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ORIGINAL ARTICLE

Comparison between enzyme immunoassay and chemiluminescence methods for the quantification of thyroid hormones

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Keywords:
immunoassay; luminescence; thyroid hormones; sensitivity and specificity (Source: MeSH - NLM).

ABSTRACT

Objective. To compare the sensitivity and specificity of enzyme immunoassay versus chemiluminescence for the quantification of thyroid hormones. **Methods.** This was a quantitative, observational, and cross-sectional study. The population consisted of 407 blood samples from patients seen at the outpatient endocrinology clinic, with a final sample of 211 patients selected based on eligibility criteria. Thyroid hormone levels were measured using both enzyme immunoassay and chemiluminescence methods. A comparative analysis was performed to calculate sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV). **Results.** The chemiluminescence method identified 68% of patients as negative and 32% as having thyroid disorders, all consistent with clinical findings. The enzyme immunoassay reported 79% as negative and 21% as positive, of which 19% were true positives and 2% false positives. Additionally, 66% were true negatives and 13% false negatives. For the enzyme immunoassay, sensitivity was 57.9% and specificity 96.5%. The PPV was 88.9%, and the NPV was 83.6%. In contrast, the chemiluminescence method showed sensitivity, specificity, PPV, and NPV values exceeding 98%. The Kappa index was 0.6, indicating moderate agreement, where a value closer to 1 is ideal. **Conclusions.** The chemiluminescence method proved to be more sensitive and specific than enzyme immunoassay, making it the preferred method for quantifying thyroid hormones.

Comparación entre el método enzimoinmunoensayo y la quimioluminiscencia, para la cuantificación de hormonas tiroideas

Palabras clave:
inmunoensayo; luminiscencia; hormonas tiroideas; sensibilidad y especificidad (Fuente: DeCS - BIREME).

RESUMEN

Objetivo. Comparar la sensibilidad y especificidad del método de enzimoinmunoensayo frente a la quimioluminiscencia, para la cuantificación de hormonas tiroideas. **Métodos.** El estudio fue de enfoque cuantitativo, de tipo observacional y transversal. La población estuvo conformada 407 muestras de sangre de pacientes atendidos en consulta externa de endocrinología, mientras que la muestra estuvo conformada por 211 pacientes, siguiendo los criterios de selección. La cuantificación de las hormonas tiroideas se realizó mediante el enzimoinmunoensayo y la quimioluminiscencia, realizándose una comparación e interpretación entre ambos métodos para calcular: sensibilidad, especificidad, valor predictivo positivo (VPP) y valor predictivo negativo (VPN). **Resultados.** Por quimioluminiscencia se obtuvo un 68 % de pacientes negativos y un 32 % de pacientes con patología tiroidea, todos compatibles con la clínica del paciente. Por el enzimoinmunoensayo un 79 % son negativos y un 21 % son positivos, de los cuales un 19 % coincidieron como verdaderos positivos y un 2 % como falsos positivos. Así mismo, un 66 % coincidieron como verdaderos negativos y un 13 % como falsos negativos. La sensibilidad y especificidad calculada para el método de enzimoinmunoensayo fue: un 57,9 % de sensibilidad y 96,5 % de especificidad. El VPP fue de 88,9 % y el VPN de 83,6 %; mientras que para la quimioluminiscencia la sensibilidad, especificidad VPP y VPN fueron mayores al 98 %. El índice Kappa obtenido fue de 0,6, lo que indica una moderada concordancia, cuando lo ideal es cerca de 1. **Conclusiones.** El método de quimioluminiscencia demostró ser más sensible y específico frente al enzimoinmunoensayo, por lo que este método es el ideal para la cuantificación de las hormonas tiroideas.

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INTRODUCTION

Thyroid diseases are among the most common medical conditions seen in endocrinology consultations. According to the Bolivian Society of Endocrinology, they primarily affect middle-aged women and are characterized by elevated or insufficient levels of thyroid hormones [1-3].

In the Clinical Laboratory of Hospital Obrero No. 5 [4], Potosí (Bolivia), the most frequently requested tests include pituitary thyrotropin (TSH), and free and total T3 and T4. The synthesis and secretion of thyroid hormones are regulated by TSH, which in turn is controlled by thyrotropin-releasing hormone (TRH), secreted by the hypothalamus [2,5]. Most guidelines and protocols recommend ordering only TSH levels in the initial evaluation of thyroid function. The determination of free T4 (FT4) would be the second test to assess thyroid function and diagnose thyroid diseases [6,7].

Hypothyroidism is a disorder in which the thyroid gland does not produce sufficient amounts of thyroid hormones, manifesting with fatigue, lack of concentration, among other symptoms. Subclinical hypothyroidism is the most common form of hypothyroidism, often with few symptoms, and is associated with hypercholesterolemia and subtle cardiac alterations. Hyperthyroidism results from increased synthesis and secretion of thyroid hormone, triggering a state of hypermetabolism [1,8-10]. The concentrations of these thyroid hormones are extremely low. Therefore, it is essential to use techniques with high detection capacity and high specificity [11].

Thyroid hormones are quantified by enzyme-linked immunosorbent assay (ELISA), which uses an enzyme as a marker to mediate the formation of antigen-antibody complexes. This complex becomes immobilized on a substrate, and the enzyme's action on the substrate produces a color that can be observed by colorimetry. Another method used is chemiluminescence immunoassay (CLIA), where the emission of light is caused by the products of a chemical reaction, using acridine ester as the chemiluminescent agent. Among its advantages are sensitivity and speed [12-15].

Sensitivity and specificity are inherent characteristics of any diagnostic test and indicate its effectiveness. Sensitivity refers to the test's ability to detect disease. Specificity, on the other hand, refers to the proportion of non-diseased individuals in a negative test. Tests used to confirm a diagnosis must have high specificity to avoid false positives [16-18].

When two different methods are available for the same measurement on different instruments, a method comparison should be conducted to better understand their similarities and differences, using rigorous procedures and analyzing the results to obtain an accurate correlation with the presumptive diagnosis [19].

This method comparison study aims to identify the ideal method with the highest sensitivity and specificity for the quantification of thyroid hormones, since the laboratory diagnosis will determine the treatment and follow-up of patients with thyroid disorders at Hospital Obrero No. 5, Potosí (Bolivia).

The objective of the study was to compare the sensitivity and specificity of the ELISA method versus CLIA for the quantification of thyroid hormones.

METHODS

Study type

This was a quantitative, observational, and cross-sectional study conducted in the Clinical Laboratory of Hospital Obrero No. 5, located in the city of Potosí (Bolivia), during the year 2024.

Population and sample

The study population consisted of 407 outpatients who attended Hospital Obrero No. 5 and were seen in the endocrinology clinic with suspected thyroid disease. The sample included 211 patients selected through non-probabilistic convenience sampling. Inclusion criteria included: patients with a presumptive diagnosis of hypothyroidism, hyperthyroidism, or thyroid disease; patients aged between 20 and 60 years; and those whose test request included thyroid hormone determination (TSH, T3, T4, and FT4). Endocrinology patients with other presumptive diagnoses and hospitalized patients with other underlying pathologies were excluded.

Variable and data collection instruments

The study variable was thyroid hormones (TSH, T3, T4, and FT4), which were quantified by ELISA and CLIA methods. The obtained results were used to calculate the specificity and sensitivity of both methods, ultimately

Table 1. Reference values for thyroid hormones according to ELISA and CLIA

Insert values	Analyte	Unit	Low normal	High normal	Sensitivity	Detection range
Enzyme Immunoassay (ELISA)	TSH	µUI/ml	0.39	6.16	0.10	0.1 - 61
	T3	ng/ml	0.52	1.85	0.04	0.15 - 8.0
	T4	µg/dl	4.4	11.6	0.128	0.8 - 25
	FT4	ng/dl	0.8	2.0	0.314	0.1 - 8
Chemiluminescence (CLIA)	TSH	µUI/ml	0.4	4.0	0.03	0.01 - 41
	T3	ng/ml	0.86	1.8	0.126	0.01 - 7.3
	T4	µg/ml	4.5	12.5	0.1	0.5 - 28
	FT4	ng/dl	0.8	2.0	0.28	0.11 - 6.8

comparing the two to determine which method is more sensitive and specific for this determination.

The measurement instrument used was content analysis and data extraction from the hormone laboratory's result record book for enzyme immunoassay and chemiluminescence methods, from which the data for this study were collected.

According to the reagent inserts, the following reference values are provided for ELISA and CLIA, which help correctly interpret the results and guide an accurate diagnosis (see Table 1).

Techniques and procedures for data collection

Results from patients who underwent thyroid hormone testing using both methodologies—ELISA and CLIA—were included. ELISA-based thyroid hormone determination was conducted on serum samples using the Stat Fax 4700 equipment.

Thyroid hormone quantification by CLIA was performed on serum samples using the LUMATIC® device. In specific cases, patient medical records were reviewed to identify diagnostic criteria for thyroid diseases. Quantification of thyroid hormones used enzyme immunoassay and chemiluminescence techniques from the Monobind line.

Data analysis

Results obtained via ELISA were compared with those from CLIA to classify true positives, true negatives, false positives, and false negatives. To analyze the diagnostic validity of both methods, sensitivity (S), specificity (E), positive and negative predictive values (PPV, NPV), and positive and negative likelihood ratios (PLR, NLR) were calculated using a 2x2 contingency table.

For variables previously transformed into dichotomous qualitative data, the degree of agreement was assessed using the Kappa coefficient to determine concordance between both methods.

Statistical analysis was conducted using Excel software to evaluate and compare the methods under study.

Ethical considerations

This research did not include any personal patient information; however, informed consent was obtained from all participants prior to the study. Furthermore, the study was approved by the Hospital's Ethics Committee and was designed in accordance with the ethical principles for human research outlined in the Declaration of Helsinki.

RESULTS

According to the results obtained through Chemiluminescence (CLIA), 68% corresponded to euthyroid or negative patients and 32% to those with some form of thyroid disease, showing a stronger correlation with clinical presentation and making this method more accurate for diagnosis. Using the enzyme-linked immunosorbent assay (ELISA) method,

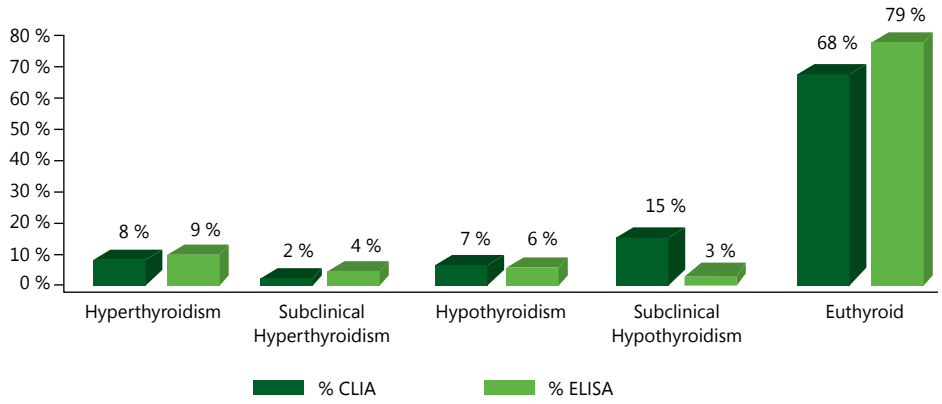

Figure 1. Comparison of TSH and FT4 results obtained using the Chemiluminescence method versus the ELISA immunoassay method

79% were classified as negative and 21% as positive, of which only 19% were true positives, while 2% were false positives. Lastly, 66% matched as true negatives and 13% as false negatives (see Figure 1).

On the other hand, when calculating the sensitivity and specificity of the ELISA method, the following results were obtained: 57.9% sensitivity and 96.5% specificity. This implies that although the method is highly specific, its ability to correctly detect positive cases is relatively low. The positive and negative predictive values (PPV and NPV) were 88.9% and 83.6%, respectively, indicating a good likelihood of correct diagnosis. For the CLIA method, both sensitivity and specificity exceeded 98%, demonstrating the method's reliability in identifying both positive and negative cases. Moreover, the PPV and NPV values were also high, at 98.5% and 99.7%, respectively. The Kappa index of 0.61 indicates good agreement between the ELISA (enzyme-linked immunosorbent assay) and CLIA (chemiluminescence immunoassay) methods. This value suggests a significant level of agreement, although not ideal, which would be closer to 1 (see Table 2).

DISCUSSION

The sensitivity and specificity results obtained with the CLIA method showed percentages greater than 95%, indicating greater reliability compared to the ELISA method. Similar findings were reported in a

Table 2. Evaluation and comparison of sensitivity, specificity, PPV, and NPV between the ELISA and CLIA methods

Diagnostic test results	ELISA		CLIA	
	fi	%	fi	%
True negatives	138	66.0	142	68.0
True positives	40	19.0	66	31.0
False negatives	27	13.0	1	0.5
False positives	5	2.0	1	0.5
Specificity		96.5		99.3
Sensitivity		57.9		98.5
Negative Predictive Value (NPV)		83.6		99.3
Positive Predictive Value (PPV)		88.9		98.5
Kappa Index		0.61		

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comparison conducted by Salirrosas Rodríguez [20], who demonstrated that CLIA had a sensitivity of 100% compared to 89.6% for ELISA. This highlights the accuracy of CLIA in clinical practice. However, it is crucial to acknowledge that both ELISA and CLIA may be affected by interferences, which could alter diagnostic outcomes.

Regarding the ability to detect thyroid disorders, this study shows that CLIA has greater sensitivity than ELISA, which aligns with the observations of Forero et al. [2] and Espitia [21]. These authors note that the CLIA method can detect levels as low as 0.01 to 0.02 µIU/mL and is characterized by ultrasensitivity. In contrast, the sensitivity of the ELISA method reflects higher concentration thresholds. Since the detection of analytes at very low concentrations—such as thyroid hormones—is often required, ELISA assays present limitations in terms of sensitivity.

The CLIA method did not present any false negatives or false positives, demonstrating high sensitivity and specificity for the quantification of thyroid hormones. Garcia et al. [15] describe the advantages of CLIA over other methods, stating it offers high sensitivity and speed in testing. The combination of methodological strengths in CLIA has led to the development of a reaction system involving an enzyme, thereby providing high analytical sensitivity. This enables the execution of diverse diagnostic profiles across nearly all areas of the Clinical Laboratory, yielding highly reliable results.

The need for more sensitive and specific methods to detect thyroid disorders supports the implementation of such methodologies in diagnostic settings. According to Diaz et al. [22], the incorporation of techniques like CLIA has lowered detection limits to as little as 0.001 µU/mL, thus qualifying them as ultrasensitive methods. As a result, it becomes possible to distinguish hyperthyroidism from euthyroidism with precision. Given the high accuracy of current TSH measurement techniques, it is considered the screening test of choice for the general population.

Sensitivity, specificity, and predictive values are traditional criteria used to assess the predictive capacity of a diagnostic test. According to Lino-Villacreses et al. [16] and Vizcaíno-Salazar [23], these metrics—when applied in the Clinical Laboratory—provide confidence in the proper implementation of each diagnostic technique across all testing areas.

This study provided objective and documented evidence regarding the performance of both methods, which is a significant strength for both the Laboratory and the Hospital. This supports the justification for using ultrasensitive methods, which ensures accurate diagnosis and appropriate monitoring of thyroid disorders.

One limitation of this study was the lack of available data in our context, which prevented a more rigorous and comprehensive evaluation, as such an analysis was beyond the scope of the original objectives.

Conclusions

When determining thyroid hormones, the chemiluminescence method (CLIA) provides more reliable results compared to the enzyme immunoassay method (ELISA).

In this study, the chemiluminescence method (CLIA) showed greater sensitivity than the enzyme immunoassay method (ELISA), confirming that this technique is as good as or superior to ELISA, and therefore more appropriate for diagnosing thyroid disorders.

Recommendations

Given the importance of diagnosing thyroid diseases, the methods used should have high sensitivity and specificity. It is recommended to use methodologies with these characteristics, such as the CLIA method.

This study is considered a starting point for future research related to method comparison and studies concerning the quality assurance of laboratory tests.

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The author declares no conflicts of interest.

Authorship contribution

Conceptualization, methodology, formal analysis, research, resources, writing—original draft, writing—revision and editing, and visualization.