



The Immunological Role of Anti-Sperm Interleukin-6 Antibody in Primary Male Infertility

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Abstract: Infertility is a multifactorial condition in which genetic factors such as chromosomal and single gene alterations account for 20-30% of the cases. It can be classified as mild (between 10-15 million sperm per ml), The total number of samples is one hundred samples (100) divided as follows in fifty (50) Iraqi infertility patients and fifty (50) apparently healthy subjects. Blood samples were collected from Al-Ansja Laboratory. The percentage of sperm concentration was significantly ($p \leq 0.01$) lower in oligozoospermic patients than in apparently healthy subjects. Serum interleukin-6 (IL-6) levels significantly ($p \leq 0.01$) increased fifty folds in infertility patients when compared with apparently healthy subjects. It was concluded anti-sperm antibody levels in seminal plasma were significantly ($p \leq 0.01$) increased in oligozoospermic patients when compared with apparently healthy subjects.

Keywords: IL-6, antibody antisperm, male infertility.

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Introduction

Infertility: A disease (an interruption, cessation, or disorder of body functions, systems, or organs) of the reproductive tract which prevents the conception of a child or the ability to carry a pregnancy to delivery. It is defined by the failure to achieve a successful pregnancy after 12 months or more of appropriate, timed unprotected intercourse or therapeutic donor insemination. Earlier evaluation and treatment may be justified based on medical history and physical findings and is warranted after 6 months for women age 35 years or older (1).

Infertility, a male or female reproductive system disease, is defined by the failure to conceive within one year of regular, unprotected sexual intercourse. World Health Organization

(WHO) statistics reveal that infertility affects 50–80 million people worldwide and male factors account for approximately 20–30% of all infertility cases (2).

Male factors are implicated as the cause of roughly half of cases of infertility, and the presence of antisperm antibodies (ASA), (IL6) may be responsible for some of these. Their presence is associated with a reduction in natural conception and live birth and impacts the success of assisted reproductive technologies (3).

Materials and methods

This study has been conducted on a group of patients at Baghdad of Iraq during the period from September 2021 to September 2022. A total number of seventy (100) males, sixty (60) with

primary infertility of age range (22-49) years and compared with thirty (40) apparently healthy fertile males of age range (27-50) years as control group.

Sample collection and storage collection of blood samples

Blood samples (5 ml) were obtained by vein puncture using a sterile disposable syringe from patients and the control group. The blood sample was dispensed in a gel tube and left for 10 minutes at room temperature to clot. Then, it was centrifuged at 3000 rpm for 10 minutes to collect serum and kept in the refrigerator (-20 °C) until use unless used immediately to analyze the immunological parameters that has been detected in this study.

Assay procedure

Amount of 50µl substrate solutions A was added to each well and then add 50µl substrate solution B to each well. Incubated the plate, covered with a new sealer for 10 minutes at 37 °C in the dark. After that 50µl of stop solution was added to each well, the blue color will change into yellow immediately. The optical density (OD value) was determined of each well immediately using a microplate reader set to 450nm within 10 minutes after adding the stop solution.

Data management and analysis

Statistical Analysis: The Statistical Analysis System- SAS (2018) program was used to detect the effect of difference factors in study parameters. T-test was used to significant compare between means. Chi-square test was used to significant compare between percentage (0.05 and 0.01) probability.

Result and discussion

Seminal plasma ASA concentration

The results showed an increase in the concentration of seminal plasma ASA in infertility in comparison with the control groups with high significant differences ($p \leq 0.01$). This concentration of ASAs indicated that one cause of infertility was immunological; the main cause of immunological infertility is the formation of antisperm antibodies (ASA) which affect the capability of fertilization of spermatozoa (5).

Infertility can result from antibodies either directly binding to sperm or affecting the spermatogenesis due to allergic orchitis. ASA can interfere with sperms motility by immobilizing the sperm, interfering with sperm- mucus interaction or disturbing sperm transport. Sperm antibodies are considered to be the cause of infertility (6).

In the current study, 19 of 40 patients were found to be positive for ASA. Positive ASA of seminal plasma samples showed a decrease in motility, concentration, and morphology of sperm, the immune response created by ASA was associated with obvious changes in the standard parameters and alteration of the quality of semen which agree with study of AL -yasiri (2018) who found that, there were 56 cases out of 100 cases having the ASA level positive it seems that most of the ASA (Ve+) cases were oligospermia compared to ASA (Ve-) and the control group. This means that ASAs develops when the patient's own immune system identifies the sperm cell as a result of a disruption in its environment because sperm-specific antigens are not present during the development of immunological tolerance. Therefore

generate antisperm antibodies, which may decrease the motility and inhibit their transport and fertilization of an ovum and ultimately interfere with their function (Ulcova, 2006), also, in this study, a correlation was observed with sperm agglutination and ASA (7,8). Where they clarified in both their researches a high percentage of agglutination in the ASA positive cases these results demonstrated in table (1).

Show a significant increase in the concentration of IL-6 in primary infertile male groups in comparison with the control group with a high significant ($p \leq 0.01$). With also increase. The Antisperm Antibody mean levels in infertile patients.

Interleukin 6 (IL-6) concentration

The results of this part of the study revealed a high level of mean of IL-6

causes high significant ($p \leq 0.01$). Whereas there are 9 of infertile males with primary infertility with high mean level of IL-6 which reach to (51.96 pg/ml). These results indicated that the elevation of IL-6 maybe have a crucial in immunological infertility, the main cause of immunological infertility is the elevated levels of these cytokines (IL-6) which lead to negatively effect on the endocrine glands later and thus to decrease the hormones released by these glands (mainly LH and FSH) which regulate the spermatogenesis process (4). IL-6 is a pro-inflammatory cytokines which could directly affect the cells of the testis, and thus also effect on the spermatogenesis. On the other hand, these pro-inflammatory cytokines could directly effect on differentiated spermatogonia and thus to affect its functions or viability (5).

Table (1): Comparison between control and patients group in IL-6 and antibody anti-sperm.

Group	Mean \pm SE	
	IL-6 ()	Antibody anti-sperm
Control	1.46 \pm 0.19	31.25 \pm 1.14
Patients	6.60 \pm 0.20	45.66 \pm 0.46
T-test	0.568 **	2.162 **
P-value	0.0001	0.0001
** (P \leq 0.01).		

Conclusion

The elevation of Anti-sperm antibody (ASA) level more than 60U/ml in some cases in primary infertile males can consider as indicator of immunological primary infertility in these cases and the elevation of IL-6 levels over 4.18 pg/ml can consider as a feature of primary males infertility in certain cases.

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