TSH were measured till May 2003, but after that only the level of TSH was measured and recall was per-

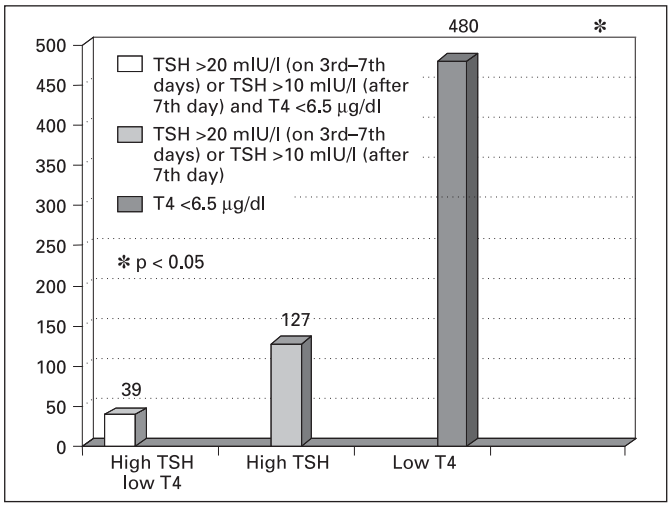

Fig. 3. Distribution of recalled neonates using the first protocol, by the criterion used for recall (only low T4, only high TSH and both low T4 and high TSH).

formed according to serum TSH level (2nd protocol). Neonates with TSH >20 mIU/l on the 3rd to 7th days of birth and TSH >10 mIU/l after 7th day of birth were recalled. Among recalled neonates, if the level of TSH at the first measurement was above 40 mIU/l, then secondary laboratory tests and treatment would be performed simultaneously, but in the case of a TSH concentration between 20 and 39 mIU/l in the first measurement, only secondary laboratory tests would be performed [3–7].

Secondary laboratory exams, performed on the 7th to 28th days of birth, included measurement of both TSH and T4 level and neonates were considered as hypothyroid if T4 was <6.5 μg/dl and TSH was >10 mIU/l [4].

In recalled neonates, if weight-adjusted T4 was low, then T3RU (T3 resin uptake) and FTI (free T4 index) would be measured in addition to previous laboratory exams [8] and according to laboratory findings, treatment would begin, in hypothyroid neonates.

Before treatment, it was recommended to perform thyroid scintigraphy in neonates with CH in order to identify the etiology of the hypothyroidism.

Laboratory Methods

Serum T4 and TSH were measured by radioimmunoassay (RIA) and immunoradiometric assay (IRMA) methods, respectively, using Iran Kavoshyar Co. Kits (Tehran, Iran) and by gamma counter of Isfahan Endocrine & Metabolism Research Center (Berthold LB 2111-12). To confirm the quality of the tests, some samples were selected randomly and their T4 and TSH levels were measured in other approved laboratories of the city and the results were compared with those of Isfahan Endocrine and Metabolism Research Center Laboratory.

Statistical Analysis

Data were analyzed using SPSS Version 10. χ^2 statistical test was used to compare the proportions.

Results

Overall, 86,660 neonates were referred and the coverage percent of the project was 87%. Among all newborns studied, 96.9% were full-term and 3.1% were premature (GA <37 weeks). The nationality of 97.4% of all cases was Iranian, 2.5% had Afghani nationality and 0.1% was of other nationalities.

The mean TSH in all referred neonates was 2.83 ± 2.79 mIU/l (median 2.1 mIU/l, range 491.98) and the mean T4 (measured till May 2003) was 10.8 ± 4.44 μg/dl (median 10.7 μg/dl, range 21.3). From all studied neonates, 29,425 were referred till May 2003. Overall, 992 neonates (1.1%) were recalled. Among cases that were recalled, 90.6% were full term and the rest were premature. The rate of parental consanguinity was 27.9% in all neonates.

Neonates recalled when the first protocol applied was 646 neonates (recall rate = 2.2%, 646/29,425), whereas 346 of them were recalled after May 2003, by using the second protocol (recall rate = 0.6, 346/57,235). Recall rate was significantly higher when T4 and TSH (first protocol) were used for recall, as compared to the time span of second protocol usage, when only serum TSH level was applied to recall ($p < 0.05$).

Detailed data from neonates who were recalled till May 2003 (according to T4, TSH or both of them) are presented in figure 3. During the mentioned period, most of the neonates were recalled because of low serum T4 level alone (74.3%, recall rate = 1.63%) than high TSH level (19.7%, recall rate = 0.43%) or both low T4 and high TSH (6%, recall rate = 0.13%) levels ($p < 0.05$).

From recalled neonates, 682 (68.8%) were categorized as healthy neonates and 247 (90 and 157 in 1st and 2nd stages of the study, respectively) were diagnosed and treated as hypothyroid cases (24.9%). Four neonates had secondary hypothyroidism and 60 (6.7%) recalled neonates didn't follow secondary or complementary exams and were classified as unknown cases. The prevalence of CH in the target population was 2.85/1,000 or 1/350 livebirths (1/327 and 1/364 for the first and the second stages, respectively; $p = 0.7$). There was no parental consanguinity among 151 (61.1%) hypothyroid neonates, whereas the parents of 71 (28.8%) neonates had first-cousin consanguinity and in 25 (10.1%) patients, second-degree consanguinity between parents had been recorded.

Using the first recall protocol, the means of TSH and T4 among recalled neonates were 6.35 ± 2.5 mIU/l and 19.8 ± 40.4 μg/dl, respectively, and the mean of TSH

Table 1. Distribution of serum TSH levels in all recalled neonates on their first measurement

TSH level	n	%
0–9.9 mIU/l	501	50.5
10–19.9 mIU/l	172	17.3
20–49.9 mIU/l	250	25.2
50–99.9 mIU/l	32	3.2
≥100 mIU/l	37	3.7
Total	992	100

Table 2. Distribution of serum T4 levels in recalled neonates on their first measurement, according to the first recall protocol

T4 level	n	%
0–0.5 μg/dl	506	78.3
6.6–9.9 μg/dl	79	12.2
10–14.9 μg/dl	54	8.4
15–19.9 μg/dl	6	0.9
≥20 μg/dl	1	0.2
Total	646	100

among neonates recalled according to the second protocol was 18.9 ± 32.5 mIU/l.

Tables 1 and 2 show the distribution of T4 and TSH levels in recalled neonates, respectively.

Discussion

Findings of the present study show that the recall rate was significantly higher when using both T4 and TSH for screening compared with the 2nd method, in which recall was performed only according to primary TSH measurement (2.2% vs. 0.6). In the case of using low T4 or high TSH or both, the high percent of recalled neonates had low T4 (74.3%). Indeed, recalling neonates regarding to low T4 level alone increases the rate of false-positive results [2].

The prevalence of congenital hypothyroidism was 1:350 living births, which was approximately 8.6- to 11.4-fold of that reported from developed countries. It was higher than the prevalence of congenital hypothyroidism in our region too (1/914 in Tehran and 1/1,433 in Fars province) [8, 9]. The screening project for congenital hypothyroidism in Isfahan had some advantages compared with previous studies in Iran. It was implemented in a large scale as mass screening and was a centralized program. Besides, though we didn't use filter paper for our blood spot specimens, but we used trained nurses to draw venous blood samples for measuring serum TSH and T4.

Different surveys have been implemented to study recalls and factors affecting recall rate in CH screening programs [2, 10–15]. The different recall rates in various screening programs are suggested to be due to several factors [1]: the use of T4 or TSH level or both for screening [2], differences in sample collecting methods and laboratories [3], different established criteria for recalling that is related to cultural, regional, and social factors of a community.

The rates of recall in other countries, according to the accepted criteria of screening on the 3rd to 5th day of birth, vary from 0.2 to 3.3. It was 0.16% in the Philippines, 0.35% in Austria, 0.3% in Greece, 0.28–0.29% in Hungary, 2.3% in Turkey and 3.3% in Estonia [15–20]. In a study in Thailand, the recall rate according to the cord TSH values greater than 30 mIU/l was 1.1% [21].

According to the results of an investigation in India, in which serum TSH was used for screening, the recall rate was 2.81% [22]. In our study, when using serum TSH level alone for screening and recall, the recall rate was lower than the mentioned survey.

In the Azizi et al. [12] study performed before iodine fortification of salt in Iran, TSH concentration of cord blood samples was determined. Results showed that the recall rate was 5%, therefore the study was stopped. Following the fortification of salt with iodine in Iran, the recall rate decreased to 1.6%, as showed by using cord blood TSH level in Ordookhani et al. [12] study. They concluded that iodine deficiency was an important factor in increasing of recall rate. Another study in our country by Karamizadeh and Hakimi [9] in 1990 showed that the recall rate was 7.3% according to cord T4 level. After implementation of the national iodine supplementation programme, conducted to improve iodine deficiency through salt idolization, this problem has been solved in Iran and one study in Isfahan has shown that urinary iodine exertion was in the optimal range in neonates and their mothers [23, 24]. Comparing the results of the mentioned studies with our findings, the recall rate in the present study was lower (0.6 vs. 1.6% when using only TSH

and 1.63% vs. 7.3% when applying low T4 for recall), although we used serum samples and they used cord blood specimens.

A study in Italy showed that if neonates were recalled based on the T4 level, the recall rate was 2.5%, but if both T4 and TSH level were measured, the recall rate would decrease to 0.11% [11]. These findings are similar to our results. During the first period when both T4 and TSH were used for recall, the recall rate was 1.63% by low serum T4 level and was 0.13% when using both T4 and TSH level.

Recall rate according to the T4 concentration of less than or equal to 6.5 μg/dl has been reported to be 3% in the newborn thyroid screening in a municipal hospital [2]. They concluded that although recalling neonates based on low T4 level alone increased the rate of falsely abnormal results, it detected newborns with secondary and tertiary forms of hypothyroidism. In our study, when only low T4 was considered for recalling during the first stage, the recall rate was lower as compared to the abovementioned study, which may be due to differences in the T4 assay.

Three thyroid-function detection methods were evaluated concomitantly in the study of Walfish [14] for possible application as routine tests for early diagnosis of CH. The study concluded that cord TSH as an initial screening test had a higher specificity and sensitivity for the diagnosis of CH than the two other tests.

Another study in Slovakia evaluated the accuracy of different laboratory methods in CH screening programs and the effect of assay method on recall rate. They measured the TSH level using both RIA and IRMA. Recall rate was 2.34% using RIA and 0.89% using IRMA [10]. IRMA was more accurate and this method was used in our screening program.

A study in Belgium evaluated the effect of povidone iodine used during delivery, specially C/S, epidural anesthesia and breast or artificial milk feeding, on recall rate. The results showed that the rate of recall was significantly increased among deliveries using povidone iodine and also among neonates who were breast-fed [13]. According to the announcement of the Isfahan CH screening project committee, maternity hospitals were advised not to use povidone iodine as far as possible during the course of delivery, but it cannot be ascertained that this recommendation has been accepted and implemented carefully.

In conclusion, the recall rate in our study was not as high as in Estonia, even when both T4 and TSH were used for screening, but it was more acceptable when only TSH was used as the recall criteria. Although it may be attractive to identify all additional patients with secondary or tertiary forms of neonatal hypothyroidism, problems of excessive recall costs, waste of professional time and psychological and emotional burden to families should be considered for implementing successful screening and recall program. Therefore, in accordance with some studies that used TSH as the most useful index for both screening and recall (specially when measured on the 3rd to 5th day of birth for reducing false-positive results), we concluded that using TSH alone for recall is more effective and feasible according to the conditions of our community.

Considering the high prevalence of CH in our investigation, further studies are needed to establish more practical methods of both screening and recall, including filter paper blood spot TSH measurement.

Acknowledgements

This project was funded by the Research Bureau, Isfahan University of Medical Sciences and Health Services (Project No. 80350).

The excellent assistance of Dr. Ramin Talaei, Mrs Kiani, Mrs Shaiesteh, Mrs Foroughifar and Mr. Abyar is gratefully acknowledged.

References

1 Wu LL, Sazali BS, Adeeb N, Khalid BA: Congenital hypothyroid screening using cord blood TSH. Singapore Med J 1999;40:23–26.
2 Harris Pl, Dreyfus NG: Newborn thyroid screening in a municipal hospital. Am J Dis Child 1982;136:248–250.
3 Fisher DA: Disorders of the thyroid in the newborn and infant; in Sperling MA (ed): Pediatric Endocrinology, ed 1. Philadelphia, Saunders, 1996, pp 51–70.
4 Screening for congenital hypothyroidism [editorial]. Thyroid 2003;13:87–94.
5 Hung E: Thyroid disorders of infancy and childhood; in Becker KL (ed): Principles and Practice of Endocrinology and Metabolism, ed 3. Philadelphia, Lippincott Williams & Wilkins, 2001, pp 463–471.
6 Klein RZ, Mitchell ML: Hypothyroidism in infants and children; in Braverman LE (ed): The Thyroid, ed 8. Philadelphia, Lippincott Williams & Wilkins, 2000, pp 973–988.
7 Frank JE, Faix JE, Hermos RJ, Mullaney DA, Mitchell ML, et al: Low birth weight infant: effects on neonatal hypothyroidism screening. J Pediatr 1996;128:548–554.
8 Ordookhani A, Mirmiran P, Hedayati M, Hajipour R. Azizi F: Screening for congenital hypothyroidism in Tehran and Damavand: an interim report on descriptive and etiologic findings, 1998–2001. IJEM 2002;4:153–160.
9 Karamizadeh Z, Amirhakimi GH: Incidence of congenital hypothyroidism in Fars Province, Iran. Iranian J Med Sci 1992;17:78–80.
10 Dluholucky S, Hornova V, Bucek M, Langer P: Studies on congenital hypothyroidism and results of three and half years of compulsory screening program in Slovak, Socialist Republic. Endocrinol Exp 1998;23:125–135.
11 Berardi R, Baracchi MR, Borgogni P, Margollicci MA, Mettei R, Fois A: Results of a screening project for congenital hypothyroidism in 4 years of experience. Pediatr Med Chir 1982;4:657–660.
12 Ordoukhani A, Mirsaiid Ghazi AA, Hajipour R, Mirmiran P, Azizi F: Screening for congenital hypothyroidism: before and after iodine supplementation in Iran. IJEM 2002;2:93–98.
13 Chanoine JP, Boulvain M, Bourdoux P, Pardou A, Van Thi HV, Ermans AM, Delange F: Increased recall rate at screening for congenital hypothyroidism in breast fed infants born to iodine overloaded mothers. Arch Dis Child 1988;63:1207–1210.
14 Walfish PG: Evaluation of three thyroid-function screening tests for detecting neonatal hypothyroidism. Lancet 1976;i:1208–1210.
15 Fagela-Domingo C, Padilla CD, Cutiongco EM: Screening for congenital hypothyroidism (CH) among Filipino newborn infants. Philippine Newborn Screening Study Group. Southeast Asian J Trop Med Publ Health 1999; 30(suppl 2):20–22.
16 Moslinger D, Frisch H, Strobl W, Stockler-Ipsiroglu S: Neonatal screening for congenital hypothyroidism. Acta Med Austr 1997;24:162–164.
17 Mengreli C, Yiannakou L, Pantelakis S: The screening program for congenital hypothyroidism in Greece: evidence of iodine deficiency in some areas in the country. Acta Paediatr 1994;(suppl 394):47–51.
18 Peter F, Blatniczky L, Kovacs L, Tar A: Experience with neonatal screening for congenital hypothyroidism in Hungary. Endocrinol Exp 1989;23:143–151.
19 Yordam N, Calikoglu AS, Hatun S, Kandemir N, Oguz H, Tezio T, Ozalp I: Screening for congenital hypothyroidism in Turkey. Eur J Pediatr 1995;154:614–618.
20 Mikelsaar RV, Zordania R, Viikmaa M, Kudrjavtseva G: Neonatal screening for congenital hypothyroidism in Estonia. J Med Screen 1998;5:20–21.
21 Mahachoklertwattana P, Phuapradit W, Siripoonya P, Charoenpol O, Thuvasethakul P, Rajatanavin R: Five year thyrotropin screening for congenital hypothyroidism in Ramathibodi Hospital. J Med Assoc Thai 1999; 82(suppl 1):S27–39.
22 Desai MP, Colaco MP, Ajgaonkar AR, Mahadik CV, Vas FE, Rege C, et al: Neonatal screening for congenital hypothyroidism in a developing country: problems and strategies. Indian Y Pediatr 1987;54:571–581.
23 Azizi F, Sheikholeslam R, Hedayati M, Mirmiran P, Malekafzali H, Kimiagar M, et al: Sustainable control of iodine deficiency in Iran: beneficial result of the implementation of the mandatory law on salt iodization. J Endocrinol Invest 2009:23:409–413.
24 Hashemipoor M, Amini M, Gheisari A, Salarifar S, Iranpour R, Aminoroaya A: Comparison of urinary iodine exertion in neonates and their mothers in Isfahan, Iran. Endocr Pract 2002;8:347–350.