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Comparison of the Profile and TSH Levels from Several Types of Blood Collection Tubes

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ABSTRACT

Thyroid-Stimulating Hormone (TSH) is an important parameter in diagnosing thyroid disease which uses serum according to the World Health Organization's (WHO) recommendations. The use of plasma can help improve the Turn Around Time (TAT); however, the discrepancy with serum is unknown. A cross-sectional study using 89 blood samples was performed to compare TSH levels using serum tubes with clot activator (Tube I), plasma tubes with heparin (Tube II), and plasma tubes with heparin-gel separator (Tube III); and to overview of TSH levels according to gender and age. The median of TSH levels in Tubes I, II, and III were 1.380 (0.032-7.420) µIU/mL, 1.380 (0.030-7.480) µIU/mL, and 1.360 (0.030-7.460) µIU/mL, respectively. There were no statistically significant differences in TSH levels of the three tubes. The median TSH levels differences of Tubes II and III compared to the tube I were -0.9% (-7.2-2.2) and -1.7% (-8.0-1.6), respectively. Measurement bias observed in this study was following the specified desirable bias according to Ricos. The median TSH levels of the male and female groups were 1.500 (0.032-4.250) µIU/mL and 1.345 (0.058-7.420) µIU/mL, respectively. Median TSH levels of 31-40 years old age group and >61 years old age group were 1.190 (0.609-3.240) µIU/mL and 1.730 (0.088-5.760) µIU/mL, respectively. Specimens from three tubes could be used to examine TSH levels. Measurement of TSH levels showed a higher median in the male and older group.

Keywords: Thyroid-stimulating hormone, serum, plasma, clot activator, separator gel, clinical significance

INTRODUCTION

Thyroid-Stimulating Hormone (TSH) along with free thyroxine hormone or free T4 are important laboratory parameters in diagnosing abnormalities in the thyroid gland.[1,2] Disorders of thyroid gland function would cause significant changes in TSH levels; thus TSH level is a sensitive parameter to detect impaired function of the thyroid gland.[3] Thyroid-stimulating hormone levels are generally measured using immunology test with serum or plasma samples.[4] World Health Organization (WHO) recommended serum samples for measurement of thyroid hormone. The use of plasma with heparin anticoagulants showed no difference in results compared to serum.[5-7]

The use of plasma and gel separator has several advantages. The use of plasma can help improving laboratory Turn Around Time (TAT) because it requires no clotting time and minimizes the interference of free fibrin formation due to inadequate clotting time or the use of anticoagulant therapy. The use of gel separator may able to prevent contamination by blood cells after separation by centrifugation and reduce the use of another tube to separate plasma or serum from blood cell components during sample storage or measurement in automatic devices. However, there is a possibility of interference if plasma samples or gel separators are used. Plasma samples which have more turbidity than serum may reduce analytes because they are trapped in the gel separator, and measurement interference is highly possible due to non-specific reactions.[7]

Thyroid-stimulating hormone and free T4 level to assess the function of the thyroid gland are the hormone measurement which is widely performed in the Laboratory of Dr.Cipto Mangunkusumo General Hospital. Measurement of TSH uses serum samples from serum tubes containing clot activator without separating gel.[8] Thyroid-stimulating hormone measurement requires no rapid TAT; however, it is frequently carried out along with clinical chemistry assays which require rapid TAT. The use of plasma with Li-heparin anticoagulants for clinical chemistry may help improve TAT performance and shows minimal interference compared with other anticoagulants. Specimens collection with two

different blood tubes may cause patients discomfort; thus the use of a single specimen is preferred in laboratory service. There has been no experience of using other blood tubes at the Laboratory of Dr.Cipto Mangunkusumo General Hospital; thus, there is no finding of an effect of another type of specimens and blood tubes in TSH measurement. Therefore, information about the results of TSH assay and other immunological assay results using plasma samples with Li-heparin anticoagulants is needed. This study was expected to provide data on TSH measurement using a serum tube with clot activator without gel separator, plasma separator tube with heparin anticoagulant without separating gel and plasma separator tube with heparin anticoagulant with gel separator.

In addition to the selection of serum or plasma specimens, TSH levels are also influenced by gender and age. According to the National Health and Nutrition Examination Survey (NHANES, 2002), there was an increase of TSH levels along with the increasing age and higher TSH levels in females in the United States.[9] Increased TSH levels comparable to age were also reported in the report by Vadiveloo. A report by Vadiveloo also found that TSH levels were relatively higher in the male group. In the aspect of demographic data, this study was expected to provide an initial description of TSH levels based on sex and age. This study aimed to obtain specimens and storage tubes for the examination of TSH levels that can be used with other clinical chemistry assay and to overview TSH levels based on sex and age.

METHODS

This research was a cross-sectional study with consecutive sampling. Subjects were volunteers and patients who visited Dr. Cipto Mangunkusumo General Hospital in September-October 2018. Subjects were ≥ 18 years old and willing to participate in this study by signing informed consent. Specimens with conditions of hemolysis, jaundice or lipemia were not used in this study. The sample was blood taken from cubiti veins collected in serum tubes containing clot activator without separating gel (Tube I), plasma tubes containing heparin anticoagulants without separating gel (Tube II), and plasma tubes containing anticoagulant heparin with gel separator (Tube III). A blood tube containing heparin anticoagulant (Tube II-III) was flipped eight times to homogeneouslymix anticoagulant with blood and centrifuged at 3,000 rpm for 15 minutes to obtain plasma. A blood tube containing a clot activator (Tube I) was flipped 5 times to homogeneously mix-blood with the clot activator and was allowed to clot at room temperature for ± 30 minutes and centrifuged at 1,500 rpm for 10 minutes to obtain serum. Specimens of the three tubes were measured for the TSH level using Cobas e601. The research was performed at the Laboratory of the Clinical Pathology Department of Dr. Cipto Mangunkusumo General Hospital in September-October 2018.

Statistical analysis was performed using SPSS ver. 20.0. The difference of median TSH level between three tubes was analyzed using Kruskal-Wallis and post hoc analysis Mann-Whitney. P-value < 0.05 was considered statistically significant, with a 95% confidence interval. The difference of TSH levels after using Tubes II and III to Tube I was also compared to desirable specifications for bias according to Ricos.

The study protocol was approved by the Ethical Committee of Health Research of University Indonesia, Jakarta, Indonesia with number No.0924/UN2.F1/ETIK/2018.

RESULTS AND DISCUSSION

Thyroid-stimulating hormone levels from three types of tubes were obtained from 89 research subjects. The results of TSH levels measurement from serum tubes with clot activator and without gel separator (Tube I), plasma tubes with heparin anticoagulants and without separating gel (Tube II), and plasma tubes with heparin anticoagulants and separating gel (Tube III) are presented inTable 1.

The Kruskal Wallis test showed p=0.95, suggesting that there were no significant differences in TSH levels from the three types of blood tubes. Thyroid-stimulating hormone assay using several types of blood tubes without significant difference was also reported by Chance and Ercan. A study by Chance, use the same blood tube as this study; however, a greater number of research subjects were involved. Ercan reported that there was no difference in TSH levels from serum tubes using separating gel to serum tubes without separating gel.[10] Comparison of the results of this study with another research is presented in Table 2.

The difference in TSH levels from Tubes II and III to Tube I was determined. The biggest difference TSH levels Tube II to Tube I was -0.29 µIU/mL (-7.2%). The biggest difference of TSH levels Tube III to Tube I was -0.32 µIU/mL (-8%). The difference in TSH levels between Tubes II and III (-7.2% and -8%) was in

accordance with desirable specifications for bias (14.14%) according to Ricos. There fore, it was concluded that there was no clinical significance to the difference in TSH levels between Tubes II and III compared to Tubes I.[11] The use of desirable specifications for bias as a comparison of the clinical significance of TSH assay has not been obtained in other research reports. The difference in TSH levels between blood tubes was reported in the study by Chance. A comparison of differences in TSH levels obtained is presented in Table 3.

The difference in TSH levels in this study was proportionally greater than the study by Chance. This difference was probably caused by the smaller distribution of TSH levels in this study compared to the study by Chance. In this study, the lowest and highest TSH levels were lower than the study by Chance. It resulted in a proportionally higher difference of low TSH levels in this study compared to the study by Chance showing higher TSH levels.

Thyroid-stimulating hormone levels from serum tubes with clot activator without gel separator (Tube I) were grouped by sex. Of the 89 research subjects, there were 52 females (58.4%) and 37 males (41.6%). A higher median of TSH levels in the male group was found, compared to that in the female

Table 1. Thyroid-stimulating hormone levels from three types of blood collection tubes

		n	TSH (µIU/mL)	Bias toTube I	
				Absolute (µIU/L)	Proportional (%)
Tube	I	89	1.380 (0.032 – 7.420)	-	-
	II	89	1		.380 (0.030 – 7.480) -0.01 (-0.29 – 0.06) -0.9 (-7.2 – 2.2)
	III	89	1.360 (0.030 – 7.460)	-0.02 (-0.32 – 0.04)	-1.7 (-8.0 – 1.6)

Table 2. Comparison of TSH levels from some type of blood collection tubes

Research	n	Mean±SD or Median (min - max)			
		Serum tube with clot activator	Serum tube with clot activator and gel	Plasma tube with heparin	Plasma tube with heparin and gel
Chance [7]	32	2			.729±4.747 - 2.717±4.682 2.720±4.684
Ercan [10]	55	1.62 (0.19 -15.11)	1.8 (0.20 -16.59)	-	-
This research	89	1.380 (0.032 -7.420)	-	1.380 (0.030 -7.480)	1.360 (0.030 -7.460)

Table 3. Comparison of the difference in TSH levels from some type of blood collection tubes

Research	N	TSH level (mIU/L)	Mean (95% CI) or Median (min-max)	
			Plasma tube with heparin – serum tube (%)	Plasma tube with heparin and gel-serum tubes (%)
Chance [7]	32	0		.19 – 23.24 0.2 (-0.8; 1.1) 0.2 (-0.7; 1.2)
This research	89	0		.032 – 7.420 -0.9 (-7.2 – 2.2) -1.7 (-8.0 – 1.6)

Table 4. Comparison of TSH levels based on gender

	Age	N	Mean ± SD or Median (min-max)		P
			Male	Female	
This research	18-83	89	1.50 (0.032-4.25)	1.345 (0.058-7.42)	
Vadivelo[2]	18-90	153.127	1.72	1,70	<0.001
Suzuki[12]	15-92	2.456	2.44±2.14	1.99±1.91	
Hadlow[13]	51.6 (18.9)	120.403	3		.8 (1.4-5.0) 3.3 (1.1- 4.9) <0.001
Ahmed[14]	11-60+	498	1.35±0.60	1.45±0.65	

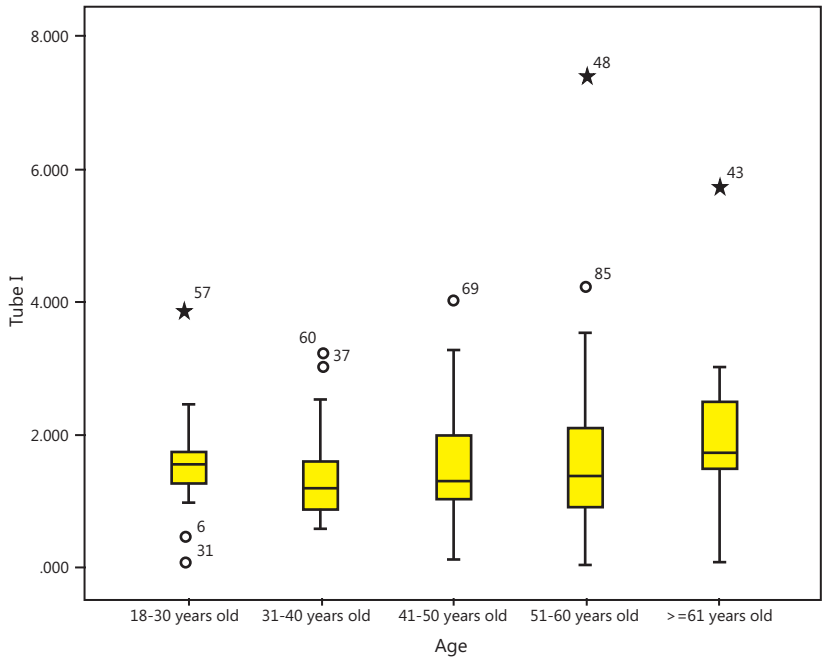

Figure 1. TSH level based on age groups

group (1,500 µIU/mL vs. 1,345 µIU/mL). According to several studies, median TSH levels in both groups in this study approached the mean TSH levels in a study by Ahmed and were relatively lower when compared with other studies (Table 4). The lower TSH levels in this study and Ahmed may be due to a younger age range and a lower number of research subjects than other studies.

The profile of TSH levels by sex in this study was in accordance with the studies of Vadiveloo, Suzuki, and Hadlow which showed that TSH levels in males were higher than those of females.[12,13] The opposite results were found in a study by Ahmed with the lower average TSH levels in males than females. The effect of sex on thyroid function remains uncertain, but there was a hypothesis suggesting that the influence of sex hormones on males increases levels of thyroid-binding globulin affecting the circulating thyroid hormone levels.[14] Increased levels of thyroid-binding globulin will increase thyroid hormone which is bound and will reduce free thyroid hormone levels. Decreased levels of free thyroid hormone in the circulation will cause a negative feedback mechanism to the pituitary gland and TSH production would increase.[15]

The age range of the subjects in this study was 18-83 years. Thyroid-stimulating hormone levels based on age were obtained by grouping TSH levels by age group. The age of subjects was grouped into five age groups and TSH levels in each group are presented in Figure 1.

A high median TSH level was found in the age

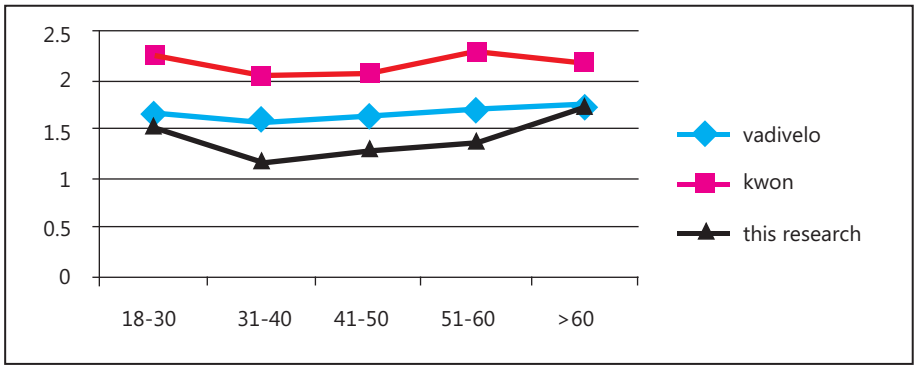

Figure 2. The pattern of median TSH level based on age group

group of 18-30 years but it decreased in the next age group (31-40 years) and gradually increased in the next age group. Patterns of changes in TSH levels in age-based TSH levels based on age groups from several studies are presented as graphs in Figure 2.

The pattern of changes in TSH levels by age group in this study was similar to the results of the Vadivelo and Kwon studies.[2,16] Young adults had higher TSH levels, then declined in the next age group but slowly increased with age. Ahmad found that TSH levels were relatively higher at young adults, possibly related to high T3 levels with relatively lower T4 levels than adult age groups.[14] Thyroid-stimulating hormone secretion is influenced by free T4 and T4 levels in circulation. Lower levels of T4 relatively trigger the feedback mechanism in the pituitary gland and increase TSH production.[17] Increased TSH levels in the older age group were suggested as a result of the aging process. The aging process would cause a decrease in endocrine function including the thyroid gland.[18] The aging process decreases thyroid hormone production, decreases sensitivity in target organs, and increases peripheral degradation of thyroid hormones. This situation triggers the negative feedback mechanism in the pituitary gland and increases TSH secretion.[19]

CONCLUSION AND SUGGESTION

There was no significant median difference of TSH levels from three types of blood tubes. The difference of TSH levels between tubes had no clinical significance and was in accordance with desirable specifications for bias according to Ricos. Heparin-plasma samples could be used for TSH assay to improve turn around time of clinical chemistry assay, while the use of gel separators in tubes could help reduce the use of additional blood tubes. A higher median value of TSH levels was obtained in males compared to females and increased TSH levels were found in the higher age groups.

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