Original Article

The Effect Of Recombinant FSH Treatment On Ceruloplasmin Activity In Infertility Women Undergoing IVF/ICSI

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Abstract

Infertility can be resulted by an underlying medical problem that damages the fallopian tubes, effects with ovulation, or cause hormonal complications. One of the most effective treatments of this disorder is the IVF/ICSI reproductive technique which can be considered as a first-stage treatment strategy for women over age (<48) years. The aim of this study to estimate some antioxidant levels in IVF undergoing recombinant FSH (rFSH) treatment. During infertility treatment by ovarian stimulation, gonadotropins are used to ripen follicles and induce ovulation, Female infertility was induced in the subjects (n=32) and healthy women (n=30), all women were undergoing IVF/ICSI, and all samples collected from the Iraqi Specialty Center for IVF. Ceruloplasmin Total ferroxidase (activity and specific activity), ceruloplasmin concentration, ceruloplasmin oxidase (activity and specific activity), and level of copper were measured in all participants before and after recombinant FSH therapy. rFSH therapy was included to be associated with a significant decreased ($P<0.05$) ceruloplasmin Concentration, Ceruloplasmin oxidase specific activity, and significant increase ($p<0.05$) of ceruloplasmin total ferroxidase activity, but show no-significant differences ($P>0.05$) with Ceruloplasmin oxidase activity, and Copper concentration whereas showed no-significant difference ($p>0.05$) with ceruloplasmin ferroxidase specific activity.

Keyword: Ceruloplasmin, Copper, recombinant FSH, IVF

1.Introduction

The failure to bear children after a year of frequent sex is referred to as "infertility." Infertility affects approximately 13% of women and 10% of males (Dohle, Colpi et al. 2005). Infertility in females is results by an underlying illness which might risk for the fallopian tubes, interrupt ovulation, and may be cause hormone imbalances. Medical disorders involve a variety of health issues such as pelvic inflammatory disease, uterine fibroids, premature ovarian failure, polycystic ovarian syndrome, and endometriosis. While clinical study show that male infertility is caused by poor sperm quality, this is not the case for more than 40% of couples seeking assisted reproduction treatment. Though conventional sperm analysis is incapable of distinguishing between the spermatozoa of fertile and infertile men, it is a closely related procedure that may aid in the diagnosis of male fertility (Lewis, Aitken et al. 2013) Indeed, congenital and acquired genitourinary problems, genetic abnormalities, infections of the male accessory glands, endocrine diseases, increased scrotal temperature (varicocele), and immunological variables all contribute to a decrease in male fertility (Schmid, Kirchengast et al. 2004).

Age, dietary antioxidants, and oxidative stress, particularly psychosocial difficulties, are all factors that influence fertility. For example, Matorras et al. reported on the impact of age on fertility, stating that the number of kids born in a population of women drops exponentially from the ages of 35 to 39 years (Matorras, Prieto et al.

2011). It has also been shown that as a result of a shift in the redox activity balance, the number and quality of oocytes drop with age. As a result, age plays an essential role in lowering oocyte function and affecting female fertility (Akhbardeh 2009). While little is known about the variables that influence fertility and early pregnancy loss, there is enough data to suggest that dietary antioxidants and oxidative stress (OS) can affect the timing and maintenance of a healthy pregnancy. (Alam, Khan et al. 2019).

Other fertility illnesses, such as polycystic ovarian syndrome (PCOS), can be caused by psychosocial issues, resulting in a variety of symptoms. PCOS is a common endocrine disorder that affects female fertility(Schmid, Kirchengast et al. 2004). PCOS is characterized by an increase in androgen production, which leads to high concentrations of luteinizing hormone (LH) and decreased levels of follicle-stimulating hormone (FSH), which prevents follicles from generating mature eggs. (Olooto, Amballi et al. 2012). Obesity, infertility, hirsutism, and oligo/amenorrhea are all symptoms of PCOS that can be detrimental to a female's quality of life and view of her body shape. PCOS, infertility, and fertility treatment may all have an effect on the quality of female (Angin, Yoldemir et al. 2019).

Treatment for infertility must begin with the treatment of any medical disorders that may be causing reproductive problems. There are several treatment approaches for restoring fertility, including lifestyle measures (eating a nutritious diet, stopping smoking, and timing sexual behavior with the ovulation cycle), however administration of medicines for increase ovulation ability, such as gonadotropin and clomiphene, and In vitro fertilization (IVF) which consider one of assisted reproductive technologies (ART) (Kashani and Akhondzadeh 2017). Many people seek therapy for infertility using assisted reproductive technology (ART), and many couples have fewer children than originally planned (Daniluk and Koert 2013).. Since the introduction of IVF, Infertility treatment has progressed significantly in recent years.; 85 percent of them have had a successful pregnancy after three times of therapy if the female is elderly; 38years old (Morin, Patounakis et al. 2018) .Aside from IVF, sperm microinjection (ICSI) is a very efficient therapy for tubal or men infertility; however, They don't occur especially useful when infertility is caused by embryo implantation failure. When three or more high-quality embryos are transferred and the pregnancy does not continue, as most women are expected to be pregnant at this time (Tremellen and Russell 2011), implant failure is frequently diagnosed.

Ceruloplasmin (Cp) (EC 1.16.3.1) is a blue enzyme with multifunctional copper 6-7 copper (II) per molecule (Sokolov, Acquasaliente et al. 2015),Also, Cp is a glycoprotein that is considered an acute phase protein, and is one of the most important antioxidants in plasma. Furthermore, it is a multifunctional plasma protein with amino oxidase and ferroxidase activity, and it contains up to 90% of plasma copper, preventing copper from being involved in the generation of free radicals (Zowczak, Iskra et al. 2001). Copper excess or deficiency can cause aberrant or defective spermatogenasis, as well as oxidative damage to testicular tissue and spermatozoa, resulting in infertility(Tvrda, Peer et al. 2015).

Ceruloplasmin Synthesis and secrete by lungs, liver, and kidneys (Zhang, Xiong et al. 2004). Iron homeostasis, copper transport, oxidase activity, oxidation of different amines, oxidation of Fe (II) to Fe (III) (ferroxidase activity) for following absorption by ferritin and transferrin, serum antioxidant, and endogenous modulation of the inflammatory response are all functions of ceruloplasmin (Erel 1998). Its level rises with injury and is generated in response to infection, trauma, and neoplasia (Gundogdu, Kaya et al. 2007).

Copper (Cu) is an important trace element found in small amounts in many cells and tissues. It is associated with serum albumin and is transported to the liver in high concentrations ()Lucia, André et al. 2010), where a small amount is excreted in the bile and the rest is associated with ceruloplasmin and enters blood flow)Cholewińska, Ognik et al. 2018) .

The present study discusses the changes in ceruloplasmin ferroxidase and oxidase activities in infertile women using the GnRH antagonist.

2. Material and Methods:

For this study, we analyzed the data collected from the Iraqi Specialty Center for IVF (AL-Jadriya Hospital), the analyzed data included about 32 of infertile women aged 20-48 years and 30 of healthy women were used.

all female infertility was treated with recombinant FSH (rFSH) hormone therapy throughout the program period rFSH was used to treat all female infertility).

Female patients were treated with a GnRH antagonist program for 12 to 14 days on average, with their hormone levels being tracked. Ceruloplasmin total ferroxidase activity and specific activity, ceruloplasmin concentration, ceruloplasmin oxidase activity and specific activity, and serum copper content were all determined or calculated in each sample before and after recombinant FSH treatment was administered.

2.1 Ceruloplasmin Oxidase Activity Determination:

The serum ceruloplasmin oxidase activity was determined by the Rice method (Rice 1962) with such modifications that the oxidation of the substrate (p-phenylenediamine) is catalyzed by ceruloplasmin. Absorbance of a control and sample was measurement at wavelength (540 nm).

Calculation:

A difference between sample and control absorbance (A) was computed, and the following relationship was used to convert it to an international enzyme unit:

where: ΔA = the differences in the absorbance at 540 nm. while the final multiplication factor was equaled to **349** that calculated from thefollowing equation:

$$International\ Unit = \Delta A \times Final\ multiplication$$

$$Final\ multiplication\ factor = \frac{10000}{\varepsilon \times incubation\ time \times b}$$

A=Absorbance, Incubation time= 15 minutes.
ε=Molar absorptivity of the base which = 1.91 L/ mol. cm,
b= 1 cm.

2.2 Concentration of ceruloplasmin (Cp) determination:

Ceruloplasmin concentration determination multiply by Holmberg-Laurell and Ravin (HOLMBERG and Laurell 1951) (Ravin 1961) factor which was calculated to be equal to **87.5** from thefollowing equation:

CP concentration (mg/dl) = ΔA× multiplication factor

At a wavelength of 605 nm, the absorbance of each above tube sample and control was measured.

2.3 Ceruloplasmin Ferroxidase Activity Determination:

Ferroxidase activity determination by using Erel 1998 method(Erel 1998) which (3-(2-pyridyl)-5,6-bis (2-[5furylsulfonic acid])- 1,2,4-triazine) (180 mM) used as chromogen stock, and calculated activity by using final equation:

Enzyme Activity (U/L) = (C1-C2) ×38.166

C1 = Concentration of substrate at the starting of the enzymatic reaction (60 µmol/L).

C2 = Concentration substrate at the end of the enzymatic reaction(calculated from a standard curve).

2.4 Copper Concentration Determination:

Copper was determined by the spectrophotometric method to form a Blue complex by using a kit from LTA Company (Italy) (Naji 2012), and calculated from the equation:

$$\text{Copper µg/dl} = \frac{\text{Asample} - \text{Ablank}}{\text{Astander\_Ablank}} \times 200$$

Statistical analysis:

For statistical computations, the IBM SPSS version 23 statistics application was utilized. The difference between patients before and after ovarian stimulation medications was analyzed using Student's independent t-test, with a $p < 0.05$ that was considered statistically significant.

3. Results:

In this section, we will illustrate some experimental results about infertility women undergoing IVF protocol. Statistical analysis of infertile women over the age of 32 undergoing rFSH therapy was (**30.88 ± 5.511)** year, while for 30 of the healthy control group was **(32.67 ± 5.689)** year as summarized in table (1).

Table 1: Statistical analysis of ages for the studied groups:

The groups	**N**	**Mean age /year**	**± SD**
Patients under rFSH	32	30.88	5.511
Control	30	32.67	5.689

The ceruloplasmin concentration and ceruloplasmin oxidase specific activity of the studied groups before and after treatment are presented in Table 2 has a decrease significant difference, and ceruloplasmin ferroxidase activity showing increase significant differences value ($p<0.05$), while ceruloplasmin oxidase activity, ceruloplasmin Ferroxidase specific activity and copper concentration appeared no significant value ($p>0.05$) of the studied groups before and after treatment.

Table 2: Comparison of ceruloplasmin oxidase Activity, ceruloplasmin concentration, ceruloplasmin Ferroxidase activity, and copper concentration level of the groups before and after treatment for rFSH.

Parameter	**Groups**	**N**	**Mean**	**±SD**	**P-value**
CP. Concentration (mg/dl)	Before therapy	32	5.8374	1.50972	**0.002***
	After therapy	32	4.9431	1.36919	
	Control	30	5.7609	2.87286	
CP. oxidase activity (U/L)	Before therapy	32	27.1041	7.51203	0.0571
	After therapy	32	23.5242	6.53949	
	Control	30	27.772	13.245	
CP. oxidase specific activity (U/g)	Before therapy	32	0.33048	0.1028	**0.001***
	After therapy	32	0.2589	0.08272	
	Control	30	0.34163	0.139	
CP. Total ferroxidase activity (U/L)	Before therapy	32	888.0115	205.191	**0.00019****
	After therapy	32	1025.03	208.7076	
	Control	30	851.319	256.323	
CP. Total ferroxidase specific activity (U/g)	Before therapy	32	10.846	3.116	0.4528
	After therapy	32	11.264	2.989	

	Control	30	10.465	2.822	
Copper concentration (µg/dl)	Before therapy	32	104.9052	26.25933	0.834
	After therapy	32	103.9539	28.73965	
	Control	30	109.9435	36.85782	

*** significant at P<0.05 for 2-tailed.**

**** significant at P<0.001 for 2-tailed**

4. Discussion:

The results of this study corroborated those of a previous study that comprised 32 patients and 30 control groups ranging in age from 20 to 48 years old that had IVF. Iraqi Specialty IVF Center samples were obtained (al-jadrya hospital). After approval from the Institutional Review Board, people with a history of implantation failure after embryo transfer were included in a prospective, randomized trial.

Researchers observed that therapy increased ceruloplasmin ferroxidase activity, a finding that contrasts with that of Kadhum and Al-Shammaree (Kadhum and Al-Shammaree 2021), who reported no alterations in the fertility of women receiving IVF.

Other has shown that serum levels of copper were measured in 26 women with unexplained infertility and in 20 normal women as controls, and serum Cu levels were significantly higher in the infertility group (10.1 mol/L0.4 VS 7.81 mol/L0.38, P= 0.01) (Khulood and Faris 2005). The results of this analysis were then compared with the current study, which found that the infertility group's copper concentration was lower than that of the control group.

In percent study we found that the p values for Cu in the female group after treatment were 103.9±28.7, which means that it is not significant ($p > 0.05$). According to previous research, Ceruloplasmin can bind copper better than other transport proteins, which could mean it plays a role in maintaining iron and copper balance (Aisen, Enns et al. 2001). but this new study implies otherwise. In other words, it's not a good idea.

This result ties well with previous studies wherein more miscarriages have been linked to women who have low levels of estrogen and copper in their bodies, according to research. Some ladies will value knowing this. Managing the copper deficiency is a huge aid in a healthy pregnancy. Women with high or physiologically unavailable copper, on the other hand, are more likely to experience infertility. This could be because of adrenal fatigue, which leads to a copper deficiency (Ingraham, Kappel et al. 1987, Wilson 2011).

A GnRH antagonist given before and after adenomyosis surgery improved fertility, despite the small sample size. It protects proteins, lipids, and DNA against free radical reactive oxygen radicals by oxidizing extremely dangerous ferrous iron to a relatively non-toxic ferric form(Serdar, Gür et al. 2006). ceruloplasmin, another important antioxidant, is found in the human body. In the words of Gutteridge (1992).

According to Hussein research, infertile women had higher levels of serum ceruloplasmin than fertile ones (Hussein, Al-Salih et al. 2017), this rise may be due to it supporting the body's antioxidant function, for example (Winyard, Hider et al. 1989).

Similarly, another study discovered that infertile women's ceruloplasmin activity was higher than controls, and a correlation was found between copper (A Al-Kado and A Al-Helaly 2012), ceruloplasmin, and antioxidant markers, which may indicate their roles in endometriosis and oxidative stress and can be used as therapeutic indicators for a predictable future.

5. Conclusion:

To sum it up, after using recombinant FSH treatment in IVF producers, the ceruloplasmin ferroxidase activity increased and this means therapy was effective for infertility women compared to fertility women. Furthermore,

these findings provide additional information about the ceruloplasmin activity and copper levels that appeared to be lower for infertility women than fertility women, and copper concentration showing no significant value.

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