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Original Article

Biochemistry Section

Thyroid Hormone Reference Interval-Evidence from Alappuzha District, Kerala State

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ABSTRACT

Introduction: Thyroid diseases are one of the most common endocrine disorders not only in India, but worldwide. Most commonly done biochemical tests to diagnose thyroid disorders are Thyrotropin (TSH), thyroid hormones {free thyroxine (FT4) and free triiodothyronine (FT3)} in serum. The Reference Interval (RI) is influenced by a variety of factors like diet, genetics, iodine nutritional status and thyroid autoimmunity. Hence, the reference values of Thyroid function tests commonly used in the clinical laboratories are derived from data from the western population and may not be applicable for Indian population.

Aim: To establish RI for thyroid hormones in the coastal area of Alappuzha District in Kerala.

Materials and Methods: This community-based study included 228 adults in the age group 18-80 years. TSH, FT3, FT4, Thyroperoxidase antibody (TPOAb) and anti-thyroglobulin antibodies (TgAb) were analysed on an automated immunoassay system Access 2 Beckman Coulter using a direct chemiluminescence detection system. RIs were calculated according to International Federation of Clinical Chemistry (IFCC) recommendation using IBM SPSS version 20. for windows.

Results: RIs for serum TSH, FT4 and FT3 were calculated as 0.11-6.39mIU/L,0.58-4.66 ng/dl and 0.72-4.66 pg/ml. No significant age and gender difference in the RIs are noted.

Conclusion: In this study, RI established in the local reference population found to be different from those reported by previous studies conducted in other geographical areas.

Keywords: Free thyroxin, Free triodothyronin, Thyroid disorders

INTRODUCTION

About 42 million people in India suffer from thyroid diseases [1]. An early diagnosis and treatment warrants better management and outcome. Biochemical tests to assess and to diagnose thyroid disorders include TSH, T3, FT3, T4 and FT4, thyroglobulin [Tg]; Thyroperoxidase antibody (TPOAb), TSH receptor antibodies (TRAb) and anti Tg antibodies (TgAb). Measurement of TSH and thyroid hormones, FT4 and FT3 in serum is routinely done in most clinical biochemical laboratories [2]. During the last fifty years, laboratory evaluation of thyroid dysfunction has changed tremendously. Sensitivity and specificity of biochemical thyroid tests has improved greatly. The fifth generation assay systems have a sensitivity as low as < 0.004 mIU/L [3]. This has strongly influenced the clinical strategy for detecting and treating thyroid diseases.

TSH is the most sensitive marker for thyroid dysfunction especially for subclinical hypothyroidism. It also has an essential role in treating both hyperthyroidism and hypothyroidism for dose adjustment. Besides, it has a prognostic significance for tumor recurrence [4]. Clinical signs and symptoms of hyperthyroidism or hypothyroidism are often nonspecific and vague. Hence, estimation of thyroid hormones (total and free thyroxine, T4 and FT4; total and free triiodothyronine) is also important in the evaluation in thyroid dysfunction. For the diagnosis of autoimmune thyroid disease, Thyroperoxidase antibodies (TPOAb) and antithyroglobulin antibodies (Anti TG) are used. RI aids the clinician in interpreting observed values. The reference values of TFT used in clinical laboratories have been adopted from those reported for the western population. These RIs may not be applicable for Indian population since a variety of factors like diet; genetics and thyroid autoimmunity influence the thyroid hormone levels [5].

RIs for TFT are also influenced by iodine status. Iodine nutritional status either deficiency or excess, has a significant impact on the determination of RI of thyroid hormones. The programme of Universal Salt Iodisation (USI) programme was instituted in India in 1984 and various studies conducted in many parts of the country has reported iodine sufficiency [6,7]. RIs are also dependent on analytical quality parameters such as sensitivity, specificity, precision, and accuracy of the applied assay system.

RI may be established by either direct or an indirect method. In direct method, reference individuals are selected based on precisely defined criteria from a reference population according to Clinical and Laboratory Standards Institute/International Federation of Clinical Chemistry and Laboratory Medicine (CLSI/IFCC) recommendation [8]. In indirect method, instead of reference individuals, values with the required characteristics are selected from an existing database. Direct method is time-consuming and expensive and the most important step is the selection of the reference group and the standardisation of pre-analytical factors [9,10].

The studies conducted in Delhi and in Ranchi report a wider range for T4 and TSH [11,12] compared to that reported in another study from Ranchi [13]. Even though similar studies for establishment of RI was done in other parts of country, data from Kerala is found to be lacking. Thus, the aim of this study was to establish RI in a sample population from Alappuzha district in Kerala.

MATERIALS AND METHODS

This community-based cross-sectional study was conducted in selected wards of Ambalapuzha North Gramapanchayath of Alappuzha District in Kerala State, India from May 2017-August 2017. The study was approved (B3/1573(A)2010/TDMCA) by the local ethical committee.

According to CLSI C28-A guideline, 153 reference values were needed to establish a nonparametric RI with 99% confidence [14]. A total of 438 subjects were recruited to the study, after taking informed consents. A questionnaire was prepared according to the CLSI C28-A standard, for determining exclusion criteria and pre-analytic factors.

Inclusion criteria: Subjects from both sex in the age group of 18-80 years were included in the study.

Exclusion criteria: Subjects with history of thyroid disease, family history of thyroid disease, use of medications known to interfere thyroid function, visible palpable goiter and systemic illness were excluded from the study [15].

Thorough history and physical examination was done for each subject. As per recommendations from the National Health and Nutrition Examination Survey (NHANES) [14], the National Academy of Clinical Biochemistry (NACB) only euthyroid healthy volunteers were included, who were free from detectable autoantibodies against Thyroid Peroxidase (TPO Ab) or thyroglobulin (TgAb) [15,16]. Hence, final decision about a reference person was made with the help of laboratory findings.

Two wards, ward number 2 and 18 from Ambalappuzha North Grama panchayath were selected. Subjects according to the inclusion criteria were identified in each house of these wards with the help of ASHA workers. Instruction was given to the subjects to come for blood collection on specified date to the locally arranged blood collection camp at sub center of Ambalappuzha primary health center and panchayat auditorium.

Blood samples were collected from 7.00 to 9.00 am, after an overnight fasting, into vacutainer tubes. Centrifugation was performed within 1 hour of sample collection at 1500×g for 10 minutes and samples were analysed immediately. The measurements were made using the same reagents and the same instruments for all patients. Analyses of TSH, FT3, FT4, TPOAb, and TgAb were performed in an automated immunoassay system, Access 2 Beckman Coulter using a direct chemiluminescence detection system according to the manufacturer's instructions.

TPOAb and TgAb levels above the 9 and 4 IU/mL, respectively were regarded as positive according to the manufacturer's data. Assay imprecision was assessed by the use of commercial quality-control materials, Liquicheck levels 1-3 (Bio-Rad) Each control material was analysed in duplicate per run. Daily runs were performed for 5 days in a week over 3 weeks for a total of 30 replicates for each control.

STATISTICAL ANALYSIS

Data was analysed using IBM Statistical Package for the Social Sciences (SPSS) version 20. for windows. Outliers was identified by Tukey's method [17,18].Visual inspection of histogram, Q-Q plot, box plot and normality check using Shapiro-Wilkis test, it was found that FT3 and FT4 levels in the reference population was normally distributed, but TSH levels are having nonGaussian distribution even after log transformation of the data. Hence, according to International Federation of Clinical Chemistry (IFCC) recommendation, nonparametric methods were used for the analysis of RIs were expressed as median and 95% confidence interval. The levels of hormones were compared between males and females and among different age group by Mann-Whitney U test and by Kruskel–Wallis–H, respectively with a significant level for p-value as <0.05.

RESULTS

Out of 446 subjects, 129 were excluded due to the increased AntiTPO and AntiTG levels. From the remaining 317 subjects, data of 228 subjects were included for analysis after removal of outliers and excluding the data with other abnormal laboratory results like high blood glucose, abnormal liver and renal function tests and high lipid profile. There were 62 male and 166 females in the study population. The mean age was 43±12.35 years.The minimum age was 18 years and maximum age was 71 years [Table/Fig-1].

The levels of FT3, FT4 and TSH in the reference population are given in [Table/Fig-2]. None of the parameters showed Gaussian distribution even after the log transformation. Hence, nonparametric methods were used to establish the RI. The RI for sample population was determined as 2.5 percentile as lower limit and 97.5 percentile as upperlimit. A 90% confidence interval for both values was calculated by bootstrap method.

Age group	Male	Female	Total
Group 1-18-30 y	12	30	42
Group 2-31-45 y	20	58	78
Group 3->45 y	30	78	108

[Table/Fig-1]: Age and gender distribution.

Parameter	N	Lower limit-2.5 percentile (90% CI)	Non para-metric Median (IQR)	Upper limit-97.5 percentile	Manufac-ture's RI
FT3 (pg/mL)	228	0.72 (0.54-1.23)	3.11 (0.92)	4.76 (4.40-4.55)	2.5-3.9
FT4 (ng/dL)	217	0.58 (0.55-0.62)	0.83 (0.19)	4.66 (3.59-6.03)	0.6-1.1
TSH (mIU/L)	217	0.11 (0.21-0.37)	1.65 (1.94)	6.39 (5.00-7.37)	0.34-5.2

[Table/Fig-2]: Reference Interval (RI) for Thyroid Function Tests established in the present study.
IQR: Interquartile range; CI: Confidence interval; TSH: Thyrotropin; FT4: Free thyroxine; FT3: Free triiodothyronine

Few samples could not be analysed due to inadequate volume.

The levels of FT3, FT4 and TSH in males and females were compared by Mann-Whitney U test [Table/Fig-3]. No significant difference were observed for FT3 {U(Nmale=99.82, Nfemale= 109.52)=4037, Z=1.027, p=0.304}, FT4 {U(Nmale=0.931, Nfemale=109.23}= 6504, Z=0.087, p=0.931).

Parameter	Gender	n	Mean rank	U	Z	p-value
FT3 (pg/mL)	Male Female	69 159	99.82 109.52	4037	1.027	0.304
FT4 (ng/dL)	Male Female	60 157	108.4 109.23	6504	0.087	0.931
TSH (mIU/L)	Male Female	60 157	90.53 107.76	5704	1.883	0.060

[Table/Fig-3]: Thyroid parameters based on gender.
U: U test statistics (sum of mean ranks); Z: Z score (Standardised test statistics); TSH: Thyrotro-pin ; FT4: Free thyroxine; FT3: Free triiodothyronine

Krusker Wallis tests showed no significant difference among different age group [Table/Fig-4]. The tests results for FT3 shows X2 (2)=3.56 and p=0.168 with mean rank score of 96.84,11.85 and 103.76, for FT4, X2 (2)=3.067 and p=0.216 with mean rank score of 122.04,100.93 and 11.07 and for TSH X2 (2)=4.662 and p=0.097 with mean rank score of 99.76,103.68 and 92.51 in age group 1,2 and 3, respectively.

Parameter	Age group (Year)	N	Mean rank	Chi-square	p-value
FT3 (pg/mL)	18-30	38	96.84	3.563	0.168
	31-45	78	11.85		
	46-71	108	103.76		
FT4 (ng/dL)	18-30	40	122.04	3.067	0.216
	31-45	76	100.93		
	46-71	101	11.07		
TSH (mIU/L)	18-30	37	99.76	4.662	0.097
	31-45	78	103.68		
	46-71	102	92.51		

[Table/Fig-4]: Thyroid parameters in different age group.
TSH: Thyrotropin; FT4: Free thyroxine; FT3: Free triiodothyronine

DISCUSSION

The present study established a new RI for thyroid function test in a local reference population of Kerala, India. The new RI differs from that reported by the manufacturer. Even though the RI of TFT in adults are well-established, different studies report different values for FT4, FT3 and TSH which can be attributed mostly to the

Authors	Country	Published year	Method and manufacturer	Sample size	FT3 pg/mL	FT4 ng/dL	TSH mIU/mL
Marwaha RK et al., [11]	Delhi, India	2013	ECLIA Roche	1916	1.56-5.72	0.66-1.62	0.68-9.78
Arora RK et al., [12]	Jharkhand, India	2017	CLIA Abbot	153	1.18-3.79	0.54-1.48	0.80-9.78
Tannu K et al., [13]	Ranchi, India	2015	CLIA Abbot	3346	1.81-3.53	0.76-1.34	0.08-4.36
Simbita M et al., [19]	Gujarat, India	2018	ECLIA Roche	1000	----	-----	0.27-5.63
Jing C et al., [20]	China	2016	CLIA Siemens	717	2.34-3.73	0.72-1.33	0.43-5.51
Henry V et al., [21]	Germany	2005	ELIZA	3915	2.47-4.56	0.54-1.23	0.25-2.12
Imad RM et al., [22]	Sudan	2018	RIA	390	----	---	0.50-3.1
Bosa MA et al., [23]	Serbia	2017	ECLIA Roche	250	2.66-4.12	0.81-1.31	0.65-5.39
Raana A et ., [24]	Pakistan	2014	RIA	852	1.81-3.51	0.78-1.45	-----
Aydan CC et al., [25]	Turkey	2010	CLIA Beckman	619	2.62-3.84	0.61-1.06	0.41-4.25
Present Study	Kerala, India	2020	CLIA Beckman	228	0.72-4.76	0.58-4.66	0.11-6.39

[Table/Fig-5]: Reference Intervals (RI) of Thyroid hormones reported by various studies [11-13,19-25].

difference in assay techniques and reference population. RI reported by various investigators from other part of India and other countries are summarised in [Table/Fig-5] [11-13,19-25].

The lower limit of FT3 (0.72 pg/mL) was found to be very low when compared with other studies. Studies suggest that lower FT3 levels and hypothyroidism are observed in fluorosis endemic area [26-28]. Flouride has multiple mechanism to interfere the thyroid function including blocking the peripheral conversion of T4 to T3 [29]. Alappuzha district, where the study was undertaken, is a well-known fluoride endemic area in Kerala [30]. Iodine nutritional status and thyroid autoimmunity may be another possible explanation [31,32]. The present study did not observe any change in FT3, FT4 and TSH levels either across different age group and or among males and females. This may be explained by smaller number of samples in each strata. The same finding was reported by Simbita M et al., from Gujrat [19]. On the contrary, the study conducted by Tannu K et al., from Ranchi reveals variation in all the three hormones according to age and sex [13]. On the contrary, Marwaha RK et al., reported no age related changes for FT3 and FT4 levels and no age and gender related changes for TSH based on a large community based study in Delhi with more than 4000 subjects recruited after stringent exclusion criteria [11]. In this study, a comparatively wider range and lower value for the lower limit of RI was noticed for FT3, FT4 and TSH and no age and gender based differences were observed.

Limitation(s)

Sonologic evaluation of the thyroid gland was not done in the study population. Iodine status of the population also was not assessed. The possible interference by fluoride on thyroid function could have been ruled out by assessing serum fluoride level, since Alappuzha is a flourosis endemic area. Age and sex-specific RI could not be established due to inadequate sample size for each age strata.

CONCLUSION(S)

The present study was community based and established a RI for thyroid hormone in the local reference population selected based on the recommendation by the National Health and Nutrition Examination Survey. The RI established is found to be different from that reported by the manufacturer and studies reported from other part of the country. A large population based multicentric study should be done to establish age and gender specific RI in the state.

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