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# COMPARISON OF MEASUREMENT OF SERUM TSH BY TWO 3RD GENERATION TECHNIQUES

**Amandeep Kaur*, Minni Verma, Shalini Gupta, Vaneet Kaur, Brinder Chopra and Kamaljit Singh**
Department of Biochemistry, Gian Sagar Medical College & Hospital, Ramnagar, Patiala, Punjab, India.

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**Abstract:** The present study compared the measurement of serum TSH by two 3rd generation methods based on different principles i.e. Chemiluminesence (CLIA) and Fluorometric enzyme immuno-assay (FEIA). Main aim of the study was to evaluate the usefulness of the more sensitive assay method in patients with thyroid disease and non-thyroid illness (NTIs). Blood samples were withdrawn randomly from 125 subjects attending OPD and same sample was analyzed for TSH on two equipment's based on methods with different principles.i.e. CLIA (Chemiluminesence) and FEIA (AIA-360). Out of 125 serum samples, the levels of TSH were within normal range in 68 subjects, were above normal range in 35 subjects and were below normal range in 22 subjects by both the methods. A specific value for TSH could not be reported by FEIA based equipment-AIA-360 in 14 (63.6%) out of total 22 hyperthyroid subjects taken up for the study. The TSH values in 35 hypothyroid subjects obtained by two different method was also significantly ($p < 0.0001$) different. This clearly indicates that the Chemiluminesence assay system is far superior with respect to precision and sensitivity as compared to FEIA for reporting subnormal TSH levels as well as above normal TSH and can be useful in detection of SCTDs (subclinical thyroid dysfunction) and in screening of thyroid diseases.

**Key words:** Third generation TSH assay, Chemiluminesence assay / FEIA method.

## INTRODUCTION

Thyroid dysfunction affects each and every system of the body and involves each and every speciality. Likewise symptoms of other organs of the body may mimic thyroid symptoms. This results in frequent advice of thyroid function test (TFT's) by the clinicians. More often than, result do not reveal any abnormality. Symptoms like weight loss or gain, tiredness, heat or cold intolerance, increased or decreased appetite and palpitation are quite nonspecific and not specifically attributable to thyroid disease. Goitre which is specific to thyroid may yield absolutely normal thyroid functions[1].

Serum TSH evaluation is very important parameter for assessing thyroid dysfunction[3]. The methodology for measuring serum TSH has undergone dramatic changes over the last four decades with respect to functional sensitivity limits. Many methods are there for estimation of TSH and the most popular had been RIA (Radioimmunoassay), EIA (Enzyme-linked immunoassay method), ELISA (Enzyme-linked immunosorbant assay). The first generation of TSH assays used between 1965 and 1985 were based on RIA methodology that had limited functional sensitivity (~1.0 mIU /L)[4,5,6].

Further in mid-80's development lead to the second generation methods based on IMA methodology with improved functional sensitivity[7,8,9,10,11,12]. These the methods comes under second generation. But with spectacular invention of the third generation assay method, the results are more precise and better with respect to analytical, operational and clinical outcomes. Moreover; it has less operator dependence and faster sample throughput.[13]

Functional sensitivities for three generations of TSH assays measure the precision. For each subsequent generations of TSH assays, the functional sensitivity limit shifts to lower concentration by one order of magnitude. The functional sensitivity limit of first-generation assays (1 to 2µIU/mL) occurs at approximately the middle of the euthyroid range for TSH concentrations. Clearly, these assays cannot distinguish between normal and suppressed TSH levels. In contrast, second-generation assays allow quantisation of TSH in the low normal and subnormal ranges, down to 0.1µIU/mL; and third-generation assays extend the range another tenfold, down to 0.01µIU/mL. In addition, third generation assays have far superior precision in the subnormal TSH range 0.1 to 0.4 µIU/ml as compared to second-generation assays[14].

The purpose of this study is to compare the sensitivity of two methods for estimation of serum TSH for which the TSH of each subject was estimated on AIA-360 based on the principal of FEIA as well as on Liasion-Diasorin Chemilunesence based. Both are under third generation. The functional sensitivity of both is same but the analytical sensitivity of CLIA as compared to FEIA is better by ten folds.

***Corresponding Author:**
**Dr. Amandeep Kaur,**
Associate Professor,
Biochemistry Department
Gian Sagar Medical College And Hospital
Rajpura, Punjab, India.

## MATERIAL AND METHODS

### Subjects

The present study comprised of 125 randomly selected patients either attending OPD or admitted in our Hospital. The patients were enrolled for the study after informed consent and approval from ethics committee of the institute. TSH levels were assayed by immunoflurometric assay as well as by Chemiluminesence immunoassay. A detailed clinical history was taken which was correlated with the hypothyroid and hyperthyroid patients.

### Blood samples

Fasting blood sample was withdrawn and serum was separated for TSH determination by immunoflurometric assay on the AIA -360 and Chemiluminesence immunoassay on Liaison Diasorin. Reagents for AIA-360 were procured from Tosoh Corporation Tokyo, Japan and Liasion Diasorin were procured from Diasorin S.p.A. Via Crescentino snc - 13040 Saluggia (VC)-Italy.

### Statistics

Values are expressed as mean ± S.D. Statistical analysis between three groups was done using student's t- test and coefficient of variation.

## RESULTS

The patients were divided into three groups according to the levels of TSH i.e. patients with normal, increased level (more than normal range) and decreased level (less than normal range) of TSH.

Table 1 shows comparison of TSH levels in three groups measured on two different instruments which are based on the principles of immunoflurometric assay and chemiluminesence immunoassay. In group 1, i.e. patients having normal TSH levels (n=68), TSH levels were not significantly different and were comparable when estimated by both methods. In group 2, hyperthyroid patients (n=22), the serum TSH as reported by chemilumesence was significantly lower ($p<0.001$) as compared to values reported on AIA-360. Furthermore, when patient wise individual results of each subject were compared, a specific value for TSH could not be reported by FEIA in 14 out of total of 22 hyperthyroid subjects. Although both the methods are categorised under the 3rd generation but it is important to note that chemiluminesence has better analytical sensitivity up to 0.004 as compared to FEIA which has analytical sensitivity of 0.06. In group 3 i.e. in hypothyroid patients (n =35), the serum TSH on chemiluminesence is significantly high ($p<0.0001$) as compared to AIA-360.This group was further divided in 2 subgroups of patients having TSH <10 and patients having TSH >10 and the results of individual patient was compared and are shown in Table 2 & 3 respectively. It is clear from the Table 2 that the TSH values reported by two different methods are quite different. The value reported by chemiluminesence have better and higher analytical sensitivity as compared to that of the AIA-360 and will definitely help to clinicians in treatment and management of hypothyroidism.

**Table 1:** Comparison of Serum TSH measurements using chemilumenesence and AIA-360 in the study group

| Group | | CHEMILUMENESENCE Mean ± S.D. (µ IU/ml) | AIA -360 Mean ± S.D. (µ IU/ml) | |
|---|---|---|---|---|
| 1 | Normal individual n = 68 | 2.6 ± 1.6 | 2.0 ± 1.3 | P<0.0178 |
| 2 | Hyperthyroid Patients n =22 | 0.120 ± 0.014 | 0.9 ± 0.014 | p<0.001 |
| 3 | Hypothyroid Patients n = 35 | 25.06 ± 4.1 | 20.0 ± 1.6 | p<0.0001 |

**Table 2:** Comparison of individual Serum TSH levels of 13 hypothyroid patients on Chemilumenesence and AIA-360.

| CHEMILUMENESENCE Reference Range ( 0.3-3.6 µ IU/ml) | AIA-360 Reference Range (0.25 - 5.25 µ IU/ml) |
|---|---|
| 27.5 | 16.7 |
| 20.8 | 9.2 |
| 10.3 | 7.5 |
| 13.0 | 9.7 |
| 17.4 | 14.7 |
| 200 | 122 |
| 12.3 | 5.9 |
| 35.5 | 25.5 |
| 30.8 | 10.6 |
| 68.1 | 49.2 |
| 13.3 | 9.7 |
| 36.5 | 32.2 |
| 93.8 | 86.1 |

**Table 3:** Comparison of individual Serum TSH levels of Patients (borderline) Having TSH < 10 on CHEMILUMENESENCE and AIA-360.

| CHEMILUMENESENCE Reference Range ( 0.3-3.6 µ IU/ml) | AIA-360 Reference Range (0.25 - 5.25 µ IU/ml) |
|---|---|
| 7.4 | 5.0 |
| 7.6 | 4.5 |
| 6.7 | 5.1 |
| 7.2 | 4.9 |
| 6.7 | 5.5 |
| 7.3 | 5.7 |
| 5.7 | 4.7 |
| 6.8 | 5.9 |
| 8.5 | 6.4 |
| 8.9 | 5.9 |

Table 3 shows that the comparison of serum TSH that having borderline normal results .The patients who were reported normal as per the report of AIA-360 were hypothyroid as per in report of chemiluminesence. The AIA-360 values are closer to the normal range as compared to the values reported

by chemiluminesence which shows that sensitivity of a method play a significant role in the diagnosis of a thyroid disorder, specifically hypothyroidism.

## DISCUSSION

Thyroid function is the most frequently advised endocrine investigation. Diagnosis and management of thyroid disease benefit substantially from close interactions between clinicians and laboratorians. Clinicians desire reliable test values for diagnose treatment and management of patient with thyroid dysfunction[15]. The current strategy recommended by the American Thyroid Association reviews the functional performance of the TSH immunometric assay methods currently used in clinical practice[16]. The prevalent disorders involving thyroid are primary hypothyroidism and hyperthyroidism in which TSH levels increase or decrease. Primary hypothyroidism is due, in most cases, to autoimmune disease (like Hashimoto's Thyroiditis) or to a congenital deficiency of thyroid tissue. Secondary hypothyroidism is less frequent and originates to alterations of the hypothalamus-pituitary axis. Primary hyperthyroidism is due to over production of thyroid hormone[2]. Under the guidelines of American Thyroid Association and American Association of Clinical Endocrinologists serum TSH measurements has been recommended as the single most reliable test to diagnose all the forms of hypothyroidism & hyperthyroidism[17,18]. Hence, the sensitivity of the method plays a vital role in diagnosis of the thyroid disorder.

The present study supports the previous study which indicated that the chemiluminesence has better analytical sensitivity than FEIA which can distinguish between normal and suppressed TSH levels. In contrast CLIA has far superior precision in subnormal TSH range and was able to correctly diagnose patients suffering from thyroid illness, which would have been either missed by FEIA, especially in, hypothyroid patients who come for follow up or diagnosis for suppression of goiter or nodular thyroid disease or for thyroid cancer[19]. The clinical sample study conducted by Hubl *et al.*, in 2000 confirmed that the LIAISON thyroid hormone assays are sensitive methods for the differentiation of euthyroid subjects and patients with hyper- and hypothyroidism[20]. In 2004 Rawlins and Roberts and Waskiewicz *et al.*, in 2005 compared the performance characteristics of 6 Third-Generation Assays for Thyroid-Stimulating Hormone[21,22]. So comparison of different methods is important with respect to the sensitivity of individual method. The higher functional sensitivity & superior precision of third generation TSH assays can be useful in detection of SCTDs (subclinical thyroid dysfunction) and can be more useful in screening of thyroid diseases. Better analytical activity approaches CLIA towards fourth generation.

The Chemiluminesence instrument based on immunoassay method has good precision and reliability and its inter assay coefficient of variation is 5%. In conclusion, the automated thyroid hormone immunoassays on the random-access LIAISON Chemiluminesence immunoassay analyzer proved to be very satisfactory, both from the analytical and the clinical point of view.

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