Effect of Serum and Seminal Plasma of Anti-sperm Antibodies, IL6 and IL8 on semen parameters in a sample of Iraqi Males with infertility

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ABSTRACT: This study aimed to investigate the effects of serum and seminal plasma of antisperm antibodiess (ASAs) and cytokines (IL6, IL8) on the seminogram of male with infertility.

One hundred and ten male individuals (70 infertile males and 40 healthy fertile males as a control group) with primary and secondary infertility attending the Fertility Center/ Al -Hussein Teaching Hospital in Thiqar governorate " south of Iraq) from August 2015 to February 2016 were included in the study.

Out of 70 infertile males 53(75.7%) showed primary infertility while those presented with secondary infertility were 17(24.3%). Asthenospermia showed highest percentage (35.8%) within the class of primary infertility, while secondary infertility showed the highest percentage (47.1%) with the type oligospermia, but the differences were not statistically significant. Zero and < 40% formed 58 cases out of 70 samples which represent 83%, which mean that the majority of spermatozoa in the samples were immobile.

Regarding the mean concentration of antisperm antibodies in the sera of infertile males, it was 26.2307pg/ml. which was considered of no significance (negative value), the seminal plasma mean concentration of ASAs was 990.9203 U/ml. There were highly significant differences between the mean concentrations of serum and seminal ASAs of males with infertility.

The mean concentrations level of serum cytokines IL6 and IL8 were measured in both study group and control. IL6 mean serum concentration was higher in study group than control 91.5708 pg/ml and17.4359pg/ml respectively. The differences between patients and control were statistically significant (P value 0.00). Serum levels of IL8 showed no differences between patients (20.8299 pg/ml) and the control group (17.7692 pg/ml)(P value 0.346).

The mean concentration of seminal plasma of IL6 among infertile men was 21.2413pg/ml, while the control group showed 4.6433pg/ml. The difference was highly significant (P value 0.00). there was obvious effect of cytokines and ASAs on the motility, number and the type of spermia .

INTRODUCTION

Infertility is defined as" inability to achieve a recognized pregnancy after one year of unprotected intercourse"[(1)] .

Global prevalence of infertility is unknown because there are no reliable proper registration Infertility differ across the world's regions in a

percentage 8–12% of couples worldwide . A male part is mostly responsible for infertility in around 20% and contributory in another 30–40% of couples; in that limit, a male component is included in more than fifty percent of the couples [2] . Since the degree of both quality and quantity of human semen ranges over a wide scale in various geographical points, it leads to the belief of the presence of certain factors which are confined to a specific geographical landmark and is thought to be the reason of this problem[3].

World Health Organization stated normal values that should be considered for the men to be fertile. Sperm parameters below these values are confirmed as male factor infertility[4] . According to these values, sperm concentration is classified in to "oligospermia " for those sperm concentration is < 20 million/ml , "asthenospermia" for sperms with poor motility and "teratospermia" for sperms that show abnormal morphology.

Antisperm antibodies have been detected from various secretions of the body including the semen, secretion produced by the cervix, besides its determination in blood serum of males and females[5]. There are various mechanisms responsible for the formation of antibodies against the sperms, including trauma to the testes of any kind (biological, surgical intervention and others). The harmful effects of the antisperm antibodies are mainly, reduction in the motility of the sperm , inhibition of penetration property and lastly stop the acrosomal reaction and thus interfere with its ability to fertilize the ovum[6] . Proinflammatory cytokines play an important role in inflammatory processes of the male genital tract during urogenital infections [7] .

Maegawa et al. reported that there is accurate arrangement between the function of the hormones mainly "gonadotropins and testosterone" with IL6 favoring the development of the "testis" and "spermatogenesis" [8] Excess of IL-6 has been reported to decrease sperm motility in vitro; this effect seems to be due to nitric oxide overproduction[9] . The acrosome reaction in normal spermatozoa is

inhibited by IL-6 resembling IL-1and tumour necrosis factor-alpha (TNFa) of such effect[10]

Interleukin 8 (IL8) is produced by various cells including the macrophages which are the main source of IL8. Smooth muscle cells of the airway, tumor cells, epithelial and mesothelial[11] and cells with toll-like receptors [12] are responsible for production of IL8.

Materials And Methods

A case -control study involved one hundred and ten male individuals attending Fertility Center / Al -Hussein Teaching Hospital in Thiqar governorate "south of Iraq" from August 2015 to February 2016, consisting of 70 infertile patients and 40 healthy control , aged between 19-55 years. All patients submitted full reports regarding their sexual and medical status history, in addition to their infertile status (primary / secondary) infertility.

Serological test

A total 5ml of blood was obtained by vein puncture from all participants (patients and controls), serum was separated by centrifugation and stored under (-20C^{o}) until analyzed for cytokines levels and antisperms antibodies.

Seminal collection

Before semen analysis, the men were perceived with instructions of the study and requested to abstain from sexual intercourse for at least 48 hours, but no longer than 5 days, before attending the clinic. Patients were instructed to urinate prior to semen sampling to ensure minimum amount of debris in the sample and reduce the number of leukocytes[13] . For semen storage and preservation, containers which were used, showed no cytotoxic activity, approved for preservation of spermatozoa produced by humans and that was according to WHO (2010) standards. Participants were instructed to avoid inner contamination of container. The semen was left for approximately 30 minutes at 37° to liquefy and be ready for examination.

The semen examination was performed manually in accordance with the (WHO standards 2010). Seminal plasma samples were collected from completely liquefied semen and were centrifuged at 1000×g for 20 minutes. Then the samples were frozen in -20 centigrade to prepare them for evaluation of the levels of (IL 6, IL 17) and anti- sperm antibodies .

Evaluation Of The Cytokines and antisperm antibodies Levels:

Enzyme linked technology (immunosorbent assay kits) provided by **Elabscience Biotechnology Co.,Ltd,** were utilized to detect the level of the cytokines in serum and semen, while antisperm antibodies (ASAs) kits were provided by **(Demeditec Diagnostics GmbH and DRG Diagnostics)** to detect the level of antisperm antibodies in seminal plasma and serum respectively. The samplers were examined according to the manufacturer's instructions.

Statistical analysis

The data were analyzed using descriptive statistics (mean and standard deviation), Chi-square (χ^2) , Independency sample T test and Fisher test. The level of significance was set at $P< 0.05$. SPSS (Statistical Package for Social Sciences) version 20.

RESULTS

Classification of infertility

Infertility was classified into primary and secondary infertility. In table1 primary infertility showed higher percentage **(75.7%)** while only **(24.3%)** for secondary infertility.

Table 1:Distribution of infertility according to types

Types of infertility	Frequency	Percent
Primary infertility	**53**	**75.7%**
Secondary infertility	17	24.3%
Total	70	100.0%

Types of infertility	**Types ofspermia**	**Total**

	Oligos-permia	Astheno-spermia	Terato-spermia	Azoo spermia	
Primary infertility	16 30.2%	**19** **35.8%**	11 20.8%	7 13.2%	 100.0%
Secondary infertility	**8** **47.1%**	3 17.6%	4 23.5%	2 11.8%	17 100.0%
Total	24 34.3%	22 31.4%	15 21.4%	9 12.9%	70 100.0%
FET = 2.546			**P = 0.329**		

Seminal analysis

Asthenospermia showed highest percentage **(35.8%)** within the class primary infertility, while secondary infertility showed the highest percentage **(47.1%)** with the type oligospermia. However the observed variations was not significant (p = 0.329).

Sperms motility percentages of infertile males

Seminal analysis regarding the motility is demonstrated in the following table. Out of **70** samples obtained from infertile males,**50** samples showed **< 40%** motility with a percentage of **71.4%.**

Table 3: Frequency and percentages of sperm motility

Motility	Frequency (no)	Percent (%)
Zero	8	11.4
< 40%	50	71.4
>40%	12	17.1
Total	70	100.0

Anti-sperm antibodies detected from sera and semen of infertile males

Mean concentration of antisperm antibodies in the sera of infertile males was **26.2307 U/ml**. which is considered of no significant value (negative value), but there were highly significant differences between the mean concentrations of sera and seminal ASAs of males with infertility; the mean concentration was higher in the seminal plasma **(90.9203 U/ml)** than that detected in the serum **(26.2307 U/ml).**

Table4: Mean concentration ASAs in serum and semen of patients.

ASAs in serum &seminal plasma	Number	Mean (U/ml)	P value
ASAs in serum	70	**26.2307**	0.000
ASAs in seminal	70	**90.9203**	

Seminal anti sperm antibodies distribution

Anti-sperm antibodies were detected in the seminal plasma of the infertile males in a percentage of **61.4%.**While those who showed no antibodies against their sperms were **38.6%.**

Table 5: Frequency of seminal plasma anti sperm antibodies among infertile group

	Frequency	Percent
ASA (+) in seminal plasma	43	**61.4 %**
ASA (-) in seminal plasma	27	**38.6 %**
Total	70	100.0 %

Distribution of positive ASAs according to types of infertility

There were 43 infertile patients with positive ASAs which include both primary and secondary infertility. Among patients with primary type, **32 (74.4%)** showed positive ASAs as compared to **11 (25.6%)** positive cases among patients with secondary infertility

Table 6: Frequency of ASAs according to types of infertility

	Primary infertility	Secondary infertility	Total
ASAs (positive)	**32 (74.4%)**	**11(25.6%)**	**43(61.4%)**
ASAs (negative)	21(77.8%)	6(22.2%)	27 (38.6%)
Total	53(75.5%)	17(24.3%)	70(100%)
P value =			

Distribution of ASA according to types of spermia

oligospermia represented the highest percentage **37.2%,** followed by, asthenospermia, teratospermia and azoospermia in percentages **27.9%, 18.6%** and **16.3%** respectively that were associated with positive ASAs, but the different did not reach statistical significance (p = 0.542).

Table 7: Frequency of ASA according to types of spermia

Types of spermia	ASA in semen		Total
	ASA (+)	ASA (-)	
Oligospermia	**16** **37.2%**	8 29.6%	24 34.3%
Asthenospermia	**12** **27.9%**	10 37.0%	22 31.4%
Teratospermia	**8** **18.6%**	7 25.9%	15 21.4%
Azoospermia	**7** **16.3%**	2 7.4%	9 12.9%
Total	43 100.0%	27 100.0%	70 100.0%
X^2 = 2.148	**df = 3**		**P=0.542**

Distribution of ASAs according to their motility (progressive + non progressive)

The majority of patients' samples showed high percentage of sperms with poor motility. Out of 43 infertile males diagnosed as positive ASAs, **33** samples **(76.7%)** displayed less than **40%** motility as illustrated in table 6

Table 8: Frequency ASAs according to total motility (progressive + non progressive)

	Motility			Total
	Zero	<40%	>40%	
ASA (+) in semen	6 14.0%	**33** **76.7%**	4 9.3%	**43** **100.0%**
ASA (-) in semen	2 7.4%	17 63.0%	8 29.6%	27 100.0%
Total	8 11.4%	50 71.4%	12 17.1%	70 100.0%
X^2 = 5.061	df =2		P = 0.080	

Mean serum level of IL 6 and IL8 in both study group and the control.

The mean concentrations level of serum IL6 and IL8 were measured both in study group and control. IL6 was higher in study group than control, with serum level **91.5708** pg /ml and **17.4359pg** /ml

respectively. The differences between patients and control were statistically significant (P value0.00). Mean serum level of IL8 showed no differences between patients **(20.8299**pg/ml**)** and the control group **(17.7692**pg/ml**) (Pvalue0.346).**

Tale 9: Mean serum level of IL6 and IL8 in both study group and the control pg/ml.

	No	IL 6	IL 8
Patients	48	**91.5708**	20.8299
Control	40	17.4359	17.7692
P value		**0.000**	0.346

Mean concentration of IL6 in seminal plasma of patients and control group

The mean concentration of seminal plasma of IL6 among infertile men was **21.2413 pg/ml**, while the control group showed **4.6433 pg/ml.** The difference was highly significant(P value 0.001).

Table 10: IL6 concentration in semen of patient with fertility and fertile men

IL 6 concentration in seminal plasma	Number	Mean concentration pg/ml.	P. value
Infertile (patients)	48	21.2413	0.001
Fertile (control)	40	4.6433	

Mean serum concentration of IL6 and IL 8 according to types of spermia

The highest concentrations of IL6 was seen in those patients with teratospermia **111.8769 pg/ml**. Highest mean concentration of IL8**(26.2436 pg/ml.)**associated with **Teratospermia**, but the differences were not statistically significant

Table 11 : Mean serum concentration of IL6 and IL8 in relation to types of spermia

	Oligo-spermia No.17	Astheno-spermia No.12	Terato-spermia No.13	Azoo-spermia No.6	Normo-spermia No.40	P value

IL 6	94.4353	86.4667	**111.8769**	49.6667	17.4359	0.822
IL8	24.8431	12.4722	26.2436	14.4444	17.7692	0.133

Mean serum concentration of serum IL6 and IL8 according to the motility:

The highest mean concentration of IL6 was **103.8647 pg/ml** which falls within the category of **< 40** motility, while IL8 values showed no significant contrast within motility categories

Table 12: Mean serum concentration of IL6 and IL8 in relation to motility

Motility	IL6 no (mean conc.).	IL8 no (mean onc.).
Zero	5 (59.2400)	5 (12.8000)
<40	34 **(103.8647)**	34 (21.5049)
>40	9 (63.0889)	9 (22.7407)
Total	48(91.5708)	48 (20.8299)

P value	0.607	0.563

DISCUSSION

Infertility was classified into primary and secondary types, the former showed higher frequency than the later in percentages 75.7%and 24.3%respectively.

Semen analysis was done routinely and classified into oligozoospermia, asthenozoospermia, teratozoospermia, and azoospermia. In the current study, asthenospermia represented the highest percentage in primary type **(35.8%)** which might reflect the high rate of ASAs associated with primary infertility. Regarding sperms motility, the highest percentage

(71.4%) of defect in motility was found in those with the motility of < 40%. Normal motility of sperms is essential for their migration in the ovarian tubes to be involved in the process of fertilization. Therefore, apparent association subsists between motility of sperms and the chance for natural pregnancy[14]

Presence of ASAs is good indicator for diagnosis of immunological infertility[15] .

Positive ASAs were found in both primary and secondary infertility with a rate of **(74.4%)** and **(25.6%)** respectively (P 1.0), these findings provide a hint that ASAs may be behind the problem of primary infertility "the immunological etiology of the infertility". ASAs develop when the patient's own immune system identifies the sperm cells as a result of disruption in its environment. As a consequence, sperms lose their ability to perform vital functions such as penetration into the cervical mucus and binding to the zona pellucida (eag coat) [16] Besides the fact that the sperm coated with antibodies may become more susceptible to phagocytosis in the reproductive tract of the women[17] . Other immunologic effects of ASAs on fertility are interference with spermatogenesis resulting in oligospermia

which represent a high percentage 37.2% and asthenospermia in a percentage 27.9% as shown in table 7.

In the current study positive ASAs of seminal samples showed apparent decrease in the motility with a percentage 76.7% for those with motility less than 40% ,. but did not reach statistical significance. (Chi-square analysis may be affected by the low sample size).The motility of the sperms was affected by the presence of ASAs which inhibit their transport and fertilization of ovum and ultimately interfere with their functions. [18] The mean serum levels of IL-6 (91.5708 pg /ml) and (17.4359 pg /ml) were estimated in patients and control group respectively which demonstrated highly statistical differences between them.

No significant differences were observed in the mean serum level of IL8 between patients and control group. Al-saimary reported that mean serum concentration of interleukin 8 in males with infertility was higher than that detected in control group , so that findings was not in agreement with ours[19] .

The most accurate and sensitive method to trace the infertility is by proper monitoring of cytokines in seminal plasma of the patients ''infertile men'' .

In the current study the mean concentration of IL6 was higher in seminal plasma of male with infertility than that of the study group in a percentage21.2413pg /ml and 4.6433 pg /ml respectively (P 0.001) and in agreement with the finding reported by (Eggert-Kruse W. 2001). High level of interleukin 6 ''released in semen during inflammation of the genitourinary tract'' leads to persistent sperm damage that affect fertility. Since IL6 is one of the proinflammatory cytokines, so its level in semen should be assessed to evaluate the inflammation in of the male genital tract[20].

we concluded that seminal plasma ASAs were better marker than the sera mean concentration and seminal cytokines are better markers than serum cytokines.

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