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Impact Of Time Variation In Sample Collection On Thyroid Assay-An Observational Study.

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ABSTRACT

Subclinical Hypothyroidism is a common thyroid disorder with a prevalence of 6% to 15% in India. There is a persistent elevation in serum thyroid stimulating hormone (TSH) concentration (12 weeks or longer) with serum free thyroxine (fT4) concentration within reference interval. Its diagnosis is mainly dependent on the laboratory diagnosis, especially when the TSH value is above 4 mIU/L. The objective of the study is to determine the impact and importance of the time of blood collection for TSH and fT4 assays. An Observational study with prospective data collection was conducted on 40 study participants attended the OPD of Stanley medical college and hospital for a period of 2 months from January 2018 to March 2018. All the study population were not known to be a case of any thyroid illness or any thyroid related medications. Blood sample for TSH and Ft4 were collected in fasting state before 8 am in the selected population and again sample was collected after 10 am on the same day. The samples were processed by ECLIA method in e411 Immuno-analyzer. The statistical analysis was done by using SPSS 2.0 software. There was significant difference with a p value of 0.0131 for the TSH assay collected at 8 am and 10 am respectively. The ft4 assay did not show much significant differences with a mean of 1.377 and 1.325 at 8 am and 10 am collection respectively. We conclude stating that the timing of the tests affects TSH values and this should be noted in making decisions in diagnosis of subclinical hypothyroidism.

Keywords: Thyroid stimulating hormone, Subclinical Hypothyroidism, Immuno-analyzer, time of collection.

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INTRODUCTION

There is a common question as whether a person should fast for a routine thyroid testing (TSH Thyroid stimulating hormone and free T4). There is as well a doubt as whether the sample collection should be done in the early morning hours that is before 8 am or the sample collection could be done at any time of the day. Circulating TSH follows a normal circadian rhythm with a peak between midnight and 8 am and nadir levels between10 am to 3 pm as well 9-11 pm [1]. The low amplitude of pulses and longer half-life of TSH result in only modest variations in blood levels [2]. This time of collection becomes very significantly important in entities like subclinical hypothyroidism, in which the diagnosis mainly relies on the TSH values. These conditions may go under or over diagnosed based on a single value [3]. In special situations, like pregnancy narrower and stricter cutoffs for TSH have been laid to define Euthyroidism. So, it is necessary to follow a uniform and standard testing conditions. We have proposed this study to evaluate whether there is difference in the TSH measured was related to the time of sample collection.

SUBJECTS AND METHODS

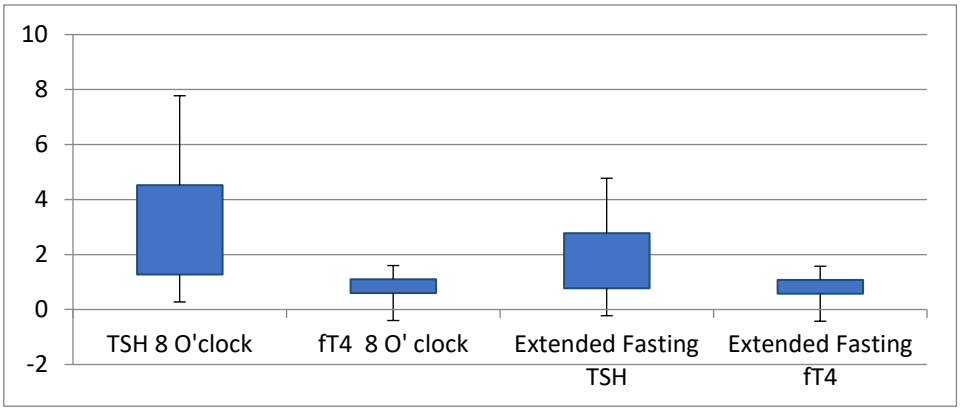
We have done a prospective collection of data from 40 participants who were not known to have any thyroid disorder and were not on any thyroid related medications. Blood samples for TSH and fT4 were collected in two sets. First sample were collected from all the subjects in the fasting state before 8 am and the second sample was collected in the fasting state after 10 am. Values were compared to find out the effect of the time of sample collection. The samples were processed by eCLIA. TSH assay in eCLIA is a three-step sandwich immunoassay. The chemiluminescent reaction is electrically stimulated to produce light at 620 nm. The amount of light produced is directly proportional to the amount of TSH in the sample. fT4 is measured by eCLIA by competitive immunoassay.

Statistical Methods

Continuous variables were tabulated and the mean, standard deviation (SD) were calculated. Comparison of means was carried out using paired *t*-test and unpaired *t*-test to find out significant differences.

RESULTS

The mean TSH of the fasting 8 am samples were 2.388 and that of after 10 am sample was 1.632 which was very significant(p=0.0131) using Box PLOT Graph comparison shown below.



Graph 1: Box plot comparison of mean TSH and fT4 at the two different timings of 8 am and 10 am and both were taken as fasting sample.

The mean fT4 value of the fasting samples that were collected before 8 am was 1.380 and that of fasting sample that were collected after 10 am was 1.330. This shows there was no significant difference among the t two groups with regards to ft4 (p< 0.446). [Table 1]

	Sample Before 8 am	Sample after 10 pm	P(paired t-test)
TSH (mIU/L)	2.388	1.632	0.0131
fT4	1.380	1.330	0.446

Table 1: Thyroid stimulating hormone and free T4 values: Comparison between the samples collected before 8 am and after 10 am (both in fasting state)

DISCUSSION

Thyrotropin-releasing hormone (TRH), is a tripeptide amide, which has a critical role in the central regulation of thyroid hormone stimulating hormone (TSH) by acting on the anterior pituitary. TSH subsequently stimulates the secretion of thyroid hormone from the thyroid gland. The circulating levels of thyroid hormones is maintained by the hypothalamic-pituitary-thyroid axis. The thyroid hormones are essential for the biological functions including brain development, food intake and energy expenditure, regulation of cardiovascular, bone and liver functions.

In addition to its role in regulation of TSH secretion, TRH is a potent regulator of prolactin (PRL) secretion via the tuberoinfundibular dopamine neurons.

The activity of TRH neurons is regulated by negative feedback effect of thyroid hormone as well through the neuronal inputs from several brain areas. TRH is a tripeptide neurotransmitter. In addition to its capacity to stimulate the release of thyroid stimulating hormone (TSH), It also stimulates prolactin secretion from the anterior pituitary. Its hypothalamic content shows a circadian rhythm in rodents, which is in its timing light-dark cycle dependent. TRH interacts with other circadian periodic neurotransmitters like 5-hydroxytryptamine, dopamine and norepinephrine.

The TRH acts via specific membrane receptor that results in the de novo synthesis of TSH. After binding with TRH, the receptor is degraded slowly. A short pulse of TRH leads to a prolonged secretion of TSH from pituitary cell. Thyroid hormones exert a powerful negative feedback control over the pituitary response to TRH.

The pulsatile stimulation of TSH secretion is due to the hypothalamic oscillator which is modulated by inhibitory hypothalamic influences of dopamine and somatostatin. Triiodothyronine (T3) secreted by the thyroid or converted from T4 in peripheral tissues. These thyroid hormones inhibit the synthesis and secretion of TRH and stimulates the release of inhibitory factors like dopamine and somatostatin. T3 inhibits the secretion of TSH secretion by interacting at the level of thyrotrope cells in Anterior pituitary.

Tri and tetra iodothyronins exert negative feedback on TSH levels. T3 is the predominant inhibitor of TSH secretion. Because TSH secretion is so sensitive to minor changes in free T4 through the negative feedback loop, abnormal TSH levels are detected earlier than those of free T4 in hypothyroidism and hyperthyroidism. Since there is a log-linear relationship between T3/T4 and TSH, even minor changes in T3/T4 can lead to significant changes in TSH.

In cases of both hypothyroidism and hyperthyroidism, TSH is the first-line screening test because the changes in TSH occur earlier than the changes in T3/T4. If the values are outside the range of 0.4 to 4.5 milliunits per litre (mIU/L), a measurement of T3 and T4 should follow. In primary hypothyroidism, TSH levels are elevated because of the loss of negative feedback inhibition on the thyrotropes of anterior pituitary.

Subclinical hypothyroidism (SCH) represents a state with increased values of thyroid stimulating hormone (TSH) with normal or near normal values of thyroxine (T4) and triiodothyronine (T3) [4]. In SCH the patient is asymptomatic, and the diagnosis is very much dependent on the results of laboratory findings when the level of TSH value is above 4.0 mIU/L [5].

Here in subclinical hypothyroidism, the values of thyroid hormone are normal and the increased level of TSH represents a compensatory mechanism that stimulates the thyroid gland to produce sufficient amounts of thyroid hormones. The disorder can eventually progress to overt hypothyroidism (OH) which is characterised by increased values of TSH but reduced values of thyroid hormones [6].

The entity of Subclinical hypothyroidism is usually asymptomatic, but in some patients may still appear symptoms that would indicate hypothyroidism. The most common symptoms are dry skin, poor memory, slower thinking, weakness and muscle cramps, fatigue, hoarseness and deep voice. In the US Colorado Thyroid Disease Prevalence Study, which included 20,862 examinees, patients with SH more frequently reported symptoms compared to euthyroid examinees but less frequently than patients with OH [7] . This study also showed that, most of the patients with subclinical hypothyroidism had TSH levels between 5.1 to 10.0 mIU/L. This shows that most of these patients have mainly a slight disturbance of the function of the thyroid gland which is the case in 90% of patients with SH in general population [8].

Thyroid hormone exerts direct effect on various organs. It exerts a direct effect on heart and blood vessels. In subclinical hypothyroidism, there is disruption of both systolic and diastolic function of the left ventricle. There is increased arterial stiffness and endothelial dysfunction [9,10].

As shown in many studies done in patients with subclinical hypothyroidism, there is increased levels of total cholesterol and low-density lipoprotein (LDL) [7,11]. Patients with SCH are believed to be at increased risk of atherosclerosis. This correlation has been shown in study done at Rotterdam, where around 1149 women aged over 55 years with TSH >4mIU/L were examined. This study showed increased risk of atherosclerosis and occurrence of myocardial infarction in these subjects [12].

In a study done by Scobbo *et al* [13], an observation was made that TSH was supressed post prandially. TSH secretion is heavily dependent on the factors including thyrotropin -releasing hormone and somatostatin. TRH stimulates TSH secretion while Somatostatin inhibits its release. In the current study, we noticed that there was significant decline in TSH values when the sample was collected after 10 am, even though the sample was collected in the fasting state. This finding is consistent with the observations of Ehrenkranz *et al* [1] that there is nadir of TSH beginning at 10 am which may be the physiological explanation for TSH suppression seen in this present study. In a study done by Mahadevan*et al* the same parameters were processed by different assay methodologies [14].

But the TSH values did not differ significantly between any of the assay methods. This indicates that the decline in TSH value is more likely due to biological factor alone.

Thus, the timing of sample collection is important for TSH assay, especially while dealing with minor variations in TSH. Even minor variations could be clinically relevant in the diagnosis of Sub clinical Hypothyroidism, pre-pregnancy counselling and subfertility. It is preferred to do an early morning sample collection whenever there is a requirement to follow a narrower and stricter reference ranges for TSH, especially in the two above mentioned settings.

CONCLUSION

This study emphasises on the early morning sample is better timing for TSH analysis especially in cases of crucial diagnosis as in subclinical Hypothyroidism and during pregnancy.

Limitations Of the Study

The testing was done in only a small population and the comparison with the post-prandial sample testing was not carried out.

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