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International Journal of Clinical Biochemistry and Research

Journal homepage: https://www.ijcbr.in/

Original Research Article

A multi-centric study on establishing reference interval for TSH, TT4 and TT3 in Western India

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ARTICLE INFO

Article history:
Received 22-01-2022
Accepted 29-01-2022
Available online 11-03-2022

Keywords:
Western India
Multicentric sites
CLSI guideline EP 28 A3C
Reference interval
Reference limits
Partitioning

ABSTRACT

Direct sampling strategy was used to establish the reference interval, with a total of 425 healthy subjects who were screened medically and by lab tests to exclude cases of subclinical thyroid dysfunction. As per guidelines published by CLSI, non-parametric method was used with ranking of values to get the central 95th percentile as reference interval. The sample size and the need for partitioning gender-wise & age-wise were verified as per tests in CLSI guidelines.

Following conclusions were drawn from the results of the study. (a): The sample size was appropriate. (b): The difference between the study and kit insert reference limits were more at the lower limits for TSH and at the upper limits for TT4 and TT3. (c): The difference between the genders was statistically significant for TT4 indicating need to adopt gender-wise reference intervals. (d): Effect of age in the group of 18 to 30 years was significant for TT3; no significant effect of age on TSH and TT4 reference intervals. (e): The upper reference limits for TSH, TT4 and TT3 in the current study, are seen to be more than the international findings and more pronounced for TT4. (f): Based on the NACB's recommendation for lowering the cut-off of TSH to 2.5 mIU/L, it was observed that there was a significantly high proportion in population with values of TSH more than 2.5 mIU/L.

1. Introduction

Ethnicity, iodine - intake and analytical methods underline the need for redefining the TSH reference interval in central laboratories in different countries.[1] Adopting a manufacturer's reference interval for thyroid tests may not be conducive to correct diagnosis and interpretation,[2] especially for subclinical thyroid disease.[3] Typically, laboratorians adopt reference intervals of manufacturers, neither establishing the intervals themselves nor even verifying the applicability of those intervals to their patients.[4] There are only few studies published on reference ranges for Indian population. Age may play an important role in reference intervals as in TSH increasing with age.[5]

2. Aims and Objectives

1. To establish reference interval for western-India population through multi-centric sites.
2. To examine requirement of partitioning of the established range gender-wise / age-wise based on statistical tests recommended in CLSI EP28 A3C.
3. To compare the obtained reference range with manufacturer's kit insert and also compare with published studies in India.

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https://doi.org/10.18231/j.ijcbr.2022.009

4. To examine proportion of population studies with values of TSH higher than 2.5mIU/L, which seem to indicate non-thyroidal abnormalities.

3. Materials and Methods

The study was approved by the ethical committee of the participating hospitals after obtaining informed consent from the participating research participants and from adult patients from whom blood samples were withdrawn. The references numbers are as follows:

Samples were collected from three different institutes in western India as shown below for the period of study between months of November 2019 to March 2020.

Sample size table for the multi-centric study

Location	Total Samples accepted
Sun Pathology Laboratory and Research Institute, Ahmedabad	197
Dr L.H. Hiranandani Hospital, Mumbai	156
Ruby Hall Clinic, Pune	72
Total	425

3.1. Sample size verification

Test for sample size (N = 425), to see if it was enough to derive the reference interval (RI), was done based on the data obtained, as cited in CLSI guidelines[6] applying the criteria recommended by Harris and Boyd i.e. Range of 90% Confidence interval (CI) at each Reference limit (RI) divided by the central 95th percentile RI should be less than 0.2. As can be seen from Table 3 in results section, sample size taken for this study has been verified to be adequate.

3.2. Method of recruitment

This study used direct sampling strategy wherein, healthy reference individuals were selected randomly by questionnaire. Healthy subjects were recruited in the age group 18 to 65 years, after taking informed consent. Thorough physical examination was done and exclusion criteria were applied as mentioned in table below. Blood samples were collected from selected subjects who were fasting for minimum 8 hours and serum separated, aliquots were prepared and kept frozen till study.

Direct selection of reference individuals is the only method that agrees more with the concept of reference values as recommended by the IFCC.[3] Care was taken to exclude cases of subclinical thyroid dysfunction by way excluding individuals positive for of anti-TPO test. Generic exclusion criteria were followed for selection of subjects as per CLSI EP23.

The following exclusion criteria were used for serum sample selection:

1. Exclude samples that do not have sufficient volume to complete testing for study
2. Exclude grossly Hemolyzed, Icteric or Turbid samples as indicated by HIT values from the instrument or samples with visible particulates that are not removed after centrifugation.
3. Exclude samples which have been subjected to more than one freeze thaw cycles.
4. Fresh samples stored for longer than 24 hours at 2-8°C.
5. Abnormal results on anti-TPO test

The information in Figure 1 provides the gender-wise and age-wise distribution of samples selected. Applicability of partitioning was examined gender-wise and age-wise.

The method most often recommended for establishing reference intervals is the non-parametric approach, as mentioned in CLSI guideline EP 28 A3C, For both analytes, a non-parametric method was applied to estimate the indirect reference intervals. The 90% confidence intervals for lower and upper limits were calculated according to the recommendations of the CLSI.[6]

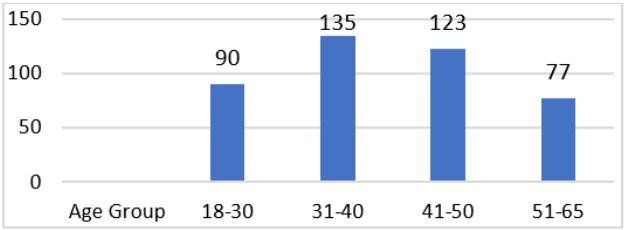
Analysis of samples was done on VITROS immunoassay based on enhanced chemiluminescence platform. The samples were collected in closed blood collection system using vacuum tubes with gel separators. Before analyzing the data, the calibration accuracies and QC data were examined and accepted. The following analyses were done on the reference interval obtained.

1. The data obtained in this study was subjected to the Box and Whiskers plot for the three hormone markers to study the nature of the distribution and the character of the outliers.
2. The requirement of partitioning between genders was examined by applying the following tests:
   (a) Test prescribed by Sullivan et al, cited in CLSI guidelines[6]
   (b) Two-tailed Z score test – test prescribed by Harris & Boyd as cited in CLSI guidelines, using an online calculator at www.statskingdom.com.
   (c) Statistical p-value as cited in CLSI as well as in publications
   (d) Ratio of standard deviations in sub-classes as cited in CLSI
3. New method of Lahti et al based on proportions of outliers of the sub-classes in the total group[7] Subsets of age groups studied for significant difference in mean value by applying the CLSI recommended Harris Boyd method and p value in order to evaluate need for partitioning by age groups for TT3, TT4 and TSH.
4. Since reference intervals are known to vary between different populations and races, the obtained reference

interval was compared with those published from different parts of India.

4. Results

Figure 1 shows that maximum number of patients were between the age 31 to 50.



*Males: 206; Females: 219.

Fig. 1: Age-wise distribution

Figures 2, 3 and 4 showing the box and whiskers plot of values of the derived reference range indicate that there were more outliers above the upper reference limit for TSH, TT3 and TT4.

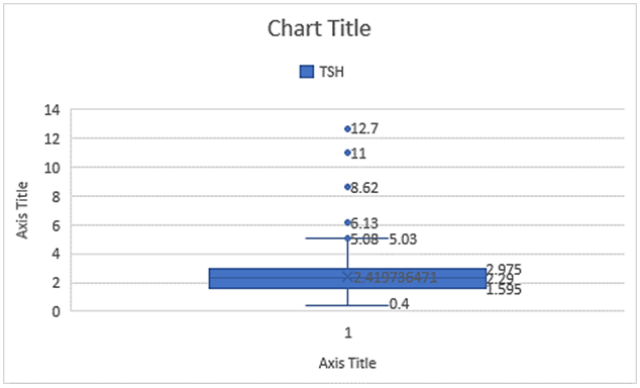


Fig. 2: Box and whiskers plot for TSH

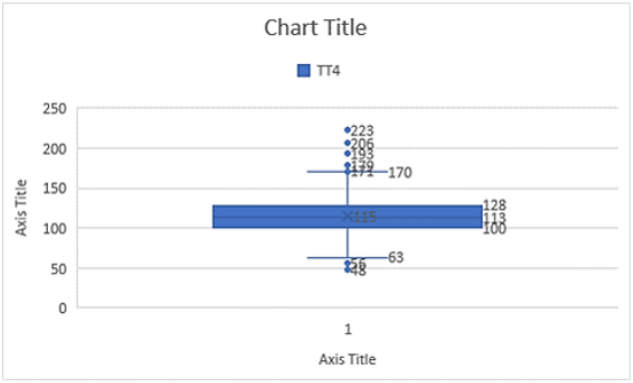


Fig. 3: Box and whiskers plot for TT4

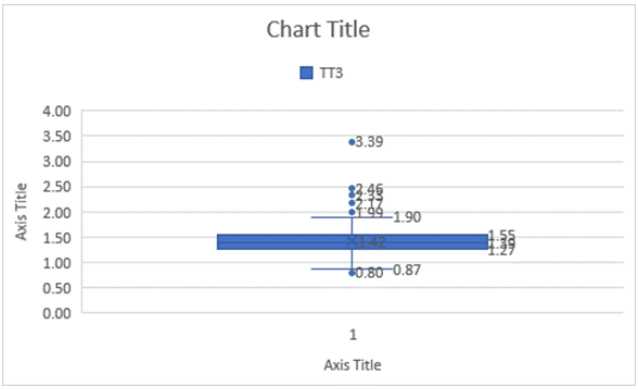


Fig. 4: Box and whiskers plot for TT3

Figure 5 shows the distribution of the values of TSH, TT4 and TT3. It can be seen that while there is near normal distribution for TT4 and TT3, it is skewed towards upper side for TSH.

Table 1 compares the reference range obtained in the current study versus manufacturer's kit insert.

Table 2 compares the lower and upper limits of the reference limits between the current study and manufacturer's kit insert and provides 90% confidence interval in the current study, for the lower and upper limits as required to be mentioned by the CLSI guidelines.

Table 3 shows the results of verification of the sample size considered in the current study for all three hormones, as per CLSI guidelines.

Table 4 shows the result of portioning test considered for gender, as per CLSI guidelines. The p-value for gender-wise difference in TT4 only is significant with $p = 0.00025$ ($p<0.05$). As per Harris & Boyd two-tailed Z score test, the gender differences are statistically significant only for TT4.

Table 5 shows result of partitioning gender-wise based on outliers as per Lahti A et al[7] cited in CLSI guidelines. It can be seen that partitioning is required due to the outlier test failing (result > 4.1) for the lower limit of TT4 in males only. This serves to confirm the findings by a different method as seen in previous Table 4.

Table 6 shows the reference limits after partitioning gender-wise separately for males and females with 90% confidence intervals of lower and upper limits.

Table 7 and Table 8 show the results of tests for partitioning age-wise for the three hormones. It can be seen that age-wise partitioning is not required for TSH and TT4 but TT3 shows significant difference between age group 18-30 versus other age group slabs.

Table 7 compares the findings in the current study on reference intervals in western Indian population with other Indian population studies for the thyroid hormones.

Table 1: Central 95^{th} percentile based reference intervals (RI) for total population studied

Assay / test (Mean value)	Current Study (Total N= 425) Reference limits		Manufacturer (package insert) (Total N= 512)
	As per Box and whiskers plot	As per CLSI, 95th percentile ranking method	
TSH (2.35 mIU/l)	0.4 mIU/mL - 5.08 mIU/L	0.675 – 4.80 mIU/L	0.465 – 4.68 mIU/L
TT4 (115 nmol/L)	63 - 173 nmol/L	76 – 167 nmol/L	71.2 – 141 nmol/L
TT3 (1.41 ng/mL)	0.87 - 1.90 ng/mL	1.07 – 1.85 ng/mL	0.97 – 1.69 ng/mL

Table 2: Central 95^{th} percentile reference limits with 90% confidence interval in the current study

Sample position	Values as per current study (90% Confidence interval) (CI)			Package insert values of manufacturer of kit used in the study		
	TSH mIU/L	TT4 nmol/L	TT3 ng/mL	TSH mIU/L	TT4 nmol/L	TT3 ng/mL
Median	2.29	113	1.39		Not given	
2.5th Percentile (lower)	0.675 (0.527 – 0.82)	76 (67 – 80)	1.07 (0.97 – 1.10)	0.465	71.2	0.97
97.5th percentile (higher)	4.80 (4.59 – 5.4)	167 (161 – 179)	1.85 (1.82 – 1.99)	4.68	141	1.69

Table 3: Test for sample size (N) as per CLSI guidelines

Formula and target: CI / RI should be less than 0.2 for each of the Reference limit			
	TSH	**TT4**	**TT3**
At 2.5th lower limit of RI	0.071	0.143	0.1667
At 97.5th upper limit of RI	0.196	0.1978	0.2179
Result for appropriate N	PASS	PASS	Lower limit -PASS

CI/RI values derived from Table 2 and Table 1

Table 4: Test for partitioning gender-wise

Assay	Difference between two sub-populations (Males and females) as per CLSI vis-à-vis targets			Mean in Male population (mIU/L)	Mean in female population (nmol/L)	Observed difference in this study (ng/mL)	P value Target <0.05
	Sinton et al: Mean difference Target: <25% of RI	Harris & Boyd - Z score (Target <3)	Ratio of SD (target >1.5)				
TSH	0.06/4.125 =1.4%	0.697	1.0	2.32	2.38	0.06 (2.5%)	0.486
TT4	7/91=7.6%	3.654*	1.1	111	118	7 (6%)	0.00025*
TT3	0.02/0.78=2.5%	1.184	1.0	1.42	1.40	0.02 (1.4%)	0.236

*Note: Highlighted value indicates requirement ofpartitioning.

Table 5: Test for partitioning gender-wise for non-parametric distribution using ratio of outliers for males and females in total group[7]

Marker Assay	% outliers below Lower limit of RI Males/Females		% outliers above higher limit of RI Males/Females		Remarks Targets - < 0.9 and > 4.1 for partitioning
TSH	4.08	1.44	2.04	2.89	No Partitioning required
TT4	4.59	0.96	1.53	3.38	Partitioning required
TT3	2.55	2.90	2.04	3.38	No Partitioning required

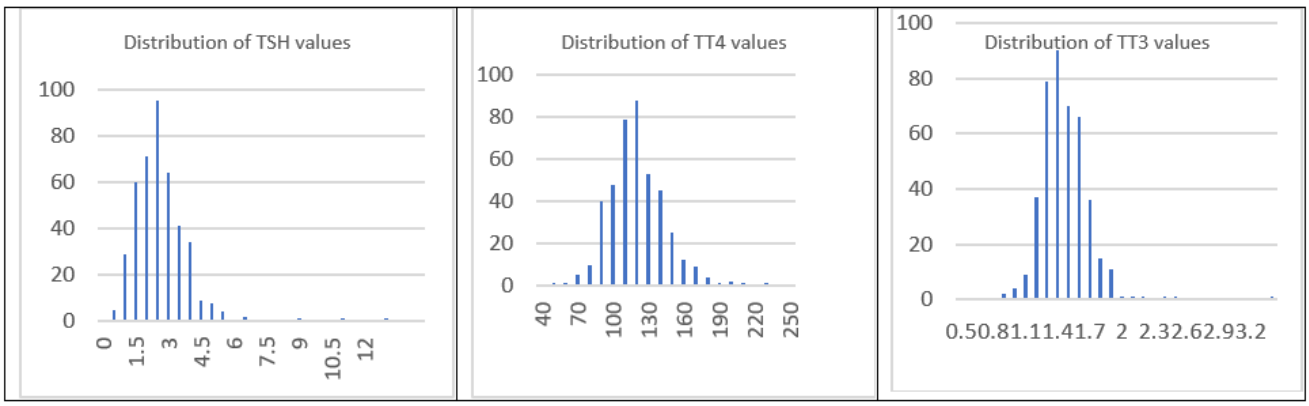


Fig. 5:

Table 6: Central 95^{th} percentile Reference range gender-wise for all three markers – Sample Size = N

Marker Assay	Males N = 206 (90% CL) [7]	Females N = 219	Remarks to use gender-wise Reference Interval (RI)
TSH	0.527 - 4.72 mIU/L (0.46 - 0.81) (4.09 – 6.13)	0.86 4.81mIU/L (0.66 -0.94) (4.47 – 11)	Applicable as per user opinion for lower limits due to border line score in partitioning test of Lahti et al
TT4	71 - 167 nmol/L (63 – 76) (154 – 193)	84 - 170 nmol/L (67 – 86) (161 – 206)	Applicable as per some tests for partitioning
TT3	1.1 - 1.84 ng/mL (0.96 - 1.13) (1.76 - 2.33)	1.07 - 1.86 ng/mL (0.91 - 1.11) (1.82 - 2.03)	Not applicable as per partitioning tests

Table 7: Mean, median and SD values of age subgroups exclusive of outliers

Age subgroup in years (sample size)	TSH(mIU/L) All age reference range 0.675 – 4.8				TT4 (nmol/L) All Age reference range 76 – 167				TT3 (ng/mL) All Age reference range 1.07-1.85			
	Range	Mean	Median	SD	Range	Mean	Median	SD	Range	Mean	Median	SD
18-30 (90)	0.86-4.59	2.17	2.12	0.82	81-162.2	115.4	112.0	19.08	1.19-2.03	1.484	1.485	0.18
31-40 (135)	0.8 – 5.08	2.29	2.25	0.92	72.9-167	114.5	113.5	19.71	1.07 to 1.84	1.41	1.39	0.17
41-50 (123)	0.675-4.74	2.56	2.45	0.91	79.4 – 162.2	114.8	114.0	17.71	1.05 – 1.82	1.39	1.38	0.15
51-65(77)	0.63-4.8	2.34	2.40	1.00	71.4-171.2	114.1	109.7	23.49	1.02-1.82	1.36	1.35	0.19
41-65(200)	0.63 to 4.8	2.47	2.42	0.96	71.4 to 171.2	114.4	112.4	20.13	0.97 to 1.82	1.38	1.37	0.17
31-65 (TT3 only)	0.66 – 4.85	2.40	2.3	0.94	71.4 - 167.3	114.4	113	19.87	1.02 – 1.83	1.39	1.38	0.17

5. Discussion

5.1. Reference interval

As can be seen from Figures 1, 2 and 3 the range of values in the current study after removing outliers as in the Box and whiskers plot differ from the central 95^{th} percentile method (Table 1). The difference between the study and kit insert reference limits were more at the lower range for TSH and at the upper limits for TT4 and TT3.

On examining the outliers in this study for TSH it was seen to up to 12.7 mIU/L of TSH (Figure 2), for TT4 values up to 48 on lower side and 223 on the higher side (Figure 3) and for TT3, values up to 0.80 ng/mL on lower side and up to 3.39 ng/mL on the higher side (Figure 4). It is worth mentioning that the outliers for TSH and TT4 were more at the lower end of the reference interval in males as compared to females, after partitioning for gender. Ioannis Legakis et al[10] postulated that women with either anti-

Table 8: Evaluation of age subgroups to determine need for partitioning by age group (derived from Table 7)

Age subgroups	TSH		TT4		TT3	
	Harris & Boyd two-tailed Z score	P value	Harris & Boyd two-tailed Z score	P value	Harris & Boyd two-tailed Z score	P value
18-30 Vs 31-40	1.12	0.264	0.34	0.73	2.89	0.004**
18-30 Vs 41-50	3.30**	0.0007**	0.23	0.82	3.80**	0.0001**
18-30 Vs 51-65	1.26	0.206	0.39	0.70	4.16**	0.00003**
31-40 Vs 41-50	2.36	0.018	0.13	0.89	1.008	0.313
31-40 Vs 51-65	0.35	0.719	0.13	0.90	1.94	0.052
41-50 Vs 51-65	1.57	0.117	0.22	0.82	1.19	0.234
18-40 Vs 41-65	2.70	0.007	0.141	0.89	3.63**	0.0003**
18-30 Vs 31-65	2.29	0.022	0.437	0.66	4.26**	0.00002**

Table 9: Review of reference range studies in India

Author	Region	TSH range	TT4 range	TT3	No of subjects	Method used
Marwaha S et al [8]	Gujarat	Males: (0.37 -5.19) mIU/L, Females: (0.27-5.63) mIU/L	Males: 57.35 - 143.81 nmol/L Females: 62.46-158.52 nmol/L	Males: 1.15-2.55 ng/mL, Females: 1.07-2.55 ng/mL	1000	Retrospective study using electro-chemiluminescence
Bose A et al [9]	Indore	0.35-4.94 mIU/L	4.5-12.6 μg/dl (58 – 162 nmol/L)	60-181 ng/dl (0.6 – 1.81 ng/mL)	28677 OPD patients	Retrospective study using Abbott Architect chemiluminescence
Current Study	Western India	0.675 – 4.80 mIU/L Males: 0.527- 4.72 mIU /L Females: 0.86 - 4.81mIU/L	76 – 167 nmol/L Males: 71 - 167 nmol/L Females: 84 - 170 nmol/L	1.07 – 1.85 ng/mL Males: 1.1 - 1.84 ng/mL Females: 1.07 - 1.86 ng/mL	425 medically screened healthy individuals	Prospective study using VITROS® enhanced chemiluminescence

TPO or anti-Tg showed higher levels of TSH. In this study while samples which were anti-TPO positive was excluded, possibility of inclusion of women with anti-Tg cannot be ruled out. E.Arseneau and C.M. Balion have opined that outliers may represent a natural variability within a given group of individuals, more so in the elderly. [11]

Table 2 shows the reference limits with their 90% confidence limits. This ensures practical applicability of the given reference range for clinical use, based on statistical probability for trueness.

The test for sample size as per CLSI (Table 3) shows that the sample size (N = 425) in the present study was appropriate. It can also be noticed from Table 3 that there is a wide gap between the passing ratio of lower and upper limits of reference limits for TSH, as compared to TT3 and TT4, which probably indicates that the sample size is critical for ensuring reliable upper limit of TSH in the reference interval.

5.2. Need for partitioning gender-wise

The tests for partitioning in current study indicate the need to partition the obtained reference interval gender-wise for TT4 only while there is no need for gender-wise partitioning for TSH and TT3 assays. Sedat et al [1] found gender bias in females which was higher at the median for TSH, which was statistically different with $p<0.05$. Camilla SJ et al in their study, [12] after excluding TPOAb-positive subjects and outliers, in a reference population of 511 subjects, found no statistically significant gender- or age-specific differences in mean TSH and reference intervals. Hubl W et al [13] found no difference in TSH and TT3 between the genders but that significant difference in TT4. These findings [12,14] agree well with the findings of the current study.

6. Limitation of the Study

In 2005, as cited by B Biondi, [14] the National Academy of Clinical Biochemistry (NACB) recommended lowering of the upper limit for TSH to 2.5 mIU/L based on a large

epidemiological survey, which included ultrasonography. However, ultrasonography could not be included in this study and is probably a limitation in this study.

6.1. Proportion of population above NACB recommended cut-off

It has been proposed by Jee-young Oh et al[15] that population with TSH above 2.5 units should be investigated for metabolic syndrome even in a normal healthy population. Interestingly, as if to support these facts, data indicating that African-Americans with very low incidence of Hashimoto thyroiditis have a mean TSH level of 1.18 mIU/liter[4] whereas the mean TSH was 2.35mIU/L (Table 1) in current study.

Jee-young Oh et al.[15] observed in their study that the high-TSH group (>2.5 mIU/L) represented 16.4% of the normal female population, whereas in the current study, the incidence was 37%, which is significantly higher. This higher incidence in the current study can have profound significance as Jee-young Oh et al.[15] found prevalence of metabolic syndrome significantly higher in the high-TSH group Vs low-TSH group (7.5% vs. 4.8%, p = 0.016) with a 2-fold greater risk of metabolic syndrome than subjects in the low-TSH group after adjusting for age and BMI (odds ratio, 1.9; 95% confidence interval, 1.1 to 3.2).

However, this should be viewed with respect to the observation by Brabant G et al[16] that it is still recommended to maintain the TSH reference interval of 0.4–4.0 mU/l. Classifying subjects with a TSH value between 2 and 4 mU/l as abnormal, as well as intervening with thyroxine treatment in such subjects, is probably doing more harm than good. In fact, in the current study, the median itself was 2.29mIU/L, very near the upper limit for TSH suggested by NACB (Table 2).

Waise A and Price HC[17] caution that the upper limit of the reference range for thyroid-stimulating hormone should not be confused with a cut-off to define subclinical hypothyroidism, especially in the light of the NACB's recommendations cited earlier. It is opined that reducing the upper limit of the TSH reference range would result in an increase in the proportion of the population diagnosed with subclinical hypothyroidism, frequently including those with non-specific symptoms probably unrelated to thyroid disease.

6.2. Effect of age on reference interval

The magnitude of difference in reference intervals between age groups 18-40 and 41-65 years for TSH was high but not statistically significant to warrant partitioning, though there was slight increase in TSH mean and median as age increased (Table 7 and Table 8). The range of TT3 in age group 18-30 years differed significantly from other slabs of age groups and was higher than the consolidated group of 31 – 65 years (Tables 7 and 8). This may warrant considering a separate reference interval for TT3 in age group 18 – 30 years of age as per this study to be clinically relevant.

Review of literature provides mixed evidence on this. Increasing TSH on ageing was observed for both men and women in study by Salman Rizvi et al[18], wherein they found the lowest TSH levels in middle-aged individuals, with higher values in younger and older age groups. Kratszch et al[19] reported that age was independently and inversely associated with TSH, when considering <40 and more than 40 years. Similarly, Hoogendoorn et al,[20] reported that serum TSH decreases gradually with age, whereas serum free T4 increases with age in individuals older than 60 years. In larger age- groups, Fontes et al[5] found that there was significant difference in TSH between two subset of population, i.e. 20 to 60 years of age 60 to 80 years of age and 80 years or more (0.4 - 6.7 mU/L).

However, studies have demonstrated that age-specific reference intervals for TSH in healthy adults may have minimal impact in the assessment and management of thyroid disorders.[21]

6.3. Comparison with other published studies

Based on the fact that TSH values vary based on ethnicities, it is important to consider studies within a country. Interestingly, even such studies within a country, show variation in recommended reference intervals as found in a Danish study.[4]

Two studies in India could be accessed for review of reference intervals of thyroid hormones.[8,9] The findings in current study (Table 9) which includes population in Gujarat, Mumbai and Pune (the western region) match TSH reference interval upper limit only with the two studies. The reference interval for TT4 in the current study is different and higher than seen in Gujarat and Indore (Table 9). However, there is a similarity in the fact that reference interval for TT4 is higher in females than males as seen in this study and Gujarat study[8] and hence worthwhile considering establishing separate reference intervals gender-wise for TT4 in western India. In the current study TT3 had narrower range vis-à-vis other studies.

Marwah S et al[8] and Bose et al[9] found that the value of serum Total T3 & serum Total T4 was higher as compared to the published international values while that of serum TSH was similar. These findings are in agreement with current study, when compared with manufacturer's (international) reference interval for TT4 and TT3 (Table 2).

7. Conclusions

A total of 425 samples used in this study was just appropriate to develop a clinically relevant reference interval which passed the statistical test for sample size, for

the thyroid harmones.

It was found that this reference interval differed from manufacturer's kit insert (USA) especially on the lower limit for TSH and upper limits for TT4 and TT3; this finding is in agreement with two other studies published in western / central India.

Tests for partitioning was applied gender-wise and age-slab wise. Use of separate reference intervals for males and females is recommended for TT4 only among the three hormones tested, as the difference between the genders was statistically significant. While different age groups did not impact TSH and TT4 reference interval, the reference interval for TT3 in age group of 18 to 30 years was found to be significantly different from others.

The NACB recommended lowering the cut-off of TSH to 2.5 mIU/L based on a large scale epidemiological survey. In fact, it is reported that high proportion of values of TSH beyond 2.5 mIU/L in a population indicates higher prevalence of metabolic syndrome. In light of these facts, proportion of subjects above 2.5 mIU/L was examined in the current study. The results showed a significantly high proportions of 37% and 39% in female and male populations with values of TSH more than 2.5 mIU/L and the median itself was 2.29 mIU/L, very near the upper limit for TSH suggested by NACB.

It is seen that while the reference ranges in current study differ from published studies from western India, they match for the upper limits of TSH. The upper reference limits for the three thyroid markers, in the Indian studies including the current study, are seen to be more than the international findings/ manufacturer's kit insert, more pronounced for TT4. Hence, each lab in India using international kits for thyroid markers, viz. TSH, TT4 and TT3, would do well to establish their own reference limits. While manufacturers may not provide gender-wise reference intervals, it is important to establish gender-wise reference intervals especially for TT4 in Western India.

8. Source of Funding

None.

9. Conflict of Interest

The authors declare no conflict of interest.

References

1. Motor S, Erden G, Sahillioğlu B, Erdoğan S, Yıldırımkaya MM. Direct versus indirect strategies for thyroid hormone reference intervals established in a middle-aged and elderly population on an immunoassay analyzer. *J Clin Exp Investig*. 2010;1(3):161–7.
2. Thienpont LM, Uytfanghe KV, DeGrande LAC, Reynders D, Das B, Faix JD, et al. Harmonization of Serum Thyroid-Stimulating Hormone Measurements Paves the Way for the Adoption of a More Uniform Reference Interval. *Clin Chem*. 2017;63(7):1248–60.
3. Horowitz GL. Reference Intervals: Practical Aspects. *EJIFCC*. 2008;19(2):95–105.
4. Wartofsky L, Dickey RA. The evidence for a narrower thyrotropin reference range is compelling. *J Clin Endocrinol Metab*. 2005;90(9):5483–8.
5. Fontes R, Coeli CR, Aguiar F, Vaisman M. Reference interval of thyroid stimulating hormone and free thyroxine in a reference population over 60 years old and in very old subjects (over 80 years): comparison to young subjects. *Thyroid Res*. 2013;6(1):13.
6. Clinical and Laboratory Standards Institute (CLSI). EP 28-A3c – Defining, Establishing and Verifying Reference Intervals in the Clinical Laboratory; Approved Guideline – Third Edition; 2010. Available from: https://clsi.org/media/1421/ep28a3c_sample.pdf.
7. Lahti A. Are the common reference intervals truly common? Case studies on stratifying biochemical reference data by countries using two partitioning methods. *Scand J Clin Lab Invest*. 2004;64(4):407–30.
8. Marwah S, Mehta M, Raval C, Trivedi A, Haridas N. Establishment of reference intervals of thyroid function test in healthy individuals. *Int J Clin Biochem Res*. 2018;5(2):257–62.
9. Bose A, Sharma N, Hemvani N, Chitnis SD. Hospital Based Prevalence Study on Thyroid Disorders in Malwa region of Central India. *Int J Curr Microbiol App Sci*. 2015;4(6):604–11.
10. Legakis I, Manousaki M, Detsi S, Nikita D. Thyroid Function and Prevalence of Anti-Thyroperoxidase (TPO) and Anti-Thyroglobulin (Tg) Antibodies in Outpatients Hospital Setting in an Area with Sufficient Iodine Intake: Influences of Age and Sex. *Acta Med Iran*. 2013;51(1):25–34.
11. Arseneau E, Balion CM. Statistical methods used in the calculation of geriatric reference intervals: a systematic review. *Clin Chem Lab Med*. 2016;54(3):377–88.
12. Schalin-Jäntti C, Tanner P, Välimäki MJ, Hämäläinen E. Serum TSH reference interval in healthy Finnish adults using the Abbott Architect. *Analyzer Scand J Clin Lab Invest*. 2000;71(4):344–9.
13. Hubl W, Schmieder J, Gladrow E, Demant T. Reference Intervals for Thyroid Hormones on the Architect Analyser lin. *Chem Lab Med*. 2002;40(2):165–6.
14. Biondi B. The Normal TSH Reference Range: What Has Changed in the Last Decade? Editorial. *Clin Endocrinol Metab*. 2013;98(9):3584–7.
15. Oh JY. Elevated thyroid stimulating hormone levels are associated with metabolic syndrome in euthyroid young women. *Korean J Intern Med*. 2013;28(2):180–186.
16. Brabant G, Beck-Peccoz B, Jarzab P, Laurberg P, Orgiazzi J, Szabolcs I, et al. Is there a need to redefine the upper normal limit of TSH? *Eur J Endocrinol*. 2006;154(5):633–7.
17. Waise A, Price HC. The upper limit of the reference range for thyroid-stimulating hormone should not be confused with a cut-off to define subclinical hypothyroidism. *Ann Clin Biochem*. 2009;46(Pt 2):93–8.
18. Razvi S, Bhana S, Mrabeti S. Sindeep Bhana, and Sanaa Mrabeti Challenges in Interpreting Thyroid Stimulating Hormone Results in the Diagnosis of Thyroid Dysfunction. *J Tyroid Res*. 2019;2019:8. doi:10.1155/2019/4106816.
19. Kratzsch J, Fiedler GM, Leichtle A, Brügel M, Buchbinder S, Otto L, et al. New reference intervals for thyrotropin and thyroid hormones based on National Academy of Clinical Biochemistry criteria and regular ultrasonography of the thyroid. *Clin Chem*. 2005;51(8):1480–6.
20. Hoogendoorn EH, Hermus AR, Vegt FD, Ross HA, Verbeek A, Kiemeney L, et al. Thyroid function and prevalence of anti-thyroperoxidase antibodies in a population with borderline sufficient iodine intake: influences of age and sex. *Clin Chem*. 2006;52(1):104–11.
21. Kahapola-Arachchige KM, Hadlow N, Wardrop R, Lim EM, Walsh JP, Age-Specific.

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Cite this article: Shetty SS, Munshi NK, Patil AA, Joshi MD, Joshi PM. A multi-centric study on establishing reference interval for TSH, TT4 and TT3 in Western India. *Int J Clin Biochem Res* 2022;9(1):44-52.