Establishment of reference ranges for thyrotropin and free thyroxine for neonates of Bangalore city

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ABSTRACT

Introduction and Aim: Congenital hypothyroidism (CHT) is a condition of thyroid hormone deficiency present at birth. To diagnose this condition, estimation of TSH and FT4 are essential. There is an urgent need to develop reference ranges for thyroid hormones for neonates because hypothyroidism is the most frequent thyroid disease encountered in pediatric age group and it requires early diagnosis to prevent the consequences, which can be lifelong. No reports exist in Indian literature regarding the establishment of reference ranges in India for thyroid profile for neonates and thus this study was carried out.

Subjects and Methods: 100 neonates were included in this longitudinal study. Serum TSH and freeT4 levels were estimated by using Electro-chemiluminescence, based on the Sandwich principle (Cobas, USA). The statistical analysis was performed using MedCalc software.

Results: Mean and standard deviation of TSH in males and females were 4.33 ± 4.25 (µIU/mL) and 4.33 ± 3.99 (µIU/mL) respectively. Mean and standard deviation of free T4 in males and females were 22.44 ± 5.31 (pmol/L) and 22.49 ± 7.47 (pmol/L) respectively. The reference ranges of TSH in males are 0.18- 15.76 µIU/mL and in females is 0.17-15.68 µIU/mL. The reference ranges of free T4 in males 14.19 – 32.69 p mol/L and females 9.65-36.25 pmol/L.

Conclusion: The reference range of TSH obtained in this study correlates with that of the western population and hence, it may be used as a stable parameter in the screening of neonates for congenital hypothyroidism.

Keywords: Congenital hypothyroidism; thyroid hormones; reference range; neonates.

INTRODUCTION

Thyroid profile testing is one of the useful investigations to assess the functioning of the thyroid gland. This assessment is very essential in neonates for the early diagnosis of any congenital anomalies of the thyroid gland such as defects in hormone synthesis, which includes congenital hypothyroidism. The incidence of congenital hypothyroidism in all over world is 1:4000 live births and in India 1:2500-2800 live births (1).

Thyroid hormone deficiencies contribute to severe retardation of growth and maturation of almost all organ systems. Most infants with congenital hypothyroidism (CH) are asymptomatic at birth. The clinical features of CH develop in neonates by 3-6 months of age and at this age neurodevelopmental, defects cannot be reversed. The prevalence of CH in Europe and USA is 1: 3500 and 1:4000 respectively. In African American population, it is 1:11,000 (2). During the first trimester, T4 in the circulation is of maternal origin, as the fetal thyroid does not produce significant amounts of T4 until the second half of pregnancy (3). Thereafter, the elevated levels of serum T4 concentrations in second half of pregnancy is due to an increase in hepatic production of thyroxine-binding globulin (TBG). At term fetal serum T4 concentration increases from 2g/dL at 12 weeks to 10g/dL and free T4 (4) increase progressively, from a mean value of approximately 0.1 ng/dL at 12 weeks to 2 ng/dL at term.

As on date, most of our laboratories are using the textbook reference values, which give reference ranges of the western population (5). Indian population should have own reference ranges for thyroid profile because it differs from other countries in terms of population, lifestyle, body mass index, food habits, environment, and basal metabolic rate. It is mandatory for any laboratory to adopt reference ranges.

There is an urgent need to develop reference ranges for thyroid hormones for neonates because hypothyroidism is the most frequent thyroid disease encountered in the pediatric age group and it requires early diagnosis to prevent the consequences, which can be lifelong. Hence, this study aims to establish reference ranges for thyroid hormones in neonates of Bangalore city with reference to particularly Thyrotropin/thyroid-stimulating hormone (TSH) and

free thyroxine (FT4). American Thyroid Association has proposed that thyrotropin/thyroid-stimulating hormone (TSH) is the best single measurement of thyroid status because of its high sensitivity (6), there is a considerable difference in reference ranges for FT4, and TSH have been reported between countries (7). Reliable reference intervals are important in the interpretation of laboratory data, and it is incumbent on each laboratory to verify that the ranges they use are appropriate for the patient population they serve (8). There is a limited number of studies in Indian literature regarding the establishment of reference ranges in India for thyroid profile for neonates. Hence, the present study was aimed at establishing reference ranges for thyroid profile in neonates.

Prompt intervention can prevent permanent adverse neurological effects caused by neonatal hypothyroidism. A well-defined established age specific biological reference interval for TSH, FT4 and FT3 is not available to help in interpretation of thyroid profile in neonates (9).

SUBJECTS AND METHODS

The present study was a duration based longitudinal type of study conducted for a period of three months on one hundred neonates of both the gender in the age group between two days after birth (there will be a surge of thyroid hormones from the maternal circulation to the foetal circulation) to one month after birth in a tertiary care hospital in Bengaluru. Ethical Committee clearance was obtained from the institute and the informed consent was taken from the parents of the subjects involved in this study and there was no financial liability on them.

Neonate's mother with the history of/on medication for thyroid disorders, autoimmune disorders, diabetes mellitus, and gestational diabetes, preeclampsia were excluded. Premature babies, neonates with congenital abnormalities were excluded from the study.

Serum TSH and free T4 levels were estimated by using Electrochemiluminescence, based on the Sandwich principle (Cobas, USA).

Statistical analysis

The statistical analysis was performed using MedCalc software. Mean and standard deviation was calculated using formulas. Distribution of TSH and FT4 was expressed in percentage

RESULTS

Out of 100 subjects included in the study, 56% were within the age group of 2-4 days, 38% were within 5-8 days and 6% of the study group was 9 -14 days (Table 1). In our study, 55% of neonates were males and 45% were females. Distribution of serum TSH and free thyroxine values are depicted in Tables 3 and 4. Mean and standard deviation of TSH in males and females were 4.33±4.25 and 4.33± 3.99 respectively. Mean and standard deviation of free T4 in males and females were 22.44 ± 5.31 and 22.49±7.47 respectively (Table 5). The reference ranges of TSH in males are 0.18- 15.76 µIU/mL and in females is 0.17-15.68 µIU/mL. The reference ranges of free T4 in males 14.19 – 32.69 p mol/L and females 9.65- 36.25 p mol/L (Table 6).

Table 1: Age distribution in study subjects

Age in days	Number of subjects	%
2-4	56	56
5-8	38	38
>8	06	06
Total	100	100

Table 2: Gender distribution among study subjects

Gender	Number of subjects	%
Male	55	55
Female	45	45
Total	100	100

Table 3: Distribution of serum TSH values in neonates

TSH (pmol/L)	No. of Subjects	%
<1	25	25
1-2	15	15
2-5	22	22
>5	38	38

Table 4: Distribution of FT4 values in study subjects

FT4 (pmol/L)	No. of Subjects	%
< 20	40	40
21-30	46	46
31-40	13	13
Total	100	100

Table 5: Mean and standard deviation for TSH and FT4 values

Subjects	TSH (µIU/mL)	FT4 (pmol/L)
Male	4.33 ±4.25	22 .44 ±5.31
Female	4.33 ±3.99	22.49 ±7.47
Overall	4.33 ±4.12	22.47 ±6.34

Table 6: Reference range of TSH and FT4

Subjects	TSH (µIU/mL)	FT4 (pmol/L)
Male	0.18-15.76	14.19-32.69
Female	0.17-15.68	9.65-36.25
Overall	0.20-15.65	12.43-34.89

Table 7: References ranges for the present study and laboratory

References	TSH (µIU/mL)	FT4 (pmol/L)
Laboratory	Up to 20	No standard value
Present study	0.20-15.65	12.43-34.89

DISCUSSION

Thyroid hormones are required for development of brain and central nervous system. Hence, neurodevelopmental defects as mental retardation arises from abnormal thyroid function (10). The reference ranges of thyroid profile are dependent on age, ethnicity, gender and the methodology adopted

to estimate the thyroid hormones (11). Since adult reference intervals could not be used as screening in paediatric disease, several age-specific, population-based reference intervals for TFTs are suggested in different regions.

Several paediatric reference intervals (RIs) for thyroid function tests (TFTs) have been published (12). The neonatal RIs described in some of these studies were established based on relatively small numbers of subjects or using analysers with diverse measurement principles and analytical performance. For example, in earlier studies (13), ultrasensitive immunoassays were not used to measure thyroid-stimulating hormone (TSH). The incidence of congenital hypothyroidism also depends on the test methodology adopted to estimate TSH and by using ultrasensitive immunoassays a very low concentration of TSH can be determined which lead to the introduction of diagnosis such as subclinical hyperthyroidism (14).

In primary hypothyroidism serum thyrotropin (TSH) level increases and is the most sensitive diagnostic marker (15). On the first day of birth thyroid sampling should be avoided as there will be TSH surge in the first 30 minutes of life and these values should not be considered for establishment of reference range. The hypothalamus, pituitary gland, and the thyroid all play a part in the feedback and regulatory mechanisms involved in the production of thyroxine (T4) and triiodothyronine (T3) from the thyroid gland. There are limited number of articles published on neonatal RIs for TFTs due to the difficulty in sampling, consent and in enrolling neonates. The CALIPER study, which was carried out in Canada and has so far enrolled 8500 children from birth to 18 years of age, has established RIs for many analytes including TFTs (16). Establishment of RI study is very challenging as there is need to standardize all phases of laboratory cycle. Hypothalamus stimulates the production of the polypeptide TSH from the anterior pituitary and then TSH stimulates the production and release of T4 and T3 from the thyroid gland. Once released, T4 and T3 (active form) then exert a negative feedback mechanism on TSH production, T4 is the main hormone produced by the thyroid. T3 is mainly produced by peripheral conversion (in liver and kidney mainly) of T4. T3 and T4 are largely protein-bound in the plasma, mainly to thyroxine-binding globulin (TBG -90-95% is T4). Only the unbound or 'free' portion (FT3, FT4) is active. T3 and T4 both act via nuclear receptors to increase cell metabolism. The half -life of T4 is 6-7 days and of T3 is 1-2 days. It is shortened in hyperthyroidism and prolonged in hypothyroidism. 100 healthy babies were considered in the age group ranging from 2 days – 14 days, out of which 55 were males and 45 were females. The majority of them belonged to 2-4 days of age. In a healthy newborn there is a TSH surge in the first hours of life as an adaptation to the extra uterine environment. To be valid, a specimen is to be collected when the infant is at least 24 hours of age. Their blood samples were drawn and their TSH and FT4 values were evaluated using chemiluminescence technology and the same was tabulated. The majority of the babies (38 in number) showed a TSH value of >5µIU/mL. Only 15 babies had TSH value in the range between 1 – 2 µIU/mL. The overall reference range we obtained for TSH was 0.20 – 15.65 µIU/mL. There is a slight gender variation in the reference range for TSH value. Males had a range of 0.18 – 15.76 µIU/mL while the females had a range of 0.17 -15.68 µIU/mL. In the study conducted by Kapelari *et al*., 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 (n = 23) for TSH and free T4 (17). In a study of Jayasuriya *et al.*, TSH and FT4 were substantially high in the first 24 h after birth followed by a rapid decline over the subsequent 168 h. There is a need to establish an hour-based RIs in newborns which is more accurate in identification of neonates who are at risk of hypothyroidism (7).

Recent studies indicate that exposure to certain environmental contaminants may also interfere with maternal thyroid status during pregnancy and with thyroid function in newborns (18). Hence, it has been speculated that some of the neurotoxic effects of early exposure to environmental chemicals may result from thyroid disruption. Nonetheless, the influence of early exposure to endocrine disruptors (ED) on thyroid function and therefore children's neurodevelopment remains to be elucidated (19).

According to updated AAP guidelines on newborn screening and therapy for congenital hypothyroidism (20), the upper limit of TSH value to categorize the baby as hypothyroid should be >40 µIU/mL. The value we obtained falls well within this category. As per the clinical study published in the European Society of Endocrinology, the value obtained in this was 0.24 - 17 µIU/mL (21). However, there is a slight variation in both lower limit and upper limit, their value being on the higher side due to the various factors like lifestyle, body-mass index, food habits, environment, and basal metabolic rate of the mother. According to UK newborn screening program, babies with TSH value >20 µIU/mL were considered to have a positive screening result for congenital hypothyroidism (CHT) and babies with values <10 µIU/mL are considered to have negative screening result for CHT. We found that the reference ranges obtained in this study correlate the reference values that being followed by the British Society for Paediatric Endocrinology and Diabetes. (22). The reference ranges of serum TSH levels obtained from this study were compared to the reference range of

the laboratory, the results found to be comparable with the reference range of the laboratory in which the samples were analysed. Previously, few studies have reported in establishing cut off values of cord blood TSH in Ethiopia. A study from Iran shows the reference range of TSH concentration ranged from 0.77 to 24.91µlU/mL with a mean value of 7.09, which had lower values compared to the present study. However, the time of establishment shows it was done with older methods, which may have lower sensitivity. This implies the importance of method-specific and timely updated reference values. One study from India reported the 97th percentile result of TSH as 25.8 mIU/L (23) which was comparable but lower than the result of the present study.

The majority of the babies (46 in number) showed an FT4 value of 21 -30 pmol/L. There is quite a variation seen in the lower limit of FT4 values between males and females. Males have a range of 14.19 – 32.69 pmol/L. Fem ales have a range of 9.65 -36.25 pmol/L. Overall, the reference range of FT4, in general, is 12.43- 34.89 pmol/L. NACB (National Academy of Clinical Biochemistry, Washington, DC) has recommended the FT4 reference range as 22 -49 pmol/L (24). The reference ranges obtained in this study do not co-relate with this reference range. This might be due to the fact that we have collected samples up to 14 days of age, by which FT4 levels would start decreasing. The race may have also played a role in FT4 production (25). Due to this wide difference in values in FT4, TSH is considered to be a more sensitive screening test for CHT. There are no studies done to establish a reference range for FT4 among neonates of Bangalore city. We could not compare the reference range obtained from this study with the reference range used by the laboratory in which we carried out our test as they do not have any standard reference range.

CONCLUSION

The reference ranges of TSH in male neonates are 0.18- 15.76 µIU/mL and in female neonates is 0.17-15.68 µIU/mL. The reference ranges of free T4 in male neonates 14.19 -32.69 pmol/L, female neonates 9.65- 36.25 pmol/L. In view of the fact that FT4 has a wide range of variation, it is concluded that it cannot be used alone as a screening test for CHT and sampling of a large population of Indian neonates are required to establish FT4 reference range.

Compliance with ethical standards

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Institute`s ethical permission was obtained for the project.

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