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Evaluation of seminal plasma IL-17 and IL-25 and its relation with serum anti-sperm antibody among infertile men

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ABSTRACT

The structural elements like the "blood-testis barrier" (BTB) and systemic and local tolerance mechanisms help to maintain testicular homeostasis, which shields germ cells and mature sperms from immune attack. Any damage to this barrier could result in an immune attack and may lead to infertility. This study aims to look at the correlation between male infertility and immunological factors (IL-17, IL-25, and anti-sperm antibody). During the months of October 2021 and July 2022, a total of 144 samples were taken from patients who sought to have infertility tested. The subjects' semen specimens were taken in order to perform the ELISA tests for IL-17, IL-25, and ASA. The levels of ASA, IL-17, and IL-25 differed significantly ($P \leq 0.05$) across study groups, according to the results of immunological parameters.

The results of the correlation analysis revealed a strong positive association between IL-17 and IL-25, but not between those two interleukins and ASA. Although there was theoretically a positive link in this study, it was very weak and not significant. The findings suggest that IL-17, IL-25, and ASA influence seminal fluid parameters, which in turn affect male fertility.

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KEYWORD

Infertility, Male Infertility, Interleukin 17, Interleukin 25,Anti-Sperm Antibody

1. Introduction

According to the "World Health Organization" (WHO), infertility is a condition that affects either the "male or female reproductive system" and is characterized by the inability to conceive naturally following a continuous twelve-month period of unprotected sexual activity. Any health problem that prevents ejaculation (aspermea or oligospermea with a low sperm count or no sperms at all in the sample), anomalous spermatozoa function characteristics, or blockages to ejaculation are all examples of male infertility. Injury, disease, lifestyle choices, and chronic morbidity are just a few of the many factors that lead to the development of this condition (Agarwal et al., 2021).

Since "Philip Rümke in the Netherlands and Leo Wilson in the United States" first discovered the existence of "ASAs in the seminal plasma and blood serum of infertile men" in 1954, many researchers have concentrated on understanding ASA (AS et al, 2019). Since ASA was identified as one of the direct causes of infertility and since infertile men had greater levels and incidence of ASA than fertile men, an immunological form of infertility has drawn more attention in recent years (Lu et al., 2019). There is currently a belief that pathologically enhancing the immune system's auto-reactive reaction to reproductive system antigens (Ags) might result in infertility, and ASAs are an immunological criterion for evaluating men's diminished fertility (Parida, 2019). Immunoglobulins called anti-sperm antibodies bind with spermatozoa ags, causing the spermatozoa to malfunction, which has a significant impact on male fertility (Azizi et al., 2015). It is now impossible to say with certainty that the etiology of ASA has been thoroughly defined, despite the possibility of its significant relevance. ASA may have numerous effects on male fertility. The sperm

motility capacitation or reduction, spermatozoa agglutination, impaired spermatozoa ability to penetrate through cervical mucosa or inhibit spermatozoa capacitation, acrosome reaction, sperm and egg interaction, as well as the early stages of embryo development, are the effects with the best documentation (Al-Daghistani 2020).

In the beginning, it was believed to be produced entirely by "leukocytes", interleukins (IL) are a "type of cytokine" that have since been discovered to be produced by numerous different bodily cells. They are crucial for the "proliferation, maturation, migration, adhesion, and the activation and differentiation of immune cells". They also possess both "pro- and anti-inflammatory" qualities. Therefore, interleukins' main role in inflammatory and immunological responses is to regulate proliferation, differentiation, and activation (Vaillant et al., 2020).

Since it innovated discovery about 30 years ago, "IL-17" has gained prominence as a critical "cytokine" for host defense against "mucosal infections" as well as a significant pathogenic cytokine and therapeutic target in a variety of autoimmune and inflammatory illnesses (Monin and Gaffen, 2018). According to Verma et al. (2017), IL-17 is best known as "a host-defensive cytokine at barrier mucosal tissues". According to McGeachy et al. (2019), IL-17 also promotes inflammation in several autoimmune diseases. Chemokines, inflammatory chemicals, and AMPs are typically produced as a result of IL-17A-mediated downstream pathways. In addition to playing a major role in the activation of innate immune defenses, IL-17A prompts significant effects on host defense, immunological modulation, cell trafficking, and tissue healing (Kim et al., 2023). In order to increase the production of chemokines, such as "granulocyte-colony stimulating factor (G-CSF), chemokine

(C-C motif) ligand CCL-20, CXCL-1, CXCL-2, and finally CXCL-8", IL-17A first stimulates non-hematopoietic cells like epithelial cells before acting alone or in concert with other pro-inflammatory mediators (Fossiez et al., 1996). The significance of IL-17 in male infertility is still being researched, however, it has been linked to several autoimmune and inflammatory disorders, including rheumatoid arthritis, psoriasis, Crohn's disease, and systemic lupus erythematosus (Alamiri, et al., 2021).

By using sequence alignment to compare data from human genomic DNA sequences, IL-25 was first discovered. It is now recognized as a novel proinflammatory cytokine that is activated by nuclear factor B (NF-B) (Lee et al., 2001). Recent research has examined the roles of IL-25 as a "barrier surface" cytokine in epithelial immunology and disorders of the airways (Borowczyk et al., 2020). IL-25 is released from the cell in the form of disulfide-linked dimers, just like other IL-17 family members. To drive downstream signaling cascades in the target cells, the binding receptor for IL-25, IL-17RB, has to associate with IL-17RA (Suto et al., 2018). When compared to other members of the IL-17 family, IL-25 performs a different function from other IL-17 cytokines and shares only a small amount of sequence similarity with IL-17A. For signal transduction, IL-25 binds to its receptor, which is made up of "IL-17 receptor A (IL-17RA) and IL-17 receptor B (IL-17RB)". In the pathophysiology of T-helper 17-dominant illnesses, IL-25 also has a pro-inflammatory impact (Deng et al., 2021).

2. Patients and Methods

A "prospective" study was conducted between October 2021 and July 2022. It involved the collection of 144 subjects' seminal plasma for the measurement of IL-17, IL-25, and serum for the measurement of anti-

sperm antibodies. The seminal plasma was separated by centrifuging the seminal fluid at 3000 rotations per minute (Rpm) for 15 minutes after liquefaction and seminal fluid analysis for all seminal fluid samples, and the "plasma and sperms" were stored at -36°C until analysis.

Immediately following the liquefaction of the semen sample, the concentration and motility of sperms were evaluated together with the other sperm function parameters to limit the detrimental effects of dehydration, pH fluctuations, and temperature variations on motility. In five fields away from the edge of the coverslip, the quantity of sperm and the motility of spermatozoa were counted and recorded automatically. The number of progressive, non-progressive, and immotile spermatozoa on each slide was counted, and the sperms were then divided into three categories based on (WHO, 2010) criteria: progressive, non-progressive, and immotile.

Using an enzyme-linked immunosorbent test (ELISA), the levels of IL-17, IL-25, and serum ASA in seminal plasma were determined.

Statistical Analysis

For the present cross-sectional study, statistical analysis was carried out using GraphPad Prism ver.7 and Microsoft Excel 2016. The presentation of numerical data involved the description of medians and 95th percentiles, while categorical data were expressed as counts and percentages. To compare between two groups, the Mann-Whitney U test was employed. The threshold for the accepted level of statistical significance was set at or below 0.05. Additionally, Spearman correlation analysis was utilized to estimate the correlation between each variable, following the methodology outlined by (Elliott & Woodward in 2007[11]). This method allowed for the examination of potential relationships between different variables in the dataset.

2. Results

The average and standard deviation of IL-17 were (20.16±5.46) for normozoospermic men, (16.92±4.75) for azoospermic men, (23.46±8.4) for oligozoospermic men, and (20.18±6.71) for asthenozoospermic men. The oligozoospermea patients had the greatest amount of IL-17 and were much more affected than the other groups, according to the data, which were significant at ($P<0.05$). As can be seen in Table (1), men with normozoospermea and asthenozoospermea had interleukine levels that were nearly identical to those of azoospermea patients, who had lower levels of IL-17.

The "mean and standard deviation" of IL-25 were (30.8±5.61) in normozoospermic men, (29.16±5.42) in azoospermic men, (31.49±4.15) in oligozoospermic men, and (33.38±6.29) in asthenozoospermic men. Significant differences between the study groups and the control group were present. According to Table (1), it is evident that asthenozoospermic males have the greatest levels of IL-25, whilst azoospermia has the lowest levels.

Men who were asthenozospermic had a mean and standard deviation of (49.7±15.29), "azoospermic men (59.69±12.89), oligozoospermic men (45.14± 14.22)", and normozoospermic men (48.64±13.21). As shown in Table (1), the findings revealed a statistically significant difference between the study groups and the control group ($p<0.05$).

Twenty-three males with ASA levels more than 60 U/ml in serum participated in this investigation; they were divided among the four study groups in the following proportions: five had normozoospermea, or 10.63%, eleven had asthenozoospermea, or 20.75%, three had oligozoospermea, or 9.37%, and four had azoospermea, or 33.33%. The overall percentage of men who tested positive for ASA was

15.97%, with the lowest positive result being 60.03 U/ml and the highest being 92.56 U/ml. The study's findings demonstrated a strong relationship (r=0.38) between IL-17 and IL-25. In contrast, the ASA statistic correlation study was carried out by excluding all men with negative ASA results in order to compare their findings and determine how they related to other study characteristics. The results of serum ASA and seminal plasma immunological parameters showed that ASA and IL-17 and IL-25 did not significantly correlate. While there was no significant association between ASA and sperm concentration, there was a mild negative correlation between ASA and sperm motility, even if the results were not significant. This was demonstrated by the correlation analysis between ASA and sperm function parameters. As can be observed in table (2) and figure (1), there was a non-significant connection between ASA and round cells in the seminal fluid at the same time.

Table (1): comparison of IL-17, IL-25 and ASA in study groups

Study groups	M ± SD		
	IL-17	**IL-25**	**ASA**
Normal	$20.16^{b} \pm 5.46$	$30.8^{c} \pm 5.61$	$48.64^{b} \pm 13.21$
Azo.	$16.92^{c} \pm 4.75$	$29.16^{d} \pm 5.42$	$59.69^{a} \pm 12.89$
Oligo.	$23.46^{a} \pm 8.4$	$31.49^{b} \pm 4.15$	$45.14^{d} \pm 14.22$
Astheno.	$20.18^{b} \pm 6.71$	$33.38^{a} \pm 6.29$	$49.7^{c} \pm 15.29$
f-test	3.35*	2.83*	3.09*
HSD	4.81	4.04	10.28
p-value	0.02	0.04	0.02

B: * (P<0.05), ** (P<0.001)

Table (2): The correlation between ASA and other study parameters

Correlation groups	Correlation coefficient	p-value
IL-17 & IL-25	0.38**	0.001
ASA & IL-17	0.13^{NS}	0.55
ASA & IL-25	0.25^{NS}	0.24
ASA & Concentration	0.17^{NS}	0.43
ASA & grade A Motility	-0.10^{NS}	0.67
ASA & Grade B Motility	-0.22^{NS}	0.31
ASA & Grade C Motility	-0.18^{NS}	0.41
ASA & R-Cells	-0.22^{NS}	0.31
ASA & Anomalies	-0.40^{NS}	0.08

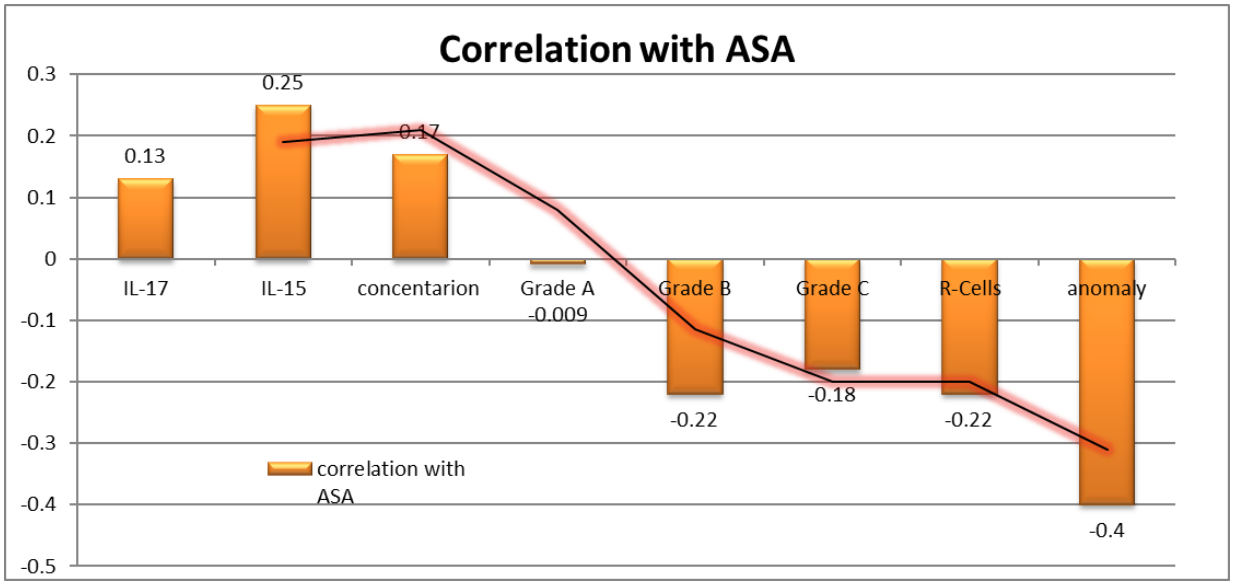

Figure (2): Correlation of ASA with study parameter

4. Discussion

On the day of pickup and the day of embryo transfer, there was a statistically significant difference in endometrial thickness between the pregnant and non-pregnant groups (p-value = 0.003, 0.049). There is a higher chance of getting pregnant when the endometrium is thicker (Kovacs et al., 2003[12]). The thickness of the endometrium during fresh IVF cycles was a better indicator of endometrium receptivity than the thickness of the endometrium during a frozen embryo transfer (FET) cycle (Zhang et al., 2018[13]). The endometrial thickness increased or stayed the same on the day of embryo transfer after progesterone administration compared to that on the day of progesterone administration. However, an endometrial thickness of less than 6 mm was linked to a dramatic decrease in live birth rates in both fresh and frozen embryo transfer cycles (Mahutte et al., 2022[14]). These findings are consistent with previous research demonstrating the important role the endometrium plays in a pregnancy's prognosis.

5- Conclusion

the present study's results are consistent with existing literature, collectively reinforcing the significance of endometrial thickness as a critical factor influencing pregnancy outcomes in assisted reproductive techniques. These findings emphasize the importance of monitoring and optimizing endometrial thickness in the management of infertility treatments.

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FAuthor Contribution

Fahad Oleiwi performed the study and Chateen Pambuk, Mousa Al-Humesh supervised the work .

Conflict of Interest

The authors declare no conflict of interest .

Ethical Clearance

The study was approved by the Ethical Approval Committee.

Financial Disclosure

There is no financial disclosure.

Ethical Clearance

The study was approved by the Ethical Approval Committee

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