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Comparative analysis of ELISA, FEIA and CLIA techniques in diagnostic immunoassays for thyroid stimulating hormone: A cross-sectional study form Morang, Nepal.

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ABSTRACT

Background

TSH and circulating thyroid hormones are measured in serum as part of a thyroid function test (TFT), which evaluates the thyroid gland's ability to produce and regulate thyroid hormones. The objective of the current study was to compare the technical performance between ELISA, FEIA and the currently marketed automated CLIA for measuring the TSH level.

Material and methods

A total of 1,200 participants were enrolled in the study. Subjects were chosen randomly, either OPD patients or inpatients of Birat Medical College Teaching Hospital. Chemiluminescence Immunoassay (CLIA), Enzyme-linked Immunoassay (ELISA) and Fluorometric Enzyme Immunoassay (FEIA) technologies were performed.

Results

The total number of patients (1200) was categorized into group I, normal TSH (n = 680); group II, elevated TSH (n = 300); and group III, decreased TSH (n = 220). CLIA and FEIA based results were significantly more sensitive compared to ELISA results for TSH detection.

Conclusion

Chemiluminescence assay system, when compared to FEIA and ELISA, is far superior in terms of accuracy and sensitivity for reporting both subnormal and above normal TSH levels and can be helpful in the detection of subclinical thyroid dysfunction (SCTD) and in the screening of thyroid diseases.

Keywords

Chemiluminescence Immunoassay (CLIA), Enzyme-linked Immunoassay (ELISA), Enzyme-Linked Immunoassay Method (EIA), Fluorometric Enzyme Immunoassay (FEIA), Nepal, Thyroid

Background

The pituitary gland secretes thyroid-stimulating hormone (TSH). Clinicians use TSH as a marker of thyroid status since it is secreted in a feedback loop relating to the level of thyroid hormones. The thyroid function test (TFT) evaluates the ability of the thyroid gland to produce and regulate thyroid hormones. The TSH and circulating thyroid hormones in serum are considered as a part of a TFT, which is used to diagnose and track treatment progress for thyroid gland dysfunctions. These biochemical tests are highly clinically relevant for their analytical sensitivity and specificity [1].

Symptoms of hyperthyroidism and hypothyroidism, like weight gain or loss, exhaustion, aversion to heat or cold, changes in appetite, and palpitations, aren't always related to thyroid disease. A thyroid-specific goiter may result in normal thyroid function [2].

Despite TFTs' widespread use and excellent clinical efficacy, the best diagnostic approach for thyroid dysfunction and follow-up is still challenging. Evaluating serum TSH is a crucial biochemical parameter in determining thyroid dysfunction [3]. Rapid advancements in diagnostic technology have been made in the last decade, including antibody detection, bringing this field of diagnosis closer to automated clinical chemistry laboratories [4]. Regarding functional sensitivity limitations, the methodology for estimating serum TSH levels has experienced significant changes during the past two decades. There are numerous ways to estimate TSH, namely Radioimmunoassay (RIA), Enzyme-linked immunoassay (EIA), and Enzyme-linked Immunoassay (ELISA).

Between 1960 and 1990, the first generation of TSH assays was in use. The first immunoassay, RIA, was the predecessor of the current immunoassay [3], but it had a low functional sensitivity (1.0 mIU/L) [5].

The second generation of approaches built on the immunometric assay (IMA) methodology with better functional sensitivity was further developed in the middle of the 1980s [6, 7]. TSH assays of the third generation are often the most sensitive tests for detecting primary hypothyroidism. The results are more accurate and superior regarding clinical, operational, and analytical outcomes. Additionally, it has a faster sample throughput and requires fewer operators [8]. Chemiluminescence Immunoassay (CLIA) is now widely used for screening hepatitis C antibodies (anti-HCV antibodies), particularly in high-volume clinical laboratories for detecting anti-HCV antibodies.

Although CLIA is gradually replacing ELISA, there is not enough Nepalese published data on the comparative evaluation of ELISA, FEIA, and CLIA for the TSH measurement. Hence, this study aimed to compare the technical performance between ELISA, FEIA, and the currently marketed automated CLIA for measuring the TSH level.

Material and methods

Study design and the participants

The current study was conducted between June and September 2022. A total of 1,200 participants were enrolled in this research. Subjects were chosen randomly either OPD patients or inpatients of Birat Medical College Teaching Hospital, Tankisinuwari, Nepal.

Data collection

After obtaining a thorough clinical history of the patients, blood was collected by maintaining all the aseptic precautions, and the serum was separated. We conducted three immunoassay investigations for TSH, which included CLIA, FEIA, and ELISA in the same blood samples. Snibe Diagnostics' Maglumi-X3 Chemiluminescence was used for CLIA analysis. The AIA-360 (based on the FEIA principle) analyzer was used, which utilizes a competitive fluorescent enzyme immunoassay performed entirely within small, single-use test cups containing all necessary reagents. BeneSphera™ diagnostic solutions ELISA was used in this study.

According to the TSH levels obtained from CLIA, FEIA, and ELISA, the patients were divided into three groups: normal (group I), elevated (group II), and lowered (group III) (based on the reference range), and data analysis was done. Group II patients were separated into two subgroups with TSH levels below and above 10 μIU/ml, and the outcomes for each patient were compared.

Inclusion criteria

The present study included all participants who agreed to consent and participate in this research.

Exclusion Criteria

Subjects unwilling to participate and who had not given consent were excluded.

Data management and statistical analysis

The student's t-test and coefficient of variation were used to compare the three groups statistically. Statistical Package for the Social Sciences (SPSS) version 21 was used to analyze the data. The P value < 0.05 was considered statistically significant.

Ethical committee approval

Ethical committee approval was obtained from the institutional review committee of Birat Medical College Teaching Hospital for this study. The methods we used were in accordance with the guidelines of the Declaration of Helsinki.

Results

The total number of patients was categorized into group I, normal TSH (n = 680); group II, elevated TSH (n = 300); and group III, decreased TSH (n = 220).

The mean values of TSH were 2.6, 2.0, and 1.7 µIU/ml, respectively, obtained by the CLIA, FEIA, and ELISA from the group I patients. The mean values of TSH were 25.06, 20.0, and 18.1 µIU/ml (FEIA and ELISA obtained TSH values were significantly lower compared to those from the CLIA-based instrument in Group II patients). In group III patients, the mean values of TSH were 0.12, 0.09 and 0.34 µIU/ml determined by CLIA, FEIA and ELISA, respectively. CLIA and FEIA-based results were significantly more sensitive than ELISA results (Table 1).

Further, to compare the sensitivity towards the upper extremities, Group II patients were separated into two subgroups with TSH levels below and above 10 µIU/ml, and the outcomes for each patient were compared. The first subgroup of Group II consists of patients with TSH levels above the reference range but below 10 µIU/ml. At this point, the mean values of TSH were 7.2, 5.3 and 4.7 µIU/ml (Figure 1).

Another Group II subgroup comprises patients with TSH levels above the normal range (>10 µIU/ml). The mean values of TSH were 30.7, 20.8, and 14.6 µIU/ml, respectively, as obtained from CLIA, FEIA, and ELISA. Comparing FEIA and ELISA results to CLIA, the extent of TSH elevation was significant (Figure 2).

We also found substantially less TSH in four patients (n = 4; 1.8 %), as detected by CLIA, and these critically low TSH levels were undetectable using the other FEIA-based AIA-360 equipment.

Table 1: Comparison of TSH level measured by CIA, FEIA, ELISA in different study groups

Group	CLIA Mean (SD) [µIU/ml]	FEIA Mean (SD) [µIU/ml]	ELISA Mean (SD) [µIU/ml]	P value in comparison to CLIA
Group-I, n=680	2.6 (1.6)	2.0 (1.3)	1.7 (1.1)	FEIA=0.043* ELISA=0.021*
Group-II, N=300	25.06 (4.1)	20.0 (1.6)	18.1 (1.6)	FEIA=0.047x ELISA=0.031*
Group-III, n=220	0.12 (0.014)	0.09 (0.014)	0.34 (0.07)	FEIA=0.083x ELISA=0.045*

*p<0.05, statistically significant, xp<0.05, statistically not significant

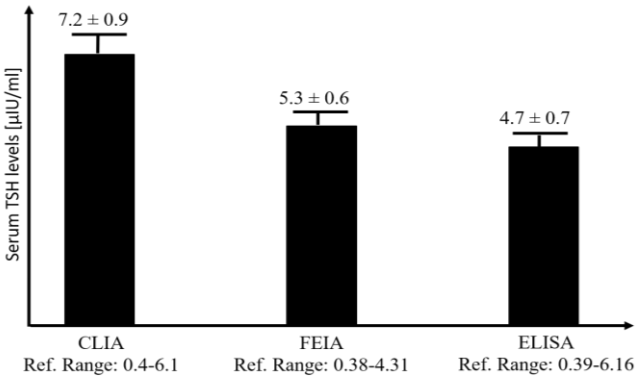

Figure 1: Comparison of individual serum TSH levels of patients who had borderline TSH values < 10 µIU/ml using CLIA, FEIA and ELISA techniques.

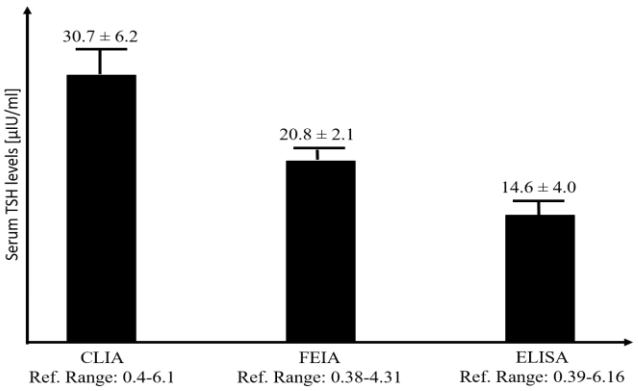

Figure 2: Comparison of individual serum TSH levels of patients having TSH values > 10 µIU/ml using CLIA, FEIA and ELISA techniques.

Discussion

TFTs are among the most requested blood tests when thyroid disease is suspected. Despite their widespread use and excellent clinical efficacy, the best diagnostic approach for the diagnosis and follow-up of thyroid dysfunction is still challenging. The most trustworthy single marker for ruling out primary thyroid dysfunction is the measurement of serum TSH levels [1]. The American Thyroid Association's most recent recommendation examines the functional effectiveness of the current TSH immunometric assay techniques in clinical practice [9]. Therefore, the method's sensitivity is key to diagnosing a thyroid disorder. The current study is in accordance with previous research, which has shown that CLIA has more analytical sensitivity than FEIA and ELISA and can discriminate between normal and suppressed TSH levels. On the other hand, CLIA has much better accuracy in the subnormal TSH range and could correctly identify patients with thyroid disease who would not have received a diagnosis using the other two methods. CLIA is very efficient, especially for hypothyroid patients who come in for follow-up or diagnosis for goiter suppression, nodular thyroid disease, or thyroid cancer [10]. The MAGLUMI-X3 thyroid hormone assays are sensitive procedures for distinguishing euthyroid people from patients with hyper- and hypothyroidism [10, 11].

Evidence suggests that the functional sensitivity limit of first-generation tests (1 to 2 IU/mL) is reached at TSH concentrations roughly in the center of the euthyroid range, unable to differentiate between normal and reduced TSH levels. However, second-generation assays enable quantification of TSH in the low average and subnormal ranges, down to 0.1 IU/mL, and third-generation assays further expand the range by a factor of ten, down to 0.01 IU/mL. Third-generation assays are much more precise than

second-generation assays in the subnormal TSH range of 0.1 to 0.4 IU/ml [12]. The functional sensitivity limit of TSH tests shifts by one order of magnitude to a lower concentration for each consecutive generation [13]. Our data indicated that CLIA has more sensitive indices towards the TSH analyte's lower and higher extremities.

Conclusion

In conclusion, both from an analytical and clinical standpoint, the automated thyroid hormone immunoassays on the random-access MAGLUMI-X3 CLIA analyzer proved to be highly satisfactory. Third generation TSH assays can be more effective at screening for thyroid illnesses due to their better functional sensitivity and superior precision. SCTDs can be detected using these assays. The fourth generation of CLIA is approached through better analytical activities. With a 5% inter-assay coefficient of variation, the CLIA equipment based on the immunoassay method has good precision and reliability.

Limitation and future scope of the study

Our study used ELISA, FEIA, and CLIA techniques to measure TSH with a limited sample size. Future studies are welcomed with more subjects with advanced techniques and a complete thyroid profile.

Relevance of the study

The present study is relevant clinically to determine thyroid dysfunction with the best diagnostic test available with complete precision.

Abbreviations

Chemiluminescence Immunoassay (CLIA), Enzyme-linked Immunoassay (ELISA), Enzyme-Linked Immunoassay Method (EIA), Fluorometric Enzyme Immunoassay (FEIA), Hepatitis C antibody test (HCV), Immunometric assay (IMA), Milli-international units per litre (mIU/L), Radioimmunoassay (RIA), Statistical Package for the Social Sciences (SPSS), Thyroid function test (TFT), Thyroid-stimulating hormone (TSH)

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Authors' contribution

a. Study planning: RLM, CPG
b. Data collection: RLM, CPG
c. Data analysis/ interpretation: RLM
d. Manuscript writing: RLM
e. Manuscript revision: RLM
f. Final approval: RLM
g. Agreement to be accountable for all aspects of the work: RLM, CPG

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Availability of data and materials

This article includes all the study's data and materials.

Competing interests

The authors declare no conflict of interest.

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