EFFECT OF PREANALYTICAL VARIABLES ON SERUM THYROID STIMULATING HORMONE ANALYSIS BY CHEMILUMINESCENCE METHOD
Ambreen Rehman, Naveed Asif, Saima Shakeel Malik, Waqas Sheikh, Quratulain, Muhammad Tahir Khadim
Armed Forces Institute of Pathology/National University of Medical Sciences (NUMS) Rawalpindi Pakistan

ABSTRACT

Objective: To identify effect of pre-analytical variables on serum thyroid stimulating hormone.
Study Design: Cross sectional study.
Place and Duration of Study: Armed Forces Institute of Pathology (AFIP) Rawalpindi, Department of Chemical Pathology & Endocrinology, from Mar 2018 to Aug 2018.
Methodology: Hundred subjects with ages ranging from 18 to 34 years, irrespective of gender, were randomly selected for this study. Five milliliters venous blood sample was collected from each subject in a serum separator and divided into two aliquots. First aliquot was centrifuged and analyzed immediately for TSH, while second aliquot was stored for 24 hours and was then analyzed. TSH was measured by third generation assay using chemiluminescence technique on ADVIA Centaur® XP. Serum TSH levels were also analyzed twice daily; in the morning (0800 to 0900 hours) and afternoon (1400 to 1600 hours). Data was analyzed using SPSS version 24. Frequency and percentages were calculated for qualitative variables like gender and pre-analytical variables. Test of significance Mann-Whitney U-test was applied and p-value <0.05 was taken as significant.
Results: Mean age of subjects was 23 ± 3.4 years. Change in circadian rhythm was observed in 17 (28%) males and 14 (36%) females. Statistically significant association was found between morning and evening TSH levels, while no change was observed in TSH level by early and late centrifugation of samples.
Conclusion: TSH levels vary significantly between blood samples collected at different timings of the day from the same person. TSH is resistant to degradation, immunologically stable, and reasonably insensitive to potential problems associated with routine specimen handling, when measured by immunoassay technique. Therefore, it is helpful in large epidemiological studies and small size laboratory, which require long transportation time and storage.
Keywords: Centrifugation, Preanalytical Variables, Thyroid stimulating hormone.

INTRODUCTION
Thyroid hormone (TH) is involved in regulating many important metabolic processes, which are vital for normal growth and development. Thyroid disorders are not very rare and are being managed by primary health care providers worldwide. In majority of cases, thyroid function workup is straightforward and easy to interpret for accurate diagnosis of various thyroid disorders including hyperthyroidism and hypothyroidism. However, in many patients results may not be obvious, either due to lack of correlation with clinical picture or because they are inconsistent with each other like high TSH, but with normal thyroid hormones; high thyroid hormones (TH) but with non-suppressed thyrotropin (TSH). In such cases it is essential to re-evaluate the clinical picture and to focus on probable confounding factors e.g. physiological variations (like pregnancy), non-thyroidal illnesses, and drugs intake (e.g. amiodarone, thyroxine and heparin). After ruling out these possibilities, common laboratory errors involved in thyroid hormone analysis should be assessed resulting in avoidance of further needless and futile workup.

A laboratory error is defined as any defect during whole testing process i.e. from advising investigations to reporting results, and in any manner affecting the quality and productivity of laboratory performance. In the recent past, fair amount of attention has been given to pre-
analytical quality in laboratory setups. Previously, analytical phase has gained more importance but with passage of time, it has become more evident that both pre- and post-analytical phases are quite vulnerable. Of the total laboratory errors, pre-analytical phase errors have been found to be 46-68.2%. Among the pre analytical variables, centrifugation time and circadian rhythm are most important that may affect TSH assay. Serum clot contact time is important pre-analytical condition that may affect the analyte stability, irrespective of patient’s physiological state. Thus, specimens should be centrifuged immediately after sampling to separate serum and plasma. It was recommended that serum must be separated from plasma within 2 hours of sampling. Different analytes have different tolerance levels and many of them are stable for longer duration as well.

TSH secretion follows diurnal variation. Peak level occurs between 0200 to 0400 hours, while low levels are seen between 1700 to 1800 hours 10. Circadian variation in TSH level is about ± 50%. However, levels do not vary beyond 10% between 0900 and 1600 hours.

Studies have been carried out in different regions and populations to assess the effect of various preanalytical variables on TSH measurements. However, our local data is sparse in this regard. Therefore, primary aim of this study was to identify effect of pre-analytical variables on serum TSH like effect of delayed separation by measuring the change in concentration of TSH. Furthermore, impact of diurnal variation and importance of timings for blood sampling were investigated.

METHODOLOGY

This cross-sectional study was conducted in Department of Chemical Pathology and Endocrinology, Armed Forces Institute of Pathology (AFIP), Rawalpindi, Pakistan from March 2018 to August 2018 after approval from Institutional Review Board (IRB). Disease free subjects with age ranging from 18 to 34 years, irrespective of gender, were included in study after taking written informed consent. Sampling technique used was non-probability convenience sampling. Sample size was calculated using EPI info Statistical calculator by using frequency of pre-analytical variables affecting thyroid assay published in article Basanta et al in 2017. Individuals with history of chronic illness, obstetric event (i.e. miscarriage or delivery) within nine months of thyroid hormone measurement and individuals taking drugs like amiodarone, iodine, dopamine agonists, glucocorticoids, anabolic steroids, somatostatin, gonadotropins, gonadotropin-releasing hormone, or any psychotropic medications were excluded from study.

A total of hundred subjects visiting AFIP for thyroid function tests were randomly selected for this study. Five milliliters venous blood sample was collected from each subject in a serum separator (yellow top vacutainer) by venipuncture and divided into two aliquots. First aliquot was centrifuged immediately, and serum was separated. Second aliquot was stored at ambient temperature (22ºC) for 24 hours in a temperature controlled air-conditioned laboratory before separation of serum. From the first aliquot, TSH was measured on the same day by third generation assay employing anti-fluorescein isothiocyanate (FITC) monoclonal antibody for chemiluminescent detection on ADVIA Centaur® XP Random Access Immunoassay System, Siemens Healthineers. Serum was separated from second aliquot after 24 hours and TSH level was measured on the same day. Serum TSH levels were analyzed twice daily, in the morning (0800 to 0900 hours) and afternoon (1400 to 1600 hours) after collection of blood samples. Data was analyzed using SPSS version 24. Kolmogorov and Shapiro Wilk test were applied to check for normality of data. Data was found to be non-parametric as it showed skewness that’s why all quantitative variables computed for non parametric statistics. Descriptive statistics like median and IQR were computed for non-parametric quantitative variables, i.e., Morning and evening TSH levels and late and early centrifugation TSH levels (table). Frequency and percentages were calculated for
qualitative variables like gender and pre-analytical variables. Test of significance Mann-Whitney U-test applied among four groups (TSH level in Morning, TSH level in evening, TSH level on early centrifugation and on late centrifugation) and p-value ≤0.05 was considered as significant.

**RESULTS**

Out of hundred study participants, 61 (61%) were male and 39 (39%) were female with mean age of 23 ± 3.4 years. Centrifugation change was observed in 11 (18%) males and 1 (2.5%) female, while change in circadian rhythm was observed in 17 (28%) males and 14 (36%) females. Changes in both variables were noted in 21 (34%) males and 22 (56%) females, whereas no change in both variables were noted in 12 (20%) male and 2 (5.1%) females as illustrated in figure. There was significant difference between TSH level and time (morning/evening) p=0.001, and there was non significant difference between early and delayed centrifugation p=0.0761 (table). A p-value <0.05 for morning and evening TSH levels while early and delayed centrifugation changes were insignificant.

**DISCUSSION**

In order to improve patients’ result, laboratory testing must be precise and accurate. Proficiency testing has improved laboratory performance especially analytical quality. However, it is not reflected by reduction in the number of analytical and organizational errors. Pre-analytical errors comprise of the largest bulk in laboratory errors in most of the studies performed recently and the most important aspect is that they are not detected even by the most stringent quality control procedures. Current study is a hospital based experimental study conducted to analyze the effects of short-term storage and delayed separation on TSH stability. Hormones stability in samples are influenced by many factors, some of which include storage temperature, length of time after sample collection and number of freeze thaw cycles. In current study, quite significant difference was observed in TSH values between blood samples collected at different times of the day from the same person, which is in concordance to the study carried out by Bosa et al in Bosnia et al proved the hypothesis that the timing of patients’ blood sampling has an impact on TSH values, requiring urgent standardization. This variable is of vital importance in patient care and diagnostics. In 2018, Liyanage et al conducted a study in Srilanka, which concluded that TSH in the early morning was higher as compared to that in the later hours of the day. This finding has been found to be consistent with our study. Similar results have also been reported in USA by Roni et al in 2014.

We found no difference across centrifugation timings in serum TSH concentration upto 24 hours after blood collection. Similar findings were also documented by Basanta et al in a study conducted in Nepal in 2017. This study revealed that, TSH measurement might be applicable in large epidemiological studies, which require very long transportation time to reach samples to the

Table: Descriptive statistics of quantitative variables affecting TSH.

| Variable | Morning Median, (IQR) | Evening Median, (IQR) | p-value |
|----------|-----------------------|-----------------------|---------|
| TSH Level (mIU/L) | 1.40, (0.78) | 1.50, (0.7) | 0.001 |
| Variable | Early Centrifugation | Delayed Centrifugation | p-value |
| TSH Level (mIU/L) | 1.40, (0.78) | 1.30, (0.6) | 0.761 |

Figure: Frequency of pre analytical factors with gender. In
laboratory. Study with similar results by Gro et al\textsuperscript{16} conducted in Norway in 2017 showed that various handling conditions have no effect on TSH in plasma kept for up to 72-hours at room temperature. Recently Gang et al reported that although interpreting results of thyroid function tests becomes straightforward but, it needs to be corelated with pre-analytical and post-analytical variables in order to improve efficacy and ignorance to these factors may lead to complications in patient’s health\textsuperscript{17}. Kyle et al. recommended to have some specific laboratory tests at particular times of the day to ensure the effect of pre-analytical variables such as sample for evaluation of testosterone should be taken early in the morning like 7am to 10am. One of the reason behind early sampling is that peak testosterone levels typically reported at the start of the day\textsuperscript{18} and found to be substantially lowered down as the day passes and remains approximately upto 50\% in the evening\textsuperscript{19,20}.

Although current study has proved its importance by revealing the effects of most common pre-analytical variables on TSH analysis but some limitations to our study includes small sample size, a fact that it was carried out in a single centre and consideration of only two among various factors affecting TSH levels.

CONCLUSION
TSH levels vary significantly between blood samples collected at different timings of the day from the same person. Time of blood sample collection has an impact on TSH results. Sample collection time must be regulated for TSH measurement standardization and harmonization. TSH is resistant to degradation, is immunologically stable, and reasonably insensitive to potential problems associated with routine specimen handling when measured by immunoassay technique. Therefore, it is helpful in large epidemiological studies and small size laboratory that requires long transportation time and storage.

CONFLICT OF INTEREST
This study has no conflict of Interest to be declared by any author.

REFERENCES
1. Mullr R, Liu YY, Brent GA. Thyroid hormone regulation of metabolism. Physiol Rev 2014; 94(2): 355-82.
2. Dietrich JW, Landgraef G. TSH and thyrotropic agonists: key actors in thyroid homeostasis. J Thyroid Res 2012; 2012: 351864.
3. Sheehan MT. Biochemical testing of the thyroid: TSH is the best and, of ten times, only test needed--a review for primary care. Clin Med Res 2016; 14(2): 83-92.
4. Soldin OP, Chung SH, Colie C. The use of TSH in determining thyroid disease: how does it impact the practice of medicine in pregnancy?. J Thyroid Res 2013; 2013: 146757.
5. Koulouri O, Moran C, Halsall D, Chatterjee K, Gurnell M. Pitfalls in the measurement and interpretation of thyroid function tests. Best Pract Res Clin Endocrinol 2013; 27(6): 745-62.
6. Green SF. The cost of poor blood specimen quality and errors in preanalytical processes. Clin Biochem 2013; 46(13-14): 1175-79.
7. Andersen IB, Braesen CL, Christensen H, Noehr-Jensen L, Nielsen DE, Brandslund I, Madsen JS. Standardised resting time prior to blood sampling and diurnal variation associated with risk of patient misclassification: Results from selected biochemical components. PLoS One 2015; 10(10): e0140475.
8. Atay A, Demir L, Cuhadar S, Saglam G, Unal H, Aksun S, Arslan B, et al. Clinical biochemistry laboratory rejection rates due to various types of preanalytical errors. Biochemia Medica 2014; 24(3): 376-82.
9. Basanta G, Gupta SP, Gautam S, Ashwini KN. Effect of delay separation and short term storage of serum on Thyroid Stimulating Hormone (TSH). Open J Thyroid Res 2017; 1(1): 001-09.
10. Sviridonova MA, Fadeyev VV, Sych YP, Melnichenko GA. Clinical significance of TSH circadian variability in patients with hypothyroidism. Endocrine Res 2013; 38(1): 24-31.
11. Fisher DA. Physiological variations in thyroid hormones: physiological and pathophysiological considerations. Clin Chem 1996; 42(1): 135-39.
12. Yilmaz FM. An underestimated preanalytical error source: Centrifugation temperature. Turk Biyokimya Dergisi 2013; 38(3): 1-5.
13. Mirjanic-Azaric B, Stojakovic-Jelisavac T, Vukovic B, Stojanovic D, Vujnic M, Uletilovic S. The measurement of time of sample collection on the measurement of thyroid stimulating hormone values in the serum. Clin Biochem 2015; 48(18): 1347-49.
14. Liyanage YS, Siriwardhana ID, Dissanayake M, Dayanath BK. Study on diurnal variation in TSH and free T4 levels of healthy adults. Sri Lanka J Diab Endocrinol Metab 2018; 8(1): 1-4.
15. Atalay R, Ersoy R, Demirezer AB, Akin FE, Polat SB, Cakir B. Day night variations in thyroid stimulating hormone and its relation with clinical status and metabolic parameters in patients with cirrhosis of the liver. Endocrinol 2015; 48(3): 942-48.
16. Villanger GD, Learner E, Longnecker MP, Ask H, Aase H, Zoeller RT, et al. Effects of sample handling and analytical procedures on thyroid hormone concentrations in pregnant women’s plasma. Epidemiol 2017; 28(3): 365-69.
17. MKGarg NM, Kumar KH. Laboratory evaluation of thyroid functions: dilemmas and pitfalls. Principles and Practices of Thyroid Gland Disorders 2017; 2017: 22.
18. Guer WG, Narayanan S, Wissler H, Zawta B. Samples: from the patient to the laboratory: the impact of preanalytical variables on the quality of laboratory results. John Wiley & Sons; 2008.
19. Brambilla DJ, Matsumoto AM, Araujo AB, McKinlay JB. The effect of diurnal variation on clinical measurement of serum testosterone and other sex hormone levels in men. J Clin Endocrinol Metab 2009; 94(3): 907-13.
20. Kyle C. Pathology handbook: a guide to the interpretation of pathology tests. New South Wales: Sonic Healthcare 2014.
