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ORIGINAL ARTICLE

Which quantitative method in determination of the thyroid hormone levels is more consistent with the clinical symptoms of the thyroid disorders?

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Abstract Various methods have been used to determine thyroid-stimulating hormone (TSH) and thyroid hormone concentrations in medical diagnostic laboratories. Selection of a suitable method for diagnosis and monitoring thyroid disorders is necessary. The study aimed to compare chemiluminescent assay (CLA), radioimmunoassay (RIA), and enzyme-linked immunosorbent assay (ELISA) methods for the determination of thyroid hormone levels in human serum. Blood samples were taken from 137 patients with thyroid disease and 58 healthy subjects. The sera were analyzed simultaneously to determine the concentration of TSH, thyroxine, and triiodothyronine by CLA, RIA, and ELISA methods. Significant correlation ranges from 0.663 to 0.876 were found between the methods. CLA was the most sensitive method (100 %), but RIA was the most specific method (100 %) according to the clinical symptoms. RIA was the most specific method for the diagnosis of the thyroid diseases, and CLA assay was the most sensitive method in the detection of thyroid diseases except for measuring T4 concentration in hypothyroidism. RIA showed the highest specificity when it comes to the diagnosis of hypothyroidism. RIA had the highest sensitivity, and CLA showed the highest specificity for all three tests in euthyroid group. Each of the methods showed good sensitivity, specificity, and accuracy. ELISA was found to be suitable for the initial screening of the thyroid disorders. The specificity and sensitivity of CLA and RIA were equally high, proposing them as valuable methods for monitoring patients. However, apart from the hazardous effects of ionizing radiation for environment, RIA is the most reliable method for the quantification of thyroid hormones.

Keywords Chemiluminescent assay (CLA) · Enzyme-linked immunosorbent assay (ELISA) · Radioimmunoassay (RIA) · Thyroid hormones

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Introduction

Thyroid gland is one of the most important endocrine glands in the body (Medifocus.com 2012). The thyroid gland secretes thyroxine (T4) and triiodothyronine (T3) (LaFranchi 2006). These hormones are critical in regulating the growth and differentiation of many tissues and organs, as well as for energy homeostasis and numerous key metabolic pathways (Jugan et al. 2010). Thyroid disease is common at the entire world, and about 300 million people in the world have thyroid dysfunction problem (Aryal et al. 2010). Hypothyroidism is the most common thyroid disease in which thyroid-stimulating hormone (TSH) is elevated and thyroid hormones are

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Table 1 Correlations between RIA, CLA, and ELISA methods for measurement of thyroid hormone concentrations

Parameters	Group	*N*	Mean±SE	*r*	*p* value
TSH (mIU/L)	RIA, CLA	195	9.96±1.04	0.877	<0.0001
	RIA, ELISA	195	13.85±1.76	0.859	<0.0001
	CLA, ELISA	195	7.72±0.79	0.825	<0.0001
T4 (mcg/dL)	RIA, CLA	181	8.89±0.24	0.758	<0.0001
	RIA, ELISA	181	9.22±0.25	0.571	<0.0001
	CLA, ELISA	181	9.16±0.28	0.627	<0.0001
T3 (mg/dL)	RIA, CLA	120	151.45±3.53	0.635	<0.0001
	RIA, ELISA	120	145.90±4.22	0.695	<0.0001
	CLA, ELISA	120	152.10±4.55	0.775	<0.0001

RIA radioimmunoassay, *CLA* chemiluminescent assay, *ELISA* enzyme-linked immunosorbent assay, *SE* standard error (α=0.05)

decreased. Hyperthyroidism, which is defined as increased thyroid hormone levels, is less common than hypothyroidism (Goldman 2013).

The laboratory diagnosis and monitoring of the thyroid diseases are based on the determination of TSH, the thyroid hormones (T4, T3, both total and free), and thyroglobulin (Preedy et al. 2009). Various methods have been used to measure TSH and thyroid hormone concentrations in serum, such as ultrafiltration (UF) (Christofides and Midgley 2009), chromatography (Wang et al. 2003), liquid chromatography-tandem mass spectrometry (LC/MS) (Kunisue et al. 2011), radioimmunoassay (RIA) (Hemmati and Pishva 2009), bioluminescent immunoassay (BLIA) (Frank et al. 2004), equilibrium dialysis (ED) (Yue et al. 2008), time-resolved fluorometry (TRFIA) (Zhou et al. 2012), fluoroimmunoassay (FIA) (Hertzberg et al. 2010), enzyme-linked immunosorbent assay (ELISA) (Islam et al. 2011), chemiluminescent assay (CLA) (Huang et al. 2010), and electro chemiluminescent immunoassay (ECLA) (Zhang et al. 2012). Among these methods, the conventional analytical RIA method is an accurate, reliable, fast, and sensitive technique but requires lengthy incubation time and specially trained persons on specialized instruments and radioactivity. Furthermore, it exposes the operators to the biohazards of using radioactive-labeled reagents. Problem of the radioactive waste disposal and the short half-life radioactive labels are other disadvantages of RIA method. Therefore, these disadvantages make the use of RIA in clinical practice increasingly less desirable. Newer and safer methods such as CLA and ELISA are increasingly preferred over RIA, and they are being routinely used in medical diagnostic laboratories and biomedical researches (Eshratkhah et al. 2010, 2011a; Jin et al. 2009; Lin et al. 2008; Nayak and Nayak 2007; Xiao et al. 2010).

The diagnosis and monitoring of the thyroid disorders need reliable quantitation assays for measurement of TSH, T4, and T3 levels. Nowadays, there are many different methods used by medical laboratories for the determination of such hormones, which do not meet the proper diagnostic value. Therefore, reliability index of these methods, particularly in our country, needs to be evaluated. The present study deals with the sensitivity, specificity, and accuracy of three main methods for the determination of TSH and thyroid hormone levels in human serum.

Materials and methods

The study was carried out on blood samples collected from 195 subjects (23 males and 172 females) that were referred to Jahad Daneshgahi Mashhad Laboratory. Of which, 137 patients had thyroid diseases and 58 were healthy subjects. All individuals were informed about the objective of the study before their participation. Each subject had full authority to participate in the study. An informed consent was taken from each of them. The study was approved by the Research Deputyship of Iranian Academic Center for Education, Culture & Research (ACECR, No: 89.48.2566). All procedures followed were in accordance

Table 2 Sensitivity, specificity, positive and negative predictive values (PPV, NPV), and accuracy of RIA, CLA, and ELISA for measuring thyroid hormone levels in the whole population

Test type	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Accuracy
TSH RIA	97.67	100	100	95.65	98.45
TSH CLA	100	98.48	99.23	100	99.48
TSH ELISA	96.12	98.48	99.20	92.85	96.92
T4 RIA	96.36	100	100	98.43	98.89
T4 CLA	96.22	96.87	92.7	98.41	96.68
T4 ELISA	94.33	96.09	90.90	97.61	95.58
T3 RIA	90.47	100	100	98.01	98.33
T3 CLA	100	99	95.23	100	99.16
T3 ELISA	95	94	76	98.94	94.16

PPV positive predictive value, *NPV* negative predictive value, *RIA* radioimmunoassay, *CLA* chemiluminescent assay, *ELISA* enzyme-linked immunosorbent assay (α=0.05)

Table 3 Sensitivity, specificity, positive and negative predictive values (PPV, NPV), and accuracy of RIA, CLA, and ELISA for measuring thyroid hormone levels in the hyperthyroid group

Test type	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Accuracy
TSH RIA	95.74	100	100	98.66	98.97
TSH CLA	97.87	99.32	97.87	99.32	98.97
TSH ELISA	95.74	100	100	98.66	98.97
T4 RIA	93.54	100	100	98.68	98.89
T4 CLA	100	100	100	100	100
T4 ELISA	93.54	98	90.62	98.65	97.23
T3 RIA	88.23	100	100	98.09	98.33
T3 CLA	100	100	100	100	100
T3 ELISA	93.75	99.03	93.75	98.09	98.33

PPV positive predictive value, *NPV* negative predictive value, *RIA* radioimmunoassay, *CLA* chemiluminescent assay, *ELISA* enzyme-linked immunosorbent assay (α=0.05)

with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975.

The age of participants ranged from 14 to 86 years old. Patients who used medicine were excluded from this study. Data about personal details, history of any current illness, use of current medication, and symptoms and signs of the disease were collected from each subject. Blood samples were taken and centrifuged at 2500 rpm for 15 min at room temperature to obtain serum. Sera were stored at −20 °C prior to analysis. The levels of T4, T3, and TSH in serum were measured with the following kits and machines: DiaSorin kit (Italy) by the LIAI SON analyzer machine, Immunotek RIA kit (Prague) by the wizard analyzer machine, and Pishtaz Teb Zaman ELISA kits (Iran) by the Awareness ELISA reader.

Diagnostic reliability including sensitivity, specificity, positive and negative predictive values, and accuracy was evaluated in all subjects. CLA and RIA methods and clinical symptoms of the patients were used as a gold standard test. The data were analyzed by independent sample *t* test and Pearson's correlation methods; in addition, the linear regression analysis was performed using SPSS/ver. 18 software. All values are shown as mean±standard error (SE). *P* values <0.05 were considered statistically significant.

Results

Significant correlations between RIA, CLA, and ELISA methods for measurement of the thyroid hormone concentrations (TSH, T4, and T3) are presented in Table 1. Sensitivity, specificity, positive and negative predictive values, and accuracy of RIA, CLA, and ELISA methods for measuring thyroid hormone concentrations in the whole population, hyperthyroid, hypothyroid, and euthyroid groups are shown in Tables 2, 3, 4, and 5. The sensitivity and specificity of RIA method for TSH test in the whole population were 97.67 and 100 %, respectively (Table 2). The specificity of RIA method was better than CLA and ELISA methods, but its sensitivity was lower than CLA method and better than ELISA method.

Table 4 Sensitivity, specificity, positive and negative predictive values (PPV, NPV), and accuracy of RIA, CLA, and ELISA for measuring thyroid hormone levels in hypothyroid group

Test type	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Accuracy
TSH RIA	100	100	100	100	100
TSH CLA	100	100	100	100	100
TSH ELISA	96.34	100	100	97.41	98.46
T4 RIA	100	100	100	100	100
T4 CLA	90.90	99.37	95.23	98.75	98.34
T4 ELISA	95.45	98.74	98.30	99.36	98.34
T3 RIA	100	100	100	100	100
T3 CLA	100	99.15	80	100	99.18
T3 ELISA	100	96.63	50	100	96.74

PPV positive predictive value, *NPV* negative predictive value, *RIA* radioimmunoassay, *CLA* chemiluminescent assay, *ELISA* enzyme-linked immunosorbent assay (α=0.05)

Table 5 Sensitivity, specificity, positive and negative predictive values (PPV, NPV), and accuracy of RIA, CLA, and ELISA for measuring thyroid hormone levels in euthyroid group

Test type	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Accuracy
TSH RIA	100	100	100	100	100
TSH CLA	100	100	100	100	100
TSH ELISA	98.27	100	100	99.27	99.48
T4 RIA	100	100	100	100	100
T4 CLA	94.73	100	100	97.63	98.34
T4 ELISA	100	100	100	100	100
T3 RIA	100	100	100	100	100
T3 CLA	100	100	100	100	100
T3 ELISA	97.91	100	100	98.68	99.18

PPV positive predictive value, *NPV* negative predictive value, *RIA* radioimmunoassay, *CLA* chemiluminescent assay, *ELISA* enzyme-linked immunosorbent assay (α=0.05)

In addition, positive predictive value of RIA for TSH test was 100 % and was better than positive predictive value of CLA and ELISA methods. On the other hand, negative predictive value of RIA for TSH test was 95.65 % which was relatively higher than ELISA (92.85 %) and lower than CLA (100 %). Furthermore, CLA had the best accuracy for TSH and T3 tests, but RIA had the best accuracy for T4 test. Although the sensitivity of RIA and CLA for T4 test was equaled and better than that of ELISA, RIA specificity was better than the two other methods (Table 2). CLA sensitivity for T3 test was the best; in contrast, specificity of RIA for T3 test was the best.

In hyperthyroid group, CLA had the most sensitivity for three tests and had good specificity (Table 3). RIA specificity for all three tests was 100 %, but its sensitivity was similar to ELISA; even for T3 test, it was lower than that (Table 3). Among the three methods evaluated in hypothyroid group, RIA showed the best performance (sensitivity, 100 %; specificity, 100 %). CLA showed 100 % sensitivity for TSH and T3 tests, but its sensitivity for T4 test was (90 %) lower than ELISA (Table 4). Finally, as shown in Table 5, in euthyroid group, all three methods had 100 % specificity. RIA had the highest sensitivity, and ELISA had the lowest ones. CLA sensitivity for TSH, T4, and T3 test were 100, 94.73, and 100 %, respectively.

Discussion

Various methods have been used to determine thyroid hormone levels in human serum, and some differences in the results of thyroid diagnostic tests have been reported. Hence, the selection of an accurate, sensitive, and cost-effective method can help the physicians make the correct diagnosis. In the present study, RIA, CLA, and ELISA were compared for the determination of thyroid hormone levels. Significant correlations were found for measurement of T4, T3, and TSH concentrations between ELISA, RIA, and CLA methods with correlation coefficients ranging from 0.571 to 0.877. The highest correlation for the measurement of TSH concentration was observed between RIA and CLA methods (r=0.877). Moreover, the highest correlation for measurement of T4 levels was observed between RIA and CLA methods (r= 0.758), and the highest correlation for measurement of T3 concentration was found between CLA and ELISA methods (r=0.775). The lowest correlation was found between RIA and ELISA for the measurement of T4 levels (r=0.571).

The results reported by Woon Young Kyu were consistent with our findings. They showed that CLA and RIA had high correlation coefficients ranging from 0.9494 to 0.998 (Woon Young Ryu 1992) These higher correlations in the study could be due to the methodology of the studies. Eshratkhah et al. in a comparative study on the determination of poultry thyroid hormone levels in serum by CLA and ELISA methods have found that these methods were significantly correlated only for fT4 (p<0.0001, r=0.798) concentration (Eshratkhah et al. 2011b).

Regarding the comparison of the three methods, the present study shows that the highest specificity (100 %) belongs to RIA method for all three tests (TSH, T4, and T3) and the highest sensitivity (100 %) belongs to CLA method for T3 and TSH tests in the whole population. CLA had the highest sensitivity and negative predictive value for TSH test, but the specificity and positive predictive value of CLA method for TSH test were lower than those of RIA method. ELISA method had the lowest sensitivity and specificity compared to the other methods (Table 2). In general, according to our findings, CLA was the most sensitive method, but RIA was the most specific method. Furthermore, RIA was the most specific method for the diagnosis of the thyroid diseases and the most sensitive method for diagnosis of the hypothyroidism. In contrast, CLA was the most sensitive method for detection of hyperthyroidism.

These results are comparable to the other researches such as in a study conducted in 2003 on the comparison of CLA

method and immune radiometric assay (IRMA). Jian-Ying Yu et al. reported that CLA is significantly correlated with IRMA (r=0.98, p<0.01). Moreover, CLA method was more valuable than IRMA assay in the diagnosis of both hyperthyroidism and hypothyroidism (Yu and Lin 2003).

Qiao Hai-ying et al. in comparison of CLA and RIA methods for the determination of thyroid hormone levels reported that the accuracy of CLA method was significantly higher than that of RIA in detecting T3 and T4 hormones (p<0.01), and the results of CLA method were significantly correlated with those of RIA method (Hai-ying. 2005).

Similar studies were carried out by different researchers. Eshratkhah B et al. found a significant correlation between RIA and CLA methods for the determination of thyroid hormone levels (T4: p<0.01, r=0.677; T3: p<0.05, r=0.538). RIA method had an acceptable limit of sensitivity and precision, and it was more appropriate than CLA method for research application in claves (Eshratkhah et al. 2010). In the present study, the coefficients of variations for TSH, T4, and T3 tests were 1.4, 3.2, and 0.8 % with RIA method, 1.7, 3.1, and 0.8 % with CLA method, and 1.4, 3.1, and 0.8 % with ELISA method, respectively.

In summary, each of the three methods showed good sensitivity, specificity, and accuracy. ELISA had the lowest but acceptable sensitivity and specificity among the other methods. Therefore, ELISA method is suitable for the initial screening of the thyroid disorders due to the simplicity, availability, and low cost of equipment. In contrast, CLA and RIA methods showed the same specificity and sensitivity suggesting these methods as valuable techniques for monitoring patients. However, apart from hazardous effects of ionizing radiation on the environment, RIA seems to be the most reliable method for the quantification of thyroid hormones.

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