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DETECTION OF ANTI-SPERM ANTIBODIES AND COMPLEMENT 5A IN PRIMARY INFERTILITY MALES IN SALAH AL-DIN, IRAQ

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Abstract

The objective of this study was to examine the immunological markers of anti-sperm antibody (ASA) and complement5a (C5a). The individuals seeking medical care are referred to Urology Department at Tikrit Teaching Hospital in Salah Al-Din Hospital (Iraq) and a private laboratory. A total of 110 patients and controls were included in the study, with recruitment taking place between May 2022 and June 2023. Samples of semen and blood were obtained from both infertile males and control subjects in order to conduct an immunological examination of seminal fluid. The control group comprised 50 individuals who were randomly selected from the population of Tikrit city during the study period. The patients were distributed into three groups: Azoospermic (AZO), Asthenozoospermia (AST) and Oligozoospermic (OLI) . Anti-sperm antibody (ASAs) and C5a levels were assessed in seminal plasma fluid and serum by Enzyme Linked Immuno Sorbant Assay (ELISA). The immunological investigation during present study, showed that among 60 samples (patients), there were 25 (41.6%) with positive anti-sperm antibody in their seminal fluids, while 35 (58.3%) patient with negative anti-sperm antibody in their seminal fluid plasma. Additionally, the study demonstrated a notable disparity in serum C5a levels between those with normospermia and those with oligozoospermia. A statistically significant difference (P value < 0.05) was seen in the levels of serum C5a between individuals with normospermia and azoospermia. The study found that out of the 110 specimens analysed, 50 samples exhibited normal seminal analysis in fertile individuals, while the remaining 60 samples were obtained from infertile individuals displaying conditions such as oligozoospermia, asthenozospermia, and azoospermia. A notable

association was seen between the presence of anti-sperm antibodies and aberrant semen parameters, particularly asthenospermia.

Keywords: Male infertility, azoospermia, oligozoospermia, asthenozoospermia.

Introduction

Infertility is defined as the lack of conception after 12 months of unprotected intercourse. On evaluation, roughly 50% of affected couples have causal or associated male factors as a cause of infertility [1]. Evaluation of the infertile men requires a complete medical history, physical examination and laboratory investigation. Usually 80% of couples are able to conceive within the first year of marriage [2]. Male infertility has several different possible causes which are primary or secondary testicular failure, infection and obstruction, but the most common diagnosis is idiopathic infertility, which accounts for about 60-70% of the patients [3]. Primary infertility is a term used for those couples who have never conceived while; secondary infertility is a term that refers to those couples who have at least one conception but currently unable to achieve pregnancy [4].Immunological factors have been suggested to be involved in the etiology of male infertility, and much concern has been focused on anti-sperm antibodies (ASAs) and C5a, and their effects on semen quality have been questioned [5]. Immune privilege in the testis is essential to maintain immunological tolerance to male germ cells during their development into spermatozoa, but immunity to sperm through the production of ASAs is thought to contribute to infertility, with 9-36% of infertility in couples being attributed to an immunological mechanism [6]. The immune privilege in the testis is maintained by blood-testis barrier, constitutive expression of anti-inflammatory cytokines and by testicular macrophages. In addition, testicular macrophages present in the interstitial tissue are thought to be essential for male reproductive function by regulating testosterone production by leydig cells [7]. It has been understood that proteins of human spermatozoa may act as auto antigens in human males. They are produced during spermatogenesis in the testis or may be attached to the sperm membrane during the passage through the seminal tract (sperm- coating antigens) [8].Recently there has been a lot of discussion about the role that the immune system plays in fertility. The immune system generally works to protect the body from foreign cells and bacteria [9]. Sometimes though problems within the immune system prevent it from working properly. Many couples facing infertility issues may actually have immune system dysfunction which is preventing them from conceiving. Luckily new tests are now available to pinpoint these immune factors in infertility [10]. Anti-sperm

antibodies present in about 10% of infertile men, Anti-sperm antibodies can affect the chances of becoming pregnant by interfering with the quality or function of the sperm [11]. Complement and its regulation are important in reproduction, many study reported CD5 was significantly expressed only in testis and played a role in sperm acrosome activation and motility [12]. There was no evidence of antibody or complement fixation by viable spermatozoa. It had been found that antibodies present in the serum of women that bind to nonviable spermatozoa (ASA) belong to the IgG and IgM class then Complement fixation occurred via the classical (antibody-mediated) and alternative pathway. This indicated that viable spermatozoa may possess antigenic properties different from nonviable spermatozoa. This leads to lack of immunological reaction of women to viable spermatozoa [12].

Patients and methods

The present cross sectional study was carried out in the Urology Department at Tikrit Teaching Hospital and a private laboratory at Salah Al-Din, Iraq. A total of 110 participants were enrolled in the study, with recruitment taking place from May 2022 to June 2023.The study participants ranged in age from 20 to 50 years. Samples of semen and blood were obtained from a male individual experiencing infertility, which is defined as the inability of a couple to achieve pregnancy after one year of consistent and unprotected sexual intercourse. These samples were then subjected to examination of seminal fluid in order to investigate the presence of anti-sperm antibodies (ASA). Serum samples collected to analyze the presence and activity of complement 5a (C5a) in the blood. In order to make a comparison, a group of fifty guys was included as a control to assess their seminal fluid analysis and serum levels. The semen samples were subjected to centrifugation for duration of 15 minutes at a rotational speed of 3000 revolutions per minute (rpm). The subsequent step was the prompt and meticulous freezing of the recovered and positioned supernatant of seminal plasma at a temperature of -20°C, in order to facilitate the subsequent determination of ASA, zinc, and fructose levels. Peripheral venous blood aspiration was performed on each male participant, with a volume of five milliliters collected. The blood samples were collected in plain tubes and allowed to undergo coagulation. Subsequently, the tubes were centrifuged at a speed of 2500 revolutions per minute (rpm) for duration of 10 minutes. The specimens were classified into several groups based on the outcomes of sperm analysis. The concentrations of blood complement 5a (C5a) were assessed using the Enzyme-Linked Immunosorbent Assay (ELISA) technique in serum analysis. The computer system SPSS (Statistical Package for Social Sciences) was applied to analyze the levels of all parameters in both seminal plasma

and serum. The data were presented as mean and standard deviation, with a significant P-value < 0.05.

Result

A total of 110 specimens were evaluated. A total of 110 samples were analyzed in this study. Among these, 50 samples (45.4%) were obtained from fertile men and exhibited normal seminal analysis. The remaining 60 samples were collected from infertile men. Out of these, 20 samples (18.1%) showed oligozoospermia, characterized by a spermatozoa concentration of less than 15 million per milliliter. Another 20 samples (18.1%) displayed asthenozospermia, with less than 40% motile spermatozoa. Additionally, 20 samples (18.1%) exhibited azoospermia, indicating the absence of spermatozoa in the ejaculate. Furthermore, approximately 33 samples (30%) demonstrated teratozospermia, which is characterized by morphological abnormalities (Figure 1). The study revealed a statistically significant distinction between normal semen examination and abnormal (infertile) semen, as indicated by a P value of less than 0.05. Cohorts of sixty male individuals diagnosed with primary infertility were examined, specifically focusing on variations in seminal fluid morphology. Out of the total sample size of 20 patients diagnosed with asthenospermia, 12 patients (60%) exhibited the presence of anti-sperm antibodies in their seminal fluid plasma. Similarly, among the 20 patients diagnosed with Oligozoospermia morphology, 6 patients (30%) demonstrated the presence of anti-sperm antibodies in their seminal fluid plasma.

Additionally, the study revealed that there were 20 patients who exhibited azoospermia morphology. Out of the total sample size, 7 individuals, accounting for 35% of the participants, exhibited the presence of positive anti-sperm antibody inside their seminal fluid plasma. The statistical analysis conducted using SPSS indicated that there was no statistically significant difference observed in the levels of positive anti-sperm antibody between individuals with azoospermia and Oligozoospermia, as compared to those with normospermia. However, a notable disparity was observed in the presence of positive anti-sperm antibodies between those with asthenospermia and those with normospermia (control group), with a statistically significant p-value of less than 0.05.

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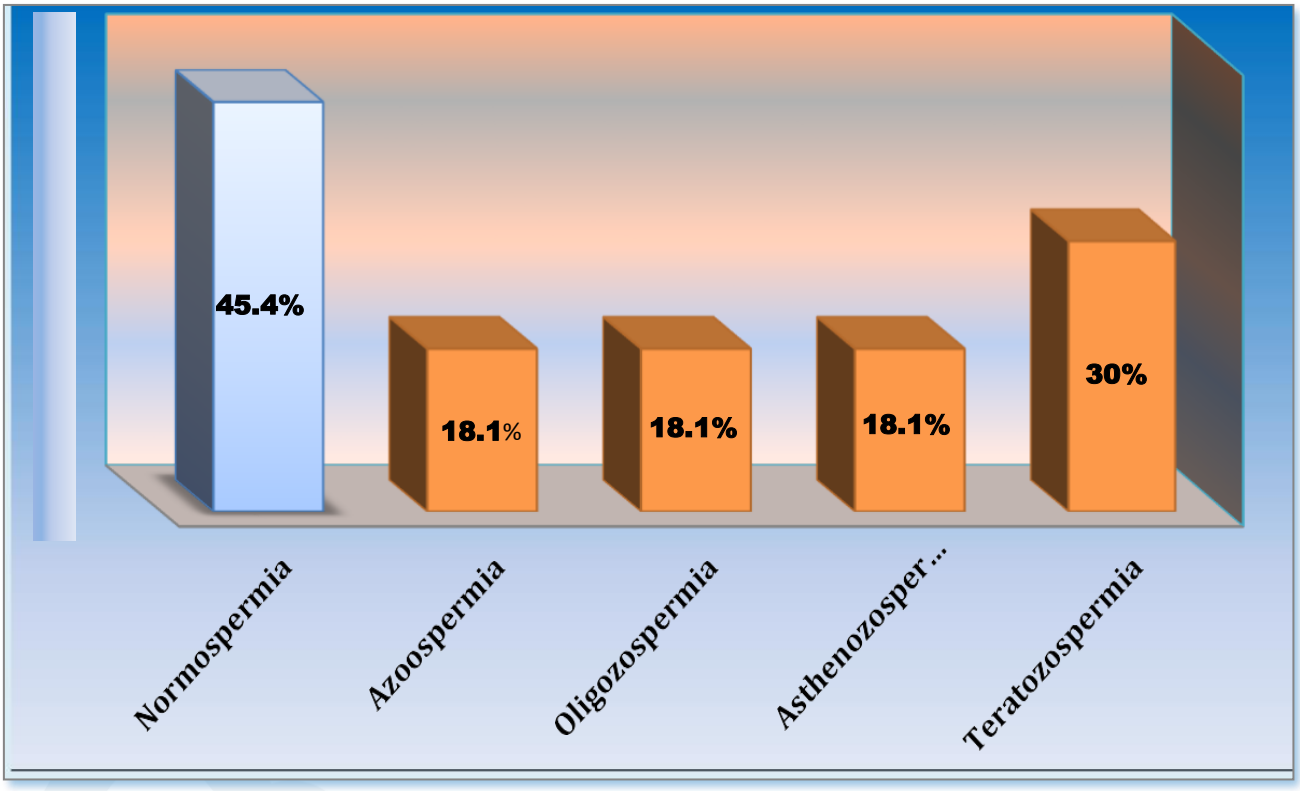

Figure 1: Interpretation of semen par

Figure 1: Semen morphological abnormalities among study population.

Table 1 : Observed percentage of ASA positive and negative in infertile patients and Controls.

Groups	NO.	Anti-sperm antibody status				P value
		positive		Negative		
		No.	%	No.	%	
Azoospermia	20	7	35	13	65	**NS**
Oligozoospermia	20	6	30	14	70	**NS**
Asthenozoospermia	20	12	60	8	40	**S**
Controls	**50**	**15**	**30**	**35**	**70**	_

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Table 2: Patients with positive and negative ASA in their seminal fluid plasma .

Total	Positive ASA	Negative ASA	P value
60	25	35	0.05

Table (3), represented the level of serum complement 5a (C5a) in infertile patients and controls, the mean level of sixty patients were included during study with different seminal fluid morphology. The mean level of serum C5a in asthenozoospermia was 70.8 ± 4.24 ng/ml , while the mean level of serum C5a in oligozoospermia was 77.2 ± 1.78 ng/ml, also the mean level of serum C5a in azoospermia was 70.8 ± 3.72 ng/ml. On other hand, it was found the mean level of serum C5a in normospermia was 42.2 ± 0.83 ng/ml. Statistical evaluation using (SPSS) program analysis revealed that there were significant difference (P value < 0.05) between serum C5a in normospermia and asthenozospermia, Also showed the presence significant difference between serum C5a in normospermia and oligozoospermia. It was also found a significant difference between serum C5a in normospermia and azoospermia (Figure 4).

Table 3: Mean levels of serum C5a in infertile patients .

Groups	C5a levels (ng/m)				P value
	NO.	Mean	SD	SE	
Normospermia	50	42.2	0.83	0.37	_
Asthenozospermia	20	70.8	4.24	1.89	**0.051**
Oligozoospermia	20	77.2	1.78	0.80	**0.053**
Azoospermia	**20**	**70.8**	**3.72**	**3.00**	**0.052**

Table 4: Mean of serum levels of complement (C5a) in infertile males.

Complement 5a Levels (ng/ml)	Study groups			P value
	Infertile male with positive anti-sperm antibodies	Infertile male with negative anti-sperm antibodies	Control	
C5a	77 ± 3.2 mean ± SD	56 ± 4.2 mean ± SD	32 ± 2.1 mean ± SD	0.01

Discussion

Among the 110 specimens were examined, fifty (45.4%) samples had normal seminal analysis from fertile men, while the results of sixty samples were taken from infertile men were as follows, 20 (18.1%) samples had oligozoospermia, other 20 (18.1%) samples had asthenozospermia, while 2 and also about 33 (30 %) samples of terato-zospermia (Morphological abnormality). Statistically, there was significant difference between normal semen examination and abnormal (infertile) semen in during study .these results agreement with the results of the other studies for regarding of semen morphology [13-15]. From those 60 patients during study, showed 25 (41.6 %) patients with positive anti - sperm antibodies in their seminal plasma and significantly p value < 0.05 effect. These results mean that there is a significant correlation between the presence of anti- sperm antibodies and abnormal semen parameters especially asthenospermia and the presence of anti-sperm antibodies in the seminal plasma may have even greater importance. This means that anti-sperm antibodies have a direct impact on infertility. These results were correspond with other studies as were of Al-Assaf et al [16], that showed positive anti-sperm antibodies in infertility males, other study done by Muhammad et al [17], showed from 155 infertile patients whose semen analyses showed asthenospermia , 75 patients (48.4%) showed with positive anti-sperm antibodies. ASA cause clumping or agglutination of sperms [18]. Anti-sperm antibodies had a significant negative effect on the sperm motility and increase the proportion of motile sperms involved in agglutination [19]. Anti-sperm antibodies could inactivate human sperm motility in the presence of complement, showing that complement-dependent inactivation of sperm motility might be the biological mechanism of male and female infertility, because incubation of motile sperm with complement-fixing immune sera resulted in a significant loss of motility, then activation of (C5a) induced alterations in sperm morphology leading to sperm lyses [20]. During the present study 21 (35 %) patients showed with positive C5a with p value < 0.05. These results are correspond with other studies as Huang HC et al [21] ,that observed the negative effect of anti-sperm antibodies and C5a in the sperm morphology and motility. Other study showed a clear inverse correlation was between sperm motility loss and the increase in sperm associated C3/C5 fluorescence. Thus assembly of the sperm-bound C3/C5 converters [22]. This is in turn might generate significant amounts of pro-inflammatory C3a, C4a and C5a peptides that could stimulate the infiltrating phagocytes to produce detrimental biological compounds harmful to sperm survival and successful fertilization in the male and female genital tract[23] .

Conclusion

A total of sixty samples were collected from a cohort of infertile males. Among these samples, 20 (18.1%) exhibited oligozoospermia, 20 (18.1%) displayed asthenozospermia, 20 (18.1%) demonstrated azoospermia, and about 33 (30%) samples exhibited teratozospermia. A notable association was seen between the existence of anti-sperm antibodies and atypical semen characteristics, particularly asthenospermia. The study also revealed notable distinctions in blood C5a levels between individuals with normospermia and those with asthenozospermia, as well as between individuals with normospermia and those with oligozoospermia. A notable disparity in serum C5a levels was observed between individuals with normospermia and azoospermia.

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