RESEARCH / INVESTIGACIÓN

Comparative study between ECL and ELISA to determine the reliable range of Estradiol in the treatment of infertility

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DOI. 10.21931/RB/2020.05.04.12

Abstract: Estradiol is one factor that can alter the outcome of the treatment of infertile couples following the application of in vitro fertilization techniques. Currently, the estradiol level is measured by two diagnostic methods Enzyme-Linked Immunosorbent Assay (ELISA) and Electrochemiluminescence (ECL). Accordingly, this study determines ELISA and ECL's sensitivity and consistency to measure different levels of estradiol and determine its reliable range and provide this information to laboratories and gynecologists. This study is performed on 250 patients of the Avicenna Fertility Center. The data of the study are analyzed in SPSS18 and MiniTab. Consent was obtained for experimentation with human subjects. Pearson correlation coefficient was used to investigate the relationship between these two methods. The results indicated a strong correlation between the two variables ECL and ELISA (r= 0.735, P-value<0.001). High numbers indicate that the decrease and increase of one variable are proportional to the other variable's fluctuation. This study shows that the results of estradiol obtained from both ECL and ELISA are similar. In the ELISA method, due to the linear values' limitation, samples with estradiol concentration above the highest standard level should be diluted and the dilution coefficient should then be applied.

Key words: Estradiol, estrogen, infertility, ELISA, ECL.

Introduction

Estradiol is a steroid hormone and is the most important sex hormone in women. It is the first type of estrogen and is produced in the ovaries. As the ovaries grow and develop, the egg follicles release estradiol to assist the onset and maintenance of the monthly cycle[1]. This hormone also affects other tissues such as bone, liver, blood vessels and reproductive tissues such as the uterus. Estradiol is one factor that can alter the outcome of the treatment of infertile couples following the application of in vitro fertilization techniques.

For this reason, in the infertility treatment cycles, the serum level of this hormone is used as a monitoring tool for ovulation induction, and the accuracy of IVF planning[2]. However, in the cycles following in vitro fertilization, the number of mature follicles is more due to the use of ovarian stimulating drugs, and hence the level of this hormone (estradiol) may be higher than its physiological range. This increase in estradiol level could raise concerns about luteal phase abnormalities, uterine tissue changes, and ovarian hyper-stimulation syndrome, a life-threatening complication of the ovulation induction cycle. Therefore, it is essential to measure the different levels of estradiol and its reliable ranges accurately[3]. Accordingly, the experience of some infertility centers suggests that by changing the method of estradiol measurement in the laboratory of an infertility center, the upper limit of this hormone, which poses the risk of OHSS, could be changed, and the state of not recognizing this level maintains the risk of consuming lower or higher doses of ovulation-stimulating drugs in infertile patients. In other words, if the physician is not informed of this change and the critical drug range, they would mistakenly increase or decrease the drug dosage due to the assumption of inadequate or excessive drug dose, which would ultimately lead to either OHSS or failure of the treatment cycle. Thus, it is essential to know that changing the method does not necessarily mean that high estradiol levels may be similar even by one unit of measurement, and this will be a critical point in the success and health of the treatment cycle.

During ovarian hyperstimulation for in vitro fertilization (IVF), serum estradiol concentrations are usually monitored daily for optimal timing of human chorionic gonadotropin (hCG) administration oocyte collection. The DRG Estradiol sensitive ELISA Kit is a solid phase enzyme-linked immunosorbent assay (ELISA), based on the competitive binding. The microtiter wells are coated with a polyclonal antibody directed towards an antigenic site on the Estradiol molecule. Endogenous Estradiol of a patient sample competes with an Estradiol horseradish peroxidase conjugate for binding to the coated antibody. After incubation, the unbound conjugate is washed off. The amount of bound peroxidase conjugate is reverse proportional to the concentration of estradiol in the sample. After adding the substrate solution, the intensity of color developed is reverse proportional to estradiol concentration in the patient sample[8].

Currently, diagnostic methods (ELISAs) and (ECLs) are used to measure estradiol levels; however, based on research studies, ECL has superiority in sensitivity, cost-effectiveness, and high precision over conventional colorimeter methods such as ELISA. On the other hand, quick and error-free answering is desirable in all situations, and to achieve this goal, changing the system from manual to machinery seems necessary. Therefore, although one of the most commonly used methods for measuring this hormone assay is ELISA, the ECL method's use is more rapid and accurate is prioritized[4,5].

Accordingly, one of the hypotheses raised in this study was that if the units of measurement were identical in the two methods, whether to obtain a standard concentration of serum estradiol level in the ECL method and to compare it with the ELISA method and to share this information with laboratories and gynecologists, is essential or not? And can the serum estradiol level measure in one person by 2 different (ELISA) methods and (ECL) be different under the same conditions? And finally, the measurement and prescription dosage of estradiol hormone should be based on the specific method (ELISA/ECL) which is used for assessing estradiol hormone.

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Based on the second hypothesis of this study, since estradiol results in high concentrations of this hormone by ELISA, which usually occurs during infertility treatment, it has no linear relationship with diluted hormone assays, therefore repeating the test with diluted samples from specified values above before reporting the result is essential. It is also crucial to specify a reliable reading range without the application of dilution. Given that assisted reproductive techniques' success is highly essential due to the financial costs and psychological problems following their failure, any studies that can increase these success rates are entirely critical. This study's main objective was to determine the sensitivity and consistency of ELISA and Electro Quantitative Luminescence (ECL) methods to measure different estradiol hormone levels and determine a reliable range to enhance assisted reproduction success and prevent complications which are dangerous and sometimes irreparable. Some other specific targets were considered to be evaluated in this paper, like determining the compatibility of two methods (ECL vs. ELISA) in measuring estradiol hormone and Determining the sensitivity of the ELISA method in measuring the high titers of estradiol and determining the reliable range for it. This study's hypothesis firstly entails some practical purposes such as changing the manual test method to an automatic one, Increasing the test's speed and accuracy, Increasing the sensitivity and specificity of the test, and increasing sensitivity and specificity of the test in high estradiol titers. This proposal's crux is to establish a satisfactory laboratory evaluation method for a clinical endocrinology hormone, estradiol.

Materials and methods

To start the practical process, inclusion criteria were established as follows. All patients with at least two previous unsuccessful IVF/ICSI cycles were admitted. After signing the informed consent and computer-generated randomization, the study was performed on 250 patients referred to specialized clinics of Avicenna infertility, and recurrent miscarriage treatment center referred to the medical diagnosis laboratory for estradiol testing. Consent was obtained for experimentation with human subjects. Out of the compilation, 250 samples were randomly selected with different concentrations and were analyzed and compared through both methods. Finally, the data were analyzed in SPSS18 and MiniTab.

$$\eta' = \left(\frac{Z_1 - \alpha\sigma}{d} \right)^2$$

$$\eta' \approx \left(\frac{(1.96)\,(200)}{25} \right)^2 = 2\,46 \approx 250$$

How to choose patients

All patients referred to the Medical Diagnostic Laboratory of specialized clinics of Avicenna Infertility and Repeated Abortion treatment center for estradiol testing during the project period were eligible for admission.

Sample size and calculation method

According to expert statistics for estimation of estradiol hormone level in Pg/ml, in two methods with 95% confidence and 25 Pg/ml, concerning standard deviation of estradiol in two methods equal to 200Pg/ml using the statistical method, approximately 250 cases who were randomly selected, were calculated.

The project started on 28 August 2018 after receiving the code of ethics: IR.ACECR.AVICENNA.REC.1397.006 from the Avicenna Ethics committee: www.ethics.research.ac.ir by Dr. Ali Sadeghi Tabar. Additionally, all applicants in this research received consent upon arrival to the program, and their information was used anonymously.

Data collection tools

Specimens were obtained from patients of the different specialized clinics referred to the Medical Diagnostic Laboratory for estradiol testing, and the results of ELISA and ECL tests were analyzed by SPSS software.

Results

Results were analyzed by the statistical expert and are as follows: Pierson correlation coefficient was used to investigate the relationship between these two methods. The results indicated a strong relationship between the two variables ECL and ELISA ($r=0.735$, P-value<0.001). High numbers mean that each variable's decrease and increase are proportional to the decrease and increase of the other variable. The distribution chart of the ECL and ELISA variables is as follows:

Discussion

Historically, quantitative methods for measuring E2 have been obtained by bioassay, mass spectrometry (MS), UV absorbance, and immunoassay. Up until now, applying any measurement method to biological specimens has required the isolation of steroids. The original immunological method for Estradiol evaluation was named RIA or indirect RIA[9]. Measurement of Estradiol in serum without its prior isolation is called direct RIA, like chromatography, as it is performed currently[10].

A group of scientists declared that estradiol must be measured at low concentrations to distinguish between suppressed levels of less than 1pg/mL and pretreatment levels[11].

Another paper suggests that the measurement of E2 should be reliable at levels of 3000pg/mL when testing performed in support of IVF programs, for ovulation induction and ovarian hyper-stimulation monitoring[12].

Several studies suggest concerns about the analytical performance in the measurement of E2 among different assays[13]. Sensitive assays for estradiol have been immunologically based, but it lacks specificity to satisfy all clinical and scientific requirements, up until recently, methods based on mass spectroscopy have been replaced[14].

ECL (ElectroChemiLuminescence) is Roche's technology for immunoassay detection of estradiol. Based on this technology, ECL delivers reliable results. The development of ECL immunoassays is based on the use of a ruthenium-complex and tripropylamine (TPA). The chemiluminescence reaction for detecting the reaction complex is initiated by applying a voltage to the sample solution resulting in a precisely controlled reaction[15].

In a study by Azim et al. (2015) on the mechanism and application of the ECL method, the results of the study showed that: different types of luminescence include quantum luminescence (cold radiation caused by a chemical reaction), bioluminescence (The biochemical reaction within an organism, such as a firefly), electroluminescence (passing electric current, such as LED lamps), electrochemical luminescence (electrochemical reaction), phosphorescence (such as lumi-

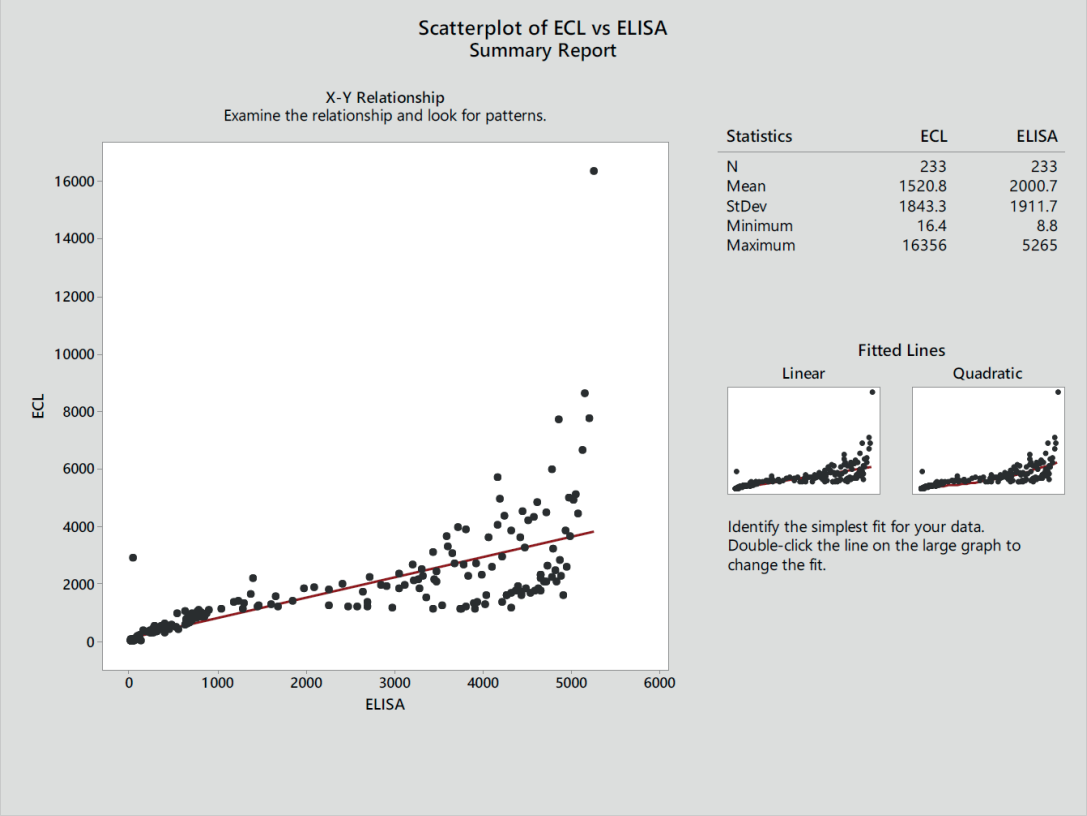

Figure 1. The distribution chart of the ECL and ELISA variables.

nous clock hands), and so on[6]. The source of radiation in ECL is often a polycyclic aromatic hydrocarbon, a metal complex, a quantum dot, or a nanoparticle. ECL's main advantage of ELISA methods is that it does not require light, and the analyte itself is the source of radiation. This small difference increases the test's sensitivity and specificity several times and makes the response or linearity of the radiation-dose curve in the ECL very wide and eliminating confounding factors in the majority of times. The concentration level of analytes in many laboratory methods is based on the light passing through the solution and its absorption rate. This process is illustrated by drawing a radiation-dose curve and that part of the curve, which has a linear correlation with the dose or concentration of the analyte of the test, can rely on[6].

In another study done by Nasiri et al. in 2013, Conducted to evaluate the compatibility of estradiol hormone levels with ELISA in two diluted and non-diluted serum of women treated by ovulation stimulation drugs concluded that in Serum estradiol level more fabulous than 2000 Pg/ml requires dilution of the samples and the results of the non-dilution assay will not be reliable. This should be taken into account in laboratories of infertility treatment centers or even other laboratories to determine the linearity of the methods used to dilute them when exposed to higher amounts[7].

Our study indicated that the estradiol results obtained from both ECL and ELISA were similar in the medical diagnostic laboratory and had a similar interpretation. It should be noted that in the ELISA method, due to the limitation of the linear values, samples with estradiol concentration above the highest standard level should be diluted, and then the dilution coefficient should be applied. This research's dilution coefficient was 1: 5, and 5µL of the sample was mixed with 20 µL of the diluent. Then the result was multiplied by 5 and compared with the ECL method.

Conclusions

As it is shown in the comparison chart of the distribution of ECL versus ELISA methods, at high concentrations of estradiol, the results of the two methods did not fit well with the low concentrations of estradiol. This means that at high concentrations of estradiol hormone, especially during infertility treatment that threatens the person with OHSS (ovarian hyper-stimulation syndrome), it is vital that the laboratory first interacts with the treatment team to allow the concentration of this hormone to be determined according to the method. Secondly, during the patients' treatment, method alternations should be avoided to evade misinterpretation of the results and overuse of ovulation induction drugs. This phenomenon is demonstrated in another way when interpreting the results of Beta-HCG concentrations in the study of pregnancy and follow-up of this molecule in infertility-treated individuals, including that it is crucial to perform the initial tests and subsequent titration in a single laboratory with a single measurement method to avoid the error of interpretation of the results due to the different specificity of the kits and different methods.

Acknowledgments

We are grateful to the respected personnel of Ebn Sina Infertility Treatment Laboratory for assisting us in this research.

Conflict of Interest

There was nothing detected as a conflict of interest by authors, and the authors have nothing to disclose.

The project started on 28 August 2018 after receiving the code of ethics: IR.ACECR.AVICENNA.REC.1397.006 from the Avicenna Ethics committee: www.ethics.research.ac.ir by Dr. Ali Sadeghi Tabar. The authors have nothing to disclose.

Abbreviations

ELISA: The enzyme-linked immunosorbent assay (analytical biochemistry assay)

ECL: Electrochemiluminescence (electrogenerated chemiluminescence)

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Received: 23 August 2020
Accepted: 4 October 2020