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RESEARCH ARTICLE
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ROLE OF SELECTED CYTOKINES AS A SUCCESS MARKER OF *IN VITRO* FERTILIZATION PREGNANCY

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Abstract

Background: Infertility is a significant global health issue and despite advancements in assisted reproductive technologies (ART) like in vitro fertilization (IVF), success rates remain relatively low.
Objective: This study aimed to explore the correlation between serum Interleukin-6 (IL-6), Leukemia Inhibitory Factor (LIF), and Tumor Necrosis Factor-alpha (TNF-α) and IVF success in women undergoing fertility treatment.
Methods: A cross-sectional study was conducted with 80 women aged 30 to 40 years undergoing IVF at a fertility clinic. Inclusion criteria included primary or secondary infertility and recurrent miscarriages, with exclusion criteria focusing on specific surgical histories and lack of histopathologic diagnoses. Blood samples were drawn during IVF procedures to measure IL-6, LIF, and TNF-α levels using enzyme-linked immunosorbent assay (ELISA) kits. Descriptive statistics and Mann-Whitney U tests were employed for data analysis using SPSS version 26.
Results: The mean age of participants was 33.42±4.74 years, and the mean BMI was 27.20±4.68. Among participants, 76 (95%) were nulliparous, and 64 (80%) had primary infertility. The most common blood group was O+ve (57.5%). The study found no statistically significant differences in mean cytokine levels between women with positive and negative pregnancy outcomes: IL-6 (55.20±28.87 vs. 47.76±21.76, p=0.436), LIF (197.96±208.31 vs. 157.41±116.67, p=0.603), and TNF-α (377.35±361.51 vs. 145.41±68.11, p=0.073).

Conclusion: Although higher mean levels of IL-6, LIF, and TNF-α were observed in women with positive pregnancy outcomes. These findings suggest that while cytokines may play a role in reproductive processes, their levels alone do not serve as definitive predictors of IVF success.
Keywords: Cytokines; Interleukin-6; Leukemia Inhibitory Factor; Tumor Necrosis Factor-alpha; *in vitro* fertilization; Pregnancy.

Introduction

Female infertility is a significant global reproductive health challenge, with prevalence rates ranging from 1.2% to 3.1% among women trying to conceive and 15% among couples. Infertility affects women of reproductive age worldwide, leading to the birth of over 8 million babies through assisted reproductive technologies like in vitro fertilization (IVF) (1). The causes of female infertility include ovulation issues (15%–20%, with polycystic ovary syndrome (PCOS) being the most common), tubal abnormalities (15%–40%), endometriosis (5%–10%), and unexplained factors (20%–30%) (2). Despite these technologies, the live birth rate per initiated IVF cycle remains low at 19 to 22%. Infertility can significantly impact physical, emotional, and psychological well-being. The complexity of reproductive biology, including the intricate interplay of hormonal, genetic, and immunological factors, contributes to these outcomes (3, 4).

Cytokines, small proteins involved in cell signaling, play pivotal roles in the immune system's regulation, particularly in the context of pregnancy. They are essential in creating a conducive environment for embryo implantation and development (5). The balance between pro-inflammatory and anti-inflammatory cytokines is crucial for successful pregnancy establishment. Disruptions in this balance can lead to reproductive challenges, including implantation failures and miscarriages (6). Notably, T-helper (Th) cells, which produce various cytokines, are significant in this immune modulation. Th1 cells, producing interleukin-2 (IL-2) and interferon-gamma (IFN-γ), and Th2 cells, producing interleukins 4, 5, and 10 (IL-4, IL-5, IL-10), need to be balanced to favor Th2 for a successful pregnancy (7, 8).

The IL-6 is a multifunctional cytokine that plays a crucial role in the regulation of immune responses, inflammation, and hematopoiesis. It is produced by various cell types, including T cells, B cells, macrophages, fibroblasts, and endothelial cells, in response to infections, tissue injuries, and other inflammatory stimuli (9). IL-6 is involved in the acute phase response and stimulates the production of acute phase proteins in the liver. Its functions extend to the regulation of metabolic, regenerative, and neural processes (10). In the context of reproduction, IL-6 is known to be involved in the processes of ovulation, fertilization, and embryo implantation. Elevated levels of IL-6 have been associated with various reproductive disorders, making it a biomarker of interest in studies of fertility and pregnancy outcomes (11).

LIF is a cytokine belonging to the interleukin-6 family, known for its diverse roles in cell differentiation, survival, and proliferation. LIF is particularly significant in the field of reproductive biology due to its critical role in embryo implantation and early pregnancy. It is produced by the endometrial glands and is essential for creating a receptive uterine environment for embryo implantation (12). LIF signaling involves the activation of the LIF receptor and gp130, leading to downstream effects that support the attachment and invasion of the blastocyst into the uterine lining. Insufficient levels of LIF or disruptions in it signaling pathway have been linked to implantation failures and recurrent miscarriages, highlighting its importance as a potential marker for successful pregnancy (13).

TNF-α is a pro-inflammatory cytokine produced primarily by activated macrophages, but also by a variety of other cell types including T cells, natural killer cells, and endothelial cells. TNF-α plays a pivotal role in the regulation of immune cells, the induction of inflammatory responses, and the apoptosis of certain cell types (14). In reproductive health, TNF-α is involved in several key processes such as ovulation, endometrial shedding, and the implantation of the embryo. Its levels need to be carefully balanced, as both excessive and insufficient TNF-α activity can adversely affect reproductive outcomes (15). Elevated TNF-α levels have been associated with conditions such as endometriosis, polycystic ovary syndrome (PCOS), and implantation failure, making it a critical

factor in the study of infertility and assisted reproductive technologies (9). This study aims to explore the correlation between the levels of these cytokines and IVF success in women undergoing fertility treatment.

Methodology

This cross-sectional study was designed to investigate the correlation between specific hormonal markers and IVF outcomes in women undergoing fertility treatment. The study involved 80 women aged 30 to 40 years, recruited using non-probability and convenience sampling techniques from the Australian Concept Infertility Medical Center in Lahore and screened at The University of Lahore. Inclusion criteria included a history of either primary or secondary infertility and recurrent miscarriages, while exclusion criteria involved certain surgical histories and lack of histopathologic diagnoses. Ethical approval was obtained from the Institute of Molecular Biology and Biotechnology (IMBB) at The University of Lahore, and all participants provided informed consent, ensuring confidentiality and ethical compliance. Comprehensive data on patient characteristics, including age, medical history, and causes of infertility, were collected from medical records. Blood samples were drawn during IVF procedures and processed to measure serum IL-6, LIF, and TNF-α levels using commercially available enzyme-linked immunosorbent assay (ELISA) kits. The ELISA procedures followed manufacturer instructions, with optical density (OD) values measured at 450 nm using a microplate reader. Quantitative variables were summarized using descriptive statistics (mean ± standard deviation), and data normality was assessed. Group comparisons were conducted using appropriate statistical tests, including Student's t-test (or Mann-Whitney test) and ANOVA, utilizing SPSS version 26 for the analysis. This methodological approach was aimed at systematically exploring the relationship between these cytokines and IVF success to enhance the understanding of their roles in reproductive health.

Results

In table 1, mean age of women was 33.42±4.74 years. Minimum and maximum age of women was 26 and 46 years. Mean body mass index of women was 27.20±4.68. Minimum and maximum BMI of women was 16 and 35. Among women 76(95%) were nulliparous and only 3(3.8%) women were prim-parous while only 1(1.3%) woman present with multiparous (Figure 1). In this study 64(80%) women present with primary and 16(20%) presents with secondary infertility. The most frequent blood group among women was O^{+ve} (57.5%) followed by B^{+ve} (31.25%), AB^{+ve} (6.25%) and A^{+ve} respectively (5%) (Figure 1). There were 40(50%) women with positive and 40(50%) with negative pregnancy outcome.

Table-1: Characteristics of Study Participants

n	80
Age (Years)	33.42±4.74 [26-46]
BMI	27.20±4.68 [16-35]
Parity	
0 (Nulliparous)	76(95%)
1 (Prim-parous)	3(3.8%)
4 (Multiparous)	1(1.3%)
Type of Infertility	
Primary	64(80%)
Secondary	16(20%)
Blood Groups	
A (Positive)	4(5%)
B (Positive)	25(31.25%)
AB (Positive)	5(6.25%)
O (Positive)	46(57.5%)
Pregnancy Outcome	
Positive	40(50%)
Negative	40(50%)

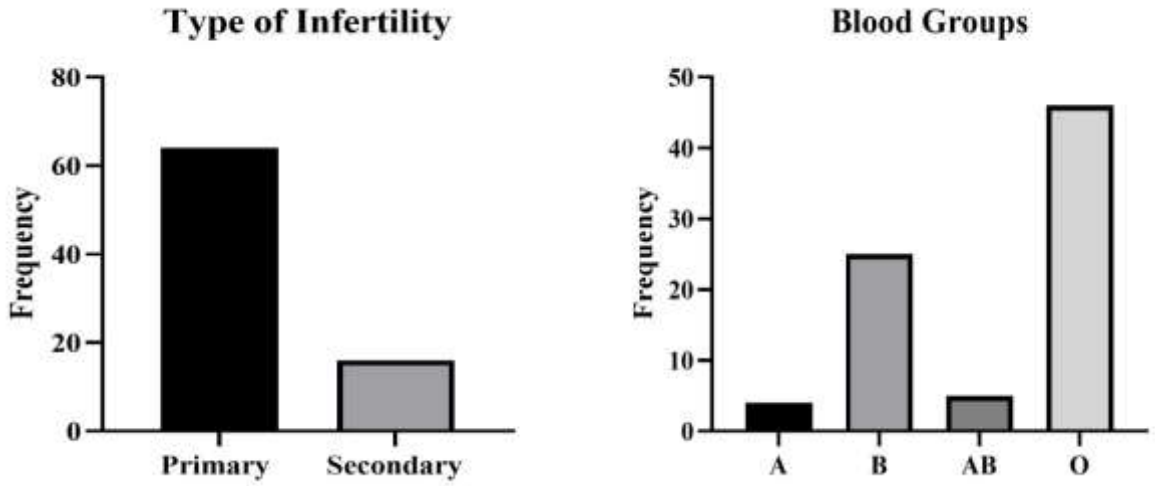

Figure 1: Characteristics of Study Participants.

Normality assessment showed that Serum IL-6 level, Serum LIF and Serum TNF- α was not normally distributed among women. i.e. p-value<0.001 (Table 2). Mean serum IL-6 level in women with positive and negative outcome was 55.20±28.87 and 47.76±21.76. Although serum IL-6 level was higher among women with positive outcome, but it was not statistically significant as p-value=0.436. Mean serum LIF level in women with positive and negative outcome was 197.96±208.31 and 157.41±116.67. Although serum LIF level was higher among women with positive outcome, but it was not statistically significant as p-value=0.603. Mean TNF-Alpha level in women with positive and negative outcome was 377.35±361.51 and 145.41±68.11. Although TNF-Alpha level was higher among women with positive outcome, but it was not statistically significant as p-value=0.073 (Figure 2).

Table-2: Comparison of Biomarkers in relation to Pregnancy Outcome

	Pregnancy Outcome		**p-value[(a)]**
	Positive	**Negative**	
	40	**40**	
	Mean±SD	**Mean±SD**	
Serum Interlukin-6 (IL-6)	55.20±28.87	47.76±21.76	**0.436**
Serum LIF	197.96±208.31	157.41±116.67	**0.603**
Serum TNF-α	377.35±361.51	145.41±68.11	**0.073**

Note: (a) Mann Whitney U Test

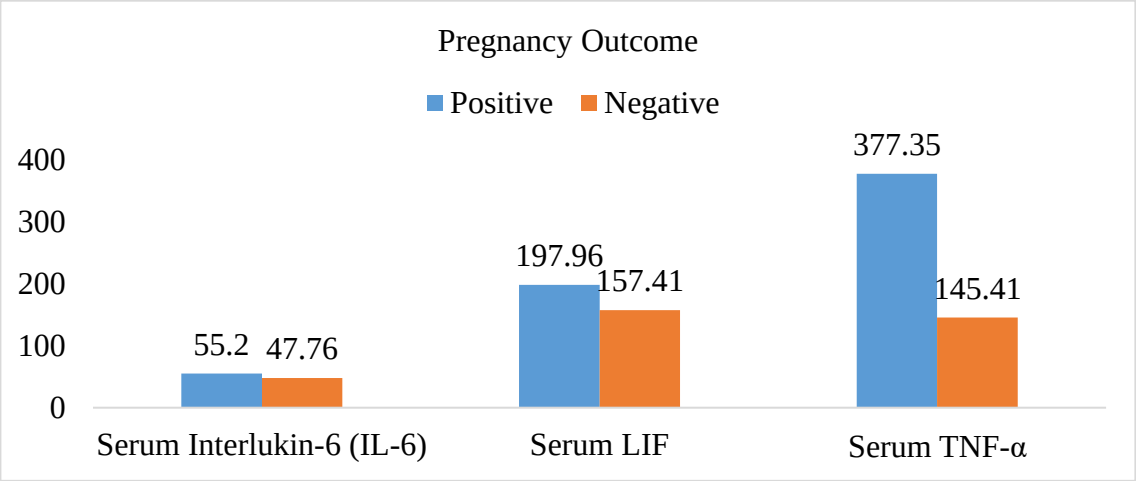

Figure 2: Comparison of Biomarkers in relation to Pregnancy Outcome

Discussion

The findings of this study, which examined the relationship between specific cytokines and IVF success, provide an intriguing insight into the complex interplay of immunological factors in reproductive health. The analysis revealed that although mean levels of Serum IL-6, LIF, and TNF-α were higher in women with positive pregnancy outcomes, these differences were not statistically significant. This discussion will compare our results with past studies to contextualize these findings and explore potential reasons for the observed patterns.

The demographic profile of our participants, including age, BMI, and parity, aligns with other studies examining IVF outcomes. The mean age of our participants was 33.42 years, with most women being nulliparous (95%) and presenting with primary infertility (80%). This demographic is consistent with the population typically seeking IVF treatment, as reported in other studies (16). The BMI of our participants averaged 27.20, slightly higher than the average in some previous studies but reflective of global trends showing increasing BMI among women of reproductive age (17).

Interleukin-6 is a cytokine involved in inflammation and immune regulation, playing a role in embryo implantation and early pregnancy (18). Our study found that mean serum IL-6 levels were higher in women with positive pregnancy outcomes compared to those with negative outcomes (55.20±28.87 vs. 47.76±21.76). However, this difference was not statistically significant (p=0.436). Previous studies have shown mixed results regarding IL-6 levels and IVF success. A study by Omidvar-Mehrabadi *et al*. (2024) found elevated IL-6 levels in follicular fluid to be associated with improved pregnancy rates, suggesting that IL-6 may enhance the implantation process (19). Conversely, other studies have reported no significant correlation between IL-6 levels and pregnancy outcomes, indicating that the role of IL-6 might be context-dependent and influenced by other factors such as the overall inflammatory environment (20).

LIF is crucial for embryo implantation, and its role in reproductive success is well-documented (5). In our study, women with positive pregnancy outcomes had higher mean serum LIF levels than those with negative outcomes (197.96±208.31 vs. 157.41±116.67), although this difference was not statistically significant (p=0.603). Past research has consistently highlighted the importance of LIF in successful implantation. Basatvat *et al*. (2021) demonstrated that higher LIF levels in the endometrium are associated with better implantation rates and successful pregnancies (21). However, serum LIF levels might not fully capture its localized activity in the endometrium, which could explain the lack of significant findings in our study. Additionally, the variability in LIF levels could be due to differences in sample size, study design, and timing of sample collection relative to the IVF cycle.

TNF-α is a pro-inflammatory cytokine involved in various reproductive processes, including ovulation, implantation, and placentation (14). Our study found that mean serum TNF-α levels were higher in women with positive pregnancy outcomes compared to those with negative outcomes (377.35±361.51 vs. 145.41±68.11), approaching statistical significance (p=0.073). The role of TNF-α in reproductive success is complex and somewhat controversial. While some studies suggest that elevated TNF-α levels are associated with adverse pregnancy outcomes such as recurrent miscarriages and implantation failure (22), others indicate that TNF-α may support implantation and placental development under certain conditions (23). The near-significant findings in our study could indicate a threshold effect, where moderate levels of TNF-α are beneficial, but excessively high levels may be detrimental. Further research is needed to delineate these dynamics.

Understanding the roles of cytokines in reproductive success is critical for improving IVF outcomes. While our study did not find statistically significant differences in cytokine levels, the observed trends suggest that IL-6, LIF, and TNF-α may still play important roles in reproductive processes. Clinicians should consider the potential influence of these cytokines when evaluating patients undergoing IVF, and future research should aim to further elucidate these relationships. Future studies could focus on larger, multicenter cohorts to enhance statistical power and generalizability. Additionally, exploring the interplay between cytokines and other biomarkers, as well as the impact of interventions such as dietary supplementation or immune modulation, could provide valuable insights. Understanding the

precise mechanisms through which cytokines influence reproductive success will ultimately help develop targeted therapies to improve IVF outcomes.

Conclusion

This study explored the relationship between specific cytokines—IL-6, LIF, and TNF-α—and IVF outcomes in women undergoing fertility treatment. Our findings indicated that while mean levels of these cytokines were higher in women with positive pregnancy outcomes, the differences were not statistically significant. This suggests that although these cytokines may play a role in reproductive processes, their levels alone are not definitive predictors of IVF success. The demographic characteristics of our study participants were consistent with those typically seen in women seeking IVF treatment, reinforcing the generalizability of our results within this population. Despite the lack of statistical significance, the observed trends align with existing literature, which highlights the complex and context-dependent roles of cytokines in reproductive health. The near-significant findings for TNF-α suggest a potential threshold effect, warranting further investigation. The non-significant differences in IL-6 and LIF levels highlight the need for larger studies with greater power to detect subtle variations. Additionally, methodological considerations such as the timing of sample collection and the focus on localized cytokine activity within reproductive tissues are critical for future research.

References

1. Mansour HAE-h. Infertility diagnosis and management. Beni-Suef University Journal of Basic and Applied Sciences. 2023;12(1):81.
2. Vander Borght M, Wyns C. Fertility and infertility: Definition and epidemiology. Clinical biochemistry. 2018;62:2-10.
3. Araya BM, Aldersey HM, Camara S, Alemu K, Dyer S, Velez MP. The varying estimation of infertility in Ethiopia: the need for a comprehensive definition. BMC Women's Health. 2024;24.
4. Wang Y, Du Y-Y, Yao W, Deng T-R, Guo N, Yin L, *et al*. Associations between phthalate metabolites and cytokines in the follicular fluid of women undergoing in vitro fertilization. Ecotoxicology and Environmental Safety. 2023;267:115616.
5. Pantos K, Grigoriadis S, Maziotis E, Pistola K, Xystra P, Pantou A, *et al*. The role of interleukins in recurrent implantation failure: a comprehensive review of the literature. International journal of molecular sciences. 2022;23(4):2198.
6. Niu Z, Zhou M, Xia L, Zhao S, Zhang A. Uterine cytokine profiles after low-molecular-weight heparin administration are associated with pregnancy outcomes of patients with repeated implantation failure. Frontiers in Endocrinology. 2022;13:1008923.
7. Dong C. Cytokine regulation and function in T cells. Annual review of immunology. 2021;39:51-76.
8. Yang X, Tian Y, Zheng L, Luu T, Kwak-Kim J. The update immune-regulatory role of pro-and anti-inflammatory cytokines in recurrent pregnancy losses. International journal of molecular sciences. 2022;24(1):132.
9. Piekarska K, Dratwa M, Radwan P, Radwan M, Bogunia-Kubik K, Nowak I. Pro-and anti-inflammatory cytokines and growth factors in patients undergoing in vitro fertilization procedure treated with prednisone. Frontiers in Immunology. 2023;14:1250488.
10. Kadivnik M, Plečko D, Kralik K, Arvaj N, Wagner J. Role of IL-6, IL-10 and TNFα Gene Variants in Preterm Birth. Journal of clinical medicine. 2024;13(8):2429.
11. He Y, Li R, Yin J, Yang Z, Wang Y, Chen L, *et al*. Influencing of serum inflammatory factors on IVF/ICSI outcomes among PCOS patients with different BMI. Frontiers in Endocrinology. 2023;14:1204623.
12. Molka B, Gwladys B, Dorian B, Lucie M, Mustapha B, Rosalie C, *et al*. Follicular fluid growth factors and interleukin profiling as potential predictors of IVF outcomes. Frontiers in physiology. 2022;13:859790.

13. Jorgensen MM, de la Puente P. Leukemia inhibitory factor: an important cytokine in pathologies and cancer. Biomolecules. 2022;12(2):217.
14. Adamczak R, Ukleja-Sokołowska N, Lis K, Dubiel M. Function of follicular cytokines: roles played during maturation, development and implantation of embryo. Medicina. 2021;57(11):1251.
15. Zhang D, Yu Y, Duan T, Zhou Q. The role of macrophages in reproductive-related diseases. Heliyon. 2022;8(11).
16. Fouks Y, Vaughan DA, Neuhausser W, Cohen Y, Penzias AS, Sakkas D. Intra-patient analysis of individual weight gain or loss between IVF cycles: cycle now and transfer later. Human Reproduction. 2024;39(1):93-101.
17. Yland J, Carvalho LFP, Beste M, Bailey A, Thomas C, Abrão MS, *et al*. Endometrioma, the follicular fluid inflammatory network and its association with oocyte and embryo characteristics. Reproductive biomedicine online. 2020;40(3):399-408.
18. Vilotić A, Nacka-Aleksić M, Pirković A, Bojić-Trbojević Ž, Dekanski D, Jovanović Krivokuća M. IL-6 and IL-8: an overview of their roles in healthy and pathological pregnancies. International journal of molecular sciences. 2022;23(23):14574.
19. Omidvar-Mehrabadi A, Ebrahimi F, Shahbazi M, Mohammadnia-Afrouzi M. Cytokine and chemokine profiles in women with endometriosis, polycystic ovary syndrome, and unexplained infertility. Cytokine. 2024;178:156588.
20. García-Juárez M, Camacho-Morales A. Defining the role of anti-and pro-inflammatory outcomes of interleukin-6 in mental health. Neuroscience. 2022;492:32-46.
21. Basatvat S, Russell JM, Saare M, Thurston LM, Salumets A, Fazeli A. Potential innate immunity-related markers of endometrial receptivity and recurrent implantation failure (RIF). Reproductive Biology. 2021;21(4):100569.
22. Dai F-f, Hu M, Zhang Y-w, Zhu R-h, Chen L-p, Li Z-d, *et al*. TNF-α/anti-TNF-α drugs and its effect on pregnancy outcomes. Expert Reviews in Molecular Medicine. 2022;24:e26.
23. Salama KM, Alloush MK. Are the cytokines TNF alpha and IL 1Beta early predictors of embryo implantation? Cross sectional study. Journal of Reproductive Immunology. 2020;137:102618.