Original Article

Serum thyroid stimulating hormone, total and free T4 during the neonatal period: Establishing regional reference intervals

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ABSTRACT

Context: Congenital hypothyroidism (CH), the most common etiology of preventable mental retardation in children, is estimated to be more prevalent among Asian population. **Aims:** Since thyroid function tests (TFTs) varied among different ages and geographical regions, in this study, the neonatal thyroid reference intervals in a healthy neonatal population is determined for the first time in Iran. **Settings and Design:** A cross-sectional study performed on 246 healthy term newborns aged between 2 days and 1 month. **Materials and Methods:** Blood samples were obtained by venipuncture from all subjects. The median, 2.5th, 5th, 95th, and 97.5th percentile of serum thyroid-stimulating hormone (TSH), as well as the total and free T4 were assessed among different age groups. **Statistical Analysis Used:** Predictive Analytics Software (PASW Statistics 18) was used for the analysis. **Results:** Serum TSH, total and free T4 concentration peaked in 5th to 7th days of life, continued over 2 weeks, then decreased and started reaching to adult reference range. A significant negative correlation between age and serum concentration of TSH ($P = 0.02$), total T4 ($P = 0.01$) and free T4 ($P = 0.01$) was found. **Conclusion:** This study yielded fairly different values for TFTs compared compared values found in other countries and also different from values reported for laboratory kits we used. These differences were assumed to be due to variations in ethnicity, age, and laboratory methods used. Due to the lack of international standardization, conducting multicenter studies helps in making a more precise evaluation of thyroid status in neonates.

Key words: Congenital hypothyroidism, infant, newborn, reference values, thyroid hormones, thyrotropin

Introduction

Congenital hypothyroidism (CH), the most common etiology of preventable mental retardation and impaired cognitive-physical development among children, is more common in Asian population.[1-4] Since its symptoms are usually concealed at birth, the importance of screening is obvious.[3,4] Regarding variations in Thyroid Function Tests (TFTs) among different ages and geographical regions,[2,5,6] determining reference interval specified by age and region is required for precise diagnosis. According to our knowledge, there is not any study on neonatal reference thyroid interval in Iran. We designed current study to determine regional thyroid reference interval specified by age in healthy neonatal population.

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Materials and Methods

Subjects

This cross-sectional study was performed on 246 healthy term neonates born at Imam hospital, Tehran, Iran during April 2011 and April 2012. All newborns were from normal pregnancies without any prenatal complications, and had apgar score of more than 7 in

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1st minute of birth. Subjects enrolled in this study met the following criteria; gestational age (GA) of 37-42 weeks, and appropriate weight, height and head circumference for GA. Newborns who had the history of congenital anomaly, intra uterine growth retardation, thyroid disease in themselves or their mothers, taking medications that affect thyroid function such as corticosteroids, dopamine or propranolol in themselves or their mothers, and pituitary disease were excluded. The study was in compliance with all principles of declaration of Helsinki. Written informed consent was taken from all parents of subjects. Newborns were classified into five subgroups based on their age: 2-4 days, 5-7 days, 8-14 days, 15-21 days, and 22-30 days.

Laboratory studies

In order to separate the serum from the cells, clotted blood samples obtained by venipuncture from all subjects were centrifuged. Samples were stored at temperature of -70°C until assayed. Serum samples were evaluated for quantitative measurement of TSH and Total/Free T4 concentration by an immunoenzymometric assay and competitive enzyme immunoassay, respectively. All parameters were measured according to manufacturer's instruction by commercial kits (AccuBind ELISA Microwells, Monobind, Inc. Lake Forest, CA, USA).

Statistical analysis

Data were analysed using predictive analytical software (PASW statistics 18.00, 2009) according to the National Committee for Clinical Laboratory Standards (NCCLS) guideline for determining reference values and reference intervals of quantitative avlinical laboratory tests. The Kolmogrove-Smirnov test was used to test the normality distribution of thyroid hormones. Due to non-normal distribution, medians, 2.5th, 5th, 95th, 97.5th percentiles for TSH, total and free T4 were reported. Extreme outliers of TSH, total and free T4 were excluded to eliminate the outliers impact.[7]

Independent-sample *t* test and one-way analysis of variance (ANOVA) (Dunett's T3 test and Tukey test) were used to compare the means of TFTs according to their age and sex. A $P < 5\%$ was considered as statistically significant.

Results

Serum concentration of TSH, total and free T4 were evaluated in 246 healthy term newborns, aged between 2 days and 1 month. About 89.8% (221/246) of these newborns were female. Although the mean serum levels of TSH and total T4 were higher in females, no significant differences were observed in serum TSH, total and free T4 levels between males and females.

Subjects were classified into different subgroups according to their age. Figures 1-3 demonstrate distribution of serum TSH, total T4 and free T4 values across age groups, respectively.

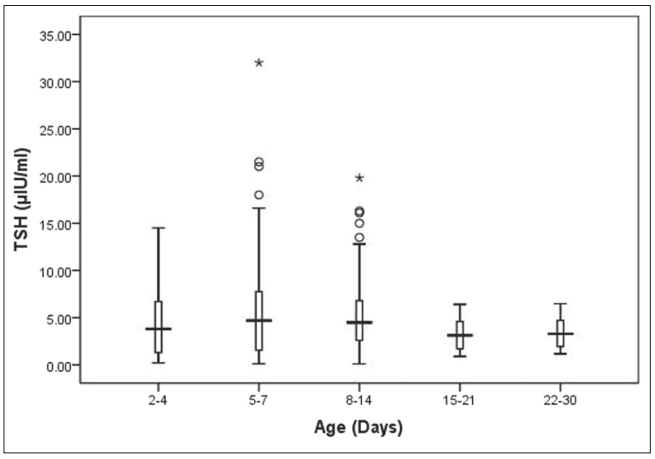

Figure 1: Distribution of serum TSH values according to age group classification

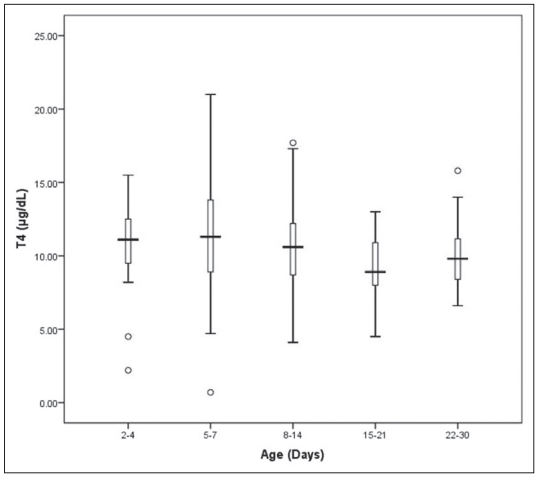

Figure 2: Distribution of serum T4 values according to age group classification

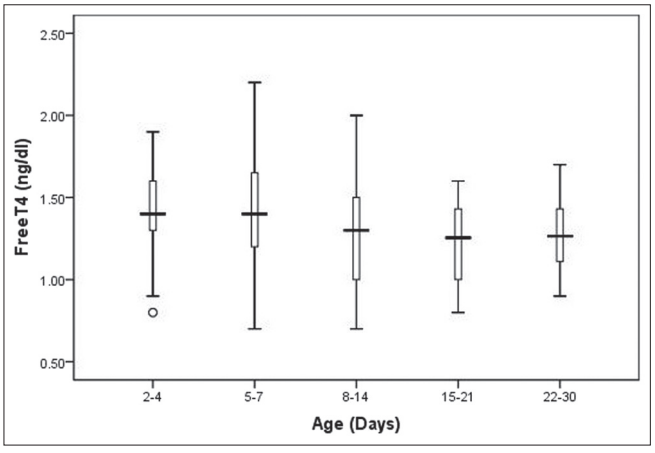

Figure 3: Distribution of serum Free T4 values according to age group classification

Table 1 shows the mean ± SE, median, 2.5th, 5th, 95th, and 97.5th percentiles of TSH, total and free T4 hormones levels, the skewness, and the number of subjects in each group. The geometric mean serum TSH in our study was 5.23 ± 0.30 ml/Ul. The mean levels of total and free T4 in newborns were 10.78 ± 0.20 μg/dL, and 1.34 ± 0.01 ng/L, respectively. The mean serum TSH concentration was significantly ($P = 0.01$) higher in newborns 5-14 days compared to those aged >2 weeks in which a decline in serum TSH level starts. The mean level of total T4 was found higher in newborns of 5-7 days and this level was significantly lower in newborns aged 2-3 weeks ($P = 0.01$). The mean free T4 level significantly decreased after 1 week ($P = 0.04$).

Consequently, TSH, total and free T4 levels peaked in 5th to 7th days of life, continued over 2 weeks, then decreased during the neonatal period and started reaching adult reference range. There is a significant negative correlation between age and serum TSH concentration ($r = -0.15$, $P = 0.02$), total T4 ($r = -0.15$, $P = 0.01$) and freeT4 ($r = -0.18$, $P = 0.01$). In newborns of 2-3 weeks of age, a significant correlation between age and TSH with the coefficient of 0.54 was observed. No more significant correlation was detected between the variables in different age subgroups.

Discussion

According to many studies, reference values of TFTs are age, gender, ethnicity, and method dependent.[1,2,6,8] Since adult reference intervals could not be used as screening in pediatric disease, several age specific, population based reference intervals for TFTs are suggested in different regions.

In this study, our main goal was to delineate the neonatal reference interval of TSH, total and free T4 for the local population using healthy newborns aged from birth up to 1 month.

Normal distribution patterns were observed in serum total T4 level, but not in serum TSH and free T4. According to laboratory kit, the 2.5th, median, and 97.5th percentile of adult TSH, total and free T4 were (0.39; 4.21; 6.16 ml/Ul), (3.1; 8.9; 16.5 μg/dL), (0.55; 1.74; 3.25 ng/l), respectively. In the present study, these measures were with, in that order for newborns aged up to 1 month. Our results show a wider variability in TSH intervals in newborns of 1 month compared to the laboratory reference intervals. We also observed that serum TSH level exceed the laboratory reference intervals in the first 14 days. Both could be explained by the age discrepancy and the hypothalamic-pituitary-thyroid (HPT) axis maturation pattern.

On the study conducted by Kapelari *et al.*,[1] to determine thyroid hormones reference interval among 1 day-18 years population, the 2.5th, median, 95th reference range in newborns up to 1 month were with, $n = 23$) for TSH and free T4.

Table 1: The Mean±SE, 2.5th, 5th, 50th, 95th, and 97.5th percentiles of TSH, Total and free T4 hormone levels, and the number of subjects in each groups

Age	*n*	2.5th	5th	50th	95th	97.5th	Skewness	Mean+SE
T4 (μg/dl)								
2-4 days	33	2.20	3.81	11.1	15.22	15.50	−1.08	10.98±0.48
5-7 days	101	4.86	5.63	11.3	17.18	19.90	0.14	11.33±0.35
8-14 days	73	4.61	5.65	10.60	16.06	17.36	0.15	10.52±0.34
15-21 days	17	4.5	4.5	8.90	13	13	−0.45	9.01±0.55
22-30 days	19	6.6	6.6	9.8	15.8	15.8	0.82	10.07±0.56
Total	243	4.7	5.64	10.8	15.88	17.29	0.17	10.78±0.20
Free T4 (ng/dl)								
2-4 days	33	0.80	0.80	1.40	1.83	1.90	−0.37	1.40±0.04
5-7 days	100	0.75	0.80	1.40	1.99	2.12	−0.01	1.41±0.03
8-14 days	74	0.78	0.80	1.30	1.80	2	0.27	1.27±0.03
15-21 days	18	0.80	0.80	1.25	1.60	1.60	00	1.21±0.06
22-30 days	20	0.9	0.9	1.26	1.69	1.70	0.02	1.26±0.05
Total	245	0.80	0.83	1.30	1.80	2	0.13	1.34±0.01
TSH (μIU/ml)								
2-4 days	33	0.20	0.41	3.8	13.45	14.5	0.86	4.83±0.71
5-7 days	99	0.25	0.40	4.7	16.6	21.25	1.68	5.98±0.57
8-14 days	69	0.17	0.45	4.5	15.55	17.17	1.37	5.36±0.50
15-21 days	17	0.9	0.9	3.13	6.4	6.4	0.37	3.57±0.55
22-30 days	20	1.18	1.19	3.29	6.46	6.48	0.33	3.48±0.36
Total	238	0.29	0.5	4.2	15	16.63	1.83	5.23±0.30

TSH: Thyroid stimulating hormone

Our study corresponds fairly close to prior published data;[1] However, the 2.5th percentile of total T4 levels were found to be lower in newborns aged up to 3 weeks.[3] Moreover, the 2.5th percentile of TSH was lower than that of other studies.[1] Among our study population, the 97.5th percentile of TSH was found to be significantly higher in newborns up to 2 weeks compared to older subjects. This percentile gradually reached to adult value after 2 weeks. Elmlinger *et al.*,[9] Hubner *et al.*,[10] and Mutlu *et al.*,[3] also reached the same result. We also observed that the reference intervals of TSH, total and free T4 become narrower as the age increases especially for TSH.

Our analysis showed that serum TSH, total and free T4 levels inversely correlated with age. These levels significantly decreased with age especially after 7th day. This result was in line with other studies. Mutlu *et al.*,[3] reported an inverse correlation between TFTs levels and age after 3rd day. Najam *et al.*,[5] mentioned that the decline in TSH and T4 levels was more marked in the first week, and this downward trend was sharper in TSH compared to T4. Zurakowski *et al.*,[11] also showed the indirect association between age and the mean serum levels of T3, TSH and free T4 in the study on 5,817 patients aged 1 month-20 years. However, there are exceptions such as the study conducted by Mansourian *et al.*,[12] on Iranian population aged 1-21 years. Except for an indirect association between serum T4 and age, they found no association between age and serum TSH and T3 levels.

In this study, females constitute the predominant gender. In spite of the higher serum level of TSH and total T4 in females, no meaningful gender differences were found in the levels of TSH, total and free T4. However, more male samples are required to deny gender-specific difference. These findings were fairly in accordance with previous studies. Mansourian *et al.*,[12] reported insignificant higher levels of TSH and T3 and lower T4 in females. Kapelari *et al.*,[1] found no significant gender differences in distribution of thyroid hormone levels except for free T3 which was higher in men. Furthermore, Mutlu *et al.*,[3] detected no gender difference in the serum hormone levels of 296 Turkish newborns of first to 28th day old. Kratzsch *et al.,*[13] mentioned no gender difference in TFTs levels in newborns aged 1 day to 1 month, although they found a significantly higher level of total T4 in 15-20 years girls. On the other side, Zurazowski *et al.,*[11] reported a more rapid decline in serum TSH and T3 levels with age and a higher level of total T4 in females compared to men, with no gender difference in free T4 levels. These differences were assumed to be due to discrepancies in ethnicity, sample size, laboratory material, and methods used.

There are different neonatal screening strategy including initial T4 assay, initial blood TSH, and concomitant TSH-T4 assay. Initial T4 measurement followed by a TSH testing is a routine protocol in the United States. Concomitant TSH-T4 measurement is the most sensitive approach as regards the higher cost and false positive rate.[4] The congenital hypothyroidism screening strategy in our country depends on TSH assay done on venous blood samples of 2-5 day newborns.[14] This method was able to detect subclinical hypothyroidism, albeit identifying central hypothyroidism and thyroid binding globulin deficiency is possibly missed by this approach.[4,6]

In order to reduce the false positive and recall rates besides minimizing the false negative rate, each country defines the conservative TSH cut points for its CH screening protocol. Based on several reports, the TSH level between 5 ml/Ul and 20 ml/Ul used as screening cut point in different regions.[14,15]

Prior studies showed high prevalence of CH in Iran in comparison with other countries. Although, CH overall was seen in one in 3,000-4,000 live birth worldwide,[16] but this ratio was reported one in 914 in Tehran.[17]

Due to the high prevalence of CH in Iran, the screening strategy attempts to minimize the false negative rate near zero withstanding higher false positive rate, by setting the TSH cut off value of 5 ml/U. The cost-benefit analysis of CH screening program in our country revealed the high potential for the implementation of this strategy.[14,18] Besides, the lower TSH cut point over estimates the recall rate. Following the pattern of HPT axis maturation with age (decrease in TSH level distinctly after 14 days), the need for a confirmatory study to re evaluate the appropriateness of current cut off seems reasonable.

Our result revealed different reference ranges for thyroid function tests comparing the values reported for laboratory kits we used. These differences were assumed to be due to discrepancies in ethnicity, age of participants, sample size, laboratory material, and methods used. Selections of participants from a single center and limited sample size are restriction of this study. Also, it seems determination of sex difference in TFTs study regard to female dominancy in our study population was faced with some challenges. Although our analysis did not show any significant difference in gender specific TFTs but it could related to lack of statistical power of study. Due to the high reported prevalence of CH in our country and the lack of international standardization, performing further multi central studies with more population helps in making a more precise evaluation of thyroid status in neonates.

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