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Evaluation of Circulation Levels of ASA and IL-6 in Infertile Men

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ABSTRACT
The elevated levels of ASA and IL-6 antibodies in semen have a fundamental role in immune sterility, so the current study was designed to compare the blood serum levels of these markers between infertile and healthy men. Serum levels of ASA and IL-6, of 46 infertile and 44 fertile males were assessed using ELISA test (standard curve). The findings showed that mean serum IL-6 and ASA levels were significantly higher in infertile male compared with control (107.4 pg/ml, and 51.4 U/ml, VS 8.3 pg/ml, 11.50 U/ml at P≤ 0.01). According to the types of infertility the result also showed there was a significant statistically difference in the level of IL-6, and ASA within comparted groups. These data concluded that the significant increase in serum ASA and IL-6, compared to the lower levels in the control, indicating that these markers may be implicated in the pathogenesis of male infertility.
Key words: IL-6, ASA, Male infertility.

Introduction
The infertility of Male is a multi-cause syndrome that includes a wide sets of disorders. More than 50% of male with infertility have idiopathic or unknown infertility, infertility cause may be congenital or acquired. Many factors are involved in infertility, including genetic, immune, imbalance in hormonal levels as well as environmental factors [1].
There are some immunological factors that are expected to be involved in promoting male infertility. The role of Anti-sperm antibodies (ASAs) and cytokines in infertility was emphasized, and their role in semen quality was questioned [2-3].
Sperm antibody (ASA) may interfere with different functions of sperm such as stimulating the reaction of acrosome prematurity and stimulating apoptosis. ASA may also impede fertilization by inhibiting penetration of cervical mucus, the fusion of spermatozoa. Ultimately these changes lead to lower pregnancy rates. The immune response to both humoral and cellular arms may be implicated in the etiology of unexplained male infertility (UMI) [4]
Cytokines account part of the autocrine / paracrine network that operates in the male reproductive system. Immune reactions induced through cytokines have beneficial and / or risky effects on male fertility. Since cytokines are released by a whole group of cells in all parts of the male reproductive system, they can also participate in various male disorders [5]. IL-6 represents a pro and anti-inflammatory cytokine which has multiple functions such as inflammation and enhancement fever in addition to regulate immunity and hematopoiesis [6]. IL-6 Produced by Sertoli may be also needed to regulate local lymphocyte functions important for immunological protection of the tissue [2]. The action of IL-6 in the testis is to low the secretion of transferrin in Sertoli cells. In addition, IL-6 forbids spermatogenesis and lowes sperm motility, and its increased level in seminal plasma has been reported linked with infertility [7]. Also, previous data indicated IL-6 have an influence on the function of sperm that possibly mediated via an increase in nitric oxide product [8].
The study was designed to compare the circulation serum levels of ASA and IL-6 between infertile and healthy men to detect the crucial role of these markers in pathogenicity of immune infertility.

Materials and Methods
This study has been conducted on a group of patients at Al-Hussein Teaching Hospital / who were referring to the Infertility Unit / Thi-Qar province / Iraq during the period from Oct. 2019 to March 2020. Sera samples from (46) male patients with infertility, and (44) healthy male as a control involved in this study. The cases were diagnosed clinically by specialist consultant at Al-hussein Teaching Hospital, blood samples were centrifuged at 1500 rpm, for 5 minutes, then the serum was frozen at -20ºC until the (IL-6), (ASA) measurement by ELISA(standard curve). The concentrations were quantitatively determined in sera of patients and healthy control subjects by means of

ELISA (Enzyme Linked Immunosorbent Assay) using ready kits manufactured by Bioassay company (China).

To conduct the research ethical permission was obtained from the hospital and from all participants in this work patients and healthy. The patients Selection were accomplished with the assistance of Physicians in the hospitals.

Results and Discussion

Table.1 illustrated the numbers and percentages of the studied infertile male and healthy male according to age stages. The age group 29-36 yrs. was predominant in infertile male. The results relatively agreed with Abdulmohsin (2018) data who founded that most infertile male were in the age range at age group (20–30) years [9]. Also, Geidam and his colleague found that 76% of the patients with infertility were in the age group 25–40 years [10] .

Table (1): Frequency of infertile patients and control regarding to age groups.

Age groups(years)	Patients		Healthy	
	No.	%	No.	%
21-28	14	30.4%	11	25 %
29-36	23	50 %	21	47.7 %
>36	9	19.6%	12	27.3 %
Total	46	100%	44	100%
Age Mean + SD = 31.1 ± 5.3. Max= 48 Min = 22				

The present data demonstrated that there was decrease in mean of sperm volume among patients age groups with no significant ،p>0.05, Whereas there was a significant reduce in sperm concentration means, and total sperm concentration among patients age groups Table (2). Also, the current finding indicated that there was an significant decrease in motility with ageing Table (3).

It is believed that the causes of reduced sperm volume and movement in infertile patients may be due to varicocele in the veins that empty the blood from the testicle, and it is also necessary to keep the testicular temperature below about two degrees from the rest of the body. When blockage occurs in these veins, the temperature of the testicle rises, which impairs the ability of sperm to move as well as their production. [11,12].

Table 2: Semen volume and concentration for patient

Parameters / P Groups	Cases Number	S. Volume M+SD	Semen C*106/ml M+SD	TSC *106 M+SD
21-28 years	14	1.96 ± 0.6	27.4 ± 5.2	51.8 ± 13.3
29-36 years	23	1.72 ± 0.5	18.7 ± 4.5	26.1 ± 6.45
Above 36 years	9	1.42 ± 0.3	12.0 ± 2.8	17.8 ± 2.26
P. Value		0.09	> 0.001	> 0.001
LSD		Non-Sig	6.29	8.23

Table 3: Percentage of grads motility according to age groups

Parameters / P Groups	No. Cases	MOT A M+SD	MOT B M+SD	MOT C M+SD	MOT D M+SD	Total MOT
21-28 years	14	5.07 ± 0.7	30.4 ± 6.7	9.64 ± 2.4	55.3 ± 8.4	44.6 ± 7.2
29-36 years	23	1.52 ± 0.4	23.2 ± 5.8	10.5 ± 2.9	48.8 ± 10	35.2 ± 9.0
Above 36 years	9	1.71 ± 0.6	12.1 ± 3.4	3.57 ±1.3	39.2 ± 4.5	18.5 ± 4.7
P. Value		> 0.0001	0.0010	> 0.0001	0.0011	> 0.0001
LSD		0.77	9.18	2.27	8.37	7.51

Also, the current findings recorded the various kinds of infertility, where asthenozoospermia was found with the highest number of patients, 23 men (50%) followed by Oligozoospermia 14 men (30.4%), and azoospermia 9 men (19.6%) with statistical difference at P<0.05. Asaimary et al. (2014) found also various types of infertility but Oligozoospermia was found in the highest number of patients 117 men (36%), and azoospermia men in the least number 41 (12%) with statistical differences among various types of infertility P<0.05 [13].

Serum levels of IL-6 and ASA:

The current finding demonstrate the high mean level for interleukin -6, and ASA concentration in serum of infertile male that reached to (107.4 pg/ml, 51.4 U/ml) in comparison with control (healthy fertile male) which was (8.3pg/ml, 11.50U/ml) with statistical differences between them at P≤ 0.01,The normal value for ASA is up to of 60 U/ml, as 13 patients were higher than this rate out of a total of 46 patients, at a rate of 28.2%, while the control group was all below 60 U / ml, Figure 1. illustrates this. Figure 2 .The findings regarding to IL-6 compatible with Alsaimary (2014) who referred that the concentration mean level of interleukin -6 was increased in infertile men sera comparison with control (fertile men) with very highly significant differences between groups [13]. Also, this data consistent with the fact that IL-6 forbid spermatogenesis and reduces sperm motility, as its increased level in seminal plasma has been reported to be associated with sterility [7]. The present result is also in line with the Al-Fahham and colleagues who found a relatively high incidence of ASA serum cases has been reported among infertile subjects [14].

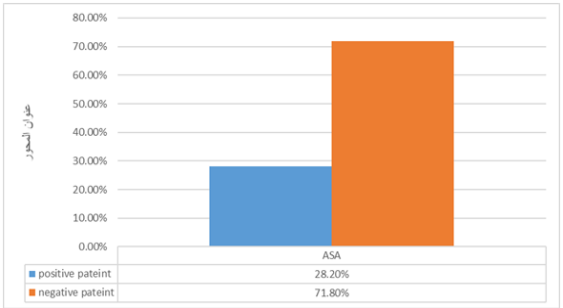

Figure (1) levels percentage ASA in infertile patients.

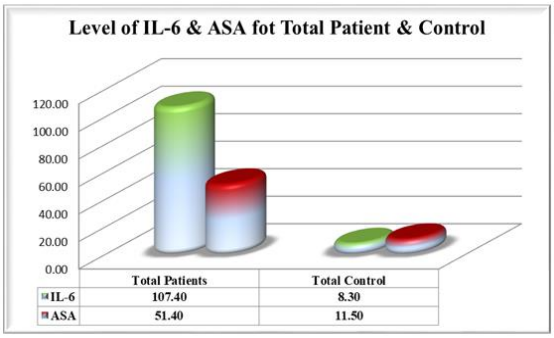

Figure (2) Serum levels of IL-6 and ASA in infertile patients and healthy.

Table 4: Level of IL-6 according to type of infertility.

Parameters Infertility Type	No.	IL-6 Mean +SD	P Value = <0.001
Primary	36	99.57 ± 23.2	
Secondary	10	115.3 ± 48.8	LSD =15.0
Control	44	8.263 ± 2.0	

According to types of infertility the result also showed there was a significant statistically difference in the level of IL-6 , and ASA within comparted groups. Where secondary infertility group recorded an increase level of IL-6 ,and ASA followed by primary groups compared with a decrease in the level of them in the control group Table 4 and Table 5 . previous data that indicated by Khatoon *et al*. (2011) appeared there is no statistical difference in the circulating antisperm incidence between primary and secondary infertility patients groups [15] .

The role of ASAs in infertility remains ambiguous. Despite its role in males, its clinical significance remains a matter of debate, and the finding of human and animal experiments confirm that ASAs can affect male fertility, in several ways including affecting sperm transfer, sperm condensation, particle interaction, and the interaction between sperm and egg. And, finally, a possible systemic effect is the presence of ASAs whereby only the generalization of immune activation and fusion with the egg membrane is reflected [16].

Table 5: Level of ASA according to type of infertility.

Parameters Infertility Type	No.	ASA M +SD	P. Value = < 0.001
Primary	36	45.2 ± 7.45	
Secondary	10	57.6 ± 11.9	LSD = 6.82
Control	44	11.5 ± 3.27	

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تقييم مستويات الدورة الدموية لـ ASA و IL-6 عند الرجال المصابين بالعقم

الملخص

أظهرت النتائج أن متوسط مستويات IL-6 و ASA كانت أعلى بشكل ملحوظ في الذكور المصابين بالعقم مقارنة بالمجموعة الضابطه 107.4).
pg/ml, and 51.4 U/ml, VS 8.3 pg/ml, 11.50 U/ml at P ≤ 0.01) وفقًا لأنواع العقم ، أظهرت النتيجة أيضًا وجود فرق معنوي إحصائيًا في مستوى IL-6 و ASA داخل المجموعات المجزأة. وجدت هذه البيانات أن الزيادة الكبيرة في ASA و IL-6في المصل الدم، مقارنة بالمستويات الأقل في المجموعة الضابطة، اذ نلاحظ ارتفاعها عند المرضى من العقم الثانوي وكذلك لها دور عند مرضى المصابين بالدوالي مما يشير إلى أن هذه العلامات قد تكون لها دور في التسبب في عقم الذكور.