Original Research Paper

Volume-9 | Issue-6 | June-2019 | PRINT ISSN No. 2249 - 555X

Biochemistry

REFERENCE INTERVAL FOR THYROID STIMULATING HORMONE ESTIMATED BY INDIRECT METHOD FROM ADULTS ATTENDING A TERTIARY CARE CENTRE IN KERALA

Dr. Asha K. Varghese Department Of Biochemistry, Government Medical College, Ernakulam

Dr. Shibu T. S.* Department Of Biochemistry, Government Medical College, Thrissur *Corresponding Author

ABSTRACT In the current study, we have taken one of the most frequently ordered biochemical parameter – TSH level estimation among adults, to check whether the provided kit insert values are applicable for our set of patient population, and also, unlike in earlier available data, if there are any gender differences in same. TSH values were determined in random blood samples from 5709 patients.Non-parametric method was used, along with suitable statistical tools. The reference interval was obtained for the entire data, then for males and females separately, and later compared with the kit insert values. Statistical tests showed inequality between the sub-groups and hence were analysed separately. The RI obtained was 0.322-9.09 μIU/L for the whole data, 0.4475-0.75625 μIU/L for males and 0.31105-9.28 μIU/L for females. The RI differs from kit insert value in upper reference limit and significant gender differences were found in both upper and lower reference limits. The emerging trend calls more extensive studies for resetting our population-specific RI for TSH, comprehensive studies to determine causes for the changing scenario and send the message across to the treating physicians, and to the general population at large.

KEYWORDS : Reference interval, indirect method, adult TSH values from in Kerala

1. INTRODUCTION

Health is a relative concept. International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) is the organisation which provide a forum for standardisation and traceability, to enhance scientific level and quality of diagnosis and therapy for patients. They have defined an individual selected for comparison using a defined criterion as the *reference individual*. *Reference value* is the value obtained by observation or measurement of a quantity on a reference individual. *Reference interval* for many laboratory tests is defined by threshold values between which the test results of a specified percentage (usually 95%) of apparently healthy individuals would fall. Reference individuals are selected by diffferent methods - direct or indirect, a priori or a posteriori, and random or non-random methods:

1.1.Direct and Indirect methods:

In direct method, individuals are selected from a parent population using defined criteria. In indirect method, individuals are not considered, but certain statistical methods are applied to the analytic values in a laboratory database to obtain estimates with specified characteristics.

1.2.A Priori and a posteriori:

A priori and a posteriori are used in direct method. A priori is when individuals are selected for specimen collection and analysis if they fulfil defined inclusion criteria. A posteriori is using an already existing database containing both analysis results and information on a large number of individuals and those fulfilling defined inclusion criteria are selected.

1.3. Random and non-random methods:

The process of selection giving each item (individual or test result) an equal chance of being chosen is termed random method. The process of selection giving each item an unequal chance of being chosen is non-random method.

Among the different methods used to estimate the reference interval, the simplest to estimate, more commonly used and that recommended by IFCC is interpercentile interval. Interpercentile interval can be calculated by parametric or non-parametric methods. Parametric method assumes a certain type of distribution and is based on estimates of population parameters, such as mean and standard deviation. Non-parametric method makes no assumptions concerning the type of distribution and does not use estimates of distribution parameters. The non-parametric method is recommended by IFCC and CLSI. When results obtained using proper application of any of the two methods are compared, it is found that the estimates of the percentiles are very similar.

Advantages of using reference interval for test result interpretation include its simplicity to use and understand. Once obtained, these are easy to store and retrieve from laboratory database and pocket notebooks. It has high degree of acceptance by the medical community through long use.

Reference interval do have certain drawbacks. If the reference population used for derivation is different from the target population, it gives misleading information of the individual's status. This can be overcome by estimating the reference interval for each population under test and by stratifying the population based on the already known variables.

Serum TSH level estimation remain the single best test for assessing thyroid function. Diurnal variations in TSH level is minimal. The hormone secretion varies with age and stays fairly constant beyond 20 years of age. It is influenced by stress, malnutrition, pregnancy and use of exogeneous thyroxine. There are no documented significant differences in the values among both genders in adults.

Reference interval used routinely in most laboratories is the ones provided in the test reagent kit insert provided by the manufacturer. It has been observed that despite satisfying recommended internal and external quality control protocols, the laboratory TSH results fail to correlate with the clinical presentation of the patient. The scenario prompted the investigators to determine the reference interval in our reference individuals to address this discrepancy in a commonly used analyte.

2. Procedure :

Objectives:

1. To determine reference interval for TSH estimation among adult population at a tertiary care centre from laboratory data
2. To determine differences in TSH reference interval between males and females

Study population: Patients coming to Government Medical College Thrissur

Inclusion criteria: All patients above age of 20 years who had given blood for TSH estimation

Exclusion criteria:

- TSH values <0.005μIU/L and >100μIU/L
- Incomplete documentation
- Age less than 20 years

Period of study: 01.01.2016 to 31.03.2016

Study design: Record based cross-sectional study.

The sample size was calculated from a study done among adult population in New Delhi for estimation of reference interval for TSH and was found to be 114.

In the pilot study done with our hospital database, 0.1% outliers were found (TSH values outside the range of 0.05-100μIU/L). Hence the sample size was recalculated to 127.

Maximum samples satisfying the inclusion and exclusion criteria were included.

Methodology:
TSH test results of study subjects obtained from the data register in Clinical Biochemistry laboratory. Cobas chemiluminescence method was used to measure the TSH level throughout the above-mentioned time period. Manufacturer advised procedure was followed. Laboratory was part of EQAS(CMC Vellore, India), and tests were acceptable when compared with the same peer group during the study period.

The data of the subjects were entered in proforma and analysed.

Data analysis:
Data analysis was carried out as per the guidelines put forward by IFCC- figure I.

3. Results
Serum TSH level estimation was done for a total of 8367 patients at our laboratory during the study period. 1957 of them did not satisfy the inclusion and exclusion criteria. Among the remaining 6410 subjects, 701 were excluded due to incomplete information, limits of detection, duplication or repeated tests done on same patient. This was done according to the study conducted by Grossi et al, by which the patients with probable thyroid illnesses were removed.

The female patients were found to be more than 4 times that of male patients.

Data was checked for normality using Kolmogorov-Smirnov (K-S) test, which is a non-parametric test of equality of distributions. K-S test was done for whole data, and for male and female. Histogram plotting the same showed a right handed skewness(table I).

Outliers were removed from the data using Horn's test and again checked for normality using K-S test. From the Male sub-class 86 outliers were detected and removed, 358 from Female sub-class and 448 from total group. Again, the p-value was found to be <0.05 and hence the assumption of normality was not satisfied, with a right-sided skewness.

Even though more attempts were made to transform data into Gaussian distribution using natural logarithm, exponential and square root value, and removing outliers, the remaining data was still found to be skewd. Hence parametric method of determination of reference interval from the indirect sample was not possible.

Further analysis was performed by non-parametric method.

The data after removing outliers (5261) was divided into 6 sub-groups to check for comparability of the data obtained over the 3 month study period. Each of the sub-groups had values obtained over consecutive 2-3 weeks.

The sub-groups were checked for normality using K-S test. The sub-groups I to V had p-values >0.05 and was Gaussian, while sub-group VI had p-value <0.05 and hence non-Gaussian. Non-parametric method was applied to estimate indirect reference interval. Rank numbers of 2.5^{th} and 97.5^{th} percentiles were computed as 0.025(n+1) and 0.975(n+1) respectively. This was done for whole data, 6 sub-groups and, for male and female sub-groups separately.90% confidence interval for lower and upper limits were calculated according to the recommendations of IFCC(table II).

The 6 sub-groups were compared using Kruskal Wallis test and, equality in distribution of gender sub-groups were analysed using Mann Whitney U test.

No significant difference was found between the 6 sub-groups, showing that the data obtained over different time periods were similar and could be combined as one. Male and female data had significant differences and were hence separately taken.

Reference interval and Confidence interval for TSH calculated from patient data (indirect reference interval) estimated by non-parametric method, and manufacturer's recommended reference interval which our laboratory currently uses were compared(table III).

4. DISCUSSION
The most important non-controllable variable influencing TSH level is age. Adult population above 20 years of age was taken to nullify this. There are no documented gender changes in the TSH level among adults. Other variables causing major variations in value were excluded by detecting the outliers from the data.

The reference value used in our Clinical Biochemistry Laboratory, provided by the Roche diagnostics, calculated using 516 healthy adults by direct method was found to be differing from our reference value using 5261 hospital patient samples by indirect method. Recent data from South Korea, based on Korea National Health and Nutrition Examination Survey(KNHANES) VI shows that the TSH reference range obtained from disease-free and total population are comparable. The significant difference in the values from the kit insert could be explained by the difference in the reference population used for both the calculations.

The data from the current study shows that though the lower limit of the reference interval is corresponding to that of the kit value, the upper limit is much higher. Also, the values obtained from male and female patients were not comparable and on analysing them separately, the lower reference limit is lesser and upper reference limit is higher for females compared to males. These could be explained by:

1. Majority of the patients analysed were females (Female: male ratio was 4.36: 1). Study from Madurai also showed a higher prevalence of sub-clinical hypothyroidism among young, otherwise normal females.
2. The adult age group considered includes the pregnant females as well. They are found to have higher TSH levels compared to non-pregnant subjects.
3. Majority of our laboratory subjects, might be having thyroid dysfunction in the form of hypothyroidism. Hypothyroidism is the commonest thyroid dysfunction.
4. Increased prevalence of subclinical hypothyroidism with TSH level upto 10μIU/mL has recently been documented from Central Kerala and Tamil Nadu studies. They found that hypothyroidism was seen even when the iodine excretion value was within normal range.
5. Changing life styles among Kerala population could be contributing in terms of goitrogenic agents in food and beverages.
6. Higher incidence of autoimmune thyroid dysfunction with high TSH levels have been detected in a previous study among population in Central Kerala.

This was indeed an eye-opener for us and is found to be consistent with recent similar studies from different parts of the country. We want to emphasize that there is an urgent need to determine the reference interval for each of our laboratory values using our own population and with suitable age or gender stratifications as required. A multi-centric study for obtaining the reference values for Kerala population is also a much-needed project.

5.Acknowledgement
Dr. Asha K. Varghese collected the information, analysed the data and wrote it up under the guidance of Dr. Shibu T.S.
All funding was by principal investigator.

6.Tables and figures :
Figure I :Algorithm to analyse data to obtain RI, as recommended by IFCC:

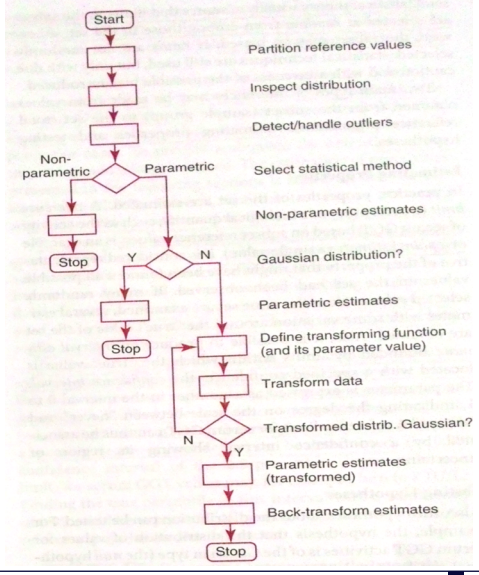

Table I: Results of Kolmogorov-Smirnov test of data:

	Raw data			*Data after removing outliers*		
Group	*N*	*K-S statistic*	*P-value*	*New N*	*K-S statistic*	*P-value*
Male	1070	11.93	0.001	984	4.88	0.001
Female	4639	23.72	0.001	4281	9.79	0.001
Whole data	5709	26.561	0.001	5261	11.04	0.001

Table II: Lower and upper reference limits of the groups:

Group/ sub-groups	*Time period*	*N*	*Median ± QD*	*Reference limit*	
				Lower	*Upper*
Total	-	5621	2.01 ± 1.06	0.322, 0.37	8.6, 9.5
I	01.01.16 - 21.01.16	1065	1.91 ± 0.9525	0.316, 0.395	6.98, 8.31
II	22.01.16 - 04.02.16	841	2.05 ± 1.105	0.346, 0.497	8.75, 10.88
III	05.02.16 - 18.02.16	883	1.99 ± 1.0875	0.337, 0.479	7.98, 10.18
IV	19.02.16 - 04.03.16	913	2.07 ± 0.9925	0.325, 0.444	7.8, 9.49
V	05.03.16 - 18.03.16	881	2.11 ± 1.16	0.251, 0.341	8.7, 11.4
VI	19.03.16 - 31.03.16	678	1.95 ± 1.1975	0.122, 0.244	8.44, 11.56
Male	-	984	1.81 ± 0.865	0.417, 0.482	7.32, 8.19
Female	-	4281	2.07 ± 1.105	0.305, 0.32	9.2, 9.32

Table III : Comparison of indirect Reference Interval of our patient population with that of kit manufacturer:

Total number	Reference interval	Lower confidence interval	Upper confidence interval	Manufacturer
5261	0.322 - 9.09	0.322, 0.37	8.6, 9.5	0.27 - 4.2
Male: 984	0.4475 - 7.5625	0.417, 0.482	7.32, 8.19	
Female:4281	0.31105 - 9.28	0.305, 0.32	9.2, 9.32	

REFERENCES

[1] Gary LH. Establishment and use of reference intervals. In: Carl AB, Edward RA, David EB, editors. Tietz textbook of Clinical chemistry and Molecular diagnostics. Elsevier 2014; p 95-119

[2] Joshi SR. Laboratory evaluation of thyroid dysfunction. Supplement to JAPI. 2011 January;59 (14-20)

[3] Jansen SW, Akintola AA, Roelfsema F et al. Human longevity is characterised by high thyroid stimulating hormone secretion without altered energy metabolism. Sci Rep. 2015; 5:11525. Published online 2015.Jun 19. doi: 10.1038/srep 11525

[4] Anne RC, Alice MA, Kendra W et al. Thyroid function in the euthyroid range and adverse outcomes in older adults. J Clin Metab. 2015 March; 100(3): 1088-1096. Published online 2014 Dec 16. doi: 10.1210/jc.2014-3586

[5] Yue Wu, Yuqing P, Wei C et al. Higher fT4 or TSH below the normal range are associated with increased risk of dementia: a meta-analysis of 11 studies. Sci Rep. 2016; 6:31975. Published online 2016 Aug25. doi: 10.1038/srep31975

[6] Ralph Grasbeck. The evolution of the reference value concept. Clin Chem Lab Med. 2004; 2005 Jun; 42(7): 692-697.

[7] James C. Boyd. Defining laboratory reference values and decision limits: populations, intervals and interpretations. Asian J Androl. 2010 Jan; 12(1) : 83-90. Published online 2010 Jan29. doi: 10.1038/aja.2009.9; PMC3739683

[8] Malati T. Review article whether western normative laboratory values used for clinical diagnosis are applicable to Indian population? An overview on reference interval. Ind J Clin Biochem. 2009; 24(2): 111-122

[9] Sikaris K. Application of the Stockholm hierarchy to defining the quality of reference intervals and clinical decision limits. Clin Biochem Rev. 2012 Nov;33(4): 141-8

[10] Katayev A, Fleming JK, Luo D et al. Reference intervals data mining: no longer a probability paper method. Am J Clin Pathol. 2015; 143: 134-42

[11] Grossi E, Colombo R, Cavuta S et al. The RELAB project: a new method for the formulation of reference intervals based on current data. Clin Chem 2005; 51::1232-40. Published online 2005. doi: org/10.1373/clinchem.2005.047787

[12] Ilcol YO, Diler A. Use of total patient data for indirect estimation of reference intervals for 40 clinical chemistry analytes in Turkey. Clin Chem Lab Med.2006; 44: 867-76

[13] Ebubekir B, Harun P, Yesim O et al. A reference interval study for common biochemical analytes in Eastern Turkey: a comparison of a reference population with laboratory data mining. Biochemia Medica 2016; 26(2) : 210-23

[14] Raman KM, Nikhil T, Mohd Ashraf G et al. Reference range of thyroid function (FT3, FT4, TSH) among Indian adults. Clin Biochem. 2013 Mar; 46(4-5): 341-45

[15] David Z, James DC, Joseph AM. Pediatric reference intervals for serum thyroxine, triiodothyronine, thyrotropin, free thyroxine. Am Asso Clin Chem. 1999 Jul; 45(7): 1087-91

[16] Tamer CI. Indirect reference intervals estimated from hospitalised population for thyrotropin and free thyroxine. Croat Med J. 2010 Apr; 51(2) :124-130

[17] Hyung HK, Hae SH, Shine YK et al. An improved auto-generation system to obtain reference intervals for laboratory medicine. Healthc Infor Res. 2010 March; 16(1): 15-21. Published online 2010. doi: 10.4258/hir.2010.16.1.15

[18] Dixon WJ. Processing data for outliers. Biometrics 1953; 9: 74-89

[19] Reed AH, Henry RJ, Mason WB. Influence of statistical method used on the resulting estimate of normal range. Clin Chem. 1971; 17: 275-284

[20] Geoffrey O, Ali K, Mary L et al. Reference intervals for thyroid stimulating hormone and free thyroxine derived from neonates undergoing routine screening for congenital hypothyroidism at a university teaching hospital in Nairobi, Kenya: a cross-sectional study. BMC Endocr Disord. 2016; 16:23. Published online 2016 May 23. doi: 10.1186/s12902-016-0107-9

[21] Catherine P et al. Defining the newborn blood spot screening reference interval for TSH: impact of ethnicity. J Clin Endocrinol Metab. 2016 Sep; 101(9): 3445-49. Published online 2016 Jul 11. doi: 10.1210/jc.2016-1822

[22] Ichihara K, Itoh Y, Lam CW et al. Sources of variation for commonly measured serum analytes among six Asian cities and consideration of common reference intervals. Clin Chem. 2008; 54: 356-65

[23] Friis- Hansen L, Hilsted L. Reference intervals for thyrotropin and thyroid hormones for healthy adults based on the NOBIDA material and determined using a Modular E170. Clin Chem Lab Med. 2008:46: 1305-12. Published online 2008. doi: 10.1515/CCLM.2008.258

[24] Dhatt GS, Griffin G, Agarwal MM. Thyroid hormone reference intervals in an ambulatory Arab population on the Abbott Architect i2000 immunoassay analyser. Clin Chem Acta. 2006; 364:226-9. Published online 2005. doi: 10.1016/j.cccn.2005.07.003

[25] Gonzalez-Sagrado M, Martin-Gil FJ. Population-specific reference values for thyroid hormones on the Abbott Architect i2000 analyser. Clin Chem Lab Med. 2004; 42:540-2. Published online 2004. doi: 10.1515/CCLM.2004.091

[26] Hubl W, Schmieder J, Gladrow E et al. Reference intervals for thyroid hormones on the Architect analyser. Clin Chem Lab Med. 2002; 40: 165-6. Published online 2002. doi: 10.1515/CCLM.2002.028

[27] Taimela E, Kairisto V, Koskinen P et al. Reference intervals for serum thyrotropin, free thyroxine and free triiodothyronine in healthy adults in Finland, measured by an immunoautomate based on time-resolved fluorescence(AutoDELFIA). Eur J Clin Chem Clin Biochem. 1997; 35: 889-90

[28] Larry JJ, Susan JM, Antony PW. Disorders of thyroid gland. In: Dennis LK, Stephen LH, Larry JJ, Antony SF, Dan LL, Joseph L editors. Harrison's principles of internal medicine. P 2285

[29] The Thyroid gland. In: Bruce MK, Bruce AS editors. Bernie and Levy physiology. Mosby 2010; p 1238-45

[30] Anthony PW. The diversity of the endocrine system. In: Victor WR, David AB, Kathleen MB, Peter JK, Anthony PW. editors. Harper's illustrated biochemistry. Mc Graw Hill 2017; p 509-11

[31] Geoff B. The definition of hypothyroidism. Endo abstracts. 2011.

[32] Douglas SR.. Symptoms and signs of thyroid disorders. Post TW ed. UpToDate. Waltham, MA: UpToDate Inc. http://www.uptodate.com

[33] Ambika G, Unnikrishnan. Thyroid disorders in India: an epidemiological perspective. Indian J Endocrinol Metab. 2011 Jul; 15(Suppl2); S78-S81

[34] Nordic Society of Clinical Chemistry(NFKK) home page http:// home. online.no/ ~rustadp/refprosj. html

[35] Rustard P. Nordic Reference Interval Project(NORIP) Available from http://www.furst.no./norip/

[36] Reference interval for children and adults. Elecsys and cobas e analyzers, Elecsys Thyroid Tests

[37] Won Gu Kim et al. Thyroid stimulating hormone reference range and prevalence of thyroid dysfunction in the Korean population: Korea National Health and Nutrition Examination Survey 2013-2015. Endocrinol Metab. 2017; 32: 106-114

[38] Kumaravel V, Sivan ASS, Unnikrishnan AG. Prevalence of thyroid dysfunction among young females in a South Indian population. Indian J Endocrinol Metab. 2015 Nov-Dec; 19(6): 781-84

[39] Menon VU, Sundaram KR, Unnikrishnan AG et al. High prevalence of undetected thyroid disorders in an iodine sufficient adult South Indian population. J Indian Med Assoc. 2009 Feb; 107(2): 72-7

[40] Iodine status worldwide. WHO global database on iodine deficiency. Department of nutrition for health and development, Geneva: WHO (2004)