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Original Research Article

Establishment of Age and Gender Specific Reference Range for Thyroid Hormones Levels among Blood Donors of Northern Indians

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Abstract

Background: In clinical endocrine practices, it is difficult to differentiate thyroid diseases from subclinical hypothyroidism (SCH). Therefore, the present study focused on the establishment of age and gender specific reference ranges of serum thyroid hormones to discriminate SCH from healthy northern Indian control.

Material and Methods: Present study was conducted on 1018 healthy individuals aged between 18-60 years who intent to participate in blood donation camps held at Medanta- The Medicity, Gurgaon, Haryana (India). Participants with clinical history of thyroid abnormality and related symptoms were excluded from the study. The circulatory levels of free triiodothyronine (FT3), free thyroxine (FT4) and thyroid stimulating hormones (TSH) were analyzed using competitive immunoassay technique on dry chemistry fully autoanalyzer.

Result: An establishment of age and gender-specific range, and corresponding 95% confidence intervals (CIs) were calculated for each age group. A significant change in thyroid hormone levels were documented with increasing age group in both genders, as well as significant correlation was also found between FT3, FT4 and TSH in respective hormones ($p<0.05$).

Conclusion: The present study concluded that circulatory thyroid hormone levels alter markedly with growing age. An establishment of reference range for thyroid hormone is need of an hour for better understanding and differentiating individuals from SCH.

Keywords: Reference range, Thyroid function test, Subclinical Hypothyroidism, Iodine Deficiency Disorders (IDD).

Background

Thyroid disorders are the commonest endocrine disorders worldwide. India too, is not exception of it. An estimate of about 42 million people in India suffer from thyroid diseases [1,2]. Serum thyroid hormone levels are the most sensitive indicator for the diagnosis of thyroid dysfunction. Thyroid hormones are affected by many factors, such as age, gender, lifestyle, ethnicity, iodine status, obesity and pregnancy [3-8]. In clinical practices, it is difficult to differentiate thyroid diseases from subclinical hypothyroidism (SCH). Subclinical hypothyroidism is the most common form of thyroid dysfunction and characterized by elevated levels of serum thyroid stimulating hormone (TSH) and normal thyroxine (T4) levels [9]. Many studies have shown that SCH is closely related to the occurrence and progression of hypertension, dyslipidemia, and even related to the risk of cardiovascular diseases (CVD) and all-cause mortality [10]. Age and gender specific reference range for serum thyroid hormone levels are the key parameter to consultant to minimize the under-diagnosis of SCH in younger adults and for prevention of over-diagnosis in the elderly.

Reference ranges altered considerably from one laboratory to another and are dependent on methodology used for assays, population diet and selection of ethnic group. Selection of reference group and standardization of pre-analytical factors are the most important stage for the determination of reference ranges. Proper clinical assessment and care of patient are virtually dependent on both the availability of specific laboratory tests and reliable reference intervals interpretation [11,12]. Although clinicians understand the importance of reference ranges for the interpretation of laboratory results and applied in day-to-day practices. Clinical biochemists indeed take keen responsibilities in providing ranges that are as robust as possible, but there are substantial difficulties in establishing reliable reference ranges. Establishment of reference ranges required sufficient number of healthy individuals distributed over the range of ages and genders for which reference range is to be provided. Thyroid hormone levels are subject to variation from range of factors like age, gender, iodine intake and climate change [3]. Therefore, the present study focuses on the establishment of age and gender specific reference ranges for thyroid hormone levels to discriminate SCH from healthy northern Indians.

Material and Methods

Study Design and sample size: The present study was held in collaboration with Clinical Biochemistry Laboratory and blood bank at multispecialty hospital Medanta- The Medicity, Gurgaon, Haryana (India). The study involved 1018 healthy individuals who voluntarily participated in blood donation camp at Medanta hospital. The current recommendation is to use the standard proposed by National Academy of Clinical Biochemistry (NACB) to establish the thyroid reference range (3, 11), which is as follows: to select at least 120 individuals 1) who have no personal or family history of thyroid disease, 2) who are negative for thyroid antibodies, 3) who have no visible or palpable goiter and 4) who have not received any treatments affecting thyroid function (except estrogen). A total of 22 subjects were excluded while 996 healthy volunteers with age group between 18-60 years were selected for the analysis and informed consent forms were filled and data was collected and stored in Microsoft excel sheet.

Specimen collection and processing- The healthy subject was divided as per age groups <20 years (n=55), 21-30 years (n=465), 31-40 years (n=312), 41-50 years

(n=130) and 51-60 years (n=34). Blood samples were collected using vacutainer gel separator tubes and centrifuged at 3000 rpm for 10 min for collection of serum samples and were immediately processed for the analysis of thyroid hormones. Method of FT3 and FT4 are based on competitive immunoassay technique (CIA) while TSH based on immunometric assay and analyzed by using VITROS 5600 clinical chemistry fully autoanalyzer (Ortho Diagnostic, USA). The Standardization of techniques prior to reference range testing and calibrating all assays, establishing Quality Control (QC) limits for each analyte. Quality control samples were processed on routine time and only when they are within limits; assessment of serum sample analysis was undertaken and validated.

Statistical Analysis

Data of 996 healthy blood donors were computerized and analyzed by using SPSS software, version 22.0. Study results were expressed in mean ± Standard deviation (SD), Median, variance, 95% Confidence Interval (CI) and percentage. The student's t-test was used to compare two groups. Pearson's correlation was used to find out the association between effect of thyroid hormone levels with advancement of age or specific for gender. p-value less than <0.05 was considered as statistically significant.

Result

In present study, the serum concentration of FT3, FT4, and TSH were assayed in 996 healthy blood donors. The study subjects were grouped as per gender- male (531, 54%) and female (465, 46%) and age-specific intervals. It was aimed to see the impact of age and gender on FT3, FT4, TSH levels respectively.

Descriptive analysis of thyroid hormones recapitulated in Table No. 01. Age and gender-specific reference interval of thyroid hormones in north Indian population was shown in Table No. 02 and 03. It is clear from the data that upper limit for FT3 increases with increasing age. The lower limit of FT4 serum value increases with age, upper limit of FT4 serum value higher in fifth decade of life and lower in age group above 51 years. The TSH values increases progressively with age. 95% CI slightly on lower side in male as compared with female. There is significant negative correlation between FT3, FT4 and TSH levels shown in Table No. 04.

Table 1: Descriptive Analysis of thyroid hormones in north Indian population

Parameters	FT3 (pg/ml)	FT4 (ng/dl)	TSH (µIU/ml)
Sample Size (N)	996	996	996
Gender M/F	531/465	531/465	531/465
Age (in years)	39.89±19.35	39.89±19.35	39.89±19.35
Mean ±SD	3.75±0.54	1.26±0.23	3.21±1.55
Standard Error	0.017	0.0073	0.0494
Median	3.72	1.245	2.33
Mode	3.53	1.11	1.5
Sample Variance	0.29	0.053	2.40
Kurtosis	44.63	11.99	3.09
Skewness	3.64	1.99	1.47
Range	3.47	2.63	10.25
Minimum	2.23	0.79	0.15
Maximum	5.7	3.42	10.4

Table 2: Age specific reference Interval of thyroid hormones in Northern Indians

Age group/ Intervals (N)	Statistics		FT3 (pg/ml)	FT4 (ng/dl)	TSH (µIU/ml)
< 20 years (N=55)	Mean ± SD		3.98±0.54	1.31±0.19	2.52±1.29
	Median		3.84	1.28	2.26
	95% CI	Lower CI	2.94	0.96	0.67
		Upper CI	5.34	1.75	6.76
21-30 years (N=468)	Mean ± SD		3.77±0.44	1.25±0.19	2.63±1.46
	Median		3.75	1.24	2.33
	95% CI	Lower CI	2.98	0.91	0.75
		Upper CI	4.97	1.67	6.61
31-40 years (N=312)	Mean ± SD		3.73±0.47	1.25±0.20	2.82±1.56
	Median		3.71	1.24	2.41
	95% CI	Lower CI	2.88	0.91	0.84
		Upper CI	4.88	1.69	6.96
41-50 years (N=130)	Mean ± SD		3.59±0.42	1.25±0.24	2.72±1.57
	Median		3.61	1.23	2.36
	95% CI	Lower CI	2.75	0.89	0.44
		Upper CI	4.41	1.89	7.36
51-60 years (N=34)	Mean ± SD		3.38±0.45	1.24±0.17	2.21±1.43
	Median		3.41	1.2	1.85
	95% CI	Lower CI	2.95	0.97	0.38
		Upper CI	4.58	1.90	5.98

Table 3: Gender specific reference Interval of thyroid hormones in Northern Indians

Gender	No.of participans (N= 996)	Statistics		FT3(pg/ml)	FT4(ng/dl)	TSH(µIU/ml)
Male	531 (54%)	Mean ± SD		3.65±0.51	1.25±0.19	2.53±1.46
		Median		3.65	1.26	2.51
		95% CI	Lower CI	2.77	0.81	0.56
			Upper CI	4.89	1.79	5.02
Female	465 (46%)	Mean ± SD		3.78±0.56	1.33±0.21	2.75±1.30
		Median		3.82	1.29	2.35
		95% CI	Lower CI	2.82	0.96	0.67
			Upper CI	5.34	1.98	6.76

Table 4: Pearson's Correlation between FT3, FT4 and TSH

Parameters	TSH (µIU/ml)	
	r value	p value
FT3 (pg/ml)	-0.281	<0.001*
FT4 (ng/dl)	-0.069	<0.05*

***p value <0.05, statistically significant**

Discussion

Reference intervals are an important pre-requisite of clinical laboratory for interpretation of subclinical diagnosis and disease patients. Reference intervals could vary considerably from one laboratory to another and were reliant not only on single factors such as age, gender, lifestyle but also on population and environmental factors such as ethnicity, climate, altitude as-well-as methodology used for assay techniques and selection of reference range [3-7]. Reference ranges are of two types: health associated and decision based. Data referred above are health associated, as it indicts that iodine deficiency disorder (IDD) status of healthy Indian blood donor. This does not necessarily interpret into decision-making efficacy. To do so, more robust data from all parts of the India can be analyzed together. Jebasingh *et al*, highlight the multifaceted ethnic makeup of our country [13]. This is the time to create a pan-India reference range for thyroid function tests not only in pregnancy but also in other age groups and gender as well [14-17]. Known fact that thyroid function test routinely used in clinical practice to diagnose thyroid disorders that are known to be influenced by age, ethnicity, geographical, climatic conditions and other biological variables including nutrition and lifestyle [1,3].

The present study highlights that reference range of serum thyroid hormone levels in different age group as well as in gender specific group. Our data analysis shows that TSH increased with age and slightly decreased in healthy elderly individuals. The findings on the association between age and thyroid profile study report consistent with previous studies [17,18]. Different studies published data adjusted for age, gender, and were found differences in results due to alterations in sample size, exclusion criteria, age range, normal limits of TSH reference values and choice of tests. In 2002, the National Health and Nutrition Examination Survey (NHANES-III) suggested that 95% of the US disease-free population had a serum TSH concentration increased with increasing age group [19]. Surks and Hollowell, 2007 also reported that distribution progressively shifts towards higher concentration with age, and the prevalence of SCH may be significantly overestimated unless an age specific range for TSH is used [20]. In present study the mean TSH value (2.82 µIU/ml) is highest in 31-40 years age group which was related with previous study of Jadav PM *et al* 2018 [21] then it gradually decreases from 2.72 µIU/ml in 41-50 year age group to 2.21 µIU/ml in 51-60 year age group. Some studies suggested that serum TSH level has been found to increase or decrease with age in relation to the iodine intake. Also, the hypothalamic-pituitary-thyroid axis may be involved in the age-related modifications of thyroid hormones and thyrotrophins [22,23].

There is ambiguity regarding the level of thyroid hormones in various age groups and gender. The present study emphasis on the fact that serum FT3 level is significantly high in early decades of life, remain unchanged in middle decade and shows subsequent declination in later decades of life. The FT3 level declination in older age may be due to decline in thyroidal FT3 secretion, increase in FT3 turnover rate and decrease in peripheral conversion of T4 to FT3. The increase in the level of FT3 in the early stage may be due to the increased metabolic activity during infancy and childhood. The reason behind that FT3 is most active and secreted by both thyroid glands as well as by the result of de-iodination of T4 in the peripheral tissues [22,23] Our finding has been substantiated that highest mean FT3 value (3.98 pg/ml) was observed in 20 years age group, and

then it declines with increases in age group above 50 years. Level of FT3 in first decade is high because of maternal estrogen induced increase in serum thyroid binding globulin (TBG) [22]. Similar findings of decrease in FT3 levels from second decade to fifth decade of life were observed in several studies [24]. Henson *et al*, reported that T3 level decreases with age in both sexes [25].

Present study shows gradual decrease in lower limit of FT4 concentration with 0.96 in <20 years old, its increases in age group above 51 year and increased in females as compared to males. However, Sawin *et al* observed incomprehensible low levels of thyroxine in the elderly [26] and Razzak *et al*, observed higher levels of serum T4 in females as compared to males before age 60 and a decline thereafter [27]. Study has also suggested this may be due to the fact that in females after age 50, there is decline in estrogen dependent TBG concentrations. Age related decrease in FT4 may be due to primary retardation in hormone metabolism that could lead to hypo-metabolism associated with aging [28].

Several studies have highlighted the clinical impacts of using inappropriate reference intervals in clinical medicine. One study found that lack of age adjusted cut offs for TSH hormone during neonatal screening for congenital hypothyroidism led to an increase in the frequency of false positives and to excessive follow- up rates [29,30]. The survey of NHANES III in the US on regular thyroid function, for establishing new TSH reference intervals on euthyroid healthy volunteers also suggested that TSH concentration increases with age. Present study conducted on establishment of reference range for thyroid hormone is need of an hour for better understanding and differentiating individuals from SCH.

Conclusion

The present study concluded that levels of FT3, FT4 and TSH have significant effects among gender and with increasing age group. It is therefore, concluded that there should be separate age and gender specific reference range among individuals so that the higher level of thyroid hormones in the early decades and the lower level in the elderly may not be interpreted as abnormal results and therefore under prediction and over prediction of results can be avoided by clinicians.

Limitation

The present **s**tudy has not analyzed the daily iodine intake, exercise, climatic changes and other environmental factors that may affect the circulatory thyroid hormone levels

References

1. Unnikrishnan AG, Menon UV. Thyroid disorders in India: An epidemiological perspective. Indian J Endocr Metab. 2011;15: S78-S81.
2. Available from: http://www.ias.ac.in/currsci/oct252000/n%20 kochupillai.PDF [Last accessed on 2011 April 2].
3. Wang X, Li Y, Zhai X, Wang H, Zhang F, Gao X, *et al*. Reference Intervals for Serum Thyroid-Stimulating Hormone Based on a Recent Nationwide Cross Sectional Study and Meta-Analysis. Front. Endocrinol. 2021. 12:660277.
4. Ehrenkranz J, Bach PR, Snow GL, Schneider A, Lee JL, Ilstrup S, *et al*. Circadian and Circannual Rhythms in Thyroid Hormones: Determining the TSH and Free T4 Reference Intervals Based Upon Time of Day, Age, and Sex. Thyroid. 2015. 25(8):954-961.
5. Li C, Shan Z, Mao J, Wang W, Xie X, Zhou W, *et al*. Assessment of thyroid function during first-trimester pregnancy: what is the rational upper limit of serum TSH during the first

trimester in Chinese pregnant women? J Clin Endocrinol Metab. 2014. 99(1):73-79.
6. Reinehr T, Isa A, de Sousa G, Dieffenbach R, Andler W. Thyroid hormones and their relation to weight status. Horm Res. 2008. 70(1):51-57.
7. Zhai X, Zhang L, Chen L, Lian X, Liu C, Shi B, *et al*. An Age-Specific Serum Thyrotropin Reference Range for the Diagnosis of Thyroid Diseases in Older Adults: A Cross-Sectional Survey in China. Thyroid. 2018. 28(12):1571-1579.
8. Zhao L, Teng D, Shi X, Li Y, Ba J, Chen B, *et al*. The Effect of Increased Iodine Intake on Serum Thyrotropin: A Cross-Sectional, Chinese Nationwide Study. Thyroid. 2020. 30(12):18101819.
9. Biondi B, Cappola AR, Cooper DS. Subclinical Hypothyroidism: A Review. JAMA. 2019. 322(2):153-160.
10. Moon S, Kim MJ, Yu JM, Yoo HJ, Park YJ. Subclinical Hypothyroidism and the Risk of Cardiovascular Disease and All-Cause Mortality: A Meta-Analysis of Prospective Cohort Studies. Thyroid.2018. 28(9):1101-1110.
11. Baloch Z, Carayon P, Conte-Devolx B, Demers LM, Feldt-Rasmussen U, Henry JF, *et al*. Laboratory medicine practice guidelines. Laboratory support for the diagnosis and monitoring of thyroid disease. Thyroid.2003;13(1):3-126.
12. Malati T. Whether western normative laboratory values used for clinical diagnosis are applicable to Indian population? An overview on reference interval. IJCB. 2009; 24(2):111-122.
13. Jebasingh FK, Salam R, Meetei TL, Singh PT, Singh NN, Prasad L. Reference intervals in evaluation of maternal thyroid function of Manipuri women. Indian J Endocrinol Metab. 2016; 20:167–70.
14. Boucai L, Hollowell JG, Surks MI. An approach for development of age-, gender-, and ethnicity-specific thyrotropin reference limits. Thyroid. 2011; 21:5-11.
15. Clinical and Laboratory Standards Institute. CLSI Document C28-A3. 3rd ed. Wayne, PA: Clinical and Laboratory Standards Institute; 2008. Defining, Establishing, and Verifying Reference Intervals in the Clinical Laboratory; Approved Guideline.
16. Demers LM, Spencer CA. Laboratory support for the diagnosis and monitoring of thyroid disease. Thyroid. 2003;13:33-44
17. Vadiveloo T, Donnan PT, Murphy MJ, Leese GP. Age- and gender-specific TSH reference intervals in people with no obvious thyroid disease in Tayside, Scotland: the Thyroid Epidemiology, Audit, and Research Study (TEARS). J Clin Endocrinol Metab. 2013; 98(3): 1147-1153.
18. Dambal AA, Padaki S, Herur A, Kashinakunti SV, Manjula R, Gurupadappa K. Thyroid Status in Relation to Age and Gender-A Cross Sectional Study. MRIMS J Health Science. 2013;1(2):37-40.
19. Hollowell JG, Staehling NW, Flanders WD, *et al*. Serum TSH, T(4), and thyroid antibodies in the United States population (1988 to 1994): National Health and Nutrition Examination Survey (NHANES III). J Clin Endocrinol Metab. 2022; 87:489-499.
20. Surks MI, Hollowell JG 2007 Age-specific distribution of serum thyrotropin and antithyroid antibodies in the US population: implications for the prevalence of subclinical hypothyroidism. J Clin Endocrinol Metab 92:4575-4582.
21. Jadav PM and Pradhan R. Establishment of reference range for thyroid hormones (T3,T4 and TSH) in adult female population at clinical biochemistry laboratory of GCS medical college and

hospital- A descriptive study. Int J Clin Biochem Res. 2018;5(3):449-452.
22. Kumari T, Prasad A, Sinha KK, Bharti MLG, Satyam K. Age and sex pecific thyroid hormone profile in euthyroid subjects. J Biochem Tech. 2015; 6(3): 1008-1012.
23. Mazzoccoli G, Pazienza V, Piepoli A, Muscarella LA, Inglese M, De Cata A, *et al*. Hypothalamus- hypophysis-thyroid axis function in healthy aging. J Biol Regul Homeost Agents 2010; 24(4):433-439.
24. Ace Lipson, Eileen L. Nickoloff, Tah Hsiung Hsu, Wayne R. Kasecamp, Helen M. Drew, Ramleth Shakir, *et al*. A Study of Age-Dependent Changes in Thyroid Function Tests in Adults: J Nucl Med. 1979; 20:1124-1130.
25. Hansen PS, van der Deure WM, Peeters RP, *et al*, The impact of a TSH receptor gene polymorphism on thyroid-related phenotypes in a healthy Danish twin population. Clin Endocrinol (Oxf). 2007;66: 827-832.
26. Sawin CT, Herman T, Molitch ME, London MH, Kramer SM, Aging and the thyroid. Decreased requirement for thyroid hormone in older hypothyroid patients. Am J Med. 1983,75:206 -209.
27. Razzak, MA. Effect of Age and Sex on Thyroid Function Tests Establishment of norms for the Egyptian Population in Developments in Radioimmunoassay and Related Procedures. International Atomic Energy Agency. 1992; 353-358.
28. Anderson S, Pederson KM, Bruun NH, Lauberg P. Narrow individuals variation in serum T4 and T3 in normal subjects: a clue to the understanding of sub clinical thyroid disease. J Clin Endocrinol Metab. 2002; 87:1068-1072.
29. Ogunkeye OO, Roluga AI, Khan FA. Resetting the detection level of cord blood thyroid stimulating hormone (TSH) for the diagnosis of congenital hypothyroidism. J Trop Pediatr. 2008; 54:74-77.
30. American Academy of Pediatrics. American Academy of Pediatrics AAP Section on Endocrinology and Committee on Genetics, and American Thyroid Association Committee on Public Health: newborn screening for congenital hypothyroidism: recommended guidelines. Pediatrics 1993; 91:1203-1209.