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Hormones affecting fertility among female in childbearing age

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Abstract
Background: Infertility defined by the failure to achieve pregnancy after 12 months or more of regular unprotected sexual intercourse. The electrochemiluminescence immunoassay is one methods intended to measure serum hormone levels.
Aim: to assess the hormonal level using cobas e411 immunoassay analyzers.
Subject and method: A case series study design was chosen to study 60 infertile women in the childbearing. The study was done at Privet Pharma Laboratory in Erbil for one-month duration during 2017. Using electrochemiluminescence immunoassay with Sandwich principle.
Result: The study revealed that women with secondary infertility formed 56.7% and women with primary infertility formed 43.3%. Two third of women in age group (15-20) year were primary infertile and 81.9% of women in age group more than 35 year were secondary infertile. P-value 0.125 and 0.062 and respectively. Three forth of study sample had abnormal level of AMH, it was 17 (37%) among women with primary infertility and it was 29 (63%) among women with secondary infertility P-value 0.071. 68.4% of women with secondary infertility had Abnormal level FSH hormone if compeer with women with primary infertility, it was 31.6%. Although LH was abnormal level among women with primary infertility if it compared with women with secondary infertility but statistically not significant. There was not very big differences between infertile women and their normal and abnormal level of prolactin hormone
Conclusion: Two third of infertile women in age group 15-20 year had primary infertility and four fifth of women in age ≥35 year had secondary infertility. Abnormal hormonal level among study sample were AMH, prolactin and LH.

Keywords: women fertility, fertility hormone, cobas e411, immunoassay analyzers

Introduction

Infertility is "a disease of the reproductive system defined by the failure to achieve pregnancy after 12 months or more of regular unprotected sexual intercourse." (and there is no other reason, such as breastfeeding or postpartum amenorrhea) [1], which can be classified into primary and secondary infertility and due to females infertility, combined infertility, and unexplained infertility [2]. Hormones in general are powerful, complex chemicals, normally produced by the endocrine system and transported through the bloodstream to stimulate or inhibit the metabolic activity of target glands or organs. In chemical terms, hormones can be divided into three classes of compounds: polypeptides, amines, and steroids. gonadal hormones (estrogen and testosterone) are of steroids type [3]. The Common hormone affecting female fertility as follow:

1. Anti-Mullerian Hormone (AMH) produces by granulosa cells in ovarian follicles [4]. AMH is a protein hormone structurally related to inhibin and activin, and a member of the transforming growth factor-β (TGF-β) family. It has a molar mass of 140 kDa. The gene is located on the short arm of chromosome 19 in humans, band 19p 13.3 [5, 6]. and can measure either by pmol/l or by ng/mL [6].
2. Follicle-Stimulating Hormone (FSH) is synthesized and secreted by the gonadotropic cells of the anterior pituitary gland [1]. FSH is consisting of two polypeptide units, alpha and beta. The alpha subunits of the glycoproteins is consist of about 96 amino acids, beta subunit of 111 amino acids (FSH β), which confers its specific biologic action, and is responsible for interaction with the receptor and is measured in International Units (IU) [7]. Molecular formula of FSH = $C_9H_{13}NO_3$. Average molecular mass of FSH = 183.204 g/mol [8].
3. Luteinizing Hormone (LH) is a glycoprotein secreted by basophilic cells of the anterior pituitary [3], of two subunits, labeled alpha and beta subunits, the alpha subunits contain 92 amino acids. The beta subunits has 120 amino acids that confers its specific biologic action and is responsible for the specificity of the interaction with the LH receptor, is measured in international units [9].
4. Prolactin Hormone is a poly peptide hormone secreted by the anterior pituitary [10]. The molecule is folded due to the activity of three disulfide bonds. There are three different sizes of prolactin: small prolactin, big prolactin and very big prolactin [11].
5. Estrogens is secreted by the ovaries under the influence of the FSH and LH [8]. ChemSpider ID: 5554, molecular formula: $C_{18}H_{24}O_2$, average molecular mass: 272.382 Da, and monoisotopic mass: 272.177643 Da [12]
6. Progesterone: An ovarian steroid hormone secreted by the corpus luteum [13]. ChemSpider ID: 5773, molecular formula: $C_{21}H_{30}O_2$, average mass: 314.462 Da, monoisotopic mass: 314.224579 Da [14].
7. Testosterone: It is male hormone in females, the adrenal glands and the ovaries secrete small amounts of it.[1] ChemSpider ID: 5791, molecular Formula: $C_{19}H_{28}O_2$, average molecular mass: 288.424 Da, monoisotopic mass: 288.208923 Da [15].

Radioimmunoassay and enzyme immunoassay are two

testing methods used by laboratories to measure serum hormone levels. But the assay method is affected by age, gender, exercise, emotional stress, nutritional status, and medications [13].

The aim of present study is to assess the hormonal level with infertility status using cobas e411 immunoassay analyzers.

Subjects and Methods

Scientific approval was received from Collage of Science Department of Chemistry in Mosul University. A case series study design was used to study the sixty infertile women seeking for pregnancy for one-month duration in Privet Pharma Laboratory in Erbil 2017, using data collection sheet consist from two part. 1st part socio demographic characteristics (age in years divided in five groups (15-20, 20-25, 25-30, 30- 35, and ≥ 35), educational status (primary, secondary, and higher education), infertility status (primary and secondary), and occupation (employed and un employed). 2nd part hormonal Assay.

Statistical analysis

The information regarding each woman was transferred into a code sheet and data entry was done using computer. Statistical analysis was done using the Statistical Package for Social Science version 23. The data were presented in suitable tables and figures. Percentages, mean and stander deviation were calculated. The association of binary outcome measured by Chi-square (χ^2). P value at or less than 0.05 was considered statistically significant.

Laboratory test: [16] Using cobas e 411 analyser is a fully automated analyser that uses a patented Electro Chemi Luminescence (ECL) technology for immunoassay analysis.

1. **Patient preparation:** Explain to the patient a blood sample will be drawn and the collecting sample takes only a few minutes and may feel some discomfort from the needle puncture, withhold medications, that may interfere with accurate determination of test results for 48 hours before the test. If these medications must be continued, the investigators note this on the laboratory slip.
2. **System information**: The electrochemiluminescence immunoassay "ECLIA" is intended for use on cobas e411 immunoassay analyzers. Electro refers to electrical stimulation, Chem indicates a chemical reaction, and luminescence means produces light.
3. **Test principle:** System usually automated, It is a disk system. Sample throughput up to 30 samples/hr using Sandwich principle. Total duration of assay: 18 minutes.

- **1st incubation:** 10-50 µL of sample according to the types of hormones analyze, a biotinylated monoclonal hormonal-specific antibody, and a monoclonal hormonal-specific antibody labeled with ruthenium complexa [Tris(2,2'-bipyridyl) ruthenium (II)-complex (Ru(bpy)] form a sandwich complex.
- **2nd incubation:** After addition of streptavidin-coated microparticles, the complex becomes bound to the solid phase via interaction of biotin and streptavidin.
- **The reaction** mixture is aspirated into the measuring cell where the micro particles are magnetically captured onto the surface of the electrode. Unbound substances are then removed with ProCell/ProCell M. Application of a voltage to the electrode then induces chemiluminescent emission which is measured by a photomultiplier.
- **Results** are determined via a calibration curve which is instrument specifically generated by 2-point calibration and a master curve provided via the reagent barcode or e-barcode.

4. **Storage and stability of Elecsys reagent kit:** Store at 2-8 °C, do not freeze, store the Elecsys reagent kit upright in order to ensure complete availability of the micro particles during automatic mixing prior to use.
5. **Specimen collection and preparation**: Serum collected using standard sampling tubes or tubes containing separating gel or Li-heparin plasma. Stable for 3 days at 20-25 °C, 5 days at 2-8 °C, 6 months at -20 °C (± 5 °C). Freeze only once. Centrifuge samples containing precipitates before performing the assay. Do not use heat-inactivated samples. Do not use samples and controls stabilized with azide. Ensure the samples, calibrators and controls are at 20-25 °C prior to measurement, and samples, calibrators and controls on the analyzers should be analyzed/measured within 2 hours.

Table 1: Calculation, measuring range, and dilution of hormones

Category	AMH[5]	Prolactin[10]	Estradiol[17]	FSH[7] and LH[9]
Calculation* (Conversion factors)	pmol/Lx0.14=ng/mL	µIU/mL (mIU/L) x 0.047 = ng/mL	pmol/Lx0.272= pg/mL (ng/L)	No Conversion factors Calculation either in mIU/mL or IU/L
	ng/mLx7.14=pmol/L	ng/mL x 21.2 µIU/mL (mIU/L)	pg/mLx3.67= pmol/L	
			pg/mLx0.00367= nmol/L.	
Measuring range	0.07-164 pmol/L	1.00-10000 µIU/mL	18.4-11010 pmol/L	0.100-200 mIU/mL
	0.01-23 ng/mL	0.0470-470 ng/mL	5-3000 pg/mL	
Dilution**	1:2. The concentration of the diluted sample must be > 71.4 pmol/L (> 10 ng/mL)	1:10. The concentration of the diluted sample must be > 50 µIU/mL or > 2.4 ng/mL.	1:10 The concentration of the diluted sample must be > 881 pmol/L (> 240 pg/mL)	Not necessarily due to the broad measuring range

*The analyzer automatically calculates the analyte concentration of each sample (either in pmol/L or in ng/mL).
**After dilution by the analyzers, the software automatically takes the dilution into account when calculating the sample concentration.

Result

1. Socio demographic characteristics of study sample

Table (2) reveals the distribution of the married women in the present investigation according to their age groups. Its noticed that one quarter (25%) of the study sample is in age group 25-30 while women in age group 15-20 years formed 15%. Distribution of women according to their infertility status is shown in Table (3). It was seen that women with secondary infertility was more common than women with primary infertility, it formed 56.7% and 43.3% respectively. Level of education among the study sample is seen in Fig. (1), it showed that women who had secondary education was 38.3%, women who had higher education was 31.7% and least percentage was among women with primary education,

it formed 30.0%.

The present study reveals that more than two third of study sample (60) was employed it formed 61.7% and un employed formed 38.3%. This is seen in Fig. (2). The present study revealed that 66.7% of women in age group (15-20) years were primary infertile and 81.9% of women in age group more than 35 year were secondary infertile. P-value 0.125 and 0.062 and respectively, this is seen in Table 4.

2. Association between fertility status and hormonal level

Percentage distribution of hormonal level status among study sample is seen in Fig. (3). It revealed that women with normal level of estrogen and FSH hormone, was 86.7% and 68.3% respectively. The least normal level was seen in AML hormone it form 23.3%.

Table (5:a) and table (5:b) showed three forth of study sample had abnormal level of AMH, it was 17 (37%) in women with primary infertility and it was 29 (63%) among women with secondary infertility P- value 0.071. Abnormal level FSH hormone among women with secondary infertility is higher than women with primary infertility, it was 68.4% and 31.6% respectively, P value not statistically significant. Regarding LH was abnormal level more prevalent among women with primary infertility if it compared with women with secondary infertility, P-value = 0.333. There was not very big differences between infertile women and their normal and abnormal level of prolactin hormone. Estrogen hormone was normal in more than four fifths (86.7%) of study sample. Normal level among women with secondary infertility was more prevalent than women with primary infertility, it was 59.6% and 40.4% respectively. P- value 0.242.

Table 2: Distribution of women according to their age groups

Age Groups	No.	%
15-20	9	15.0
20-25	12	20.0
25-30	15	25.0
30- 35	13	21.7
≥ 35	11	18.3
Total	60	100.0

Table 3: Distribution of women according to their infertility status

Infertility Status	No.	%
Primary	26	43.3
Secondary	34	56.7
Total	60	100.0

Table 4: Distribution of women according to their age group and infertility status

Age Groups in Years	Infertility Status				Total		P- Value*
	Primary		Secondary				
	No.	%	No.	%	No.	%	
15-20	6	66.7	3	33.3	9	15.0	0.125
20-25	7	58.4	5	41.6	12	20.0	0.241
25-30	5	33.3	10	66.7	15	25.0	0.367
30- 35	6	46.1	7	53.9	13	21.7	0.817
≥ 35	2	18.2	9	81.9	11	18.3	0.062
Total	26	43.4	34	56.6	60	100.0	0.160

*Using χ^2 Test.

Fig (5: a): Distribution of women according to their Infertility status and hormonal level

Hormones Status	Infertility				Total =60	
	primary		Secondary			
	No.	%	No.	%	No.	%
AMH						
Normal	9	64.3	5	35.7	14	23.3
Abnormal	17	37.0	29	63.0	46	76.7
FSH						
Normal	20	48.8	21	51.2	41	68.3
Abnormal	6	31.6	13	68.4	19	31.7
LH						
Normal	9	36.0	16	64.0	25	41.7
Abnormal	17	48.6	18	51.4	35	58.3
Prolactin						
Normal	10	45.5	12	54.5	22	36.7
Abnormal	16	42.1	22	57.9	38	63.3
Estrogen						
Normal	21	40.4	31	59.6	52	86.7
Abnormal	5	62.5	3	37.5	8	13.3

Table 5: b: Distribution of women according to their Infertility status and hormonal level

Parameters	Infertility Status								P- Value*
	Primary Total =26				Secondary Total= 34				
	No.	Control group (Normal)	No.	Patients group (Abnormal)	No.	Control group (Normal)	No.	Patients group (Abnormal)	
AMH level (ng/mL)	9	1.39±0.11	17	4.95± 4.85	5	1.36 ± 0.01	29	5.38± 4.41	0.071
FSH level (IU/mL)	20	8.05 ± 2.91	6	7.76 ± 3.96	21	7.32 ± 1.40	13	16.78 ± 16.48	0.211
LH level (IU/mL)	9	7.67± 0.60	17	7.75 ± 6.63	16	7.46± 1.39	18	10.05 ± 9.10	0.333
Prolactin level (ng/mL)	10	8.48± 2.36	16	30.59 ± 9.47	12	8.55± 2.14	22	31.93 ± 30.47	0.801
Estrogens level (pg/ml)	21	50.54 ±32.23	5	8.48 ± 2.55	31	59.74±37.54	3	8.56 ± 3.08	0.240

*Using χ^2 Tests

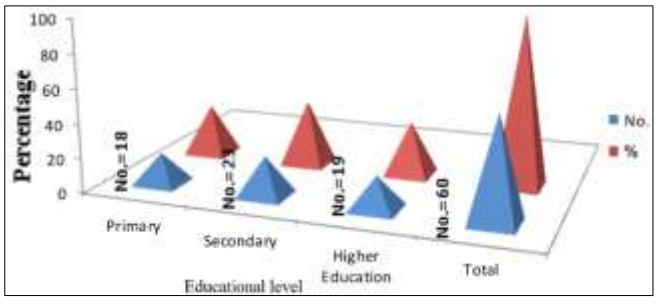

Fig 1: Distribution of women according to their level of education

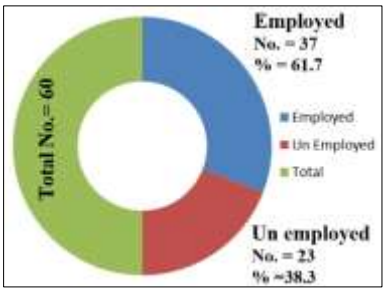

Fig 2: Distribution of women according to their occupation Status

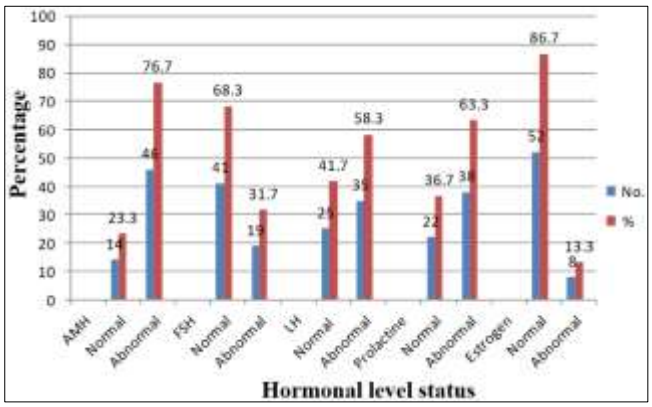

Fig 3: Percentage Distribution of hormonal level status among study sample

Discussion

An immunoassay is a biochemical test that measures the presence or concentration of a macromolecule or a small molecule in a solution through the use of an antibody (usually) or an antigen (sometimes). The molecule detected by the immunoassay is often referred to as an "analyte" and is in many cases a protein, or it may be other kinds of molecules, of different size and types. Analytes in biological liquids such as serum or urine are frequently measured using immunoassays for medical and research purposes. It is of many type depend on type of substances labeled antibody such as: Radioactive immunoassay, Enzymes immunoassay, Fluorescent immunoassay, and Electrochemiluminescent immunoassay [18]. Oldest methods for immunoassay was radio immune assay it is very useful in toxicology, oncology and endocrinology but it required up to two days to complete the test, expensive equipment due to use of gama or beta, and hazardous of radioactive. Enzymes immunoassay very useful in viral and bacterial infection, it is more sensitive and specific than previous, save because no radioactive substance, inexpensive but the enzyme activity may be affected by plasma constitute [18, 19]. Fluorescent immunoassay it is safe, sensitive, inexpensive, and simple but some time auto florescent or extra florescet occure [20]. The present study used Electrochemiluminescent immunoassay is rapid measurement, with wide measuring range, controlled reaction, precise, sensitive, and low sampling volume [16].

1. Socio-demographic characters of study sample.

The study revealed that two third of study sample were in age group less than 30 years, this high percentage attributed to the high incidence of early marriage. Early age of marriage is common among developing countries for certain socio-cultural reasons [21]. The study showed that women with secondary infertility more than women with primary infertility this attributed to the fact that 80% of the married couple get pregnant within first year and half of remaining get pregnant in second year [1]. secondary infertility occur as a result of many causes such as; sever pelvic inflammatory disease, abdominal or pelvic operation specially appendicitis, complication of previous pregnancy and labor drugs specially usage of contraceptive without medical advice [2].

66.7% of women in age group (15-20) years had primary infertile, the biological years of complete development of genital organ for female is around 20 years old [1]. 81.9% of women in age group more than 35 year had secondary infertile, this also a biological fact women's ability to conceive decrease after age of 35 year [2]. The present study showed that 61.7% of study sample were employed and more than two third were had secondary and higher education. Education and employment are a power encourages women to be independent and make a decision, and seek medical advice [22].

2. Association between fertility status and hormonal level

Abnormal level of AML was higher among women with secondary infertility than women with primary infertility, this is a fact as serum levels of AMH are barely detectable at birth in females, reach their highest levels after puberty, decrease progressively thereafter with age, and become undetectable at menopause [23]. This agree by study done among 10984 women attending infertility clinic showed that decline in AMH with age [24]. Regarding FSH women with secondary infertility had higher level of abnormal hormone, FSH level shows a peak at mid-cycle, although this is less marked than with LH. Due to changes in ovarian function and reduced estrogen secretion, high FSH concentrations occur during menopause [25]. More than half of study sample had abnormal level of LH but women with primary infertility had higher abnormal hormonal level than women with secondary infertility, this abnormal level required further investigation and repeat the test at mid cycle [26]. High levels at times outside of the LH surge can interfere with ovulation and menstruation, contributing to infertility [27]. The present study revealed that 63.3% of study sample had abnormal level of Prolactin hormone which play an important role in fertility status of the women, it is very sensitive and affected by emotional status of the women [11], Estrogen hormone was normal in more than four fifths of study sample, it has indirect role in infertility by suppression of FSH after ovulation [2]. Elevated estrogen levels may occur with conditions such as obesity, diabetes, high blood pressure, and with the use of some medications, such as steroid hormones, phenothiazines, tetracyclines, and ampicillin [28].

Limitation

Small sample size make the study not applicable to the whole population

Conclusions

The study concluded that nearly half of the infertile women are in age group 20-30 years the percentage of secondary infertile women is higher than women with primary infertility. Two third of infertile women in age group 15-20 year had primary infertility and four fifth of women in age ≥35 year had secondary infertility. Abnormal hormonal level among study sample were AMH, prolactin and LH. The most prevalent abnormal hormone among women with primary infertility were AMH, LH, and prolactin, while among women with secondary infertility were AMH, FSH, and prolactin.

Recommendation

1. Repeat the study and increase sample size to include all women in child bearing age with more specified types of studies, including cross section, case-control and cohort studies, in order to determine the possible influencing and protective factors that affect fertility status of women.
2. Using different immune assay methods and study sensitivity and specificity of each methods.

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