Original Article

# The relation between serum and filter paper TSH level in neonates with congenital hypothyroidism

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## Abstract

**Background:** the aim of this study was to determine the relation between serum and filter paper thyroid-stimulating hormone (TSH) levels in neonates with congenital hypothyroidism (CH). We also tried to determine an appropriate cutoff point of filter TSH for recalling screened neonates.

**Materials and Methods:** in this descriptive-analytic study, records of 2283 neonates who had been recalled during CH screening program in Isfahan (Iran) were studied. The relation between serum and filter paper TSH levels in the studied neonates was assessed and the best cutoff point of filter TSH and its sensitivity and specificity for proper diagnosis of CH were determined.

**Results:** among the studied neonates, 103 (4.5%) were diagnosed with CH. Using receiver operating characteristic (ROC) curve, the best cutoff point for diagnosing CH was 7.5 with a sensitivity of 74.8% and specificity of 71.3%. The rates of false positive and false negative diagnoses at this cutoff point were28.7% and 25.2%, respectively. There was a significant relationship between serum and filter paper TSH levels.

**Conclusion:** the cutoff point for recall should be changed to 7.5 for appropriate screening outcome. On the other hand, considering the low cost of filter paper and importance of missing any case of CH, changing the cutoff point is not necessary. However, further studies in different parts of Iran are required to obtain more accurate results and consider all related factors.

**Key Words:** Congenital hypothyroidism, filter paper, thyroid-stimulating hormone

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## INTRODUCTION

Congenital hypothyroidism (CH) is one of the most common endocrine and metabolic diseases in children and a preventable cause of mental retardation.[1,2] The rate of its incidence varies in different societies; however, one case per 3000-4000 births is reported on average. Mental retardation resulting from the disease is due to the pivotal role of thyroid hormone in



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brain development. Early diagnosis and treatment of the disease during the first days of life could prevent its related neourodevelopmental complications.[1] The clinical symptoms of CH are few and nonspecific in the first days of life. Delayed diagnosis of CH based on only clinical presentation is associated with higher rate of missed cases of CH and irreversible mental retardation deafness and developmental complications.

Therefore, in order to prevent mentioned adverse effects CH screening programs were developed worldwide. Appropriate detection of cases with CH due to improving of CH screening programs and its related social and economic benefits made it an important health issue in communities.[2]

Though different protocols of CH screening are implemented in different countries and regions worldwide but the basic framework is similar in all of the screening protocols. There are three different screening strategies including a primary TSH/backup T4, a primary T4/backup TSH, and a combined primary TSH plus T4 approach. Each of these mentioned approaches have their benefits as well as their related false positive or negative results. An appropriate approach is selected according to the conditions and priorities of each region.[2,3]

CH screening in Isfahan, Iran, was initiated after eliminating of iodine deficiency in Iran in 2002.The results of the screening program reveled higher prevalence rate of CH in Isfahan (1 in 370 live births). Thereafter, CH screening in Isfahan was integrated to the national CH screening in 2005 using filter paper for TSH measurement.[4]

Though the effectiveness and benefits of CH screening program in improving public health have been reported in several studies but it seems that the quality of the screening program should be improved. One of the items is the TSH cutoff point that should be set up in a way that in accordance with diagnosing all neonates, CH has an acceptable recall rate. Many studies indicated that higher recall rate not only could have high cost, but it could also result in adverse psychological effect such as parental stress on neonate's families.[5]

According to the current national CH screening protocol, newborns with TSH Filter > 5 are recalled. The aim of this study was to determine the relation between serum and filter paper thyroid-stimulating hormone (TSH) levels in neonates with CH. We also tried to determine an appropriate cutoff point of filter TSH for recalling screened neonates. However, an appropriate cutoff point would be useful in both early diagnosis and treatment of CH and cost effectiveness of the screening program.

## MATERIALS AND METHODS

In this descriptive-analytic study, records of 2283 neonates who had been recalled during CH screening program in Isfahan (Iran) and referred to Isfahan Endocrine and Metabolism Research Center from April 2005 to September 2009 were studied.

According to the CH screening program, neonates with TSH >10 were recalled and those with abnormal T4 and TSH levels on their second measurements (TSH> 10 mIU/l and T4 < 6.5 μg/dl) were diagnosed as CH patient and received treatment and regular follow up.[6]

The study was approved by Ethics Committee of Isfahan University of Medical Sciences. During the study, demographic characteristics and screening properties of recalled neonates were recorded. Recalled neonates classified according to the screening filter paper TSH level in three groups as follows; 5 < TSH ≤ 10, 10 <TSH ≤ 20 and TSH > 20.The proportion of neonates diagnosed with CH in each studied TSH group was determined. In the second part of the study, the relation between filter and serum TSH, an appropriate cutoff point for recall and its specificity and sensitivity, was determined.

The serum level of TSH and T4 was measured through IRMA and RIA methods, respectively, using Kavoshyar diagnostic kits (Tehran, Iran) by Berthold-LB2111 unit gamma counter equipment in Isfahan Endocrinology and Metabolism Research Center.

The collected data were analyzed using statistical software SPSS18. Pearson Correlation Coefficient and Regression Analysis were used to study the relationship of the TSH level between filter test and newborn serum and the ROC curve was used to determine the cutoff point and the sensitivity of filter test.

## RESULTS

During the current study, 2283 newborns studied. 1258 (55.1%) and 1025 (44.9%) of recalled neonates were male and female, respectively. Recall rate was 2.5%. Mean of filter TSH in all recalled neonates was 8.6 ± 9.2 (5-100). Mean of serum TSH and T4 in studied population was 8.4 ± 16 (0.02-224) and 13.9 ± 22.9 (0.2-199), respectively. Demographic and screening characteristics of recalled neonates in CH screening program in Isfahan are presented in Table 1. From

recalled (filter TSH >5) neonates 103 (4.5%) diagnosed as CH patients and underwent treatment with levothyroxine. The proportion of recalled neonates that diagnosed as patients with CH based on filter TSH level is presented in Table 2. The area under the curve (AUC) level for filter TSH more than 5 according to the serum TSH (gold standard) was 0.80 with CI 0.75-0.85 [Figure 1]. Based on ROC curve, the appropriate cutoff point for recall for filter TSH with acceptable sensitivity and specificity was 7.5. The sensitivity, specificity, and false positive and negative rate of determined cutoff point (7.5) is presented in Table 3. The positive and negative predictive values of the cutoff point were 11% and 98.4%, respectively [Table 3]. There was significant correlation between serum and filter TSH level ($P < 0.001$, $r = 0.63$) [Figure 2].

## DISCUSSION

The overall objective of the study was to determine the relationship between the levels of thyroid stimulating hormone (TSH) in filter and serum TSH level in a newborn with CH and to determine an appropriate cutoff point for filter TSH for recalling neonates during CH screening program in Isfahan. There was a significant relationship between the level of TSH in filter test with serums TSH in newborn diagnosed with CH and the cutoff point of filter TSH test for diagnosing CH was 7.5.

CH screening is now implemented aiming at early diagnosis and treatment of the disease and preventing mental retardation related to the disorder in most world countries such as Iran. Along with the diagnosis and treatment proceedings, it seems to be necessary that studies with the aim of optimizing

**Table 1: Demographic and screening characteristics of recalled neonates in CH screening program in Isfahan**

| Variables | |
|---|---|
| Age at screening (days) | 12.3+/−15.9 |
| Weight (gr) | 2750+/−1850.7 |
| Height (cm) | 48.4+/−5.1 |
| Head Circumferences (cm) | 35.2+/−1.7 |
| Sex (female/male) | |
| Maturity (term) | 94.1% |
| Type of delivery | |
| Normal | 30.6% |
| Caesarean | 69.4% |
| Parental consanguinity | 31.1% |
| History of thyroid disorders | |
| Mothers | 7.9% |
| Fathers | 1.6% |
| Mean of screening TSH (mlu/lit) | 8.6±9.2 |

**Table 2: The proportion of recalled neonates diagnosed as patients with congenital hypothyroidism based on the filter TSH level**

| Filter TSH level | Neonates diagnosed with CH | Neonates diagnosed as normal after recall | Total recalled neonates |
|---|---|---|---|
| 5<TSH≤10 | 7 | 1980 | 1987 |
| 10<TSH≤20 | 32 | 188 | 220 |
| TSH>20 | 64 | 12 | 76 |
| Total | 103 | 2180 | 2283 |

**Table 3: The sensitivity and specificity of filter TSH test in the cutoff of point of 7.5**

| Filter TSH test in cutoff of point of 7.5 | Recalled non-CH neonates | Recalled neonates diagnosed with CH | Total |
|---|---|---|---|
| Negative | 1554 (71.3%) | 26 (25.2%) | 1580 (69.2%) |
| Positive | 626 (28.7%) | 77 (74.8%) | 703 (30.8%) |
| Total | 2180 | 103 | 2283 |

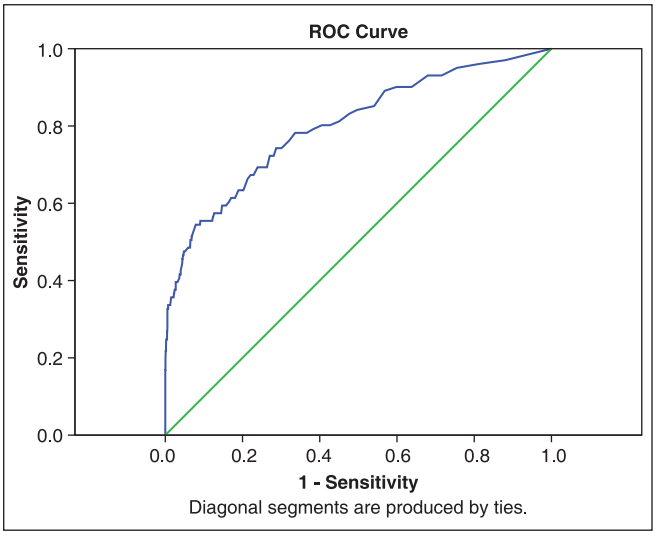


**Figure 1:** The area under the curve (AUC) level for filter TSH more than 5

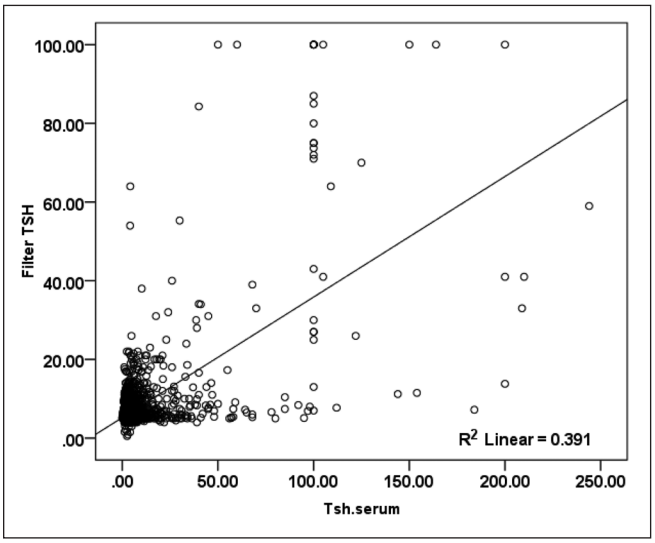


**Figure 2:** The correlation between the filter TSH test and serum TSH

the screening system in each region, particularly in countries reporting the high prevalence of the disease, be planned and implemented to achieve the goal. However, both missing cases of CH and their early treatment and high recall rate in CH screening program would consequently increase the cost of the disease.[6,7]

Screening of CH was first performed in 1970 in Canada and various studies have been conducted with the goal of improving screening system for the appropriate diagnosis and treatment of most disease cases during the past few years and screening methods have been changed several times. Thus, first, the TSH >30 was used as an appropriate cutoff point for recall. Then, the cutoff point has been changed to 20 and in recent years, given the high cases of false negative, it has even been changed to ten.[8-11] Along with these modifications, the incidence rate of disease is increased in various parts of the world and LaFranchi considered lowering the cutoff point of filter TSH test for diagnosis of CH as the reason for such increase.[12]

CH screening initiated after elimination of iodine deficiency in Iran using cord blood TSH level in Tehran Mahdie Hospital in 1997 and that according to the findings of that study, the prevalence of CH has been reported one case per 914 living births. CH screening in Isfahan was initiated in 2002 and a prevalence rate of one in 370 live birth was reported.[4,13,14] Given the high prevalence of the disease in the region, nationwide screening program was developed in Iran in 2005 and according to the protocol of the program neonates with TSH >5 are recalled.

Reviewing CH screening protocols worldwide indicated that in most of them used TSH level of 10-20 for recall. TSH >5 as recall cutoff is used in few regions such as Wales, England, in order to reduce false negative rate.[15] In a study in Italy, the role of using the lower level of screening TSH was studied for screening CH was studied and they concluded that lowering the level of TSH cutoff from 20 to 10-12 result in diagnosing more cases of CH and reducing the false negative rate.[16] Though Pryce *et al.,* in England, agreed with lowering the level of TSH cutoff level for diagnosing all suspected cases of CH, it is suggested that lowering TSH cutoff level is also related to higher cost, increased recall rate and consequently their related adverse effects.[17]

As mentioned earlier, since developing CH screening in 1970, the protocol of screening is being modified frequently for obtaining more favorable screening program, but the goal has not been achieved yet.[18] Lowering of TSH cutoff point is one of the most important causes of the increasing recall rate. Evidences showed that the recall rate is one of the crucial indicators of screening cost-effectiveness and implementation capability. Even in some cases higher recall rate led to stop the program.[19]

In this study, the recall rate was 2.5%, which is higher than that reported worldwide. The recall rates have been reported higher in other cities of Iran that used the same screening protocol. Recall rate has been reported 4.4% and 2% in Zanjan and Shiraz, respectively.[6,20,21]

Karamizadeh *et al*., in Shiraz, Iran, concluded that current cutoff point is too low for recall and it should be reevaluate for obtaining more appropriate cutoff.[21]

In our study, in 87% of recalled neonates screening TSH was between 5-10 and only 0.3% of them (7/1987) were diagnosed with CH. At higher TSH levels (TSH> 10), 32.3% of recalled neonates diagnosed with CH. Also, in Zanjan, Iran, 86.4% of recalled cases screening TSH level was between 5-10.[20]

According to our results filter TSH of 7.5 had the sensitivity of 74.8% and the specificity of 71.3%. In this cutoff point, the percentage of false positive and negative were 28.7% and 25.2%, respectively, and the positive and negative predictive values at that cutoff point were 11% and 98.4%, respectively.

Hence, considering the findings of this study filter TSH cutoff point for recalling neonates during CH screening should be increased from the current cutoff level of 5-7.5 to decrease the rate of false positive cases. It seems that increasing recall TSH cutoff point is a conflicting issue. The importance of diagnosing patients with CH for preventing its related neourodevelopmental retardation and also the lower cost of filter paper test led us to this conclusion that current cutoff point is completely reasonable and acceptable.

Considering the cost-effectiveness of the disease detection and treatment, having higher false positive rate in different screening programs could not consider a limitation.[22]

However, it seems that for determination of appropriate filter TSH cutoff point as well as obtaining better recall protocol, further studies from different regions of Iran with consideration of factors effecting on TSH level during screening is necessary evidence from previous studies indicating that various conditions such as different seasons, laboratory methods, and the size of blood spot measured on filter paper affects the level of

filter TSH.[23,24] Therefore, it seems that more studies should be conducted to study the effect of mentioned factors.

In our study, there was a significant relationship between serum and filter TSH level in newborns with CH while in a study in Philippines, Capistrano-Estrada *et al.,* observed no-relationship between the primary TSH during screening and serum TSH.[25] The causes of above findings are different screening methods and differences in the studied sample size.

There was not a similar study investigating the studied relationship between serum and filter TSH level, so obtained results could not be compared. Findings of the current study could be used for improving CH screening protocol in Iran.

In sum, it seems that considering the findings of this study the cutoff point for recall should be changed to 7.5 for appropriate screening outcome, but on the other hand considering the low cost of filter paper and importance of missing any case of CH, changing of cutoff point is not considered necessary. However, for more accurate results more studies in different part of Iran with considering its related factors are recommended.

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