

Comparison of Enzyme-Linked Immunosorbent Assay and Chemiluminescence Immunoassay for Thyroid Stimulating Hormone Analysis in Tertiary Care Hospital Lahore

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ABSTRACT

Objective: To compare enzyme-linked immunosorbent assay and Chemiluminescence immunoassay for thyroid stimulating hormone analysis in human serum.

Methodology: This cross-sectional study conducted from 17th March till 13th June. In Punjab Rangers Teaching Hospital, Lahore after approval from Institutional Review Board. Random samples from both genders, between 18-70 years of age were included. Haemolysed, lipemic or icteric specimens were excluded. The sera were assayed for thyroid stimulating hormone (TSH) by enzyme-linked immunosorbent assay (ELISA) and Chemiluminescence immunoassay (CLIA). Data was analyzed by SPSS 24.

Results: A total of one hundred and ninety-eight serum samples were processed by ELISA and CLIA in all the samples, with more females as compared to males (1:1.3). ELISA technique identified 134 subjects as Euthyroid, 40 Hypothyroid and 24 Hyperthyroid, while 122 Euthyroid, 48 hypothyroid and 28 hyperthyroid subjects by CLIA. Thyroid stimulating hormone (TSH) levels reference range was taken 0.4-4.5mU/L according to American Thyroid Association. Mean \pm SD of TSH was 1.45 ± 0.79 ; 12.27 ± 11.03 and 0.23 ± 0.17 mU/L respectively by ELISA whereas CLIA indicated Mean \pm SD to be 1.93 ± 0.936 , 16.04 ± 14.68 and 0.393 ± 0.375 mU/L respectively for Euthyroid, hypothyroid and hyperthyroid. Correlation of coefficient ($R^2 = 0.89$) was found significantly positive between both methods. There was a significant difference in hypothyroid and Euthyroid groups.

Conclusion: TSH assay by CLIA has shown a wider range of functionality, throughout and borderline cases were identified better as compared to ELISA. Turn-around-time (TAT) decreased, physician satisfaction increased and indirectly benefitting patient treatment and prognosis.

KEYWORDS: Chemiluminescence Immunoassay, Enzyme-Linked Immunosorbent Assay, Thyroid Stimulating Hormone.

INTRODUCTION

Thyroid hormones critically regulate the growth and metabolic key pathways of different organs as well as

energy homeostasis. Worldwide, 300 million people suffer from abnormal thyroid functioning. The most prevalent abnormality of it is the elevation of thyroid-stimulating hormone labelled as Hypothyroidism, while Hyperthyroidism, showing decreased levels of TSH, is less common.¹ The modern advancement in technology has categorized the enzyme linked immunosorbent assay into 3rd generation assay. TSH monitoring by enzyme-linked immunosorbent assay (ELISA) in very low and very high level of frank hyperthyroidism and hypothyroidism is very beneficial in grouping subjects according to expression of TSH level.² ELISA is a popular analytic procedure that applies a solid stage enzyme immunoassay (EIA) to identify the presence of a specific element, usually an antigen, in a serum sample. In ELISA, antigens from the test specimen adjoined to a surface are treated with specific antibody; and enzyme substrate is added as a final step. End point reaction is color change in the substrate producing a detectable signal.³ Keeping in mind the benefits, of 4th generation assays,

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Chemiluminescence immunoassay (CLIA) is widely used as a reliable, sensitive, specific, automated method which measures the immune complexes formed as a result of antigen-antibody reaction by using labelled antibodies. Substrate was added to these intact immune complexes producing light signal. The intensity of light (measured as Reactive Light Units, RLU) helps to quantify the labelled complexes. This assay significantly promotes detection of sub-minimal level of active substances in clinical diagnosis and treatment prognosis.⁴ In third world countries, there is a lack of reliable comparative searches between both techniques. The patients were undergoing over or under treatment and have to bear the brunt of discrepant results produced by different techniques.^{5,6} The Primary aim of our study is to compare values of TSH assessed by both enzyme-linked immunosorbent assay and Chemiluminescence immunoassay in a tertiary care setting.

METHODOLOGY

This cross-sectional study was conducted in Pathology laboratory at Punjab Rangers Teaching Hospital, Lahore from 17th March till 13th June 2021. After taking ethical approval from Institutional Review Board with IRB Reference number 07/2021. A total of 198 whole blood samples of both genders, between 18-70 years of age were selected randomly and included in the study. Haemolysed, lipemic and icteric specimens were excluded from the study. Blood collected in serum separator tubes was centrifuged at 2500 rpm for 15 minutes at room temperature to obtain serum. The separated serum was aliquoted in 2 fresh micro-centrifuged tubes and stored at -20°C. Samples were tested simultaneously for TSH by both ELISA and CLIA techniques. Both methods were calibrated and controls were run.⁶ Reference range to categorize the patients with thyroid disease was 0.4-4.5mU/L.

The quantitative measurement of TSH was done by ELISA and CLIA. Monoclonal antibodies coated on ELISA strips were directed against serum TSH, which reacts with the two antibodies simultaneously. This sandwich complex is immobilized to the well, and incubated for an hour and excess antibodies are removed by washing the wells. After 15 minutes, stop solution was added and the end product of colored solution was measured by ELISA reader set at 450nm.³ The standard for CLIA technique works as sandwich technique. Its principle is to employ monoclonal antibodies specifically directed against human TSH. Antibodies labelled with ruthenium

complex, which consists of chimeric construct from human and mouse specific components. This test was performed on the fully automated Roche cobase 411 analyzer using CLIA, instructions of training manual were carefully followed. Eighty six tests /hour were performed. The total duration of assay was eighteen minutes. Two-point calibration curve and master curve presented through the reagent barcode or e-barcode particularly supports the outcomes.

Statistical analysis: Data was analyzed by SPSS 24.0. Continuous variables were expressed as mean \pm SD. Mean TSH was compared by independent T-test. P-value \leq 0.05 was considered as significant. Coefficient of correlation was applied between ELISA and CLIA for euthyroid, hypothyroid and hyperthyroid TSH levels.

RESULTS

A total of one hundred and ninety-eight serum samples were processed. Table 1 shows comparison of TSH status by ELISA and CLIA in all the samples, with more females as compared to males (1:1.3). TSH assay by both techniques identified 134 and 122 subjects as Euthyroid by ELISA and CLIA, respectively. Similarly, ELISA identified 40 subjects as hypothyroid while 48 were found hypothyroid by CLIA, on the basis of reference value 0.4-4.5mU/L. The difference was highly significant in hypothyroid and Euthyroid subjects.

Table 1: Comparison of Thyroid Stimulating hormone by ELISA and CLIA (N=198).

TSH Reference 0.4-4.5mU/L	ELISA		CLIA		p-value
	N 198	Mean \pm SD	N 198	Mean \pm SD	
Hypothyroid	40	12.27 \pm 11.03	48	16.04 \pm 14.68	<.0005
Euthyroid*	134	1.45 \pm 0.79	122	1.93 \pm .936	<.0005
Hyperthyroid	24	0.23 \pm 0.17	28	0.393 \pm 0.375	0.127

TSH= Thyroid Stimulating Hormone; ELISA= Enzyme Linked Immunosorbent Assay; CLIA= Chemiluminescence immunoassay. Euthyroid* Range was identified in 134 and 122 subjects respectively, by ELISA and CLIA
P-value \leq 0.05 taken as significant.

In Table 2, the difference of mean cases with similar diagnosis is shown that 37 hypothyroid samples when tested by ELISA fall in 4.8-44mU/L, while these same samples by CLIA gave results from 4.58-38mU/L. Shifting of 24 subjects from (134, in Table 1) Euthyroid by ELISA to either hypothyroid or hyperthyroid groups; and 12 subjects from (122, in Table 1) Euthyroid by CLIA to other groups.

Similarly, the difference of hyperthyroid samples (22, as in Table 2) was shown by 2 subjects and 6 subjects by ELISA (24 as given in Table 1) and CLIA (28 as in Table 1), respectively. P-value was highly significant in hypothyroid and Euthyroid subjects, while insignificant in hyperthyroid samples. Correlation of coefficient ($R^2=0.89$) was positive between both methods (Figure 1)

Table 2: Difference in values of serum TSH by two methods(N=169)

TSH Reference	N	ELISA*	Mean ± SD	CLIA*	Mean ± SD
0.4-4.5mU/L	169				
Hypothyroid	37	4.8-44	11.9± 10.64	4.58-38	15.11± 14.73
Euthyroid	110	0.4-3.7	1.44±0.79	0.56-4.2	1.90 ± 0.92
Hyperthyroid	22	.009-.36	0.174 ±0.17	.006-.39	0.145 ± 0.12

ELISA* and CLIA* range of TSH level in N=169 subject

DISCUSSION

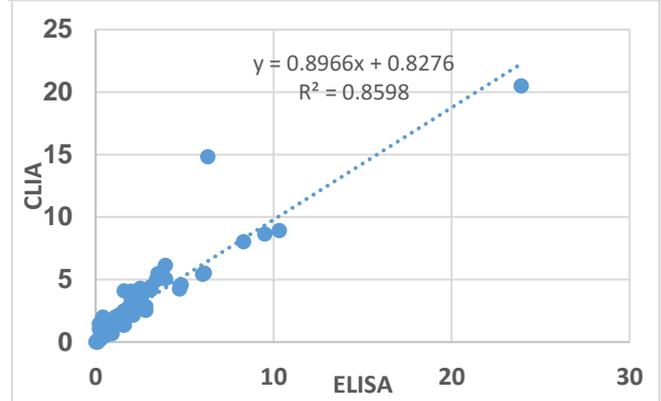
Thyroid disorders are the commonest endocrine disorders throughout the world including Pakistan.⁷ For diagnosis and management of thyroid diseases, thyroid function test is the most frequently advised endocrine investigation.⁷ The guiding principles of American Thyroid Association and American Association of Clinical Endocrinologists serum TSH measurements has recommended as a primary screening test to diagnose most types of hypothyroidism & hyperthyroidism.⁸ TSH secretion is exquisitely sensitive to minor increase, as well as decreases in serum free T₄. Abnormal TSH levels are detected earlier during developing the course of hypothyroidism and hyperthyroidism even before free T₄ abnormalities become detectable.^{8,9} Although thyroid disorders are not a life threatening disorder, but if it is not diagnosed timely and remained untreated, it may develop into life threatening disorders like cancer. Moreover, thyroid gland malfunctioning will greatly affect various functions of many other body parts which depend on the hormonal secretion of thyroid hormones for performing their normal functions.¹⁰

In our study the patients were divided into three groups according to serum TSH levels i.e., euthyroid, hypothyroid and hyperthyroid. Our results indicate significant difference in TSH levels measured by ELISA and CLIA for euthyroid, hypothyroid subjects, however the difference was insignificant by both methods for hyperthyroid subjects. Same results are documented within Pakistan by Ejaz et al and internationally by Santosh et al.^{10,3}

In our study, thyroid diseases were found to be more prevalent (56%) in females compared to males. Similar findings are reported locally by Naz et al and Shah et al^{11,12} and internationally by Paczkowska et al.¹³ In our study, 169 subjects gave same results by both methods with highly significant difference in hypothyroid and Euthyroid subjects. Statistically, all the data was analyzed for coefficient of correlation and it displayed a significant coefficient of correlation ($R^2=0.8598$) as documented locally by Naz et al and foreign researcher Hamed et al.^{11, 14}

In present study two methods, ELISA and CLIA were compared for determination of TSH. The comparison of both methods shows CLIA exhibit better and higher sensitivity as revealed by Higgins et al, with wider range (0.005-100.0mU/L) as compared to ELISA

Figure1: The coefficient of correlation between ELISA and CLIA (n=198)



(0.09-40.0 mU/L).¹⁵ The reason for this discrepancy could be technique based, errors in pipetting and washing step, fluctuation in instrument handling. Other disadvantages of ELISA are its laborious techniques with high incidence of false positive and negative results and antibody instability as shown in research by Pramila et al.¹⁶ In our set up, the clinician’s feedback was considered regarding discrepancy of results and delayed turnaround time prompted the decision to up-grade the diagnostics with advanced 4th generation autoanalyzer. Present study clearly indicates that Chemiluminescence has better analytical sensitivity than ELISA, which can differentiate between normal and subnormal TSH levels as shown by the work done in our region by Shah et al and in neighboring countries by Higgins et al and Jiang et al.^{17, 18,15} Further studies on a broader scale will yield better results for understanding the importance of diagnostic accuracy of various techniques for thyroid function tests for accurate diagnoses of thyroid disorders.

CONCLUSION

TSH assay by CLIA has shown a wider range of functionality, throughout and borderline cases were identified better as compared to ELISA. Turn-around-time (TAT) decreased, physician satisfaction increased and indirectly benefitting patient treatment and prognosis.

Conflict Of Interest: None

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Authors' contribution:

Dr. Nusrat Alavi	Conception of study design, acquisition, analysis and interpretation of data.
Dr. Aneela Khawaja	Drafting and methodology, data interpretation.
Dr. Maliha Asif	Drafting of intellectual content.
Dr. Asma Ejaz	Drafting the research work and critical revision.
Dr. Abeer Sheikh	Analysis and interpretation of data for work.
Dr. Faiqa Arshad	Analysis, acquisition and interpretation of data.
	All authors participated in study design and writing manuscript and agree to be accountable for accuracy, integrity of all aspects of the work.

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