**MEASURING PLASMA FERRITIN LEVELS WITH TWO DIFFERENT METHODS:
A COMPARISON OF ROCHE COBAS E601 VERSUS ROCHE COBAS C501
(INTEGRATED MODULAR SYSTEM ROCHE COBAS 6000)**

MERENJE NIVOVA PLAZMA FERITINA SA DVE RAZLIČITE METODE:
POREĐENJE ROCHE COBAS E601 SA ROCHE COBAS C501
(INTEGRISANI MODULARNI SISTEM ROCHE COBAS 6000)

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Summary

Background: The aim of our study is to compare plasma ferritin levels found to be high or low in terms of reference range by means of electrochemiluminescence (ECLIA) and immunoturbidimetric method and to examine whether they can be used interchangeably.

Methods: 84 patients with high plasma ferritin level and 153 patients with low ferritin level according to the reference range were included in the study. Plasma samples measured in Cobas e601 device with ECLIA were also measured as immunoturbidimetric Cobas c501 device. For method comparison, CLSI EP9-A3 Guideline was used. While the consistency between the methods were specified with Passing-Bablok regression analysis and Spearman correlation analysis, bias error between the methods (bias%) was determined through Bland-Altman analysis.

Results: Both high and low plasma ferritin levels measured with Cobas e601 module and determined high in terms of reference range were compared with the results found with cobas c501 module. The difference was found to be statistically significant ($p < 0.001$). According to regression and correlation (for low plasma ferritin levels; $r = 0.993$, $p < 0.001$, for high plasma ferritin levels; $r = 0.966$, $p < 0.001$) results, the methods were in consistency with each other. Additionally, while the bias% value was found to be 10.4% for low plasma ferritin levels, it was found to be 12.6% for high ferritin levels.

Conclusions: Accordingly, we believe that, comparison with more samples especially in terms of different clinical decision levels is required in order to examine interchangeable use of immunoturbidimetric method in integrated devices and ECLIA.

Keywords: plasma ferritin, electrochemiluminescence, immunoturbidimetric

Kratak sadržaj

Uvod: Svrha ovog izučavanja je bila da se uporede nivoi plazma feritina bilo visoki ili niski u odnosu na referentne vrednosti određeni elektrohemioluminescijom (ECLIA) i imunoturbidimetrijskom metodom i da se proceni da li mogu da se koriste prema potrebi.

Metode: U proučavanja je uključeno 84 pacijenta sa visokom vrednošću feritina i 153 pacijenta sa niskom vrednošću u odnosu na referentne vrednosti. Uzorci plazme mereni su na Cobas e601 sa ECLIA metodom kao i imunoturbidimetrijski na Cobas c501. Za poređenje je korišćen protokol CLSI EP9-A3. Takođe je primenjivana Passing-Bablok regresiona analiza i Spearman korelaciona analiza, dok je greška odstupanja između metoda (bias%) određivana Bland-Altmanovom analizom.

Rezultati: Visoke i niske vrednosti feritina u plazmi izmerene na Cobas e601 modulu su poređene u odnosu na one dobijene na Cobas c501 modulu. Nađena je statistički značajna razlika ($p < 0.001$). Prema korelacionoj i regresionoj analizi (za niske nivoe plazma feritina; $r = 0.993$, $p < 0.001$, za visoke nivoe plazma feritina; $r = 0.996$, $p < 0.001$) metode su bile u saglasnosti jedna sa drugom. Dodatno, vrednost bias% bila 10,4% za nizak nivo feritina u plazmi, a za visoke nivoe feritina u plazmi 12,6%.

Zaključak: Shodno ovim istraživanjima zaključujemo da su potrebna mnogo obimnija ispitivanja u prvom redu radi donošenja ispravnih kliničkih odluka a u vezi primene imunoturbidimetrijske metode integrisane sa ECLIA sistemom.

Ključne reči: plazma feritin, elektrohemioluminescencija, imunoturbidimetrija

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Introduction

Plasma ferritin levels are sensitive parameters used for evaluating condition of iron in the body non-invasively and for diagnostic reagents of iron deficiency anemia (1–3). Additionally, high plasma ferritin levels reflect systemic inflammation as acute phase reactants (4, 5). Although there are different methods for the measurement of plasma ferritin levels such as immunoturbidimetric method, electrochemiluminescence (ECLIA), Enzyme-Linked Immunosorbent Assay (ELISA), Radioimmunoassay (RIA), Chemiluminescence immunoassay (CLIA), the use of two methods in clinical laboratories stands out more. These methods are immunoturbidimetric method and electrochemiluminescence (ECLIA) method (6). As these measurement methods used in clinical laboratories have different test principles, their reference ranges and measurement limits are also different from each other. While anti-ferritin antibodies (rabbit) are used in immunoturbidimetric method, antibodies against human liver, spleen and heart ferritins are used in immunoassay method. The one with the highest analytic specificity among these antibodies is liver ferritin (7, 8).

Hormone and biochemistry module-integrated-systems (Roche Cobas 6000) were used in our laboratories for measurement (or determination) of plasma ferritin. While plasma ferritin levels result in 9 minutes in biochemistry module (Cobas c501) with immunoturbidimetric method, they result in 18 minutes in hormone module (Cobas e601) with ECLIA method due to long incubation period. The fact that tests examined in hormone module and analyzed in biochemistry module provides advantages for the laboratory in terms of saving time and obtaining practical results. The aim of our study is to examine whether there is a difference between plasma ferritin levels identified as high and low in terms of laboratory reference range with different methods in our hormone and biochemistry module-integrated-systems (Roche Cobas 6000), and whether they can be used interchangeably.

Materials and Methods

The ferritin levels of 153 (86 female, 68 male) patients whose sample acceptance was performed by Amasya Central Public Health Laboratory were found to be low in terms of reference range and 84 (41 female, 43 male) patients found to have high plasma ferritin levels in terms of reference range. Both values were studied in Cobas c501 biochemistry module and Cobas e601 hormone module in order to make a comparison. No additional samples were taken from the patients. The written and signed consent forms regarding their voluntary participation were received from the participants.

Regarding the method comparison, Clinical Laboratory Institute (CLSI) EP9-A3 Guidelines was used (9). Blood samples were selected among daily received blood samples between 01/08/2018 – 30/09/2018 from the patients who were suitable for the inclusion criteria of the study. Since it could affect the measurement method, patients with immunological diseases, biotin use history (at least 8 hours should pass after use), having iron treatment, with malignancy were excluded from the study. Moreover, as indicated in kit insert, patients who had RF levels higher than 2500 U/mL were excluded from the study by measuring rheumatoid factor (RF) levels of the patients. Furthermore, hemolysis, lipaemia and icterus indexes of the patients were evaluated and the samples having the level of hemolysis, lipaemia and icterus for interference were excluded from the study. Patient samples were taken to 5 mL tubes with mechanic separators and Barricor (BD, Becton Dickinson) in order to prevent artefact-based problems in gel separator tubes. After being centrifuged at 4000 RCF (g) for 3 minutes, plasma samples were measured in Cobas c501 (Roche Diagnostics, Mannheim, Germany) biochemistry module within 20 minutes following pipetting procedure in Cobas e601 (Roche Diagnostics, Mannheim, Germany) hormone module. Systematic error (bias%) comparison was carried out on the basis of data obtained from different sources (Royal College of Pathologist Australasia (RCPA): 3 µg/L (<40 µg/L for results), 7.5% (>40 µg/L for ferritin results), Wisconsin State Laboratory of Hygiene (WLSH):15%; College of American Pathologists (CAP):8%; Canadian Fixed Limits (CFX): 9.7%; American Association of Bioanalysts (AAB): 15%. Biological Variation (BV): 8.7%) (11).

Cobas c501 biochemistry module measurement method: Human-driven ferritin shows agglutination with latex particles covered with anti-ferritin antibodies in expanded particle surface immunoturbidimetric test. Precipitation was to be turbidimetric at 570/800 nm.

Cobas e601 hormone module measurement method: First incubation: sample with monoclonal antibody with biotin specific to ferritin and monoclonal antibody specific to ferritin marked with ruthenium complex creates a sandwich complex. Second incubation: After streptavidin-covered micro-particles are added, by way of interaction between biotin and streptavidin, complex is linked to a solid phase. Reaction mixture is aspired into measurement cell where micro-particles are magnetically caught by electrode surface. Voltage application on electrode causes chemiluminescence emission, and this is measured by a photon counter (photomultiplier).

Method characteristics for ferritin measurement in Cobas c501 and Cobas e601 devices are shown in Table I.

Table I Characteristic of methods for immunoturbidimetric assay and ECLIA

	Immunoturbidimetric assay (Cobas c501)	ECLIA (Cobas e601)
Testing time	9 minutes	18 minutes
Test principle	Expanded particle surface immunoturbidimetric test	sandwich
Calibration traceability	This method has been standardized against a selected manufacturer's measurement procedure (immunological method) *	The Elecsys Ferritin assay (REF 04491785) has been standardized against the Elecsys Ferritin assay (REF 11820982). The Elecsys Ferritin assay (REF 11820982) has been standardized against the Enzygum-Test Ferritin method. This in turn has been standardized against the 1st International Standard (IS) NIBSC (National Institute for Biological Standards and Control) »Reagent for Ferritin (human liver)« 80/602.
Limitations-interference	<ul style="list-style-type: none"> – Icterus: No significant interference up to an I index of 60 (approximate conjugated and unconjugated bilirubin concentration: 1026 $\mu\text{mol/L}$). – Hemolysis: No significant interference up to an H index of 500 (approximate hemoglobin concentration: 310 $\mu\text{mol/L}$). – Lipemia (Intralipid): No significant interference up to an L index of 1000. There is poor correlation between the L index (corresponds to turbidity) and triglycerides concentration. – Rheumatoid factors up to 1200 U/mL do not interfere. 	<ul style="list-style-type: none"> – The assay is unaffected by icterus (bilirubin < 1112 $\mu\text{mol/L}$) hemolysis (Hb < 0.31 mmol/L), lipemia (Intralipid < 3.3 g/L), and biotin < 205 nmol/L. – In patients receiving therapy with high biotin doses (i.e. > 5 mg/day), no sample should be taken until at least 8 hours after the last biotin administration. – No interference was observed from rheumatoid factors up to a concentration of 2500 U/mL. – There is no high-dose hook effect at ferritin concentrations of up to 100000 $\mu\text{g/L}$.
Reference range	30–400 $\mu\text{g/L}$ for males and 15–150 $\mu\text{g/L}$ for females.	30–400 $\mu\text{g/L}$ for males and 15–150 $\mu\text{g/L}$ for females.
Measuring range	5–1000 ng/mL (5–1000 $\mu\text{g/L}$)	0.5–2000 ng/mL (0.5–2000 $\mu\text{g/L}$).

* Data on file at Roche Diagnostics (kit insert).

Table II Evaluation of intra-day and intra-day method accuracy

	Cobas c501 biochemistry module				Cobas e601 hormone module			
	Intra-day mean ($\mu\text{g/L}$) (CV%)		Inter-day mean ($\mu\text{g/L}$) (CV%)		Intra-day mean ($\mu\text{g/L}$) (CV%)		Inter-day mean ($\mu\text{g/L}$) (CV%)	
	Level 1	Level 2	Level 1	Level 2	Level 1	Level 2	Level 1	Level 2
Laboratory	99.8 (0.73)	193.2 (1.3)	98.7 (1.78)	196.2 (2.8)	142.5 (1.09)	911.7 (2.25)	149.7 (4.59)	951.7 (4.1)
Manufacturer	223 (0.7)	568 (0.9)	226 (1.2)	558 (1.7)	19.4 (3)	234 (3.1)	14.7 (4)	361 (4.4)
IQC of Laboratory	99.3 (1.08)	197.9 (1.57)	98.7 (0.98)	194.1 (1.98)	161.3 (2.9)	965.3 (1.26)	158.1 (3.84)	949.5 (4.04)
IQC of Manufacturer	125 (0.8)	306 (0.6)	119 (1.1)	310 (1.3)	22.2 (2.1)	221 (1.9)	23.8 (4.3)	247 (4.9)

IQC: Internal Quality Control, CV: Coefficient of variation

Intra-day and inter-day repeatability were performed with the plasma pool obtained from patient samples. For inter-day repeatability, plasma pool was aliquoted as 21 pieces and stored under -20 degrees. The repeatability was conducted by using a single piece every day. On the other hand, intra-day repeatability was performed by using the prepared human plasma pool consecutively 21 times under the same conditions. The results were found as coefficient of variation (CV). Certainty study was performed according to EP15-3A protocol of CLSI. (10) Furthermore, different-level-controls for these two methods submitted by the manufacturer firm were repeated 5 times in 3 different days. Results were calculated as coefficient of variation (%CV) and compared to the values given by the manufacturer firm (Table II).

Statistical Analysis: For the statistical analysis, Medcalc (Mariakerke, Belgium) 18.9.1 version was used. Descriptive statistics were presented for categorical variables as number and percentage, average for numerical variable, median, standard deviation and interquartile range (IQR). Normal distribution skewness was determined by the examination of kurtosis values, Kolmogorov-Smirnov (Lilliefors Significance Correction), Shapiro-Wilk tests and distribution of histogram graphs. As the numerical variables had not met the normal distribution condition, two connected group comparisons were made with Wilcoxon Runk Sum test. Inter-methods relation was found with Spearman correlation and Passing Bablok regression analysis used for non-parametric test condition. The measurement difference between the methods were found with Bland-Altman analysis.

Results

When the results of patients grouped in terms of low ($n=153$) and high ($n=84$) plasma ferritin levels were evaluated with different devices by means of different methods, both high and low levels of plasma ferritin measured in hormone module (Cobas e601) were statistically much higher than results measured in biochemistry module (Cobas c501) ($p<0.001$). In addition, both low plasma ferritin results ($r:0.993$, $p<0.001$) and high ferritin results ($r:0.966$, $p<0.001$) of the two methods revealed a strong correlation positively. In Passing Bablok regression analysis, while $y = 1.285 + 0.767x$ (intersection confidence interval: $0.7657 - 1.6695$, slope confidence interval: $0.7292 - 0.8088$) equation was found for low level plasma ferritin method comparison, $y = 5.719 + 0.859x$ (intersection confidence interval: $-3.8540 - 16.7387$, slope confidence interval: 0.8195 to 0.9048) equation was found for high level plasma ferritin method comparison (Figure 1 and Figure 2). In Bland-Altman graph, when differences between the two methods were compared, low plasma ferritin levels measured with ECLIA method were found to be 10.4% ($1.44 \mu\text{g/L}$) (bias%) higher

Figure 1 Comparison of Cobas c501 and Cobas e601 methods for low plasma ferritin values by Passing Bablok regression analysis.

Figure 2 Comparison of Cobas c501 and Cobas e601 methods for high plasma ferritin values by Passing Bablok regression analysis.

Figure 3 Differences between Cobas c501 and Cobas e601 methods for low plasma ferritin values by Bland Altman analysis (bias%= 10.4%).

Figure 4 Differences between Cobas c501 and Cobas e601 methods for high plasma ferritin values by Bland Altman analysis (bias%= 12.6%).

compared to plasma ferritin levels measured with immunoturbidimetric method. Furthermore, high plasma ferritin levels measured with ECLIA were about 12.6% (bias%) higher than plasma ferritin levels measured with immunoturbidimetric method. While inter-methods bias value (1.44 µg/L) of low plasma ferritin level was lower than the acceptable bias value (3 µg/L) declared by RCPA for results lower than 40 µg/L, bias% value (10.4%) was found to be lower than bias% values suggested by WLSH (bias%: 15%) and AAB (bias%: 15%). However, it was found to be higher than bias% values suggested by CAP, BV and CFX (8%, 8.7%, 9.7% respectively). Inter-methods bias% value of high plasma ferritin levels (12.6%) was found lower than bias% values suggested by WLSH (bias%: 15%) and AAB (bias%: 15%). Yet, it was found to be higher than bias% values suggested by RCPA, CAP, BV and CFX (7.5%, 8%, 8.7%, 9.7% respectively) (Figure 3 and Figure 4).

Discussion

In this study, plasma ferritin levels identified as high and low were measured with both methods in Cobas 6000 modular system in our laboratory according to reference range of Cobas c501 biochemistry analyzer and Cobas e601 hormone analyzer. Whether there is a difference between different levels of plasma ferritin between two methods and whether these methods could be used interchangeably have also been evaluated.

The first result of the study regarding whether there is a difference among plasma ferritin levels according to the methods have revealed that the difference was statistically significant. Secondly, the results of the two methods were in consistency according to Passing-Bablok regression analysis and

Spearman correlation analysis. When the difference between methods were evaluated with Bland-Altman analysis, bias% value for low plasma ferritin levels was found to be 10.4% (1.44 µg/L) and bias% value for high plasma ferritin levels was found to be 12.6%. Acceptable bias% values for plasma/serum ferritin levels obtained from various sources (RCPA: 3 µg/L (<40 µg/L for results), 7.5% (>40 µg/L for ferritin results), WLSH: 15%; CAP: 8%; CFX: 9.7%; AAB: 15%. BV: 8.7%) and bias% value obtained for low plasma ferritin results were found to be lower than bias value recommended for low ferritin levels by RCPA and bias% values recommended by WLSH and AAB. bias% value found in the study regarding high plasma ferritin results was only lower than bias% values recommended by WLSH and AAB. These results show that deviation in high plasma ferritin levels between the two methods is higher than deviation in low plasma ferritin levels.

Previously, serum/plasma ferritin levels were evaluated with different methods and the results were shown below. In the study where nce et.al evaluated randomly selected patients' serum ferritin levels with unintegrated AU5800 biochemistry analyzer and Cobas e601 hormone analyzer, it was noted that there was a positive correlation between serum ferritin results evaluated with different measurement methods and indicated that the two methods can be used interchangeably as the difference between them was within clinically acceptable limits (11). In another study, Dupuy et al. (12) compared chemiluminescence and immunoturbidimetric method with radioimmunoassay (RIA) method. As a result of their Bland Altman analysis, it was highlighted that the methods of which serum ferritin levels were compared were in consistency with each other and indicated that these methods could be used instead of RIA method (12). Karakochuk et al. (13) examined serum ferritin levels in non-pregnant women having iron deficiency with four different immunoassay methods and they observed that serum ferritin results were in different concentrations in systems using different calibrator, ferritin isoforms and antibodies. Despite those different results, they stated that it correctly reflected iron deficiency prevalence (13). Zhang et al. (14) compared patient samples in different concentrations in Architect i2000 (Abbott Laboratories) and Cobas e601 (Roche Diagnostics) systems with two different methods and revealed that the average of serum ferritin levels made in Cobas e601 around 60.6 ng/mL (µg/L) were found to be higher than the average of Architect 2000 auto-analyzer. As a result, it has been concluded that both methods show correlation; however, they cannot be used interchangeably, and patients' serum ferritin results should always be observed with the same method (14, 15).

Conclusion

According to our results, the difference between high and low results depending on plasma ferritin reference range was found to be statistically significant. On the other hand, there was a coherence found between the different levels of both methods based on correlation and regression analysis. However, while bias% results were lower than WLSH and AAB % bias results, they were higher than CAP, CFX and BV's bias% results. As a result, it has been believed that, these two methods should be compared with more samples especially in different clinical decision levels in order to examine interchangeable use of immunoturbidimetric method and ECLIA in integrated devices.

Limitations

Despite the fact that the study has fulfilled its purpose, there were some limitations. Firstly, plasma ferritin levels of patients only determined as low or high depending on reference range were examined. Patient samples which are at the clinical decision limits can be compared with these two methods both for serum and plasma samples and the difference between the methods can be analyzed. Secondly, a comparison between the two methods can be performed by including normal plasma ferritin levels within the reference range for future studies.

Conflict of interest statement

The authors stated that they have no conflicts of interest regarding the publication of this article.

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