Original Article

The Effect of Clot-Activator and Storage Time on Estimation of Free triiodothyronine T3 levels in Blood Samples

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Objective: To find out the effect of clot-activator and storage time on estimation of free triodothyronine (FT3) in blood samples. .

Methods: Thirty five normal volunteers from general population were selected. Their blood samples were collected into two different types of Greigor Bio-One blood collecting tubes (BCT). Blood collecting tubes of first type were without clot activator and second type of BCT was with clot activator. Blood samples of first type of BCT were used to analyse the effect of storage time on free triodothyronine (FT3) estimation. These tubes were centrifuged and separated serum was preserved into 4 BCT without clot activator and labelled as first day, 24 hours, 48 hours and 96 hours. The serum of these tubes was estimated for FT3 at the intervals as labelled on the BCT. Free triiodothyronine estimation was done using Beckman coulter kit. To analyse the effect of clot activator on FT3 estimation, blood samples collected in BCT with clot activator were allowed to clot, serum was separated and FT3 estimation was done on the same day.

Results: Results of this study showed no statistical significance of results after 24h (p=0.256) by one way ANOVA analysis and Post Hoc test, but significant difference in values of FT3 concentration was observed after delayed analysis of 48 and 96 hours with P value(p=0.03). Paired Samples Test-BCT with and without clot activator had variation in results and significance (p =0.002) were analysed by statistical analysis.

Conclusions: This study concludes that, FT3 concentration variation was not statistically significant when it was estimated within 24 hours, but a false increase was observed after a delay of 48 to 96 hours. Samples of BCT without clot activator showed precise results of FT3 estimation when compared to the results of samples of BCT with clot activator. Therefore, BCT without clot activator should be preferred for collection of blood sample for FT3 estimation.

Keywords: Triodothyronine (FT3), thyroxine (T4), blood collecting tubes (BCT), clot activator.

Introduction

Endocrine glands secrete different types of hormones. These hormones through blood circulation reach the target organs and control their functions. The thyroid gland is also an endocrine gland which produces thyroid hormones that are classified as tyrosine derivatives.[1] Anatomically, thyroid gland consists of two lobes, lobus sinister and lobus dexter,[2] which are joined to one another by the median isthmus. In addition to that, a majority of the population (44.3%) has a pyramidal lobe, arising from isthmus or the next lobes.[3] The thyroid gland is attached to trachea via several ligaments and muscles that make it move during swallowing with the movement of trachea.[4-6] Histologically, thyroid gland is consists of epithelial cells, follicles and parafollicular cells. Epithelial cells control the T3 (triiodothyronine) and T4 (thyroxine) secretions and parafollicular cells are involved in production of calcitonin, , whereas thyroglobulin which is found in follicles (20-40 in number) helps in thyroid hormones production.[7] Spleen, kidney and liver control the 80% conversion of T4 to T3, as T3 is it's more active form (10 folds) than T4.[8-9] This conversion is done by Deiodinase enzyme followed by the release of thyroxine and triiodothyronine into the blood by protein kinase.[10] When thyroid hormone decreases in blood, TSH is released from anterior pituitary gland through feedback mechanism and binds the receptors on the thyroid follicle cells. This stimulates the production of thyroid hormones, which are released into bloodstream. In the body, only a small amount of thyroid hormones are found in free form, as FT3 (0.3%) and FT4 (0.03%). While the remaining T3 (20%) and T4 (80%) is bounded to thyroxine binding globulin, albumin and transthyretin.[11] Triiodothyronine can cross the blood brain barrier via transport protein(MCT8). It is essential for neural development during fetal period of life. It is also involved in cell proliferation, growth and development of the body.[12-13]

Abnormally high production of thyroid hormones causes hyperthyroidism which may lead to thyrotoxicosis during which iodine uptake is abnormally increased. For laboratory diagnosis of thyrotoxicosis, direct measurement of FT3 is preferable. Treatment of hyperthyroidism includes anti-thyroid drugs, radioactive iodine and thyroidectomy.[14-15] In hypothyroidism, thyroid hormone synthesis is reduced that can be due to acute thyroiditis, Hashimoto's thyroiditis, radiation treatment or drugs etc. and treatment include thyroxine replacement therapy by levothyroxin[16].

Estimation of thyroid hormones in the blood samples is improved by enhancement of techniques like radioimmunoassay, IRMA, LC-MS (Liquid Chromatography-tandem Mass Spectrometry). Immunoassays have replaced the typical protein bound technique[17-18]. But still thyroid test sensitivity, stability and specificity are creating hurdles.[19]

Detection of FT3 is easy because it circulates in the blood in its free form as compared to Total T3(TT3) and Total T4 (TT4) that is bound to several proteins. Free hormone tests are developed to find thyroid hormones impact at the cellular level where antibody labelled analysis is done to get reliable results[20]. Variations of results of FT3 are found due to pre-analytical errors and endogenous materials,[21] which may be clot activator present in the BCT. Therefore, this study was conducted to determine the stability of FT3 by analysing the effect of storage time and clot-activator present in a blood sample.

Methods

Thirty five normal volunteers from general population were selected. Their blood samples were collected into two different types of Greigor Bio-One blood collecting tubes (BCT). Blood collecting tubes of first type were without clot activator and second type of BCT were with clot activator. Blood samples of first type of BCT were used to analyse the effect of storage time on free triodothyronine (FT3) estimation. These tubes were centrifuged and separated serum was preserved into 4 BCT without clot activator and labelled as first day, 24 hours, 48 hours and 96 hours. The serum of these tubes was estimated for FT3 at the intervals as labelled on the BCT. Free triodothyronine estimation was done using Beckman coulter kit. To analyse the effect of clot activator on FT3 estimation, blood samples collected in BCT with clot activator were allowed to clot, serum was separated and FT3 estimation was done on the same day. Beckman Coulter kit (REF No. IM1579) was used for determination of FT3 concentration in serum samples. The standard value of serum FT3 in pM/L is 2.5-5.8. It follows the principle of labelled antibody immunoassay (competitive).

During the procedure, 100 micro litre of control was added in a tube; calibrator 100 micro litre was added in 5 tubes, whereas 100ul of each sample was added in a separate container. Then, 400ul of tracer was introduced into each tube and mixed completely by pulse overtaxing. These tubes were then incubated for 2 hours (20°C) on shaking incubator (350rpm) followed by aspiration of each tube. Gamma (Scintillation) counter was used to count total and bound compound. From standard curve, the concentration of FT3 samples was obtained in pM.

Paired t-test, ANOVA, Post-Hoc test and correlations were performed for analysing the statistical significance of the results. IBM SPSS statistics software (version 21) was used for all statistical analysis.

Results

False increase in levels of FT3 concentration was obtained in delayed analysis, i.e. with increase in storage time. After 24 hours of delay, no statistically significant difference (p=0.256) was seen in FT3 concentration (Fig.1). Whereas, with further delay (i.e. after 48h and 96h) false increase in levels of FT3 concentration was significant (p=0.03) when analysed by one way ANOVA **(Table.1).**

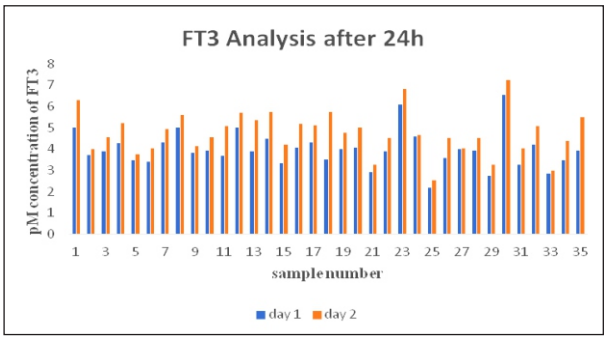

Fig-1: Showing the significance of concentration of FT3 for first day and second day (24h) of FT3 analysis. X-axis represents the no. of samples for first and second day while y-axis represents pM concentration of Ft3. It shows false increase in FT3 concentrations.

The concentration of each sample in BCT with or without clot activator was measured using the

standard technique, and the difference was observed in both the instances. The concentration of FT3 in the samples collected in BCT with clot activators were higher as compared to without clotactivators. Therefore, significant difference in the values were obtained (p =0.002) by using paired T-Test **(Table 2)**.

Significant difference in the concentration of FT3 of samples in tubes with clot activators as compared to tubes without clot activators was observed.

Table-1: Comparison of serum level of FT3 on 1st day, after 24 hours, 48 hours and 96 hours.

Parameter pM	FT3 level at 1st day Mean ± SD	FT3 level at 24 hours Mean ± SD	FT3 level at 48 hours Mean ± SD	FT3 level at 96 hours Mean ± SD
FT3 (2.5-5.8)	3.98±0.85	4.15±1.02*	4.94±1.26**	5.21±1.94**

**p = 0.256, **p = 0.03 (p<0.05).*

Table-2: Comparison of serum level of FT3 between sample without clot activator and sample with clot activator

Parameter pM	FT3 level in sample without clot activator Mean ± SD	Ft3 level in sample without clot activator Mean ± SD	p-Value
FT3 (2.5-5.8)	4.98±1.86	6.05±1.6	0.002*

**p<0.002*

Discussion

The thyroid gland secretes thyroid hormones into the blood stream. Thyroid hormones reach the target organs and control the functions of the body. Release of thyroid hormones T3 and T4 is controlled through TSH which is secreted by anterior pituitary gland. In the serum, concentration of FT3 in pico-moles is difficult to estimate as compared to the estimation of TT3 with conventional detection methods. Currently, radioimmunoassay kit method is used for this purpose as it is reproducible, easy to use, saves time and cost effective. In this study, the effect of storage time of sera and presence of clot activator in the BCT were analysed on the estimation of FT3 by using Beckmann Coulter Kit. It is a unique study and minimal research is available in the literature. Results of previous studies showed that prolonged storage time of sera and presence of clot activator affect the FT3 analysis.[22] In our study, insignificant increase in FT3 was observed after 24 hours of delay. Similarly, in another study, FT3 concentration increased within reference range after a delay of 24 hours.[23,24] This observation of our study confirms the results of previous studies. However, after a delay of 48 to 96 hours significant increase in FT3 was observed in our study. This false increase in FT3 was due to fibrin clot, inappropriate T3 formation or heparin interaction.[25] Analytical error in FT3 estimation was observed when clot activator coated BCT were used which is one of the causes of false increase in FT3. This research concludes that a delay of 48 to 96 hours in analysis of FT3 can significantly affect its estimation and false increase in its levels are observed. Therefore, for precise measurements BCT without clot activator should be preferred.

Conclusion

This study concludes that, FT3 concentration variation was not statistically significant when it was estimated within or after 24 hours, but a false increase in it's concentration was observed after a delay of 48 to 96hrs. Samples of BCT without clot activator showed precise results of FT3 estimation when compared to the results of samples of BCT with clot activator. Therefore, BCT without clot activator should be preferred for collection of blood sample for FT3 estimation.

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