

Correlation of Chemiluminescence and Electrochemiluminescence Methods for Determination of Serum Free Triiodothyronine Level in Cow

Behrad Eshratkhah, Mehrdad Mostafaei Bigham, Alireza Mirzaei and Rahim Beheshti
Department of Veterinary Medicine, Shabestar Branch, Islamic Azad University, Shabestar, Iran

Abstract: The aim of this study was to determine serum free-triiodothyronine (fT_3) level and its correlation between the CLIA and ECLIA methods in cow. Blood samples were collected from the jugular vein of 25 clinically healthy animals aged from 2 to 4 years. This study was performed in August. The separated sera were analyzed to measurement of fT_3 concentration using the CLIA and ECLIA methods. Our results indicate that there was no a significant difference between two method concerning the fT_3 concentration in cow, but its mean was higher in CLIA method. The regression analysis revealed a significant correlation between two methods: ($p = 0.022$, $r = 0.460$ for fT_3). It was concluded that the CLIA and ECLIA methods can be used as an alternative to the previous methods for assaying the fT_3 concentration in veterinary diagnostic laboratories and exhibited an acceptable of sensivity and precision for the serum fT_3 determination in cow.

Key words: Chemiluminescence immunoassay, cow, electrochemiluminescence immunoassay, free-triiodothyronine, serum

INTRODUCTION

In healthy subjects, the thyroid gland secretes T_3 about 5-10 $\mu\text{g}/\text{day}$. However, the most part of circulating T_3 produced by peripheral deiodination of T_4 by 5'-deiodinase enzyme in most tissues predominantly in liver and kidney (White, 1987; Todini, 2007). In blood, the main part of thyroid hormones are bound to carrier proteins (e.g., albumin) and there is a small part of their in free forms which are physiologically active (Keffer, 1996; Piketty, 1996). The fT_3 is approximately 0.01% of plasma total thyroid hormone in calve (Eshratkhah *et al.*, 2010a). Its level affected by different endogenous and exogenous factors such as age, sex, nutrition and physiological status, climate, season, circadian rhythms and method of determination in livestock (Rasooli *et al.*, 2004; Todini *et al.*, 2007; Eshratkhah *et al.*, 2010b, c, 2011a, b). Various methods were used for determination of fT_3 concentration in ruminant which among them, the radioimmunoassay (RIA) method is a commonest assay (Eshratkhah *et al.*, 2011b). The newer immunoassay methods such as CLIA and ECLIA are used in some researches about the determination and variation of thyroid hormones in sheep and calve (Eshratkhah *et al.*, 2010c, 2011a) and has been validated for human samples (Sanchez-Carbayo *et al.*, 1999). These methods have some advantages over the RIA method that including: low repeat costs, short assaying time, fast result turnaround, and have no a radioactive label and waste. Additionally, the recent methods are not harmful for operators in a long time (Eshratkhah *et al.*, 2011b). Overall,

chemiluminescence is observed when the excited product of an exoergic reaction relaxes to its ground state with emission of photons. The CLIA and ECLIA can be described as chemiluminescence produced as a result of chemical reaction and of electrochemical reaction, respectively. These methods have been validated for determination of plasma thyroid hormones in human samples and commonly are used in medical diagnostic laboratories. The CLIA and ECLIA methods have been used for determination of plasma thyroid hormones in sheep, calve, cow and poultry (Singh *et al.*, 1997; Eshratkhah *et al.*, 2010a-d, 2011a, b); but there are few reports with regards to the relationship and correlation between serum fT_3 concentration using the new immunoassays methods in cow and other livestock animals. The main objective of this study was to evaluate the correlation of fT_3 concentration when determined using the CLIA and ECLIA methods in cow.

MATERIALS AND METHODS

Experimental animals: This study was performed on 25 clinically healthy and non-pregnant Holstein cows which were reared in one group at the animal house of the Islamic Azad University, Shabestar branch, East Azarbaijan, Iran. The age of the cows ranged from 2 to 4 years and the experiment was carried out in the summer (in August and ambient temperature $34 \pm 4^\circ\text{C}$).

Blood sampling: Blood samples were collected from the jugular vein directly into vacutainer tubes with no an

anticoagulant (Becton Dickinson, NJ, USA) from Holstein cows at 8-10 A.M. The serum was separated by centrifugation at 750×g for 15 min and then frozen at -20°C until used.

Sample analyses and assay performance: The level of serum fT₃ were determined in duplicate by DiaSorin CLIA kits (Strada per Crescentino-13040 Saluggia (Vercelli)-Italy) with the LIAISON analyzer, and Cobas ECLIA kits (Roche Boeringer-Mannheim, USA) with the Elecsys 2010 analyzer according to the kit manufacturer recommendations. As the hypothyroidism is the commonest type of thyroid disorders in the ruminants (Gupta *et al.*, 2010), intra-assay precision of the CLIA and ECLIA methods was determined by evaluating 10 pooled plasma samples with low fT₃ level, 3 times within the same run of assay by both methods, then the percentages coefficient of variation (CV %) was calculated. For inter-assay precision, 3 pooled plasma samples with low level of fT₃ were analyzed everyday for 10 days. The assay linearity was determined using two pooled plasma samples with low and moderate fT₃ concentrations by CLIA and ECLIA methods which were diluted 1/2, 1/5 and 1/10 with saline. Then, each dilution was measured in duplicate. The evaluations were made by the percentage of difference between the expected and observed values. For recovery studies, a pooled sample with low fT₃ concentrations was selected. The level of fT₃ was determined 5 times a day by both methods. Different amounts of this serum sample were added to the two serum samples at different concentrations of fT₃. The recovery percentages were calculated by the differences between the expected and observed values.

Statistical analysis: The data were analyzed by independent sample t-test for determination of significant difference between CLIA and ECLIA values at the p<0.05 level using SPSS v. 17 software. The linear regression analysis was performed for determining the percentage coefficient of variation (CV%), coefficient of determination (r²), correlation coefficient (r), 95% confidence interval (CI) and the slope of the curve. All the values are shown as mean ±standard deviation (SD).

RESULTS

The mean±SD of the serum concentration of fT₃ and its intra- and inter- assay percentage coefficients of variations in Holstein cow are presented in Table 1 and 2, respectively.

In this study, we showed no a significant difference between two methods concerning the serum fT₃ concentration in Holstein cow, but its value was higher when determined by CLIA method.

The intra - and inter assay % CV were <20% in both method which indicated on good precision of above

Table 1: The value of serum fT₃ concentration in Holstein cows determined by Chemiluminescence Immunoassay (CLIA) and Electrochemiluminescence Immunoassay (ECLIA) methods

Analyte	Group	N	Mean±SD	p-value	95% CI
fT ₃ (pmol/L)	CLIA	25	7.1±1.0	>0.05	6.5-7.3
	ECLIA	25	6.0±0.9		5.6-6.3

SD: Standard deviation; CI: confidence interval; CLIA: chemiluminescence immunoassay; ECLIA: electrochemiluminescence immunoassay

Table 2: Precision of Chemiluminescence Immunoassay (CLIA) and Electrochemiluminescence Immunoassay (ECLIA) methods for determination of serum fT₃ concentration in Holstein cows

	fT ₃ (CLIA)	fT ₃ (ECLIA)
Number of replicates	30	30
Mean value (pmol/L)	1.7	1.6
Standard deviation	0.1	0.1
Intra-assay % CV	5.8	6.2
Number of replicates	30	30
Mean value (pmol/L)	1.5	1.6
Standard deviation	0.2	0.2
Inter-assay % CV	13.3	12.5

Table 3: Recovery analysis of the fT₃ assay using the CLIA and ECLIA methods

Analyte	Theoretical values	Measured values	Recovery (%)
fT ₃ (pmol/L) (CLIA)	3.2	3.5	108.9
	1.8	1.7	94.3
fT ₃ (pmol/L) (ECLIA)	3.0	3.3	109.5
	1.7	1.8	105.7

Table 4: Linearity analysis of the fT₃ assay using the CLIA and ECLIA methods

	Theoretical values	Dilution	Measured values	Difference (%)
fT ₃ (pmol/L) (CLIA)	1.0	1/2	1.9	102.7
		1/5	2.4	104.3
		1/10	2.7	105.2
2.8	1/2	1/2	4.5	108.7
		1/5	5.9	116.3
		1/10	6.5	118.6
fT ₃ (pmol/L) (ECLIA)	1.1	1/2	4.9	107.5
		1/5	7.2	113.2
		1/10	10.4	118.1
2.9	1/2	1/2	3.9	110.4
		1/5	4.4	116.5
		1/10	4.6	118.3

mentioned methods for measurement of fT₃ concentration in cows. The recovery and linearity analysis of fT₃ when determined by both mentioned methods are presented in Table 3 and 4. The linear regression and analysis curve of the measured fT₃ concentration by the CLIA and ECLIA methods is shown in Fig. 1.

The distribution of fT₃ was linear when CLIA values were plotted against the ECLIA values (Fig. 1). Additionally, the fT₃ (r = 0.715) values showed a significant positive correlation when it measured using the both method (p<0.001) and the slope of the curve was 0.78. The results from the Kolmogrov-Smirnov test indicated that both CLIA and ECLIA methods had a normal and relatively similar distribution.

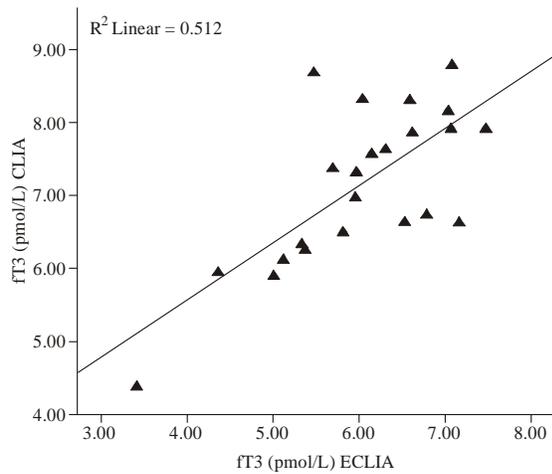


Fig. 1: Distribution of cow serum fT_3 (pmol/L) concentrations in samples determined using Chemiluminescence Immunoassay (CLIA) and Electrochemiluminescence Immunoassay (ECLIA) methods. ECLIA values (X-axis) were compared with the CLIA values (Y-axis) by linear regression analysis ($Y = 2.445 + 0.780 X$, $r^2 = 0.512$; $r = 0.715$, $p < 0.001$)

DISCUSSION

As the above mentioned, the intra - and inter assay % CV were $< 20\%$ in both method and also, we found $> 90\%$ recovery of the fT_3 which indicated on good precision of CLIA and ECLIA methods for determination of serum fT_3 concentration in cow. According to our results, the level of the fT_3 was lower than the value reported in calve using the CLIA method (Eshratkhah *et al.*, 2010a, b) and higher than the values reported in sheep using CLIA, ECLIA (Eshratkhah *et al.*, 2010c, 2011a) and RIA methods (Eryavuz *et al.*, 2007; Nazifi *et al.*, 2007). The coefficient of determination (r^2) of fT_3 was 0.512; therefore, about 51.2% of the variation in the fT_3 concentration using the CLIA method explained by the ECLIA method. These findings indicate that the regression equation of fT_3 appear to be relatively strongly useful for making prediction about its concentration using the CLIA and ECLIA methods. The principal of procedure for the determination of fT_3 using the DiaSorin CLIA kit is based on the solid phase antigen linked technique, where the free hormone binds to the polyclonal antibody and using the Cobas ECLIA kit is based on a competition principle assaying anti-antibody specifically directed against T_3 . On the basis of our results, the value of serum fT_3 in Holstein cows was higher when using the CLIA method which was inconsistent with the previous similar report in sheep (Eshratkhah *et al.*, 2010c, 2011a). As, unlike to ECLIA method, the polyclonal antibody is used in CLIA method for determination fT_3 concentration, therefore our result seem normal. Indeed, likely tends of serum fT_3 for binding

to isoluminol-antibody conjugate in CLIA method may be higher compared to the anti- T_3 antibody labeled with a ruthenium complex in cow. According to the kit manufacturer recommendations, some agents might affect on binding behaviour of binding proteins such as therapy with high biotin doses, furosemide and disalbuminemia which all the listed agents had no any effect on the results of our experiment. There is no any information about comparing the determination methods of serum fT_3 concentration in cow, particularly using the CLIA and ECLIA methods. The performance of CLIA and ECLIA methods suggested that they can be used for the quantitation of fT_3 in cow. The analysis of serum samples by ECLIA provided lower fT_3 values than did CLIA in the same samples, although both methods had approximately similar good precision for determination of the fT_3 in cow. Therefore, veterinary researchers and clinicians must be aware of the limitation of these methods for determining the serum fT_3 in cow. In a clinical setting, fT_3 values obtained by CLIA and ECLIA methods should be interpreted with reference ranges created in the respective laboratory according to the breed, age, gender, season, fasting, drug consumption and physiological states of cow in order to have reliable results.

CONCLUSION

The determination of fT_3 concentrations using the CLIA and ECLIA methods was cleared that there was a little and non significant difference between methods concerning the fT_3 concentrations and its value was lower in ECLIA method compare to the CLIA method. It could be related to use of polyclonal antibody in CLIA method; although, the both method had good precision for determination of the fT_3 values in cow.

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