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Research Article

Effect of Delay Separation and Short Term Storage of Serum on Thyroid Stimulating Hormone (TSH)

Abstract

Introduction: Thyroid stimulating hormone (TSH) regulates the level of thyroid hormones synthesized in the thyroid gland. Its measurement greatly facilitates the clinical diagnosis as well as management of pituitary-thyroid diseases. The level of analytes in biological sample is affected by pre-analytical, analytical and post-analytical factors.

Objective: To find the effect of delay separation and short term storage on serum TSH level.

Materials and Methods: A total of 15 blood samples were collected from the patients visited to the immunoassay laboratory, Department of Biochemistry, BPKIHS for thyroid function test. Serum TSH was estimated on the day of sample collection, after 24 hours in delay separated samples and after seven days in short-term stored sample by sandwich ELISA method (Eliscan, India).

Results: There were no significant difference in median serum TSH in baseline and delay separated samples (1.43 (0.18-6.52) IU/mL, vs 1.61 (0.25-6.51) IU/mL, $p = 0.069$) as well as baseline and short term stored samples 1.43 (0.18-6.52) IU/mL vs 1.57 (0.26-5.75), $p = 0.925$).

Conclusion: There is no effect of delay separation and short term storage on the serum TSH level. So, TSH measurement may applicable in large epidemiological studies which require long transportation time to transport samples to the laboratory.

Introduction

There has been increased progress on immunoassay of the thyroid stimulating hormone, over the past decades, which greatly facilitate clinical diagnosis as well as management of pituitary-thyroid disease [1]. Large-scale epidemiological studies are needed for the screening of diseases of endocrine systems. It requires the measurement of hormones in blood samples from large numbers of subjects, but there are practical difficulties in collecting and processing samples immediately in the developing country like Nepal. In large scale screening blood sample may be collected at local centers or clinic and sent unfrozen by courier or routine post to central processing and storage laboratories but analysis is not cost-effective on daily basis in most middle size laboratories. Therefore, samples are stored for a period of time [2].

If the result from a specimen is altered by serum-clot contact enough to exceed the variability expected for physiological processes. The serum-clot contact time represented a unique

pre-analytical condition that could change the analyte stability independently of any physiologic condition in the patient [2]. Prolonged contact of serum with the clot can cause pre-analytical variation [3]. So, serum or plasma should be separated from physical contact with cells as soon as possible, unless conclusive evidence indicates that longer contact times do not contribute to result inaccuracy. A maximum limit of two hours was also recommended [4].

Each analyte has a different tolerance to a delay in separating serum from clot. Many analytes are stable for much longer than two hours. In hospitals and outpatient clinics, transportation of specimens from a phlebotomy site to a laboratory sometimes takes longer than two hours. Analyte are usually grouped into time blocks in which serum-clot contact cause no changes in analyte concentrations [2].

Objective

To investigate the effect of delay separation and short term storage by measuring the change in concentration of TSH

in blood that will be held at ambient condition for up to 24 hours since venipuncture and stored at 4 °c for 7 days since venipuncture.

Material and Methods

A quasi experimental study was carried out in Immunoassay Laboratory, Department of Biochemistry, BPKIHS, Dharan, Nepal. A total of 15 individual visited to the immunoassay laboratory for thyroid function test were conveniently selected for this study and five milliliters venous blood samples were collected in a plain vial by venipuncture and divided into two aliquots. First aliquot was centrifuged immediately and serum was separated. Second aliquot was stored at ambient temperature (22 °c) for 24 hours in a temperature controlled air conditioned laboratory before separation of serum. From the first aliquot, TSH was measured by sandwich ELISA based immunoassay (Eliscan, India) [5] on the same day and remaining aliquot was stored at 4 °c for 7 days and TSH level was measured after 7 days. Serum was separated from second aliquot after 24 hours and TSH level was measured on the same day. Data was expressed as median (Inter Quartile range) and Wilcoxon Signed Ranks test was applied to test the significance level, considering p value ≤ 0.05 as statistically significant.

Result

The median (IQR) of baseline serum TSH level was not statistically significant different between delay separated (after 24 hours) sample and short term stored (4 °c for 7 days)

(1.43 (0.18, 6.52) μ IU/mL vs 1.61 (0.25, 6.51) μ IU/mL, $p=0.069$ and 1.43 (0.18, 6.52) μ IU/mL vs 1.57(0.26, 5.75) μ IU/mL, $p=0.925$).

Discussion

This is hospital based quasi experiment to see the effect of delay separation and short term storage on stability of thyroid stimulating hormone. Stability of hormones in a samples are influenced by the length of time after collection, storage temperature and the number of freeze-thaw cycles [6]. The rationale of measuring serum TSH level in clinical setting is different from that in epidemiological settings. In clinical settings, measurement used to find out the normal and abnormal values as well as to assess the degree of abnormality but moderate difference with in the reference range are of etiological interest in epidemiological studies [7]. Delays in transportation of blood specimens to the laboratory may cause systematic changes in hormone concentrations and this could obscure association in epidemiological studies [8]. It is not always practical to process blood samples immediately after collection and should be stored for further analysis in large epidemiological as well as in clinical study [9].

In this study, the baseline serum TSH level is not significantly different as compared to delay separated samples and short term stored samples (Table 1). Mannisto T et al. (2007), reported no differences in TSH, fT₄, TPO-Ab, or TG-Ab concentrations when 50 frozen and thawed serum samples were compared with 50 fresh serum samples [10]. Allen AL et

Table 1: Comparison of Median (IQR) Serum TSH level between bassline, delay separated and short term stored sample.

Group	Median IQR TSH (μ IU/mL)
Baseline Sample (n=15)	1.43 (0.18, 6.52)
Delay Separation (n=15)	1.61 (0.25, 6.51)*
Stored Sample (n=15)	1.57(0.26, 5.75)**

*Comparison between baseline and delay separated sample ($p=0.069$),

**Comparison between baseline and short term stored sample ($p=0.925$).

al. (1997), also reported statistically no significant differences ($p=0.7$) associated with the measured concentrations of baseline T₃ or T₄ and serum separated after 24 hours. The effect of long-term storage on T₃ and T₄ in male and female serum was also reported by Allen et al. (1997), [7]. There were no apparent trends or statistically significant differences in between baseline and 19 to 22 months stored T₃ concentrations either in males ($p=0.23$) or females ($p=0.52$)¹² Jones ME et al. (2007), reported that, 7.1% and 5.6% increase in estradiol concentration after one day and two days delay in processing. Progesterone concentrations showed no substantial change over the two days period but testosterone concentrations increased by 23% after one or two days delay in processing. FSH and LH concentrations increased on average by 7.0% and 3.8% per day respectively. In contrast to this, SHBG concentrations decreased on average by 5.8% per day over the two days period [11]. Oddie TH et al. (1979), reported that, significant decline in T₄, rT₃, TSH and T₃, concentration with storage interval, the mean observed rates of decline correspond to 5.3%, 3.5%, 0.9%, and 4.3% per year, respectively but TBG concentrations did not appear to change significantly during storage [12].

Conclusion

TSH is resistant to degradation, immunologically stable, and reasonably insensitive to potential problems associated with routine specimen handling when measured by immunoassay technique. So, it is helpful in large epidemiological studies and small size laboratory which require long transportation time and storage.

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