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# Evaluation of Thyrotropin and Thyroxine Levels in The First Month of Life

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**BACKGROUND/AIMS**
Thyrotropin (TSH) and thyroxine (FT4) levels in neonates are not similar to those in older children and adults. The aim of this study is to determine the TSH and FT4 hormone levels in newborns aged >37 gestational weeks.

**MATERIAL and METHODS**
The blood samples for TSH and FT4 obtained from newborns aged >37 gestational weeks were analyzed at postnatal 4-7, 8-14, 15-21, and 22-30 days and established as reference intervals for the first week of life, in addition to the mean and median levels for other observation points.

**RESULTS**
There was no significant difference according to gender, gestational age, and mode of delivery (p>0.05); therefore, a pooled analysis was performed. The lower and upper limits of the TSH and FT4 levels from the 4$^{th}$ to 7$^{th}$ day of life were 1.20-10.70 mIU/mL and 0.87-2.20 ng/dL, respectively.

**CONCLUSION**
We demonstrated that the TSH and FT4 levels change during the neonatal period without a significant sex difference.

**Keywords:** Neonate, thyrotropin, thyroxine, congenital hypothyroidism

## INTRODUCTION

Congenital hypothyroidism (CH) is one of the most common etiologies of preventable mental retardation all over the world (1) and the signs and symptoms are not very apparent at birth. Therefore, neonatal screening has been performed in many countries, including Turkey, under a nationwide neonatal thyroid screening program to diagnose and treat CH on time (2). Thyrotropin (TSH) is measured using a filter paper collection method at discharge, and elevated levels are reevaluated for confirmation as soon as possible using both serum TSH and thyroxine (FT4) values in Turkey. The American Academy of Pediatrics' (AAP) recommendations for screening are filter paper collection at 2-4 days of age or at discharge for term infants delivered in the hospital, within 7 days for term neonates staying in the neonatal intensive care unit (NICU), and within 7 days for preterm neonates and for infants delivered at home. Screen cord blood is suggested for neonates whose mothers are on thyroid medication and/or have a family history of CH. A low FT4 level and TSH concentrations >40 mIU/L are indicative of CH (3). The European Society for Pediatric Endocrinology Consensus Guidelines on Screening, Diagnosis and Management of CH recommendations suggest starting treatment if capillary TSH concentration obtained for neonatal screening is ≥40 mU/L, which is similar to AAP suggestions, and/or if venous TSH concentration is >20 mU/L even if the FT4 concentration is normal.

During the neonatal period, reference ranges of thyroid hormones and TSH are not similar to older children and adults (4). Therefore, neonatal reference values for TSH and FT4 are required for accurate assessment. The aim of this study was to determine the TSH and FT4 reference ranges in hospitalized neonates with gestation age ≥37 weeks.

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## MATERIAL and METHODS

In this retrospective study, we included 615 neonates with gestational age ≥37 weeks and hospitalized in the NICU between May 2015 and October 2017.

The gestational age was calculated by using the last menstrual date of the mother; if this date was not known, Ballard score or fetal USG measurements were used. Inclusion criteria were an Apgar score >7 at the 1st and 5th min of life, ≥37 weeks' gestation, mothers having no history of thyroid disease, and appropriate height, weight, and head circumference for appropriate gestational age (AGA). Neonates who had congenital malformations, chromosomal anomaly, metabolic disease, received any medication that might interfere with thyroid or pituitary function (e.g., corticosteroids, dopamine, or propranolol), and whose mothers had any thyroid disease were excluded.

The results of the FT4 and TSH serums were analyzed at postnatal 4-7, 8-14, 15-21, and 22-30 days in AGA neonates from the existing laboratory data. Further, the thyroid function results of neonates on L-thyroxine treatment due to congenital hypothyroidism were collected, but separately evaluated.

The analyses of the FT4 and TSH levels from the serum samples were measured by a Beckman-Coulter DxI device (Minnesota, USA) using the electrochemiluminescence immunoassay method.

Gaziosmanpasa Taksim Research and Training Hospital Ethics Committee approved the study protocol (approval number 90) and all the procedures were conducted in accordance with the Declaration of Helsinki. The study was retrospective, so informed parental consent was not obtained.

### Statistical Analysis

Normal distribution was obtained by using Kolmogorov-Smirnov tests and histogram, Q-Q plot, and box plot graphics. The data were expressed as mean, standard deviation, median, minimum, maximum, IQR, frequency, and percentage. Logarithmic transformation was used for non-normally distributed variables to provide normality. The first-week TSH and FT4 levels, which were normally distributed between two categorical variables, were analyzed with independent samples t test and the other results that were non-normally distributed were analyzed by the Mann-Whitney U test. Variables with three or more categories were compared with the Kruskal–Wallis test. Multiple comparisons were not performed as there was no significant difference. Correlation between the measurable variables was performed by Spearman's correlation test. Normality was not provided for the determination of a reference interval; therefore, the nonparametric percentile method (C28-A3, CLSI guideline) was used. The controls of the outliers were obtained by Rosner's test for multiple outliers. The statistical analysis was carried out using NCSS 10 statistical software (2015, Kaysville, Utah, USA): p values with significance of less than 5% were considered to be statistically significant.

## RESULTS

We studied 615 healthy newborns with mean gestational age of 38.61±1.89 weeks, mean birth weight was 3500±540 g, mean head circumference was 33.5±1.3 cm, and mean birth length was 49.83±1.8 cm. Here, 271 females (44.1%) and 344 males (55.9%) were included. Further, 44% neonates (n: 275) were delivered by caesarean section. Furthermore, 15% neonates (n: 93) exhibited prolonged jaundice. The FT4 and TSH levels during the 4-7th day were higher among neonates with prolonged jaundice, but none of them were diagnosed and treated for CH. There was no significant difference related to the FT4 and TSH levels for other observation points with or without prolonged jaundice. There was no significant difference according to gender, gestational age, and mode of delivery (p>0.05); therefore, a pooled analysis

**TABLE 1.** TSH (mIU/mL) and FT4 (ng/dL) levels according to postnatal day

| | 4-7th DOL | 8-14th DOL | 15-22th DOL | 23-30th DOL |
|---|---|---|---|---|
| TSH (median) | 3.67 (0.85-15.63) | 3.65 (0.93-12.07) | 3.61 (0.76-17.10) | 3.20 (1.28-13.50) |
| FT4 (mean±SD) | 1.45±0.33 | 1.3±0.20 | 1.16±0.22 | 1.11±0.30 |

TSH: thyrotropin; FT4: free thyroxine; DOL: day of life

**TABLE 2.** Reference ranges for TSH (mIU/mL) and FT4 (ng/dL) during postnatal 4-7th day

| | 95% Reference range lower limit (90%CI) | 95% Reference range upper limit (90%CI) | Median (IQR) |
|---|---|---|---|
| TSH | 1.20 (1.13-1.28) | 10.70 (10.04-11.36) | 3.67 (2.35-5.85) |
| FT4 | 0.87 (0.80-0.96) | 2.20 (2.16-2.27) | 1.45 (1.24-1.66) |

TSH: thyrotropin; FT4: free thyroxine; DOL: day of life

**TABLE 3.** Demographic variables and TSH and FT4 levels belonging to patients diagnosed with hypothyroidism

| | Patient 1 | Patient 2 | Patient 3 | Patient 4 | Patient 5 | Patient 6 |
|---|---|---|---|---|---|---|
| HC (cm) | 33 | 32 | 32,5 | 33 | 34 | 35 |
| BW (g) | 3310 | 2300 | 2580 | 2500 | 3170 | 3800 |
| BL (cm) | 49 | 46 | 47 | 47 | 49 | 50 |
| GA (week) | 39 | 36 | 36 | 36 | 383/7 | 382/7 |
| Gender | Male | Female | Male | Male | Male | Female |
| TSH (mIU/mL) | DOL 3>48 | DOL 3: 30.6 | DOL 3: 21 | DOL 3>100 | DOL 3:32.99 | DOL11>50 |
| | DOL 6>48 | DOL 7: 32.25 | DOL 6: 45 | DOL 5>100 | DOL6>46.8 | DOL16>50 |
| FT4 (ng/dL) | DOL 3: 0.35 | DOL 3: 1.53 | DOL 3: 1.36 | DOL 3: 0.36 | DOL 3: 1.24 | DOL11: 0.35 |
| | DOL 6:1.40 | DOL 7: 1.02 | DOL 6: 0.9 | DOL 5: 0.24 | DOL 6: 0.81 | DOL6: 0.23 |

TSH: thyrotropin; FT4: free thyroxine; DOL: day of life; HC: head circumference; BW: birth weight; BL: birth length; GA: gestataional age

was performed. The median TSH and mean FT4 levels during the 4-7th day (n: 482), 8-14th day (n: 131), 15-22nd day (n: 57), and 23-30th day (n: 87) are listed in Table 1. We tried to determine the 95% reference range of TSH and FT4 level during the 4-7th day by using a nonparametric percentile method (C28-A3, CLSI). The reference intervals for TSH and FT4 of the neonates during the 4-7th day of life are listed in Table 2. The median TSH level of neonates with prolonged jaundice during the 4-7th day (n: 55), 8-15th day (n: 43), 16-22nd day (n: 29), and 23-30th day (n: 30) were 4.3 (01.36-15.6), 3.66 (1.01-12.06), 3.69 (1.3-17), and 2.95 (1.3-13.5), respectively. The mean FT4 levels of neonates with prolonged jaundice during the 4-7th day, 8-15th day, 16-22nd day, and 23-30th day were 1.33±0.30, 1.30±0.30, 1.14±0.20, and 1.02±0.20, respectively. The TSH and FT4 levels measured during the 4-7th day of life were statistically different among neonates with and without prolonged jaundice (p: 0.038, p: 0.002), but the hormone values of these newborns were not as high as the values required for treatment for CH mentioned in the literature; in other words, none of these newborns were treated for CH.

In this study, we discuss the period in which we diagnosed and started the treatment for 6 neonates for CH along with pediatric endocrinology. Information regarding these neonates is listed in Table 3. The TSH level of patient 4 was the highest; an enlarged thyroid gland was distinctly visible from the outside.

**DISCUSSION**

Primary hypothyroidism is diagnosed when there is a high concentration of serum TSH and/or low concentration of serum FT4. Therefore, the determination of the reference ranges for these hormones during the neonatal period is very essential. The FT4 and TSH levels of newborns increase dramatically just after birth because of cold stress. These hormones reach their peak levels in the first 24-36 h, and these high levels continue for 2 or 3 days and slowly decrease to their adult levels after 4-5 weeks (4). Mutlu et al. (5) demonstrated that the TSH and thyroid hormones peak in the first 24 h of life and then slowly decline during the neonatal period. According to their study, both TSH and thyroid hormone levels were significantly higher during the neonatal period as compared to those in adults (5). However, Kawahara et al. (6) found that the TSH level reached adult levels after 5 or 7 days of life. They also reported that FT4 was lower at 72 h and thereafter, comparable to the first 48 h of life, but still higher than adult levels. In our unit, we did not evaluate the TSH and FT4 levels as early as these studies because of the rise in these hormone levels following birth to prevent recurrent measurements regarding high levels. We generally measured these hormones after 72 h in our NICU. Neonates whose TSH and FT4 levels were measured on the 4th day of life and thereafter were included in the present study. We investigated several factors that may affect thyroid function: gestational age, gender, and mode of birth. There was no significant difference between these hormone levels and gestational age, gender, and mode of delivery at any observation point; therefore, we decided to combine all these values. Kawahara et al. (6) and Mutlu et al. (5) also demonstrated that there was no significant difference between the hormone levels and the mode of delivery or gender. Imamoglu et al. (7) reported that the serum TSH levels at the postnatal 1 week and 1 month were not correlated with the gestational age, but the FT4 levels were correlated with gestational age at both postnatal 1 week and 1 month. Kapelari et al. (8) found no gender difference except for free T3. Kratzch reported no gender difference involving the thyroid function tests in newborns aged 1 day to 1 month (9).

Kapelari et al. (8) reported the FT4 and TSH reference intervals (2.5, 50, and 97.5%) of neonates from 1 day to 1 month as 0.66/1.56/2.36 ng/dL and 0.70/3.50/18.10 mIU/L, respectively. Elmliger et al. (10) also studied these reference intervals of TSH for neonates aged 1 to 7 days and 8 to 15 days, which were 1.79/4.63/9.69 and 1.80/3.71/7.97 mIU/L, respectively. In another study performed by Hubner et al. (11), the lower limit of TSH for neonates aged 1 to 3 days and 4 to 30 days was lower as compared to the abovementioned studies; however, the upper limit was similar to the results of Elmliger et al. (10), but significantly lower than the results of Kapelari et al. (8). Imamoglu et al. (7) obtained the serum TSH and FT4 levels at the postnatal 1 week and 1 month and determined the reference ranges for TSH and FT4. The reference ranges for TSH (mIU/L) irrespective of the gestational age at the postnatal 1 week and 1 month were 3.71 (0.57-13.11) and 3.30 (1.0-8.37), respectively. Verburg et al. (12) studied the reference ranges for thyroid function in children. They evaluated 139 newborns and determined the reference intervals (2.5, 50, and 97.5%) of TSH and FT4 at the 7th, 14th, 21st, and 28th day of life. The values of TSH at the 7th, 14th, 21st, and 28th day of life were 3.11 (0.32-2.27), 3.01 (0.34-11.44), 2.89 (0.35-10.43), and 2.80 (0.36-9.75) mU/L, respectively. The values of FT4 at the 7th, 14th, 21st, and 28th day of life were 18 (8.9-33.6), 17.9 (8.9-32.9), 17.8 (9-32.3), and 17.7 (9-31.8) pmol/L. The design of the study by Sheikhbahaei et al. (13) was similar to ours. They evaluated the TSH, total, and FT4 hormone levels during 5-7, 8-14, 15-21, and 22-30 days of life and established the reference ranges. The reference ranges (2.5, 50, and 97.5%) of TSH and FT4 during 5-7 days were 0.25/4.7/21.25 mIU/mL and 0.75/1.40/2.12 ng/dL, respectively. The FT4 levels are similar to our results, but the upper TSH level is higher and the lower TSH level is less than those in our results. The TSH level during 22-30 days of life is similar to that obtained in our study, but the FT4 level was higher when compared to that obtained in our study.

We observed that the TSH and FT4 levels decreased with postnatal age. Mutlu et al. (5) found an inverse relationship between the TSH and FT4 levels and age after the 3rd day of life. Najam et al. (14) demonstrated that the decline in the TSH and T4 levels was more apparent in the first week.

Mutlu et al. (5) found that if the TSH level is >20 mIU/L on the 3rd day, >16 mIU/L during the 5-7 days, and >5 mIU/L on the 28th day, these patients should be carefully followed-up with regard to CH. Lott et al. (15) suggested a cutoff value of TSH ≥20 mIU/L for newborns older than 72 h for CH diagnosis. In our unit, 6 patients were diagnosed for CH during the study period. The TSH serum level of our patients on the 3rd day and during the 5-7th days were >20 mIU/L and >30 mIU/L, respectively. The TSH level of 1 out of the 6 patients diagnosed for CH was higher than 100 mIU/mL on the 3rd and 5th days of life with a diffuse goiter visible from the outside.

Prolonged jaundice is defined as jaundice persisting beyond 14 days of life in term neonates and beyond 21 days in preterm neonates (16). One of the pathological causes associated with prolonged jaundice is congenital hypothyroidism (16-19).

Agrawal V et al. (19), Najati N et al. (20), Sabzehei MK et al. (17), Boskabadi H et al. (21), and Cetinkaya et al. (22) studied the etiology of prolonged jaundice in newborns and demonstrated that the incidence of hypothyroidism was 4-8%. Further, 13% (n: 93) of our cases diagnosed as having prolonged jaundice were investigated for the underlying disease. Surprisingly, none of the infants had hypothyroidism. The FT4 and TSH levels measured during the 4-7$^{th}$ days of life are higher, but not as high as levels that necessitate treatment. The FT4 and TSH levels at other observation points were in the normal range.

The main limitation of our study is that it comprises a hospital-based population. Ideally, reference intervals should be determined using blood samples, obtained from a large cohort of healthy subjects. However, due to ethical and practical considerations, reference interval determination is usually performed on the basis of the hospital database by applying appropriate selection criteria. We addressed this concern by excluding all the subjects with diagnoses and concomitant medications that might affect the thyroid function. The other limitations are that the study is retrospective and the number of subjects is small.

In conclusion, it is known that neonatal reference intervals for thyroid function tests are different from older children and adults and should not be used interchangeably. It is important to diagnose CH in a timely manner due to long-term sequelaes. Therefore, we decided to evaluate retrospective data of our unit to determine the upper and lower limits for TSH and FT4 levels during the neonatal period at different observation points. We demonstrated that the TSH and FT4 levels change during the neonatal period without a significant sex difference. Both TSH and FT4 levels were prone to decrease with postnatal age; however, higher than adult levels have been reported in most studies.

**Ethics Committee Approval:** Ethics committee approval was received for this study from Gaziosmanpasa Taksim Research and Training Hospital Ethics Committee (Approval Date: 15.11.2017, Approval Number: 90).

**Informed Consent:** Informed consent is not necessary due to the retrospective nature of this study.

**Peer-review:** Externally peer-reviewed.

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**Conflict of Interest:** The author have no conflicts of interest to declare.

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