Annals of Thyroid Research

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Research Article

TSH Should not be used as a Single Marker of Thyroid Function

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Received: August 07, 2018; **Accepted:** August 31, 2018; **Published:** September 07, 2018

Abstract

Context: Thyroid Stimulating Hormone (TSH) alone is often used as a primary marker to screen for thyroid function. Significant intra-individual variation of TSH concentrations occur in healthy individuals. Intra-individual sex and time-based variations pose the question of whether TSH can reliably screen for thyroid disorders.

Objective: To quantify the degree of diurnal fluctuations in TSH concentrations of healthy individuals and assess its diagnostic reliability. To propose preliminary sex and time dependent TSH reference intervals.

Design and Methods: Healthy volunteers (n=102) were recruited from 4 participating sites. Couplet (AM and PM) serum samples were drawn and analyzed for TSH concentration using immunoassay.

Results: Significant AM to PM increases in TSH levels for both men ($P < 0.0001$) and women ($P = 0.0003$) were noted.

Conclusion: TSH should not be used as a single marker for the assessment of thyroid function. We recommend that TSH be used in conjunction with Free Thyroxine (FT4), Free Triiodothyronine (FT3), and Total T3 measured by LCMS/MS.

Keywords: Thyroid stimulating hormone; Congenital hypothyroidism; Immunoassays; Thyroid function

Highlights

Significant differences exist in:

1. Intra-individual TSH concentrations
2. Diurnal and sex-based TSH concentrations

1 and 2 together with the many drugs and steroids that lower TSH concentrations, suggest we should question the reliability of TSH as a single marker of thyroid function.

Introduction

Currently Thyroid Stimulating Hormone (TSH) is considered one of the most sensitive screening tests for the initial assessment of Hypothalamic-Pituitary-Thyroid axis (HPT) function. A good relationship exists between TSH and both FT4 and FT3 measured by LCMS/MS. In contrast TSH correlates poorly with FT4/FT3 when the latter are measured by Immunoassays (IA) [1]. TSH measurement is performed by using third generation TSH assays with functional sensitivity of <0.02mIU/L necessary for detection of conditions causing TSH suppression [2]. TSH measurement is widely available, safe and inexpensive [3]. Nevertheless, the use of TSH as the best single test for hyper- and hypothyroidism has recently been questioned [1,4,5]. Inter- and intra-individual variation, seasonal and diurnal fluctuations, aging, heterophilic antibodies, biologically inactive forms of TSH, pregnancy, and drug-related effects, etc, can influence TSH concentration [4-14].

TSH has circadian and pulsatile secretion with fluctuations that contribute to the width of its normal reference intervals [15]. This adds to the intra-individual variation in TSH levels limiting its diagnostic use [9,10]. Like other pituitary glycoproteins, TSH exists as a mixture of isoforms with different degrees of sialylation and sulfation, which account for the heterogeneity of circulating TSH [16] and complicate its measurement by current IA's. TSH values can differ by as much as 1.0 μIU/mL due to differences in the specificity of immunoassay antibodies to human TSH [17].

Large and varied effects of medications on TSH concentration are well-documented and decrease the interpretative value of TSH measurement [6,8]. Glucocorticoids such as prednisone and other steroids can decrease TSH levels below the normal range [4,5,8,18]. Certain medications such as bexarotene show suppression of TSH after a single dose, resulting in clinically significant hypothyroidism [19]. In general, classes of medications such as amphetamines and dopamine agonists suppress TSH while others can increase TSH levels [20].

Our hypothesis is that TSH concentrations vary significantly within healthy individuals. We can quantify these changes in terms of the number of standard deviations needed to account for the change. To evaluate this, we studied healthy individuals from whom diurnal blood samples were drawn.

Subjects and Methods

This study was approved by institutional review boards at NIH (protocol 93-CC-0094) and Georgetown University (Pro0000007-

Citation: Sheikh SI, Parikh TP, Kushchayeva Y, Stolze B, Masika LS, Ozarda Y, et al. TSH Should not be used as a Single Marker of Thyroid Function. Annals Thyroid Res. 2018; 4(2): 151-154.

Table 1: Preliminary reference intervals based on a 95-percentile confidence interval of TSH concentration for healthy volunteers. Mean TSH SD factor difference and percentage of participants showing a diurnal variation > 1μIU/mL between am and pm.

	Reference Interval		Mean TSH SD Factor Difference	Percentage of Volunteers with Diurnal TSH Change > 1μIU/mL
	am	pm		
Male, n=47	0.7-3.7	0.7-4.7	4	21%
Female, n=55	0.5-4.3	0.5-6.1	5	29%

Roche FDA approved reference interval is (0.27-4.20 μIU/mL).

Table 2: TSH concentrations of 7 participants with diurnal fluctuations > 1μIU/mL.

Age	AM	PM	TSH SD Factor Difference	TSH Change
34	3.82	5.98	14	2.16
55	3.31	5.82	16	2.51
59	3.09	4.77	11	1.68
50	3.41	5.01	10	1.60
35	3.15	4.98	11	1.83
39	3.48	4.67	7	1.19
28	3.46	6.84	21	3.38
Mean	3.4	5.4	12.9	2.05

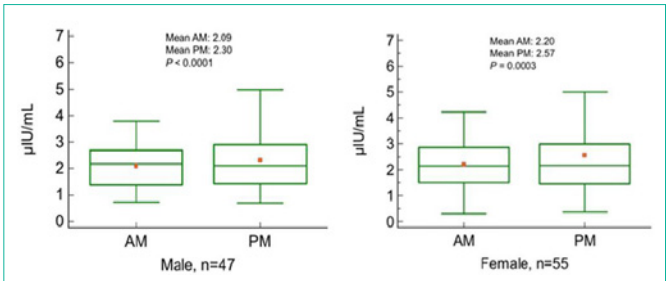

Figure 1: Box and whisker plots: AM vs. PM values of TSH for males and females, from 0 to 100%. The mean is indicated by a small square marker and the median is represented by the middle line within the box, $P < 0.0001$ for males and $P = 0.0003$ for females.

01). Blood samples were collected at 4 sites which included the National Institutes of Health (NIH), Georgetown University, Walter Sisulu University in South Africa, and Uludag University in Turkey. Morning samples were collected between 6:00-9:00 AM, and paired evening samples were collected between 6:00-9:00 PM.

A screening assessment was implemented to determine if each participant qualified as healthy for this study; this included a survey, absence of medication or supplement use, a detailed medical history, and physical examination by a medical provider. 114 volunteers were screened. All volunteers had TBG and lipid levels within the reference intervals. 5 volunteers with significantly higher AM values (> 4 μIU/mL) of TSH were excluded as they met the current diagnostic criteria for hypothyroidism. We excluded all volunteers with high TPO antibody values above 35 IU/mL (reference range 0-35 IU/mL), which is known to be associated with Hashimoto's thyroiditis [21]. TPO could be an early indicator of hypothyroidism. After the exclusions 102 healthy volunteers remained which included 47 males and 55 females.

TSH concentrations were measured on the Roche Cobas 6000 (Roche Diagnostics, Indiana).Statistical analysis was performed using MedCalc Version 17.8.6 and box and whisker plots were generated. Reference intervals were calculated using a non-parametrical percentile method with a 95% double-sided interval. The direct percentile approach for obtaining reference intervals provided similar results.

Results & Discussion

Our study revealed significant AM to PM increases in TSH levels for both men ($P < 0.0001$) and women ($P = 0.0003$), where $P < 0.05$ is considered statistically significant. For this reason, we urge that AM and PM reference intervals be reported based on sex and time of sample collection. The IFCC recommends a minimum of 120 subjects prior to publishing reference intervals. Given the high statistical significance of the differences found, we present "preliminary" reference intervals for AM and PM for both sexes as shown in Table 1. It is interesting to note that 13% of the euthyroid females in this study had TSH levels above the FDA approved Roche reference interval (0.27-4.20 μIU/mL) and would be misclassified as being hypothyroid. Simulation analysis on NHANES data [22] shows a 6% misclassification rate of females using the higher end of the current Roche reference interval.

The analytical standard deviations of the TSH assay calculated from quality control materials (1 SD = 0.16 μIU/mL) was used to determine the TSH SD factor difference between AM and PM concentrations for each volunteer. The mean SD factor differences for males and females are listed in Tables 1 and 2. The mean SD factor difference between AM and PM was 4 and 5 for males and females respectively. A mean SD factor difference of 4 represents a statistical chance of occurring in only 6 in 100,000, indicating a very small probability of variation by randomness or analytical imprecision. Instead our results suggest large, statistically significant intra-biological variation between AM and PM concentrations. A sub-cohort of 7 normal individuals displayed large diurnal fluctuations between AM and PM TSH concentrations, with a mean TSH SD difference factor of 12.9 (Table 2).

The mean PM TSH concentration is 10% higher than the mean AM TSH concentration for males. A similar diurnal increase of 17% is seen for females. The AM and PM mean TSH concentrations for females is 5% and 12% higher than that of males (Figure 1). Within individual diurnal variation has previously been reported to be as high as 0.8 μIU/mL [23]. While the mean variations in our study are similar a significant proportion of the participants, 21% of males and 29% of females exceeded the AM and PM TSH variation of 1μIU/mL (Table 1).

Between method differences in TSH measurement varies from 0-1.0 μIU/mL [17]. Analysis of the College of American Pathologists ABTH 2018 Survey shows inter-method mean TSH concentrations vary considerably above that threshold. The mean variation for

Table 3: CAP data (ABTH 2018 Surveys) showing the extreme range of TSH concentrations in 3 human serum samples reported irrespective of immunoassay method used.

Survey 2018	High Value in Measured TSH Concentration (μIU/mL)	Low Value in Measured TSH Concentration (μIU/mL)	Factor (High/Low)
ABTH-01	0.81	0.54	1.5
ABTH-02	3.74	2.64	1.42
ABTH-03	6.36	4.17	1.53
		Mean:	1.48

three CAP surveys shows 48% variability in TSH concentration measurements (Table 3). The risks of relying on TSH measurement for diagnostic purposes include a combination of diurnal, intra-individual, and inter-IA method variation.

A number of factors contribute to the diminished utility of TSH as a primary diagnostic indicator for thyroid disorders, namely, the larger than anticipated variability of TSH as well as drugs and hormones which affect its concentration (Table 1). The mean TSH SD factor differences from this study further demonstrate that the dimension of diurnal change is greater than anticipated from current literature. The frequent use of TSH alone to screen for thyroid dysfunction should be discontinued. The direct measurement of thyroid hormones via LCMS/MS more closely correlates with the patient's condition and should be preferred in clinical settings where these methods are available [1,6]. Immunoassays for the measurement of thyroid hormones have been shown to be unreliable and frequently give the incorrect diagnosis [1,6,7]. Here at the NIH, reflex testing of FT4, FT3, and total T3 is performed on all patients with TSH's > 10 μIU/mL by both IA and LCMS/MS. The mass spectrometric method correlates better with the clinical condition than the former [1,4,24]. We have also shown that IA values of FT4 wrongly classify between 50 and 65% of subclinical hypothyroid populations compared to gold standard LCMS/MS [25]. In the FT4 harmonization study recently reported, the IFCC/AACC patient classification criteria for hypo, hyper and euthyroid status was inappropriately based on IA analysis of FT4 and TSH. In addition, there was unfortunately no physician involvement in the direct assessment of the patient's clinical status [26].

Thyroid hormones measured by LCMS/MS correlate better with TSH than with IA. Both TSH and the LCMS/MS measured thyroid hormones reflect the clinical condition of an individual [1].

Our findings argue the need for reassessment of neonatal screening programs for Congenital Hypothyroidism (CH) which currently depend solely on TSH. Perinatal factors such as acute stress during labor, high steroid concentrations during pregnancy, and common medications would suppress TSH secretion in newborns giving the biochemical impression of a euthyroid neonate and potentially a false normal result [27,28]. Untreated CH in newborns results in irreversible cognitive deficiencies, which can be prevented with early diagnosis and treatment [29,30]. More reliable markers would be FT4, FT3 and total T3 measured by mass spectrometry. Studies comparing outcomes based on diagnosis of CH using TSH vs free thyroid hormones measured by LCMS/MS need to be done.

This paper demonstrates the substantial diurnal variation and sex-dependent differences of TSH concentrations. The preliminary reference intervals established in this study are based on careful inclusion and exclusion of participants to ensure that the intervals reflect a healthy population. These "preliminary" reference intervals are an improvement over the Roche FDA approved intervals, which misclassify many healthy individuals, especially females, at the higher end of the interval.

In summary, TSH alone should not serve as the gold standard for the measurement of thyroid status. More accurate assessment is afforded through additional mass spectrometric measurement of thyroid hormones in combination with physician assessment of the patient's clinical condition.

Acknowledgement

The authors thank the NIH study participants and staff for all of their work in contributing to our research.

Funding

Supported by the NIH Intramural Research grant

Authors' Contributions

Sheikh SI and Soldin SJ performed the literature review and drafted the initial manuscript, critically reviewed and edited the manuscript, and approved the final manuscript as submitted. TP and YK assisted in data analysis and manuscript editing. All authors critically reviewed the manuscript.

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Annals Thyroid Res - Volume 4 Issue 2 - 2018
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Citation: Sheikh SI, Parikh TP, Kushchayeva Y, Stolze B, Masika LS, Ozarda Y, et al. TSH Should not be used as a Single Marker of Thyroid Function. Annals Thyroid Res. 2018; 4(2): 151-154.