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Correlation of interleukin-6 gene expression and idiopathic female infertility

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Abstract

The present investigation was focused on the identification of the interleukin-6 (IL-6) role in the unexplained female infertility. According to this aim, 100 blood samples were collected from fertile (T1), 50 samples, and infertile (T2), 50 samples, women who attended Al-Nasiriyah Children and Obstetrics Hospital, Al-Nasiriyah, Iraq. Enzyme-linked immunosorbent assay (ELISA) detection was performed to recognize the IL-6 concentrations in the blood samples of those women. A real time-quantitative polymerase chain reaction (RT-qPCR) method was used to identify the gene expression (GE) of the IL-6 gene in the blood of those women. The results of the ELISA showed significant ($p<0.05$) higher concentrations of the IL-6 in the blood samples of the T2 women, 352.25pg/ml, than those in the blood samples of the T1 women, 152.35pg/ml. Moreover, the RT-qPCR revealed significant ($p<0.05$) higher relative GE of the IL-6 gene in the blood samples of the T2 women, 6.892-fold change (FC), than that in the blood samples of the T1 women, 2.488 FC. The current study indicates that IL-6 may have a major role in the unexplained female infertility.

Keywords*: ELISA, female, infertility, interleukin-6, RT-qPCR.*

I. Introduction

Infertility is a health condition in which conceiving is not produce after unprotected intercourse for one year. The evaluation analyses for the infertility problem are ideally performed after the former period of conceiving, however, this period is decreased to after-six-month period of conceiving for females older than 35 years old of age. Standard infertility evaluation (SIE) guidelines have been published by The Practice Committee of the American Society for Reproductive Medicine (ASRM). These guidelines contain female infertility evaluation procedures of ovulation assessment, hysterosalpingogram techniques, and, if recommended, examination of the ovarian reserve plus laparoscopy. The defects in ovulation can reach up to 40% of infertile female population. These defects can be reflected by the disturbances initiated in the menstrual cycle of those women which can be identified by reading the history of those women. Health conditions such as polycystic ovary, thyroid-related diseases, hyperprolactinemia, and hypothalamic effects due to changes in weight are major causes for the defects in ovulation. If the results of the SIE come normal, unexplained infertility evaluation (UIE) should be performed to identify the reasons behind this condition. The results come normal for SIE females, being of those who have unexplained infertility, can reach up to 15% to 30% of female population (Malcolm and Cumming, 2003; Practice

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Committee of the American Society for Reproductive Medicine, 2006a, 2006b; Quaas and Dokras, 2008). The present investigation was focused on the identification of the interleukin-6 (IL-6) role in the unexplained female infertility in women attended Al-Nasiriyah Children and Obstetrics Hospital, Al-Nasiriyah, Iraq (Sherwin et al., 2002).

II. Materials and methods

Patients

One-hundred blood samples were collected from fertile (T1), 50 samples, and infertile (T2), 50 samples, women who attended Al-Nasiriyah Children and Obstetrics Hospital, Al-Nasiriyah, Iraq. The samples were collected from these women at day-3 of the menstrual cycle. These samples were transferred to a Lab where they were further processed to initiate the principle tests, ELISA and RT-qPCR.

ELISA

The ELISA was performed using an IL-6-based ELISA kit from (CUSABIO Comp., China). The protocol accompanied the kit was followed.

RT-qPCR

Extraction of the total RNA was done from the blood samples using TRIzol® component constructer kit (Promegae Comp., USA) and according to the instructions enclosed with the kit. The resulted RNA was read for its quality and quantity using a NanoDrop UV/VIS (OPTIZEN POP. MECASYS, Korea).

The cDNA was produced using AccuPower® Greenstar™ qPCR PreMix component kit (Bioneer, Korea) and according to the informational protocol accompanied the kit. Exicycler™ 96 Real-Time Quantitative Thermal Block (Bioneer, Korea) was used following its protocol to perform the RT-qPCR method. The housekeeping gene used was the *GapdH* regarding the analysis of mRNA-fold change of the IL-6 gene.

III. Results

ELISA

The results of the ELISA showed significant (p<0.05) higher concentrations of the IL-6 in the blood samples of the T2 women, 352.25pg/ml, than those in the blood samples of the T1 women, 152.35pg/ml. Figure 1 shows these levels in the blood samples of both T1 and T2 females.

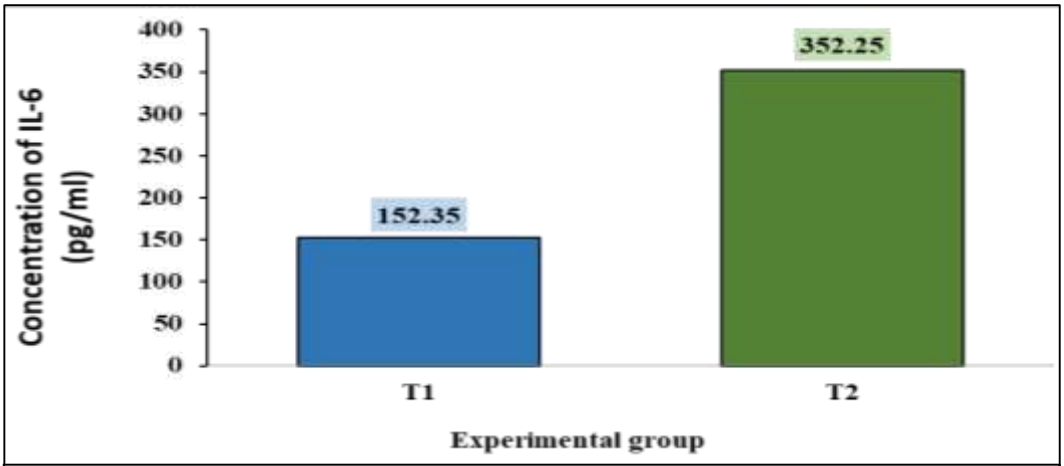

Figure 1: The concentrations of the IL-6 in the blood samples of both T1 and T2 females.

RT-qPCR

Moreover, the RT-qPCR revealed significant (p<0.05) higher relative GE of the IL-6 gene in the blood samples of the T2 women, 6.892-fold change (FC), than that in the blood samples of the T1 women, 2.488 FC. Figure 2 shows these levels in the blood samples of both T1 and T2 females.

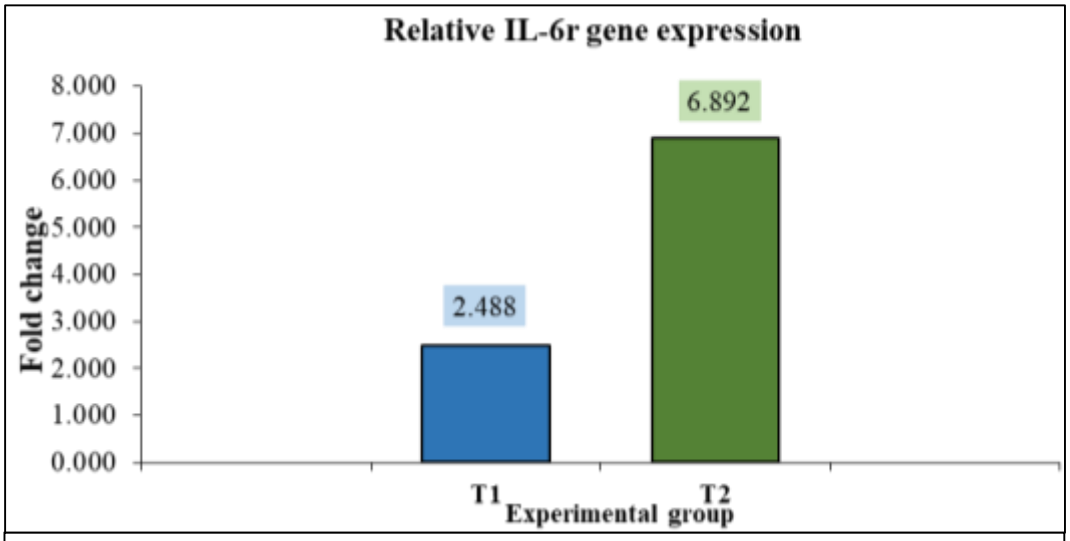

Figure 2: The relative gene expression of the IL-6 gene in the blood samples of both T1 and T2 females.

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IV. Discussion

Infertility is a health condition in which conceiving is not produce after unprotected intercourse for one year. If the results of the SIE come normal, unexplained infertility evaluation (UIE) should be performed to identify the reasons behind this condition. The results come normal for SIE females, being of those who have unexplained infertility, can reach up to 15% to 30% of female population (Malcolm and Cumming, 2003; Quaas and Dokras, 2008).

Showing no rational reasons for female infertility does not demonstrate a cause for this health problem. Detailed studies should be focused on other etiological factors other than those recommended for the SIE such as molecular changes occurred at the levels of ovarian dysfunctions, the quality of the oocytes, fallopian tube-based transport damages, the receptivity of endometrium, implantation-related failures, hormonal disturbances, and endometriosis (Isaksson and Tiitinen, 2004; HATASAKA, 2011; McGovern;, 2017).

According to the current study results of the IL-6 concentration in the blood samples of the women with unexplained infertility, higher concentrations were noticed in the blood samples of those females in compared with those in the blood samples of the health women. These results agree with the findings observed by (Demir *et al.*, 2009) who detected that the serum levels of the IL-6 were higher in the blood samples of the females with unexplained infertility suggesting an important role for this cytokine in this health problem. Moreover, (An *et al.*, 2015) found increases in the serum levels of different cytokines such as IL-2, IL-4, and IL-6 in females with unexplained infertility.

Endometriosis needs more attentions when take a deeper look at the molecular aspects of the IL-6. It has been recognized that IL-6 is increased in the endometrial layer of patients with endometriosis. According to this, the IL-6 increases in the blood of the females with unexplained infertility may have been induced due to an unapparent health problem of endometriosis in the current study infertile females (Ahn *et al.*, 2015; Lessey and Kim, 2017).

According to (Mima and Nishimoto, 2009; Sikorsky et al., 2011), it is very important to study the relationship between reproductive status and IL6 bioavailability more thoroughly to improve the precise role of IL6 in controlling non-immunological pathways and immune adaptation for proving pregnancy, maybe by studying the relation between T signal components and IL6 of cellular patterns in gestational tissue and endothelial or by investigating the impact of IL6 pharmacokinetic signal inhibits on reproductive tissues. Clark et al., (2001); Guerin et al., (2009) stated that these are some things that related with Variable parameters of maternal immune system such as preterm birth, pre-eclampsia, miscarriage and Infertility as well as there seems to be evidences that immunity factors have an important role to play behind these diseases in the underlying causal pathways.

IL6 is the nominee role in certain pregnancy complications according to the multifunctional and central role of IL6 in active interaction and as a regulator of adaptive immune response. The IL6 content of plasma and cervical mucus in women with idiopathic infertility was reported to be higher than in fertile women (Demir et al., 2009). It does not seem to be affecting endometrial tissue, where comparable rates of IL6 are observed in women with fertility biopsies and idiopathic infertility (Sherwin et al., 2002). In accordance with the above, the expression of IL6 mRNA in endometrial tissue in females with primary idiopathic infertility has not improved in the mid-secretion process (Jasper et al., 2006). On the other hand, in the mid-secretion process of infertile women, the secretion of sgp130 in the endometrium was decreased, leading to a lower ratio of sgp130 to IL6 inhibitors, potentially raising the signal through membrane related gp130 (Sherwin et al.,

2002). These data indicate that in endometrial preparation for transplantation the ratio between sgp130 and IL6 is significant and may be a better indicator of fertility than IL6 separately (Sherwin et al., 2002).

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