Short Communication

The Gene Expression of TNF-α Predicting ICSI Outcome

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Abstract

Background: According to the updated international glossary on infertility and fertility care, infertility is defined as a disease characterized by the inability to achieve a clinical pregnancy after twelve months of regular, unprotected sex. Millions of people are affected by infertility, which also affects their families and communities. **Objective:** The aim of the current study was to assess the association gene expression of tumor necrosis factor alpha (TNF-α) with implantation success in women undergoing intracytoplasmic sperm injection. **Materials and Methods:** The current study examined 150 infertile women who received intracytoplasmic sperm injection at the Tiba Center for Infertility, Babil, Iraq and Al-Kafeel Hospital, Karbala, between May 2022 and November 2022. Women with positive biochemical pregnancies were included in the first study group, and those with negative pregnancies were included in the second. Blood samples were taken from individuals for the study. The gene expression of TNF-α was examined. **Results:** The median and interquartile range of TNF-α in positive and negative pregnancy were 17.13 (27.58) and 4.23 (27.69), respectively, and the range was from 0.00 to 759.78 and 0.00 to 120.99, respectively. The difference in mean rank was statistically significant ($P = < 0.001$). **Conclusion:** Gene expression of TNF-α can affect endometrial thickness, leading to implantation failure.

Keywords: Infertility, intracytoplasmic sperm injection and implantation, tumor necrosis factor alpha

Introduction

A disorder of the male or female reproductive system known as infertility is characterized by the inability to conceive after 12 months or more of regular, unprotected sexual activity. Millions of people are affected by infertility, which also has an effect on their families and communities.[1] The primary reasons for infertility vary by nation. About 30% of infertility cases in the UK are caused by female factors, 30% by male factors, 25% by unknown causes, and 15% by combined factors or other causes.[2] Uterine factors such as leiomyomas can interfere with fertility, although the degree depends on the size and location of the lesion. Submucosal myomas directly affect embryo implantation, and intramural myomas that are larger than 5 cm may reduce fertility, according to strong evidence.[3] Endometriosis is the presence of stromal tissue and endometrial glands outside the uterus.[4] Ovulatory dysfunction has been classified into three main types and for which, hyperprolactinemia has been recognized as a further etiology:

1. WHO category I: Hypogonadotropic hypogonadism.
2. WHO category II: Normogonadotropic normogonadism.
3. WHO category III: Hypergonadotropic hypogonadism.
4. Hyperprolactinemic.

The three tubal factors are periadnexal adhesions, endosalpingeal injuries, and tubal blockage or obstruction (proximal, distal, unilateral, or bilateral).[5] Large chromosomal abnormalities, submicroscopic chromosomal deletions and duplications, and DNA sequence variations in the genes that regulate many biological processes involved in oogenesis, ovarian reserve maintenance, hormonal signaling, and anatomical and functional development of female

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reproductive organs are just a few examples of the genetic abnormalities that lead to infertility in females.[6] The endometrium, the tissue that develops on the inner lining of the uterus, must go through biological changes in order for an embryo to implant effectively in the uterus. The endometrium thickens and changes its responsiveness to potential implantation by the healthy embryo.[3,7-17]

TNF-α cancer TNF-α, a pro-inflammatory cytokine generated by macrophages and monocytes during acute inflammation, regulates a wide variety of cellular signaling processes, TNF-α is a multifunctional molecule that is secreted by both innate immune cells and the placenta and is involved in the control of a wide range of biological processes, including cell proliferation, differentiation, apoptosis, lipid metabolism, and coagulation, TNF-α have an important part in the growth and development of the ovarian follicle and is crucial in the regulation of the ovarian cycle.[7]

The aim of the study was to evaluate the expression of protein TNF-α in infertile women.

Materials and Methods

This study is a case-control study.

The current study examined 150 infertile women who received intracytoplasmic sperm injection at the Tiba Center for Infertility, Babil, Iraq and Al-Kafeel Hospital, Karbala, between May 2022 and November 2022. The patients were divided into two groups: The first group had 75 positive pregnancies while the second group had a negative group. All women involved will have a detailed medical examination including general, abdominal, genital tract for any abnormality, and baseline hormonal evaluation in the early follicular phase (day 1or 2 of the menstrual cycle): FSH, luteinizing hormone (LH), 17β estradiol (E2), progesterone P4 and TSH.[8] The female has three phases of the menstrual cycle: follicular phase (before release of the egg), ovulatory phase (egg release) and secretory phase. The embryo transfer process begins in one phase, and the action of TNF also begins in this phase. Therefore, blood was drawn at this time to determine the effect of TNF on the implantation rate.[9]

Inclusion and exclusion criteria

Exclusion criteria in females

Females aged over 40 years, moderate or severe endometriosis, polycystic ovary syndrome (PCOS), uterine fibroid or any abnormality in the genital tract, or bilateral tubal occlusion were excluded from the study.

Exclusion criteria in male

Men with oligospermia (low sperm count), asthenozoospermia (reduced sperm motility), teratospermia (presence of sperm with abnormal morphology), obstruction or absence of the vas deferens, severe varicocele, or history of cryptorchidism and anejaculation were excluded from the study.

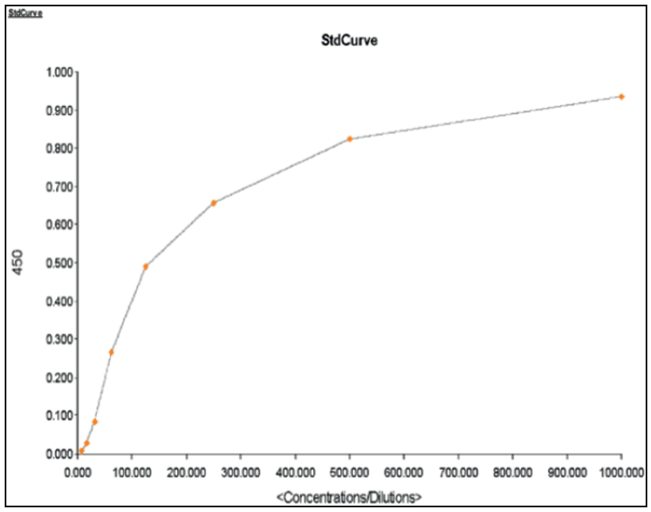

Figure 1: Standard curve of TNF-α

Table 1: Kits used in the study

Name of kit	Company	Country
TNF-α Kit	Elabscience	China

Estimation of immunological TNF-α

Measuring the concentration of TNF-α serum level as shown in Figure 1, procedure was done according to the instruction in the ELISA Kit (Elabscience, China) as shown in Table 1.

Statistical analysis

Data were collected, summarized, analyzed, and presented using Statistical Package for Social Sciences (SPSS, SPSS Inc., Chicago, Illinois, USA) version 26 and Microsoft Office Excel 2010. Numeric variables were not normally distributed and there for Mann-Whitney *U* test and the expression in the form of median (an index of central tendency) and inter-quartile range (an index of dispersion)).[18] The level of significance was considered at a P-value of equal or less than 0.05.

Ethical approval

The study was conducted in accordance with the ethical principles. It was carried out with patient's verbal and analytical approval before sample was taken. The study protocol and the subject information and consent form were reviewed and approved by a local ethics committee according to the document number EC/1278/A on May 15, 2022 to get this approval.

Table 2: Comparison of serum hormonal levels between positive pregnancy group and negative pregnancy group

Characteristic	Positive pregnancy $n = 75$	Negative pregnancy $n = 75$	*P*
E2 (pg/mL)			
Median (IQR)	529 (435)	426 (248)	0.042 M*
Range	96 -2047	40–1321	
P4 (ng/mL)			
Median (IQR)	20.99 (22.61)	13.8 (8.78)	0.001 M***
Range	0.36–40	1.23–40	
FSH (IU/L)			
Median (IQR)	7.7 (2.4)	7.2 (2.6)	0.271 M
Range	1–17.1	2.6–17.5	NS
LH (IU/L)			
Median (IQR)	4.7 (3.8)	3.7 (2.4)	0.029 M*
Range	0.2–15.2	1.2–14	

n: number of cases; IQR: interquartile range; E_2: estradiol; P_4: progesterone; FSH: follicle stimulating hormone; LH: luteinizing hormone; M: Mann-Whitney *U* test; NS: not significant; *: significant at $p \leq 0.05$; ***: significant at $p \leq 0.001$

Table 3: Comparison gene expression of TNF-α level between positive pregnancy group and negative pregnancy group

Characteristic	Positive pregnancy $n = 75$	Negative pregnancy $n = 75$	*P*
TNF-α			
Median	17.13 (27.58)	4.23 (27.69)	< 0.001 M***
IQR	0.00–759.78	0.00–120.99	

n: number of cases; IQR: inter-quartile range; M: Mann Whitney U test; NS: not significant; *: significant at $p \leq 0.05$; *: significant at $p \leq 0.01$; *: significant at $p \leq 0.001$

Table 4: Correlations of TNF-α to serum hormonal levels

Hormone	TNF	
	R	*P*
E2	0.005	0.948
P4	0.079	0.334
FSH	0.018	0.824
LH	0.127	0.122

Results

Comparison of serum hormonal levels between positive pregnancy group and negative pregnancy group

Table 2 compares the blood hormonal levels between the positive pregnancy group and the negative pregnancy group. The median and inter-quartile range of E2 with positive and negative pregnancy were 529 (435) pg/mL and 426 (248) pg/mL, respectively, and the range was from 96 to 2047 and 40 to 1321, respectively. Statistics showed that the difference in mean rank was significant ($P = 0.042$).

The median and interquartile range of P4 with positive and negative pregnancy were 20.99 (22.61) ng/mL and 13.8 (8.78) ng/mL, respectively, and the range was from 0.36 to 40 and 1.23 to 40, respectively. Statistics showed that the difference in mean rank was significant ($P = 0.001$).

The median and inter-quartile range of FSH with positive and negative pregnancy were 7.7 (2.4) IU/L and 7.2 (2.6) IU/L, respectively, and the range was from 1 to 17.1 and 2.6 to 17.5, respectively. The difference in mean rank was statistically insignificant ($P = 0.271$).

The median and inter-quartile range of LH with positive and negative pregnancy were 4.7 (3.8) IU/L and 3.7 (2.4) IU/L, respectively. Moreover, the range was from 0.2 to 15.2 and 1.2 to 14. respectively. The difference in mean rank was statistically significant ($P = 0.029$).

Comparison gene expression of TNF-α level between positive pregnancy group and negative pregnancy group

The median and inter-quartile range of TNF-α with positive and negative pregnancy were 17.13 (27.58) and 4.23 (27.69) respectively and the rage was ranging from 0.00 to 759.78 and 0.00 to 120.99 respectively; the difference in mean rank was statistically significant ($P < 0.001$) as shown in Table 3.

Correlations of TNF-alpha to serum hormonal levels are shown in Table 4. Tumer neicrosis factor alpha showed no significant correlation to any serum hormonal level.

Discussion

All these differences between the hormone levels of healthy and infertile Iraqi women show the great effect of hormones on female fertility. Because hormone levels differ depending on the menstrual phase, the majority of variables were significantly rhythmic during the follicular

phase but not during the luteal phase of the menstrual cycle. These discoveries have significant implications for our understanding of how reproductive function is controlled.

Our study shows significant differences in serum concentrations of estradiol E2 between the two groups, positive and negative pregnancies. These results were similar to the findings of a number of studies in which females with a greater number of oocytes produced greater E2, which helped in the implantation and attainment of pregnancy.[10]

Our study shows significant differences in serum concentrations of progesterone P4 between the two groups, positive and negative pregnancies. optimal P4 level is essential for successful implantation and pregnancy.[11]

According to FSH level, there was no significant difference between the positive and negative pregnancy groups, the median interquartile range of FSH 7.7 (2.4) 1–17.1 and 7.2 (2.6) 2.6–17.5 of positive and negative pregnancy, respectively. These results were similar to the findings of a number of studies.[12] Previous data indicate high levels of endometrial LH receptor expression and LH secretion during the "implantation window," which is conducive to embryo implantation.[13]

Serum TNF-α in infertile women enrolled in this study

In this study, we found that serum level of TNF-α was higher in pregnant women in comparison with non-pregnant women, and from statistical perspective, the difference was significant. In addition, it has been found that a higher TNF–α level was associated with a higher pregnancy rate, and it was established that the determination of TNF-α concentration might be a useful tool to predict embryo implantation.[14]

In Table 4, TNF-α has no effect on hormone levels, however, it may aid in increasing the development of endometrial thickness. TNF-α plays a pivotal role in early implantation and is expressed by local endometrial epithelial cells. TNF-α and other cytokines such as IL-1, IL-5, IL-17, etc. work to establish an inflammatory environment for uterine, receptivity. TNF-α also regulates the gene expression signalling by modulating the human endometrial stromal cells (HECs). This will induce a favorable environment for embryo implantation.[15,16]

Conclusion

Gene expression of TNF-α can affect endometrial thickness and lead to implantation failure.

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Conflicts of interest

There are no conflicts of interest.

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