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Comparison of Second and Third Generation Thyroid Assays.

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ABSTRACT

To meet the demand of clinical practice rapid, sensitive and specific laboratory tests are essential. Determination of TSH by third generation thyroid function assay is currently considered as the most sensitive and cost effective first line approach to thyroid function testing. In the current study we have analysed and compared the interassay and intraassay precision and sensitivity of ADVIA centaur CP CLIA and Bio-Rad ELISA.By using standardised approach during a period of one month functional sensitivity was calculated as 0.015mu/l for a sample size of 100. The third generation assay has far superior precisions in subnormal TSH level and above normal TSH range as compared to second generation assays. The results were statistically significant with p value less than 0.0001. Higher functional sensitivity & superior precision of third generation TSH assays can be useful in detection of subclinical thyroid dysfunction and can be more useful in screening of thyroid diseases.

Keywords: thyroid, TSH, assay

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INTRODUCTION

Thyroid disease is one of the most common endocrine disorders. The laboratory diagnosis and monitoring of thyroid diseases such as hypo and hyper thyroidism are based on serum TSH measurement along with serum T4 and T3 (both free and total) . The National Academy of Clinical Biochemistry (NACB) has recommended that the functional sensitivity of TSH assay be less or equal to 0.02 mIU/L. This permits patients with nonthyroid illness to be distinguished from those with primary hyperthyroidism.

The analytical sensitivity of TSH assay and its ability to reliably distinguish between euthyroid and hyperthyroid patients especially in subclinicial stages, where T4 and T3 levels are in normal range makes it a very sensitive marker of primary thyroid function abnormalities. Several years ago, the most commonly used assay for the measurement of TSH was radioimmunoassay which was considered as the first generation method with functional sensitivity of 1 mIU/L, IRMA was the second generation method with functional sensitivity of 0.1 mIU/L from the 1990s to date, and the third generation method was electrochemiluminescence assay that had been introduced with improved functional sensitivity.

Aims and Objectives

To meet the demand of clinical practice rapid, sensitive and specific laboratory tests are essential. This study compares the sensitivity of CLIA (Chemiluminensceimmuno assay) vs ELISA (Enzyme linked immuno sorbent assay).Determination of TSH by third generation thyroid function assay is currently considered as the most sensitive and cost effective first line approach to thyroid function testing [1-5].

MATERIALS AND METHODS

In this current study 100 patient were randomly selected attending sree balaji medical college and hospital. Written consent from the patients and ethical committe approval was obtained for this study. Bloodsamples was collected and serum was separated for investigation. TSH were assayed by second generation (Bio-Rad ELISA) and third generation (ADVIA centaur CP CLIA) immune assay. A detailed history was taken to correlate the values clinically. Serum specimens were used to evaluate the minimum detectable concentration and intra- and inter-assay precisions for TSH. For intra-assay run imprecision estimation, the analytes were analyzed 20 times a day and 20 different non-consecutive days in one month for the inter-assay imprecision study. Data was analysed with SPSS 18 package.

RESULTS

The patients were divided into three groups according to the levels of TSH i.e. patients with normal TSH,decreased level and increased level . The table illustrated shows a comparison of TSH levels measured on two different instruments which are based on the principles of ELISA and CLIA.The values of TSH in same serum sample obtained by the two methods was statistically significant with a signicant p-value of 0.0001 specifically in group 2 (hyperthyroid patient) and group 3 (hypothyroid patients).

The serum TSH levels estimated on ADVIA centaur CP CLIA and Bio-Rad ELISA shows a significant difference and wide range of coefficient of variation. In group 1, control group, the TSH levels were comparable on both assays. In the group 2, hyperthyroid patients, significant difference has been observed, i.e.subnormal level of TSH by third generation method has been reported with better sensitivity and precision than second generation method. In this group, 25 serum TSH levels were below normal range, of which 15 serum TSH levels were not detected by second-generation method but the third generation assay method could report which ensures the better sensitivity. In group 3, hypothyroid patients, 34 patients were having higher serum TSH levels than normal range, of which 10 patients had serum TSH more than 40 µIU/ml by second generation assay were as the value detectable by third generation immune assay was upto 300µIU/ml.So the functional sensitivity of method becomes more important in these groups of patients.

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Table 1: Comparison between CLIA and ELISA

S.NO	GROUP	CLIA-MEAN SD	ELISA-MEAN SD
1	EUTHYROID n=41	2.3 +/- 0.34	2.5+/-0.642
2	HYPERTHYROID n=25	0.05+/-0.1	0.41+/-0.312
3	HYPOTHYROID n=34	11.5+/-3.0	14.08+/-6.1

Table 2: Comparison of Higher Detectable Value of TSH in CLIA and ELISA

CLIA	ELISA
44.5	212.5
43.1	167.4
41.5	292.1
41	81.01
42.9	231.8
43.2	213.6
40.1	150.81
41.7	249.01
42.4	67.92
40.3	245.01

Table 3: Comparison of Functional Sensitivity of Imunoassay Technique

S.NO	GENERATION	FUNCTIONAL SENSITIVITY
1	FIRST	1- 2 µIU/ml
2	SECOND	0.1 - 0.2 µIU/ml
3	THIRD	0.01 - 0.02 µIU/ml

DISCUSSION

The minimum detectable concentration for TSH ADVIA centaur CP CLIA was lower than BIORAD ELISA. When zero standards were processed, the minimum TSH measurement was 0.01 & 0.1 µIU/L by ADVIA centaur CP CLIA & ELISA respectively. The reason for this difference is the use of the 3rd generation TSH by ADVIA centaur CP CLIA which is capable of measuring TSH concentration as low as 0.01 µIU/L. Another reason for this ability is that, ADVIA centaur CP CLIA immunoanalyzer is based on a new detection technology that uses an electrochemiluminescent label. This is particularly important in its ability to differentiate the subclinical and clinical hyperthyroidism states.

This analyzer shows no carry–over in the measurement which can be expected in an automated system that changes its tips and curettes with every sample. Linearity assays which were verified by diluting samples with ADVIA centaur CP CLIA buffer indicated a desirable percentage of recovery. Based on percentage recovery, the obtained ADVIA centaur CP CLIA for TSH, T3 and FT4 were more satisfactory than and Elisa.

In dilution studies performed for ADVIA centaur CP CLIA unlike Elisa; TSH, T3 and FT4 measurement results were independent of dilution factor .Certain amount of carry–over has been reported with most immunoassay systems, however, with CLIA method in which solutions are provided by the company itself , tips and curettes are changed with every sample and no carry – over has been found. In this study, we did not evaluate the effect of lipemia, hemolysis and icterus on the hormone .The possibility of false low concentration for TSH is unlikely, whereas for Elisa methods, the maximum reporting range proposed by manufacturers is 40 mIU/L (2). In pregnant women, because of high HCG concentration, there is a possibility of cross-reactivity in TSH assay, however, it has been shown that high concentrations of HCG, FSH and LH have no cross-reactivity with the ADVIA centaur CP CLIA.

Regression analysis results showed no correlation between the ADVIA centaur CP CLIA and Elisa. This method shows a high degree of reproducibility and linearity with no carry-over effect. The low detection limit for TSH by Elecsys 2010 makes it a sensitive method for detecting patients with thyroid disorders.

In conclusion, we found that ADVIA centaur CP CLIA is an automated reliable, efficient and technically excellent instrument to use in the measurement of serum TSH. The electrochemiluminescence technology of ADVIA centaur CP CLIA shows the advantages in system performance. This method is particularly superior to other laboratory methods for the measurement of serum TSH since its minimum TSH concentration detectibility of 0.01 µIU/L facilitates the diagnosis of subclinical hyperthyroidism from euthyroid state with low serum TSH.

Measuring thyroid concentrations in various patients with 3rd generation thyroid assay was found to be advantageous in the following clinical situations,

a) Detects mildly suppressed thyroid concentrations as in case of maternal diabetes there by initiating treatment and minimising overt complications in both mother and offspring
b) Patient receiving long term suppression treatment after thyroidectomy, measurement of TFT allows accurate monitoring of hormonal therapy.
c) Discriminates between moderate suppression and undetectable levels.

CONCLUSION

Due to its sensitivity of measuring thyroid function test of very low concentration CLIA is considered to be more accurate method and recommended for specialised clinical laboratories for identification of subclinical thyroid abnormalities.

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