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Association between proinflammatory cytokines (Interleukin-6 and Interleukin-17) with risk of male idiopathic infertility

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Abstract

Background: Infertility defines as the inability to get pregnant after 12 months of unprotected sexual activity. In a healthy testis, the spermatogonia cells, sertoli cells, interstitial cells, and immune cells secrete cytokines that act as intracellular signals to control the differentiation and development of hormones that control reproduction, testicular function, germ cells, and spermatogenesis. Male infertility can result from impaired cytokine production, which can impact how the reproductive system works. **Objective**: The present study was conducted to estimation of the potential role of proinflammatory cytokines (Interleukin-6 and Interleukin-17) in male idiopathic infertility. **Methods**: This study was conducted on fifty male infertile patients who were admitted to hospital from the period November /2023 – May/ 2024 and another group consisting of 50 apparently healthy individuals used as controls. **Results:** The results demonstrated significant elevation of Interleukin-6 and Interleukin-17 levels in the patients group (azoospermia and oligospermia) (14.52±6.27; 12.3±5.76); (15.65±5.32; 13.6±4.42) respectively, when compared with those of control group (7.83±0.61; 6.52±0.75), respectively. **Conclusion**: Interleukin-6 and Interleukin-17 can be as diagnostic and prognostic biomarker give more accuracy and precise results.

Keywords: Azoospermia; ELISA, Interleukin-6; Interleukin-17; Idiopathic Male Infertility.

Introduction

After a year of unprotected sexual activity, failure to become pregnant is known as infertility. It affects 15% of married couples on average. About 30% of instances involve men and 35% include women.(1) There is a growing understanding of the role played by men in the etiology of marital infertility. 45–50% of instances of infertility are reported to be caused by men. Out of them, in 30-45%, the cause has not been identified (idiopathic male infertility).(2)

Cytokines play a wide range of biological roles. In a healthy testis, the spermatogonia cells, sertoli cells, interstitial cells, and immune cells secrete cytokines that act as intracellular signals to control the differentiation and development of hormones that control reproduction, testicular function, germ cells, and spermatogenesis. Male infertility results from poor cytokine production, which can harm the reproductive system's operations.(3)

Increased IL-6 concentration in seminal fluid has also been linked to infertility because it slows spermatogenesis and decreases sperm motility(4) IncreasedIL-17A immunoreactive cell numbers and expression levels in chronically inflamed azoospermic testes suggest that overly much IL-17 A expression likely destroys healthy germ cells and spermatogenesis as well as the blood-testis barrier, which could ultimately result in azoospermia.(5)

Methods

Study design and Setting

This study included 50 male infertile patients ranging in age from 20 to 43 years, attended to the infertility and IVF center in Al-Sader City hospital for the period from November/2023–May/2024. Other (50) healthy subjects without any history of systemic disease were included as a control group. Any infertile man with reproductive system diseases, surgery history, diagnosed varicocele, patients having cryptorchidism and testicular tumors or undergoing chemotherapy and radiotherapy were excluded from this study. The sample collection was based on WHO (2010) recommendations. After a period of abstinence spanning from three to five days, each patient was requested to donate semen by masturbating. The semen fluid samples were directly incubated at 37Cº, waiting for complete liquefaction. Seminal fluid parameters were examined immediately after liquefaction and certainly within 1hr of ejaculation. Centrifuging at 1000 g for 10 minutes was done on the semen to separate the seminal plasma which was stored in the plane tube at -20 Cº, until used for immunological analysis.

Estimating of IL-6 and IL-17 concentration in seminal plasma

In accordance with the guidelines provided by the company, the reagents were prepared, and the test was performed.

Assay principle

Utilizing sandwich enzyme immunoassay in quantitative form method. A pre-coated microplate contains an antibody that is specific for IL-6 and IL-17. After samples and standards are injected into the well, the fixed antibodies absorbs all IL-6 and IL-17 that is available. A well is injected with an IL-6 and IL-17- specific biotin-conjugated antibody after any unbound compounds have been removed. After cleaning, the wells are filled with avidin-conjugated horseradish peroxidase (HRP). A substrate solution is then introduced to the wells after rinsing to eliminate any unattached avidin-enzyme reactant. Color then emerges in accordance to how much IL-6 and IL-17 were initially bound. A measurement of the color's intensity is done after the color development has ceased.

Statistical analysis

Data are either normally distributed or not normally distributed and recorded in a Microsoft excel spreadsheet, statistical research was done using the SPSS v 0.26 program. (chi-square, independent sample T-test, Spearman and Pearson correlation coefficient) after translated data into codes. The results were analyzed and assessed using appropriate statistical methods. When a less than 0.05 of a P value, the significance level is considered.

Results

Both the controls and the patients had a similar age range. The current study showed that the majority of patients (50%) belonged to the age group (less than 30) years and ages 30 to 39 made up 34% of the total patients.

Spermatozoa count, and their morphology and motility

The microscopic semen analysis indicated significant variations in semen parameters between study groups as shown in table (1).The mean sperm count in the fertile control group was ($37.43x10^6 \pm 2.11$) this was higher by a substantial amount ($p<0.05$) than the sperm count of all patient subgroups, while the mean sperm count in the oligospermia group was ($11.23\ x10^6 \pm 0.62$) this was higher by a substantial amount ($p<0.05$) than azoospermia. While the mean of sperm count in the azoospermia group was (0.0 ±0.0) which was substantial ($p<0.05$) lesser than of other groups.

Mean of progressive motility in control was (65.33±2.26) which was considerably greater than of other patients subgroups ($p<0.05$), while in (oligospermia and azoospermia) was (27.4 ± 2.1; 0.0±0.0, respectively) due to severe decline in sperm motility in patients groups. In contrast, the immotile spermatozoa in control and oligospermia (31.2±3.1 and 57.6 ± 3.2, respectively), were significantly increased in the patients and the difference between these two means was significant (P 0.05). In oligospermia patients, abnormal shape percentage of spermatozoa were considerably ($P \leq 0.05$) greater than the noticed percentage in controls (Table 1).

Table (1). Spermatozoa count, and their morphology and motility (percentage) in infertile patients (azoospermia and oligospermia) and controls.

Variables Groups	No.	Sperm Count ($x\ 10^6$) Mean ± S.E	Normal forms (%) Mean ± S.E	Motility (Mean ± S.E. %)	
				Progressive	Immotile
Azoospermia	25	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
Oligospermia	25	11.23 ±0.62	3.4± 1.8	27.4 ± 2.1	57.6 ± 3.2
Control	50	37.43±2.11	10.3± 1.4	65.33 ±2.26	31.2 ± 3.1

The amount of IL-6 present in seminal fluid among infertile and control males

The result demonstrated significant elevation of IL-6 level in the patients group (azoospermia and oligospermia) (14.52±6.27),(12.3±5.76) (Mean±SD), respectively, when compared with those of control group (7.83±0.61), as depicted in tables (2, 3).

Table (2). Seminal fluid Interluikin-6 concentration in azoospermic patients & control.

IL-6 concentration (pg/ml)	Cases – control comparison		*P value*
	Azoospermia	Healthy Control	
Range	12.5-26.8	5.8-11.2	0.03
Mean	14.52	7.83	
SD	6.27	0.61	
N	25	50	

Table (3). Seminal fluid level of IL-6 in control and oligospermic patients.

IL-6 level (pg/ml)	Cases – control comparison		*P value*
	Oligospermia	Healthy Control	
Range	7.9-29.2	5.8-11.2	0.04
Mean	12.3	7.83	
SD	5.76	0.61	
N	25	50	

The difference between infertile and control men' seminal IL-17 levels.

The findings of the present study revealed a considerable rise in IL-17 levels in the patients group (azoospermia and oligospermia) (15.65±5.32), (13.6±4.42) (mean±SD), respectively, when compared with those of control group (6.52±0.75), as depicted in tables (4), (5).

Table (4): Seminal fluid level of IL-17 in control and azoospermic patients.

Serum IL-17 pg/ml	Cases – control comparison		P value
	Cases(Azoospermia)	Healthy Control	
Range	13.2-27.4	6.3-12.1	0.02
Mean	15.65	6.52	
SD	5.32	0.75	
N	25	50	

Table (5): Seminal fluid level of IL-17 in control and oligospermic patients.

Serum IL-17 pg/ml	Cases – control comparison		P value
	Cases(Oligospermia)	Healthy Control	
Range	8.7-26.3	6.3-12.1	0.03
Mean	13.6	6.52	
SD	4.42	0.75	

N	**25**	**50**	

Discussion

Spermatozoa count, and their morphology and motility

Mahdi *et al*., (2021)[6], who found that when oligospermia was contrasted with normospermic number for each ml (51.30x1061.24), aberrant motility (22.815.8), mobility (22.815.8), quantity (3.342.31), irregular shape (25.8612.4) or regular shape (V) (p = 0.0001), there was a substantial difference (p = 0.0001).

Butt *et al*., (2013)[7], found that in comparison to normospermia, where irregular shape was 35%14.5% as well as non-motile spermatozoa percentages were 43%18%, oligospermic specimens exhibited considerably higher irregular shape (55%15.6%) and percentages of non-motile spermatozoa (62%23.9%) (p 0.0001). In terms of volume, oligospermia had a mean amount of 2.052.0ml while normospermia had a mean amount of 2.971.35ml (p 0.0001). The volume, motility and morphology go with the findings of Al-Daghistani *et al*., (2010)[8], but different in sperm count, who examined 50 fertile control males and 82 infertile patient cases found no significant change in seminal fluid volume and sperm count also revealed significant differences in motility and morphology.

Menkveld *et al*. (2011)[9] have recently challenged the fact that measuring or evaluating human sperm cells shape and its clinical importance has been and continues to be a contentious part in the semen examination for determining a male reproductive ability. However, they also emphasized that sperm shape assessment still has a significant function to show in the medical assessment of men reproductive possible. Such objection is justified by the fact that the criteria for sperm shape estimation are defined and used in a stringent manner. Additionally, the proportion evaluation provides more useful information in this regard, and defective spermatozoa morphology may affect fertility in the infertile patient group (oligospermia).

The amount of IL-6 present in seminal fluid among infertile and control males

The combination, interaction, and functioning of several types of cells in the testis during germ cell development are necessary for male fertility. Furthermore, it is closely regulated by cytokines, and men with hypo-fertility have been discovered to have dysregulated levels of these cytokines in their seminal plasma. Male genital tract immune cells, including dendritic cells, lymphocytes macrophages, and monocytes produce cytokines in response to infections and foreign antigens during chronic inflammation.[10] The current study agree with results Alsaimary (2014)[11], researchers discovered that there were extremely significant statistically variations among the two groups (P <0001), and that the mean amount of IL-6 was higher in the infertile males serum, reaching 328.75 pg/ml as opposed to fertile males, which achieved 116.24 pg/ml.

A previous study done by Seshadri *et al*., (2009)[12], who found that as contrast to the normospermic group, the oligospermia, asthenospermia, oligoasthenospermia, and germ cell damage groups all had substantially higher quantities of IL-6 (P= 0.05).According to Camejo *et al*. (2011)[13] infertile males had greater IL-6 levels in their seminal fluid than fertile men, whereas TNF- levels were similar in both study groups.

On the one hand, elevated levels of IL-6 have seen in infertile men with oligo-asthenoteratozoospermia, but on the other hand, there is no correlation among levels of cytokines and

sperm quality. The relationship among IL-6 cytokine levels and semen quality is still up for discussion.[10] The fact that a number of cytokines, such as TNF-α, IL-6, and IL-1 are cyclically generated by the spermatogenic cells or Sertoli throughout the development stages of the seminiferous epithelium suggests that their activities are essential for regulating this fundamental feature of testicular functioning.[14] The generation of transferrin by Sertoli cells is slowed down by elevated IL-6 levels. Iron is one of the key components that drives spermatocyte and spermatid division, and transferrin is responsible for moving Iron past the blood-testis barrier. This may result in a reduction in the number of sperm.[15]

The difference between infertile and control men' seminal IL-17 levels

A proinflammatory cytokine called interleukin-17 (IL-17) appears to have a role in maintaining spermatogenesis and testicular immunity. When compared to controls, IL-17 levels in instances of infertility were considerably higher ($p = 0.041$ and $p = 0.046$, respectively). This is in agreement with the results of the current study. Moreover, the substantial relationship between IL-17 and sperm shape suggests that inflammation might change the structure of sperm, limiting fertilization.[16]

AL-Msaid *et al*., (2018)[17], who found that there was a negative association among IL- 17 levels and the sperm progressive motility percentage, sperms quantity and normal shape of sperm detected in idiopathic infertile males ($r = -0.584$,$r= -0.589$ and $r = -0.544$, respectively). Previous study done by Sabbaghi *et al*., (2014)[18], showed that neither the concentration of IL-17A in seminal plasma nor in serum was significantly different in patients with astenozoospermia, oligozoospermia and azoospermia compared to the control group ($p<0.05$).

The finding of Alamiri *et al.* (2021)[19], who recognized likewise sperm quantity and seminal plasma IL-17 concentration were not significantly correlated, azoospermic individuals had the greatest average seminal plasma IL-17 concentration (2.892.27ng/l). Moreover, sperm survival and seminal plasma IL-17 concentrations did not significantly correlate with one another ($P = 0.246$ and 0.898, respectively).It is believed that IL-17 may reduce sperm motility by increasing oxidative stress in seminal plasma, which is brought on by several cytokines.[20]

Conclusions

A high significant variation and an increase in IL-6 and IL-17 concentrations were seen in the patient group comparison to the control group. Which may indicate that these cytokines can play a role in the causation of infertility.

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