STUDIES ON HORMONAL CHANGES, HOMOCYSTEINE AND LIPIDS PROFILE IN IRAQI WOMEN WITH INFERTILITY [+]

دراسة التغيرات الهرمونية والهوموسستيين وصورة الدهون في النساء العراقيات المصابات بالعقم

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Abstract:

The cases of infertility have increased in women lately. The current study was conducted in order to obtain some indicators about the hormone changes, lipid profile and shedding light on the novel risk factor homocysteine which as considered as predictive and diagnostic factor of infertility. This study included 56 infertile women; in addition to 30 healthy women volunteers represented the control group, the study period was from December 2009 and April 2010. The results in this study revealed a high significant increase (P<0.0001) in BMI and the ratio of W/H in infertile women as compared to control group. The present study referred to significantly decrease(P <0.05) in the level of FSH and LH, and increase significantly (P< 0.0001) in LH/FSH ratio and prolactin.

The present study show highly significant increase (P< 0.0001) in level of homocysteine as a risk factor in infertile women as compared to control group. The results also revealed to non significant increase in the level of cholesterol, TG and VLDL. While there was significant increase in LDL and significantly decrease in HDL. Results of the present study showed that important role of obesity, the increase in BMI, a decline in the level of sex hormones and increase in the level of homocysteine lead to infertility in women.

المستخلص:

ازداد شيوع حالات العقم لدى النساء في الآونة الأخيرة .أجريت الدراسة الحالية بهدف الحصول على بعض الإيضاحات حول التغيرات الهرمونية وصورة الدهون وتسليط الضوء على عامل الخطورة الجديد الهوموسستيين الذي قد يعد عامل تكهني وتشخيصي للعقم. شملت هذه الدراسة ٥٦ مريضة تعاني من العقم، إضافة إلى ٣٠ متطوعة من النساء السويات اللاتي يمثلن مجموعة السيطرة ، للفترة من كانون الأول ٢٠٠٩ والى نيسان ٢٠١٠ . أظهرت نتائج هذه الدراسة ارتفاعا معنويا كبيرا ($p<0.0001$) في دليل كتلة الجسم ونسبة محيط الخصر الى محيط الورك لدى النساء العقيمات مقارنة بمجموعة السيطرة . أشارت الدراسة الى وجود انخفاضا معنويا ($p<0.05$) في مستوى كل من FSH وLH وارتفاعا معنويا ($p<0.0001$) في نسبة LH/FSH ومستوى البرولاكتين .

بينت الدراسة الحالية ارتفاعا معنويا كبيرا ($p<0.0001$) في مستوى الهوموسستيين كعامل خطورة في النساء العقيمات مقارنة بمجموعة السيطرة. لوحظ ان هنالك ارتفاعا غير معنويا في مستوى كل

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من الكولسترول والكليسيريد الثلاثي والبروتين الشحمي واطئ الكثافة جدا . في حين كان هناك ارتفاعا معنويا في مستوى البروتين الشحمي واطئ الكثافة ونقصان معنوي في مستوى البروتين عالي الكثافة . بينت الدراسة الدور الكبير الذي تلعبه السمنة وزيادة معدل كتلة الجسم في انخفاض مستوى الهرمونات الجنسية وزيادة مستوى الهوموسستيين مؤديا الى حدوث العقم في النساء.

Introduction:

Infertility is defined as the inability to conceive after at least 1 full year of unprotected sexual intercourse [1]. It is estimated that worldwide, between 70 and 80 million couples suffer from infertility, and most of these are residents of developing countries, including the Middle East [2]. Infertility is a major problem in these countries and causes extensive social and psychological suffering [3]. Complex biological processes in the mammalian ovary, such as follicular development, oocyte maturation, oocyte meiosis, ovulation and corpus luteum formation and demise, or coordinately regulated by autocrine, paracrine and endocrine factors of the hypothalamic- pituitary- ovarian axis [4]. Specifically Follicle Stimulating Hormones (FSH) is a major promoter for orchestrating follicular development and differentiation in the granulosa cells of preovulatory follicles [5]. Luteinizing Hormone (LH) plays a key role in initiation of the ovulatory process of preovulatory follicles by activating multiple cellular signaling pathways [6]. Hormonal balance between estrogen, progesterone, FSH and LH is important to induce and promote fertility. The most common cause of female infertility is ovulatory disorder characterized by anovulation or by infrequent and/or irregular ovulation. [7]. The major causes of infertility includes ovulatory dysfunction (15%), tubal and peritoneal pathology (30-40%), and male fact (30 -40%) and uterine pathology. To some extent the prevalence of each varies with age. Ovulatory dysfunction is mo re common in younger than old couples, tubal and peritoneal factors have a similar prevalence [8]. An elevation of prolactin (hyperprolactinemia) is thought to be a frequent cause of chronic anovulation and infertility serum prolactin (PRL) levels were also studied as a marker of infertility [9]. Deficiencies in luetinizing hormone (LH), follicle stimulating hormone (FSH) and elevated prolatin level even slight irregularities in the hormone system can affect ovulation. The infertility causes due to insufficiency or imbalance hormones. The lack of ovulation may lead to mild enlargements of ovaries especially in obese patient. Fertility can be negatively affected by obesity. In w omen, early onset of obesity favors the development of menses irregularities, Obesity in women can also increase risk of miscarriages and impair the outcomes of assisted reproductive technologies and pregnancy, when the body mass index exceeds 30 kg/m^2 [8]. Hyper-homocysteinemia is another important risk factor for the development o coronary and thromboembolic disease, it was postulated that homocysteine levels are higher in infertile patients with PCOS than controls [10]. Homocysteine (Hcy) is a sulfur-containing amino acid formed during the metabolism of methionine. It is metabolized by one of two pathways: trans-sulfuration and remethylation [11]. Classic hyperhomocysteinemia has been characterized as the accumulation of Hcy due to defects in enzymatic pathways [12]. Elevated plasma Hcy induces oxidation of low-density lipoprotein (LDL), proliferation of smooth muscle cells, increased platelet adhesiveness and endothelial cytotoxicity [13]. Plasma lipoproteins transport cholesterol [as unesterified cholesterol (UC) and cholesteryl esters (CE)] and other lipids to and from tissues where they play critical roles in maintaining cell integrity (e.g. membrane synthesis), endocrine functions (e.g.

cholesterol is a precursor for steroid hormone synthesis), and fertility [14]. In addition, high-density lipoprotein (HDL) appears to play an especially important role as an extracellular acceptor for cholesterol efflux [15], a function that is commonly thought to underlie, at least in part, the well established association of elevated plasma HDL cholesterol with reduced risk for atherosclerotic disease (coronary heart disease and stroke) [16]. HDL may also have a particularly important role in mammalian female fertility, because in many species, including humans, HDL is the main class of lipoprotein found in substantial amounts in the follicular fluid enveloping oocytes in ovarian follicles [17]. The objectives of this study therefore was to determine the levels of follicle stimulating hormone(FSH), luteinizing hormone (LH) and prolactin (PRL), in infertile women which will help to assess the contribution of endocrine abnormalities to infertility in the study population. Estimate the homocysteine as a risk factor for infertility and attempted to find the correlation between BMI and all parameters that used in this study (Hcy, FSH, LH, PRL, and lipid profile) in infertile women.

Materials and Methods

Subjects

The present investigation was carried out at Kamal ASamarai Hospital between December 2009 and April 2010, in Baghdad, Iraq. The data collected from 56 women patients as referred by a Gynecologist for infertility investigation, mean aged 26.85±3.23 years. A total of 30 healthy females volunteers (mean aged 26.70±2.43 years) served as controls. To eliminate the factors which might affect this study, we excluded all smoking females, as well as females suffering from chronic or acute diseases, such as hypertension, diabetes mellitus, diseases of the liver, kidney, and all patients with hormonal therapy treatment.

Anthropometric Measurements

The physical examination of body weight was calculated by taking weight in kilogram(kg) [18] and height was measured in centimeters [19]. The Body Mass index was calculated from the formula; BMI = weight in kilograms / (height in squared meters). Patients were taken as obese if their body mass index was 29.9 [20]. Waist circumference (WC) in centimeters, Hip circumference (HC)in centimeters, and Waist/Hip ratio were also calculated [21].

Analysis of Samples

Fasting blood samples (10 mL) were separated at 8:00 to 10: after morning during 2-5 days of the menstrual cycle .Sample collected and placed into containing tubes. After centrifugation at 1500×g for 5 min. the serum were removed and retained for assay of all the parameters, respectively. Serum samples were stored at -20 ^{o}C until analysis. Serum concentration of Luteinizing Hormone (LH), Follicle Stimulating Hormones (FSH), and Prolactin were measured. Levels of LH, FSH, and PRL were measured by mini-VIDIS assay using kit supplied by Bio Merieux Sa- France.

Homocysteine determination

The concentration of homocysteine in the collected serum samples was determined using High – performance liquid chromatography assay according to method by Robert et al. [22].

Lipid Profile assay

The levels of total cholesterol, triglyceride, HDL, were measured by colorimetric assay using kits supplied by Spinreact in spain. LDL was calculated according to Bairaktary et al equation [23]:

LDL = 0.94 × Total cholesterol – 0.94 × HDL – 0.19 × TG

While, VLDL was estimated mathematically by using Friedwald formula [24]:

VLDL = TG/5 (mg/dL) or **VLDL = TG/2.2** (mmol/L)

Statistical analysis

All data were expressed as mean ± standard deviation (Mean ±SD). Statistical analysis was performed using a software program (SPSS 13). The comparison of mean between patients group and control group was tested by Student's t–test Results. One Way Analysis of Variance (ANOVA) was use to compare means with least significant differences (LSD) between variables of differentiated groups.

Results and Discussion:

Table (1): Demographic , Hormonal profile, homocysteine and lipid profile Data (mean ± SD) in infertile women and control group.

Parameters	Control (N=30)	Women infertility (N=56)	P
	Mean± SD	Mean± SD	
Age (years)	26.70± 2.43	26.85±3.23	NS
Hight (cm)	153.66± 25.11	157.02± 5.49	NS
Weight (Kg)	63.27± 6.29	74.92± 14.99	0.0001*
BMI (Kg/m^2)	22.57± 2.41	30.47± 6.70	0.0001*
Waist (cm)	80.60± 4.17	94.93± 10.35	0.0001*
Hip (cm)	103.80± 6.16	110.38± 10.96	0.001*
Waist/Hip ratio	0.776± 0.0219	0.852± 0.0476	0.0001*
FSH mIU/ mL	7.08± 2.52	5.06± 1.82	0.05
LH mIU/ mL	4.08± 1.12	2.67± 1.72	0.05
LH/FSH	0.549± 0.173	1.584± 0.81	0.0001*
PRL ng/mL	11.66± 5.59	22.87± 12.44	0.0001*
Hcy µmol/L	5.56± 0.88	11.58± 1.23	0.0001*
Cholesterol mg/dL	171.33± 21.11	202.64± 36.08	0.05
TG mg/dL	96.0± 19.21	132.89± 57.66	0.01*

HDL mg/dL	69.0± 11.02	64.54± 10.61	NS
LDL mg/dL	90.09± 15.58	98.68± 35.19	NS
VLDL mg/dL	19.27± 3.95	28.79± 15.57	0.01*

NS: non significant *** Significant at p≤ 0.01 in comparison with control**

Successful pregnancy results from an interaction between myriad physiological processes in both men and women. Any disruption to this interactive system, whether in a man or woman can result in an inability to have a biological child called infertility [25].

Table (1) shows the overall clinical and hormonal results of women with infertility and control group. The two groups were similar for age, but body mass index (BMI) was significantly higher ($p<0.0001$) in infertility women as compared with the control group (30.47 ± 6.70 vs 22.57 ± 2.41 kg/m²). W/H ratio was significantly higher ($p<0.0001$) in infertility women as compared to the control group (0.852± 0.0476 vs 0.776 ± 0.0219). The mean levels of LH was significantly decreased ($p<0.05$) in infertility women (2.67± 1.72 mIU/mL) than in the control group (4.08± 1.12mIU/mL), as well as FSH levels (5.06± 1.82 vs 7.08± 2.52 mIU/mL). Whereas higher significantly increased ($p< 0.0001$) in PRL levels (22.87 ± 12.44 vs 11.66 ± 5.59 ng/mL), the LH/FSH ratio (1.584 ± 0.81 vs 0.549 ±0.173) and homocysteine levels (11.58 ± 1.23 vs 5.65 ± 0.88 μmol/L) in infertility women as compared with the control group. No significant differences were detected in serum LDL and HDL levels between infertile women and control group. There was a significant increased ($p<0.05$) in total cholesterol, while the mean triglyceride, and VLDL concentration was significantly higher ($p<0.01$) in women with infertility as compared to control group.The amounts of FSH and LH released and their specific functions change as the cycle progresses. FSH stimulates the growth of follicles in the ovaries. Each follicle contains an egg and produces additional hormones. LH helps FSH to stimulate the production of these hormones, both before and after ovulation. Roughly half way through the menstrual cycle, a sudden surge of LH and FSH causes a small rupture of the dominant follicle, releasing the egg. At this stage of the cycle, LH is the most important hormone because it enables the egg to become mature and ready for fertilization [26]. This study indicate a significantly decrease in FSH levels, and this result was agreement with Jose-Miller *et al* study which, found a decrease level of FSH in infertility women that causes ovulation failure, subsequently failure in the role of genital glands and menstrual cycle which lead to infertility [27]. Several researches confirm the role of high concentration of LH after ovulation stimulates the granulosa cell of the ruptured follicle to luteinize and to form the corpus luteum which synthesizes and secrets progesterone and estradiol. Progesterone is the principle hormone of the luteal phase and prepares the endometrium for the implantation of fertilize ovum. Whereas the decreased levels of LH was accompanied with rises of prolactin levels in blood, Kallman syndrome and decrease guandotropin [28, 29]. Veena Bhaskar *et al* found that serum PRL levels is a marker of infertility and determine its relation to oxidative stress and antioxidants [9]. High circulating levels of PRL may inhibit ovarian function and ovulation by both central and peripheral mechanisms [30]. The presence of elevated LH/FSH concentrations with a decreased estradiol concentration is diagnostic of hypergonadotrophic hypogonadism [31].Hyperhomocysteinemia is a risk factor for cardiovascular diseases, it was postulated that homocysteine levels are higher in infertile patients with PCOS than control. Homocysteine is an amino acid formed by the conversion of methionine to cysteine [32]. Several intermediates of the homocysteine pathway are directly

involved in the synthesis of proteins, the synthesis and repair of DNA, and balance the degree of oxidative stress, which are critical intermediates in gametogenesis. Therefore, derangements in this pathway in both women and men resulting in hyperhomocysteinaemia, are suggested to be detrimental for reproduction [33]. Maternal hyperhomocysteinaemia is associated with adverse pregnancy outcome, such as recurrent miscarriages, pregnancy induced hypertension, abruptio placentae and several congenital abnormalities [34, 35]. Recent publications suggest that the homocysteine pathway is not only important for reproductive outcome, but for fertility as well [36]. Ebisch *et al*. measured concentration of Hcy, suggesting that Hcy is inversely related to fertility outcome [37]. Hague stated hyper-homocysteinemia has been associated with vascular disease. In normal pregnancy, Hcy concentrations fall . Eleven out of 69 studies fulfilled, which found in their results significant higher serum Hcy levels among women with a history of recurrent miscarriage [38]. According to the results in this study, there is an elevation in the level of total cholesterol, triglyceride, LDL and VLDL, while there was decreased in HDL in infertile women as compared to control group, this is due to hyperlipidimia, life style, genetic factors and this elevation is a risk factor of coronary heart diseases. These results were in agreement with several researchers which found a higher level of cholesterol associated with obesity [39]. Table (1) shows also the relationship between infertility with weight, height, and BMI.

Table (II): Serum levels of hormones, Homocysteine and lipid profile in infertility women distribution according to BMI categories.

Parameters	**Normal (N=10) (17.86%)**	**Overweight (N=26) (35.71%)**	**Obese (N=20) (46.43%)**
	Mean± SD	**Mean± SD**	**Mean± SD**
Age (years)	26.13± 4.42	26.96± 3.98	28.67± 3.93
BMI (Kg/m^2)	24.46± 0.41	27.0± 1.22	37.21± 6.55
FSH mIU/ mL	6.15± 3.89	6.05± 3.39	6.895±5.98
LH mIU/ mL	6.68± 4.57	10.37± 6.40	10.34± 9.81
LH/FSH	1.05± 0.40	1.85± 0.89	1.45±0.71
PRL ng/mL	17.90± 14.72	23.58± 11.28	24.10± 12.97
Hcy µmol/L	10.65± 1.66	11.97±0.98	11.29± 1.30
Cholesterol mg/dL	194.25± 74.66	185.33± 33.74	210.83± 18.34
TG mg/dL	114.75± 34.02	116.89± 45.56	169± 75.28
HDL mg/dL	65.0± 12.91	71.22± 14.22	71.83± 13.96
LDL mg/dL	106.50± 61.07	90.78± 32.95	105.33±16.21
VLDL mg/dL	33.50± 26.19	23.44± 9.14	33.67± 15.16

Table (III): P values of Serum levels of hormones, Homocysteine and lipid profile in infertility women distribution according to BMI categories.

Parameters	**Normal& Control**	**Overweight & Control**	**Obese & Control**	**overweight & Normal**	**Obese & Normal**	**Obese & Overweight**
	P value					
Age (years)	NS	NS	NS	NS	NS	NS
BMI (Kg/m^2)	NS	0.008*	0.0001*	NS	0.0001*	0.0001*

FSH mIU/ mL	NS	NS	NS	NS	NS	NS
LH mIU/ mL	NS	0.01	0.015	NS	NS	NS
LH/FSH ratio	NS	0.0001*	0.001*	0.007*	NS	NS
PRL ng/mL	NS	0.007*	0.006*	NS	NS	NS
Hcy µmol/L	0.0001*	0.0001*	0.0001*	NS	NS	NS
Cholesterol mg/dL	NS	NS	NS	NS	NS	NS
TG mg/dL	NS	NS	0.004*	NS	NS	0.05*
HDL mg/dL	NS	NS	NS	NS	NS	NS
LDL mg/dL	NS	NS	NS	NS	NS	NS
VLDL mg/dL	NS	NS	NS	NS	NS	NS

NS: non significant *** Significant at p≤ 0.05 in comparison with control**

Table (II) and (III) show there were non significant differences between infertile women in age, FSH, cholesterol, HDL and LDL upon classified infertile women according to BMI (Normal, overweight and obese women). ANOVA was used for different parameters and interpretation of the results. A comparison between different groups and control group found a significant increase in LH, PRL, LH/FSH ratio and Hcy in both overweight and obese infertility women as compared to control group. No significant differences were found in total cholesterol, LDL and HDL in all groups. Triglyceride was significantly higher in obese infertile women ($p \leq 0.004$) as compared to control group. Triglyceride was also found to be significantly higher ($p < 0.05$) in obese as compared overweight infertile women. Obesity is the most pervasive metabolic disease in industrialized and developed countries and increasingly in the developing world. Obesity is represented in all age categories male and female population. Obesity is associated with several health problems including diabetes, cardiovascular disease, hypertension and infertility. In recent years the association between lifestyle, weight, nutrition and fertility is gaining more public exposure [40].

The degree of obesity is most often evaluated using BMI value of 25 cut- off value most used. Values of 25-29.9 characterize on overweight individuals, while ≥ 30 classified obese according to the classification of WHO [41]. The present study was done to understand the association between BMI and infertility in all parameters used in this study. In table (II) it was observed that the percentage of obese (46.43%) and overweight (35.71%) infertile women were more than the percentage of normal (17.86%) infertile women. These results revealed to increase infertility with increasing BMI. The higher BMI in women can cause insulin levels to increase. This can cause testosterone not to be converted into estrogen. The bottom line here is that the ovaries will not release eggs without sufficient estrogen production [41].

The infertile was increased with hormonal imbalances (FSH, LH, and PRL) that have an impact on ovulation and menstruation. Catalano *et al*, found that excess weight is in not only link to increased risk of chronic disease, but also shown to increase the risk of reproductive problems [42].Several studies have shown that women with excess body weight are more likely to have fertility problems [43]. The Hcy was increased significantly in overweight and obese infertile women as compared to control group. Hcy was of non significant increase in all BMI categories in comparison between them. In this study, women were more at risk for coronary heart disease due to obesity because the homocysteine increased in BMI categories as compared to control group. Lipid profile was influenced on infertile but not to give a

clear vision on the roles in infertility women except TG which may be due dyslipidemia in women in this study.

In conclusion, Results of the present study showed that hormones play a crucial role in infertile women, as was reflected by the clear decline in the level of FSH and LH and increased of LH / FSH ratio. This study confirmed that hyperhomocysteinemia was observed in women suffering from infertility. Hyperhomocysteinemia increased with increase in BMI and obesity in infertile women. Elevated homocysteine levels may contribute to the risk of cardiovascular disease in women with infertility.

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