

## Assessment of Age Dependent Reference Range of Thyrotropin Hormone Using Immunoradiometric Assay

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**Abstract:** In total 218 suspected and confirmed male patients and 159 normal male test subjects of different age group were analyzed for thyrotropin abnormality and estimation of age depended standard reference range. Immunoradiometric methods were used for accurate thyrotropin measurement. In 4 to 10 years age group among the patients 44% were found to be euthyroid and 56% were found to have hypothyroidism. The normal reference range from this age group were obtained 2.5- 4.64 $\mu$ IU/mL. The lower limit of thyrotropin was recorded as 0.16  $\mu$ IU/mL and highest values found to be 98.55  $\mu$ IU/mL. As for 11 to 28 years age group thyrotropin range were found to be 0.18 to 56.67  $\mu$ IU/mL and normal reference range from this age group was obtained 0.7—3.56 $\mu$ IU/mL. This group was found to have 79% hypothyroidism among the suspected patients. Age group 29 to 45 showed 82% hypothyroidism among the patients and the reference range are found to be 0.82 - 4  $\mu$ IU/mL. As for 45 to 80 years age group 65% suspected patients showed hypothyroidism and the normal reference range are found to be 0.68-2.86  $\mu$ IU/mL. The present study shows that age depended hormonal parameters are important factors to consider in clinical situations such as infertility, hypo/hyper-thyroidism etc.

**Keywords:** Thyrotropin, Immunoradiometric assay, hormonal parameters, reference limit.

### Introduction

Over the last four decades serum thyrotropin (thyroid-stimulating hormone-TSH) assay methodology has undergone dramatic improvements that have revolutionized strategies for thyroid testing and firmly established thyrotropin as the first-line thyroid function test to assess thyroid hormone status for most clinical conditions [1]. In fact, serum thyrotropin has become the single most reliable test for diagnosing abnormalities in thyroid status, provided that patients are ambulatory and not receiving drug therapies that alter thyrotropin secretion [2]. The diagnostic superiority of thyrotropin measurement arises principally from the physiologic inverse log/linear relationship between circulating thyrotropin and free T4 concentrations. Thyrotropin stimulates the thyroid gland to secrete the hormones thyroxine (T4) and triiodothyronine (T3)[3]. Measurement of the serum concentration of thyrotropin, is generally regarded as the most sensitive indicator available for the diagnosis of primary and secondary (pituitary) hypothyroidism [1,2,4]. Increase in serum concentrations of thyrotropin, which is primarily responsible for the

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synthesis and release of thyroid hormones, is an early and sensitive indicator of decrease thyroid reserve and in conjunction with decreased thyroxine (T<sub>4</sub>) concentrations is diagnostic of primary hypothyroidism. At present only a single reference range is used to detect TSH level for all ages and for both male and female. But recently our literature survey shows that it is difficult to identify TSH abnormality with only one reference range; it varies with age. Another important parameter is that male and female reference range is never exactly the same. There had been several works on female population but work on male population remains nearly scarce [2]. Recent literature survey up to 2014 shows most works were done on female population. Even when surveying neonates, female children were given superior priority[5]. So in this study we are attempting to achieve standard reference range for different age group of male population.

### **Methods commonly used for TSH assay**

Several methods are used worldwide for TSH measurement. These include: Enzyme-linked Immunosorbent assays (ELISA)[5], One step ELISA[6], Chemiluminescence Immunoassay (CLIA)[7], Chemifluorescence Immunoassay (CFIA)[8], and Immunoradiometric assay (IRMA)[1]

Microplate ELISA method is most widely used in Bangladesh and India for its low price. There are available commercially in Bangladesh in varieties of brand name such as AccuBind ELISA Microwells, Human ELISA Microwells, AutoBio ELISA Microwells, Clayman ELISA Microwells, DRG ELISA Microwells etc. The later version comes as one step microchip-ELISA which is faster (10–20 min) and requires a much smaller sample volume (a few microliters) which is really inexpensive and with acceptable result[5-9]. Chemiluminescence Immunoassay (CLIA) and Chemifluorescence Immunoassay (CFIA) have largely replaced ELISA and one step ELISA which is highly accurate but very expensive. All these four methods are widely used for the analysis of TSH at a commercial and acceptable accuracy. In this study we have used immunoradiometric assay (IRMA) for TSH determination. IRMA is a method for measuring certain plasma proteins by using radiolabeled antibodies. These type of assay that differs from conventional radioimmunoassay in that the compound to be measured combines directly with radioactively labeled antibodies. We have used Gamma irradiator I-125 labeled antibody for TSH separation from blood serum and by measuring gamma radiation irradiated per minute the amount of TSH is determined. ELISA and one step ELISA uses simple absorption photometry using visible light with 450nm filter. These instruments are relatively cheap. In our study highly sensitive Gamma counter was used. This instrument employs high resolution gamma spectrometry for measuring different isotope of different energies. Compare to first four methods IRMA is most expensive but with high accuracy. The use of gamma irradiating radioactive isotope is restricted by international law for its radiotoxic nature. So very few laboratory have the authorities to use them. In Bangladesh only Bangladesh Atomic Energy Commission offers IRMA technology. Our present research was carried out under the supervision of Bangladesh Atomic Energy Commission.

**Material and method**

In total 218 male patients and 159 normal male test subjects of different age group were analyzed for thyrotropin abnormality and standard reference range. These peoples are from middle class with high protein diet. None of the normal male participants were on medication and just few subjects smoked occasionally and none reported daily consumption of alcohol. Body weight and height were measured using electronic weighing machine and their heights were determined. Weekly basis blood samples were collected from each individual to measure the natural and medicinal induced change of TSH parameter for three months. Two Immunoradiometric assay (IRMA) kits were used for more accurate thyrotropin measurement. Commercially available Thyrotropin- IRMA kits supplied by Bio-Line B-1150 Bruxelles – Belgium and TSH-IRMA kits form Beijing Atom Hightech Co., LTD Beijing, China were used for thyrotropin hormonal estimations. The Bio-Line TSH-IRMA is an immunoradiometric assay based on coated plastic tube separation. Mabs1 (anti TSH monoclonal antibodies) the captured antibodies, are attached to the lower and inner surface of the plastic tube. Calibrators or samples added to the tubes will at first show low affinity for Mabs1. Addition of Mab2, the signal antibody labeled with I-125, will complete the system and trigger the immunological reaction. After washing, the remaining radioactivity bound to the tube reflects the antigen concentration. The use of several distinct Mabs avoids hyperspecificity. Twenty zero calibrators were assayed along with a set of the other calibrators.

The detection limit, defined as the apparent concentration of the average count at zero binding plus two standard deviations, was 0.025  $\mu$ IU/ml. Cross-reacting hormones were added to a low and to a high TSH value calibrator. The apparent TSH response was measured. All assay were carried out using Multi Crystal gamma counter from BERTHOLD TECHNOLOGIES. One disadvantage of employing thyrotropin as a diagnostic screening test for thyroid function is that it can often fail to detect the presence of pituitary and/or hypothalamic disease [central hypothyroidism or TSH secreting pituitary tumors (TSHomas)] [11-13]. In these conditions serum thyrotropin can be paradoxically within the normal reference limits because current assays cannot distinguish between normal and biologically altered thyrotropin isoforms that may be present in these states. For example, thyrotropin isoforms with impaired biologic activity are typically secreted in central hypothyroidism whereas thyrotropin isoforms with enhanced biologic activity are often secreted by TSH secreting pituitary tumors [14-16]. These abnormal thyrotropin isoforms can result in paradoxically normal or high thyrotropin being reported in the face of clinical hypo- or hyperthyroidism, respectively.

**Result and Discussion**

In total 218 suspected and confirmed patients and normal test subjects of different age group were analyzed for thyrotropin abnormality and standard reference range. In 4 to 10 years age group, 44% suspected patients were found to be euthyroid and 56% were found to have hypothyroidism. The normal reference range from this age group were obtained 2.5- 4.64 $\mu$ IU/mL. In this age group lower limit of thyrotropin was recorded as 0.16

$\mu\text{IU/mL}$  and highest values found to be  $98.55 \mu\text{IU/mL}$ . As for 11 to 28 years age group thyrotropin range were found to be  $0.18$  to  $56.67 \mu\text{IU/mL}$  and normal reference range from this age group was obtained  $0.7\text{--}3.56 \mu\text{IU/mL}$  (Table 1,2,3). This group was found to have 79% hypothyroidism among the suspected patients. Thyrotropin levels for children normally start out much higher. The difference of values of case and control is due to the Thyroid disease. According to the National Academy of Clinical Biochemistry (NACB) in the United States recommended age-related reference limits starting from about  $1.3$  to  $19 \mu\text{IU/mL}$  for normal-term infants at birth, dropping to  $0.6\text{--}10 \mu\text{IU/mL}$  at 10 weeks old,  $0.4\text{--}7.0 \mu\text{IU/mL}$  at 14 months and gradually dropping during childhood and puberty to adult levels,  $0.4\text{--}4.0 \mu\text{IU/mL}$ . [15]

**Table 1:** Comparison of thyrotropin range between patients and control

Age	Number of patient	Number of normal test subjects	Experimental thyrotropin range from lower to upper limit $\mu\text{IU/mL}$	Normal Reference thyrotropin range $\mu\text{IU/mL}$ obtained
4-10	54	42	0.16-98.55	2.5-4.64
11-28	68	64	0.18-56.67	0.7-3.56
29-45	46	35	0.13-88.00	0.82-4
45-80	50	28	0.15-75	0.68-2.86

**Table 2:** Lower thyrotropin range between case and control

Age	Thyrotropin range of suspected patient (lowest) $\mu\text{IU/mL}$	Thyrotropin range of confirmed patient (lowest) $\mu\text{IU/mL}$	Normal Reference thyrotropin range of lower limit obtained $\mu\text{IU/mL}$
4-10	0.16-1.5	0.19-2	2.5
11-28	0.18-0.55	0.14-0.45	0.7
29-45	0.12-0.80	0.13-0.65	0.82
45-80	0.15-0.45	0.15-0.30	0.68

**Table 3:** Higher thyrotropin range between case and control

Age	Thyrotropin range of suspected patient (highest) $\mu\text{IU/mL}$	Thyrotropin range of confirmed patient (highest) $\mu\text{IU/mL}$	Normal Reference thyrotropin range of higher limit obtained $\mu\text{IU/mL}$
4-10	8.56-85.54	8.52-98.55	4.64
11-28	5.3-54.44	8.95-56.67	3.56
29-45	7.00-57	6.5-88	4.5
45-80	5-75	9.57-75	2.88

Age group 29 to 45 showed 82% hypothyroidism among the patients and the reference range are found to be  $0.82$  to  $4 \mu\text{IU/mL}$ . As for 45 to 80 years age group 65% suspected patients showed hypothyroidism and the reference range are found to be  $0.68\text{--}2.86 \mu\text{IU/mL}$  (Table 1,2,3). TSH levels for children normally start out much higher [5]. The NACB also stated that it expected the normal (95%) range for adults to be reduced to

0.4–2.5  $\mu\text{IU/mL}$ , because research had shown that adults with an initially measured thyrotropin level of over 2.0  $\mu\text{IU/mL}$  had an increased odds ratio of developing hypothyroidism over the following 20 years, especially if thyroid antibodies were elevated. Our present studies have suggested that thyrotropin increases with age and that a mild thyrotropin elevation in elderly individuals may even convey a survival benefit, although other reports dispute this [11-15]. These reports have led to the suggestion that age-specific thyrotropin reference limits should be considered [16,17]. There appears to be a positive correlation between age and thyrotropin concentrations in iodine sufficient populations [18,19] the opposite is the case for iodine deficient populations in which there appears to be no TSH increase with age, or even a decline [19-21]. Complicating these questions is the fact that current thyrotropin IRMAs differ in specificity for recognizing circulating thyrotropin isoforms and that this can give rise to a full 1.0 mIU/L difference in thyrotropin values reported by different assays – a difference that in some cases is greater than the influence of many of the other variables. Because hypothalamic TRH modulates thyrotropin molecular glycosylation and biological activity, a rise in thyrotropin with age could represent an increase in the secretion of biologically inactive thyrotropin, yet immunologically detected isoforms. The blunting of the thyrotropin response to TRH and decreased amplitude of the thyrotropin nocturnal peak would be consistent with this premise [22-25]. In contrast, in areas of iodine deficiency the inverse relationship between thyrotropin and age could represent a failure to exclude individuals with autonomously functioning nodules [24,25]. Thus the thyrotropin upper reference limit for non-pregnant subjects remains a contentious issue so that it is difficult for manufacturers to cite a thyrotropin reference range that could be universally adopted across different populations in different geographic areas This has led to guidelines proposing the adoption of an empiric thyrotropin upper limit of 2.5 -3.0  $\mu\text{IU/mL}$ , which is in accord with the thyrotropin interval associated with the lowest prevalence of thyroid antibodies [22, 25].

## **Conclusion**

The present study shows that the adult thyrotropin population reference range does not apply to neonates or children. Serum thyrotropin values are generally higher in neonates and then gradually decline until the adult range is reached after puberty. This necessitates using age-specific thyrotropin reference ranges for diagnosing thyroid dysfunction in these pediatric age categories. The significance of reference hormonal levels lies in the fact that hormonal parameters are important factors to consider in clinical situations such as infertility, hypo/hyper-thyroidism. In developing countries like Bangladesh, industrialization and urbanization with economic development have resulted in lifestyle changes and nutrition transition increasing the risk of chronic disease. Such changes are known to influence hormone level. Further, international guidelines have recommended the establishment of reference intervals for every country and the lack of such studies in country like Bangladesh has been reported. At present study thyrotropin hormone values measured using IRMA were within the physiological range for each age group in subjects between 4 to 80 years of age. Although diet was similar in all the study subjects, it has

been reported that high dietary salt influence thyroid hormone level. These findings may help in our understanding of the prevalence and pattern of thyroid hormone reference. There are few limitations of our present study, small number of test subjects from a specific region may be the major factor. A wide spectrum of study and more test subjects both suspected thyroid patient and normal people might be needed for more accurate determination of normal reference range for thyrotropin hormonal parameter.

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