Original Article

Study The Pharmacologic Effect Of Both Gonadotropins And Letrozole On The Level Of Serum Glycoproteins (Inhibin B, Amh, And Hrg) In Infertile Women And Their Effect On Stimulating Ovulation

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Abstract

Objective: To see how letrozole and gonadotropin alter the levels of serum glycoproteins (HRG, AMH, and Inhibin B)**.**

Methods: As a control group, 33 infertile women and 22 healthy (fertile women) were recruited from an outpatient women's clinic (OWC). They are between the ages of (50) and (44). HRG, inhibin B, and AMH glycoproteins were evaluated using the ELISA technique.

Results: According to a research that evaluated the blood levels of inhibin B, AMH, and HRG in infertile and healthy women, Infertile women's serum inhibin B levels are substantially lower than healthy women's ($p = 0.05$). Despite the fact that HRG and AMH concentrations were not significantly different ($p > 0.05$), the quantity and size of oocytes increased significantly ($p = 0.000$) in both the right and left ovarian infertile women after therapy as compared to the infertile ladies who were infertile before therapy.

Conclusion: Letrozole and gonadotropin medicines have an effect on HRG and AMH levels in infertile women after treatment.

Key-words: HRG, Inhibin B, AMH, Letrozole, and Gonadotropin are all used to treat infertility in women.

INTRODUCTION

Infertility is a reproductive system illness in which following a period of unprotected sexual interaction of at least twelve months, a clinical pregnancy is not attained and the Iraqi ecosystem was severely harmed as a result of the conflicts, both before and after 2003. A large number of chemicals and radioactive material were taken and destroyed from unskilled individuals, resulting in numerous injuries and deaths, with the survivors contracting cancer or becoming infertile. Only a few research has looked directly at the impact of conflict on infertility [1].

HRG (histidine-rich glycoprotein) is a multi-domain protein that has a role in angiogenesis, the immune system, and coagulation, everything of which are crucial on behalf of establishing a pregnancy [2]. Inhibin B is a transforming growth factor (TGF-) superfamily member generated by the granulosa cells of the female ovary. Rather than serving as a possible indicator of male and female poverty, it serves as a catalyst for change, it is now known to act as paracrine ovarian and testicular regulators in the utero-placental unit, with diverse paracrine effects. [3].

AntiMüllerian Hormone -AMH is a glyco-protein that is generated through the granulosa cells of small antral follicles, prenatal and assists in folliculo-genesis guidance (FGG) [4]. It's essential for cell growth and differentiation [5]. Gonadotropins (luteinizing hormone (LH) and follicle-stimulating hormone (FSH)) are hormones injected into a woman's ovaries to promote the formation of follicles, which contain an egg [6]. Treatment with gonadotrophins has been linked to a greater likelihood of multiple pregnancies, especially in women who have an ovulatory cycle [7]. Letrozole inhibits the conversion of androgens to estrogen, removing estrogen's negative feedback from the hypothalamic-pituitary axis and enhancing FSH release from the anterior pituitary [8].

MATERIALS AND METHODS

The research included 33 infertile women who were recruited from an outpatient women's clinic between October 2016 and April 2017. They are between the ages of fifteen and forty-four. The control-group included twenty-two healthy fertile women who were free of symptoms and sickness signs, and their ages were matched to the ages of the infertile women. A practical phase took place at Kerbala's AL-Hussein Teaching Hospital (KA-HTH), where a survey was created toward collect data going on female infertility. On the second day of menstruation, blood samples were obtained from infertile women and the control group to evaluate the levels of glycoproteins Inhibin B, AMH, and HRG. After that, the infertile- women were given medication, letrozole with gonadotropin for four months, and a blood sample was taken to assess the same glycoprotein levels again. All of the glycoproteins under examination were identified using the Enzyme Linked Immuno Sorbent Assay (ELISA) method (Bio- Tek Instruments 217337, U.S.A.). Pelvic ultrasonography was used to determine the size and number of oocytes.

STATISTICAL ANALYSIS

The data is shown as Mean Standard Error (SE). The results were analyzed using (SPSS version 22.0), which contains a student t-test and Pearson's- correlation coefficients. Statistical significance was defined as a (P-value) of less than (0.05) [9].

RESULTS AND DISCUSSIONS:

Infertile women's clinical features:

This study employed a control group of thirty-three infertile women and twenty-two fertile women. The number of infertile women, as well as their age, type of infertility, BMI, residency, causes for infertility, family-history, abortion, menstrual control, and education, were all noted table (1).

The table (1): Infertile- women have certain clinical features.

Variables	No. = 33	%
Age(years)	**24**	
(15 - 29)	**6**	**72.72**
(30 - 40)	**3**	**18.18**
(41 - 45)		**9.09**
BMI(Kgm/m^2) :	**12**	**36.36**
Normal (18.5 to 24.9)	**14**	**42.42**
Over weight (25 to 29.9)	**7**	**21.21**
Obese (> 35)		
Family history:	**10**	
With family	**23**	**30.30**
Without family		**69.69**
Residency:	**24**	
City	**9**	**72.72**
Urban		**27.27**
Education:	**21**	
Uneducated	**12**	**63.63**
Educated		**36.36**
Type of infertility	**13**	
Primary	**20**	**39.39**
Secondary		**60.60**
Regulation of menses	**16**	**48.48**
Regular	**17**	**51.51**
Irregular		
Abortion:	**10**	**30.30**
With abortion	**23**	**69.69**
Without abortion		
Causes of infertility:	**7**	**21.21**
Tubal factor	**4**	**12.12**
Endometriosis	**22**	**66.6**
Unexplained		

GLYCOPROTEINS WERE MEASURED IN INFERTILE WOMEN AND CONTROL GROUPS

HRG, inhibin B, and AMH glycoproteins were all tested. The results demonstrated a substantial ($p = 0.05$) drop in serum inhibin B concentration in infertile women's sera compared to control groups, whereas no significant changes ($p > 0.05$) were seen in AMH and HRG levels (Table 2).

Table (2): Infertile and control groups' HRG, Inhibin B, and AMH levels.

Parameters	Infertile women No.= (33) Mean ± SE	Control No.=(22) Mean ± SE	P-Value
HRG /(ng/ml)	24.87 ± 7.17	48.46 ± 2.21	0.08
Inhibin B/ (pg./ml)	241.95 ± 20.43	319.34± 6.55	0.05
AMH /(ng/ml)	5.30 ± 0.68	3.70 ± 0.48	0.06

Follow up of infertile women:

After four months of therapy with pharmaceutical drugs (Gonadotrophin and Letrozole), 33 infertile women were the parameters under investigation were examined, and followed up on.

Measurement of glycoprotein (HRG, Inhibin B, AMH):

Infertile women's sera exhibited a substantial ($p = 0.04$) drop in AMH concentration and a significant ($p= 0.01$) there was a substantial increase in HRG concentration after therapy compared to infertile women's sera before treatment, However, there were no statistically significant variations in inhibin B concentrations ($p > 0.05$), table (3).

The table (3): Infertile- women's (Inhibin B, AMH, and HRG) concentrations after and before therapy.

Parameters	Before treatment No.= (33) Mean ±SE	After treatment No.= (33) Mean ±SE	P-value
HRG / (ng/ml)	4.89 ± 7.17	57.96± 10.77	0.01
Inhibin B/(pg/ml)	242.00 ± 20.43	283.77± 19.70	0.14
AMH (ng/ml)	5.55 ± 0.65	3.87± 0.49	0.04

In both the left and right endothelial growth factors, the size of follicles that matured into mature oocytes increased significantly ($p = 0.000$) on pelvic ultrasonography. Fibroblast Growth Factor (FGF) and (VEGF) were not raised in infertile women before treatment when compared to infertile women before treatment, table (4).

The table (4**)** :The quantity and size of infertile women's follicles after and before therapy.

	Before treatment No. = (33) Mean ±SE	After treatment No.= (33) Mean ±SE	P-Value
Size of follicles	8.015 ± 0.21	20.03 ± 0.53	0.000
No. of follicles	19.15 ±1.11	25.4 ± 1.50	0.000

DISCUSSION:

The low levels of inhibin B are associated to reduced pregnancy rates, a higher chance of miscarriage, and ovulation issues, according to study [10]. Conferring to Groome et al. (1994), follicular growth may be aided by inhibin B, a granulosa cell product, with serum concentrations perhaps reflecting follicular function and oocyte number. [11].

According to Klein et al. (1996), lower inhibin B production in older women is caused by a smaller ovarian follicular pool [12]. According to (Magoffin and Jakimiuk), the amount of both inhibins generated in follicular fluid increases as follicles expand (1997), due to the strength in a higher fluid volume, in the biggest follicles, their concentrations may drop somewhat [13]. Inhibin B(I-B) is a crucial predictor of ovarian reserve (the ovary's ability to react to gonadotropin stimulation), retrieval size, and gonadotropin dosage for Assisted Reproductive Technologies (ART) [14]. Inhibin B in follicular fluid FF was found by Chang et al. (2002) to be a follicular development marker. [96]. Although HRG interacts with other Angio-Genic (AG) factors such as Fibroblast Growth Factor (FGF) and Vascular Endothelial Growth Factor (VEGF), not any study has yet been done to determine how (HRG) impacts angiogenesis in the follicle. Other (VEGF) isoforms, as well as various (FGF) family members, have been linked to the regulation of oocyte production and Folliculo-Genesis (FG) [15]. HRG's specific bimolecular function is unknown, hence its exact involvement in reproduction has yet to be determined [16]. HRG may be implicated in a number of important processes that lead to conception, although the specific role of HRG infertility is yet unknown [17].

The amount of AMH in the infertile women group was greater than in the control group, despite the fact that there was no significant difference in AMH concentrations in this study. Despite recent research in the both FSH and AMH are still employed as ovarian reserve tests, despite evidence that AMH can be an excellent predictor of ovarian reserve and IVF success rates. [18].

Takahashi et al. (2008) found no connection between AMH levels and oocyte number in their research [19].

Ovarian stimulation for infertility therapies relies heavily on gonadotropin therapy. Efforts have been made to improve gonadotropin preparations during the previous century. Current gonadotropins, without a doubt, have safety profiles and higher quality, in addition to clinical effectiveness, than previous ones. The development of recombinant technology for the production of follicle-stimulating hormone, luteinizing hormone, and human chorionic gonadotropin was a huge step

forward [20]. Gonadotropins influence oocyte growth and maturation by influencing the release of numerous chemicals by granulosa cells (e.g., hyaluronic acid) [21] and the treatment of infertility [22]. The physiological concept behind gonadotrophin therapy (GT) is that a transient increase in (FSH) above a threshold dose can initiate and maintain follicle growth, similar to the effects of clomiphene citrate on the level of (AMH) slowly drops after gonadotropin treatment during controlled ovarian stimulation. This reduction might be explained by (FSH's) direct or indirect deleterious impact on (AMH) ovarian production. Exogenous FSH therapy boosts estradiol, which may be the cause of (AMH) reduction, because estradiol inhibits (AMH) regulation in the ovary. The (GnRH) antagonist regimens and low initial gonadotropin doses are recommended. It's also crucial to bring women to fertility treatment clinics as soon as possible so that they may select the best treatment option for them [23]**.** Letrozole has become a key weapon in our arsenal for treating infertility, but it has received relatively little attention in terms of improving its efficacy [24]. Aromatase inhibitors like letrozole have a negative feedback effect on the hypothalamus and pituitary glands in the early follicular phase, triggering GnRH, LH, Letrozole production and ovarian follicular growth stimulation FSH and its drug class look to be safe, trustworthy, and affordable therapeutic options. Letrozole unlikely to have the same Despite the fact that it may produce conception, clomiphene citrate has unfavorable effects on the endometrium [25].

According to (Durlinger et al.), granulosa cells produce (inhibin B) and (AMH), which appear to have both paracrine and autocrine effects within the follicle (2002). The oocyte expands in size when the follicle creates new layers of Granulosa Cells (GC) in the Pre-Antral Follicles (PAF). The latter stages of follicle maturation, from antrum formation through ovulation, are heavily influenced by cyclical (FSH), (LH) oscillations and are gonadotrophin-dependent. The main oocyte does not expand much throughout these gonadotrophin-dependent phases, but it undergoes cytoplasmic and nuclear maturation] 26].

Progesterone levels are similar throughout comparing the Luteal Phase (LP) of letrozole-associated Controlled Ovarian Stimulation COS cycles started by Human Chorionic Gonadotrophin (HCG)) to normal COS without letrozol. Letrozole was shown to be useful in maintaining Infra-Physiological Estrogen (IPE) levels while collecting multiple mature oocytes for future oocyte or embryo vitrification [27].

CONCLUSION:

Inhibin B, rather than AMH, is regarded to be a stronger predictor of infertility. HRG and AMH are connected to leterozol and gonadotrophin therapy because HRG levels rise and AMH levels fall in infertile women after treatment. The antral follicle grew into a mature oocyte after therapy with gonadotrophin and letrozole.

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