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Role of chlamydia trachomatis infection and anti-sperm antibody in male infertility

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Abstract--There are many different factors that affect male infertility, including bacterial infections and immunological factors. The current study examined two factors that negatively affect male fertility. These factors include detection of *C. trachomatis* IgG antibodies and evaluation the presence of antisperm antibodies. Blood samples were collected from 63 infertile male with age of 33.37 ± 0.86 years and 80 fertile male as control group with age 34.39 ± 0.61 , tests were conducted for them to detect for *chlamydia trachomatis* IgG antibody and presence of antisperm antibody measured by ELISA technique. *C. trachomatis* was demonstrated in the blood samples of (61,9%) infertile male were seropositive and all control samples were negative for anti chlamydia IgG. The seropositive for antisperm antibody was 20.6% in infertility male while seropositive for control group was negative this lead to suggest that *chlamydia trachomatis* and antibody against sperm play role in pathogenesis of fertility.

Keywords--Male infertility, *chlamydia trachomatis*, antisperm antibody.

Introduction

Infertility is defined as "a disease of the reproductive system defined by the failure to achieve a clinical pregnancy after a period of 12 months or more of regular unprotected sexual intercourse with no other cause" (Barbu, 2021), male infertility is considered as a multifactorial disease with an incidence rate of 50% of infertile couples (Jenkins., *et al* 2016). Sperms develop after immunocompetence is established, but if it is disrupted and sperms are exposed to blood, an antigenic response can result. Risk factors for development of antisperm antibodies (ASAs) occur in approximately 6% of men presenting with infertility including previous genital infection by *chlamydia trachomatis* (Turek, 1999). Antibodies bound to sperm may be clinically relevant since they can

interfere with sperm motility or prevent fertilization (Vazquez., *et al* 2014). *Chlamydia trachomatis* infection, which most frequently is asymptomatic, is the most common bacterial sexually transmitted infection and a major public health concern globally, patients with *C. trachomatis* urogenital infections often remain undiagnosed and untreated and has been known for some time to have a significant effect on human reproduction (Gimenes., *et al* 2014). Antisperm antibodies are immunoglobulins that interact with sperm antigens, hindering the function of the male gamete, and thus playing a role in fertility (Azizi *et al.*, 2015). In fact, it has been described that the presence of these antibodies can be involved in male fertility impairment (Verón *et al.*, 2016; Al-Daghistani 2020).

The aims of this study were to determine the effect each of anti-*chlamydia trachomatis* and anti-sperm antibody in blood sample on male infertility.

Materials and method

One hundred and forty-three males participated in this study, which was divided into two fundamental groups: infertile and fertile. Infertile group, which included 63 patients, their ages was 33.37 ± 0.86 years as well as 80 healthy fertile male with age 34.39 ± 0.61 years each of them has at least one child the sample were collected examined at Kamal Al-Samarrai Hospital for infertility and In vitro Fertilization from April 2021 until January 2022. Ethics committee of Baghdad University, College of Medicine, approved the present research. At first the aim of study was explained for all participants and after obtaining their oral and signed consent they have been studied. two milliliters of venous blood were drawn from the infertile and fertile control groups using a plastic disposable syringe then put it in polyethylene tube and allowed to coagulate for ten minutes in a water bath at 37°C before being centrifuged for ten minutes at 12,000 rpm (3000 rpm). The clear serum was obtained and kept frozen at -20°C until it was used to calculate the various parameters in this study. The serum sample was separated into two equal halves. The first one was used to determine the presence of

chlamydia trachomatis IgG antibody and the second portion was used to determine the presence of anti sperm antibodies IgG in the infertile and control by ELISA test in serum samples.

Determination of Human AS-IgG (anti sperm- Immunoglobulin G) ELISA kit.

The first portion of serum was used to determine presence of anti-sperm IgG Abs kit was based on indirect enzyme-linked immune-sorbent assay technology (Al-shkairate establishment for medical supply/ Jordan).

Determination of *chlamydia trachomatis* IgG using Enzyme-Linked Immunosorbent Assay

The last portion of serum was used to determine the presence of IgG antibody in the infertile and control serum sampl according to manufacturing kit (DRG/ Germany).

Statistical analysis

Data were expressed as counts with percentages in parentheses or mean \pm SE * Statistical analyses were performed by independent samples T-test. **Statistical analyses performed by the Chi-squared test; NS, no significant difference.

Results and discussion

The result of present study showed that there was no significant difference between studied groups as in table (1).

Table 1: The mean of age for the two groups

Groups	Age mean \pm S.E (years)	P
Infertility patients	33.37\pm0.86	0.320 NS
control	34.39\pm0.61	

The ages of the men in this study were comparable, indicating that age had no effect on infertility. The absence of age differences in these groups reflect the absence of an age effect on male infertility, but rather that the age categories were uneven in relation to one another. Elbashir *et al.* (2018) made the same observation, concluding that this age had no effect on reproductive status.

Table 2: Frequencies of anti-chlamydia IgG between studied group.

Anti-chlamydia IgG antibody	Infertile patients' frequency (%)	Control frequency (%)	P
Positive	24 (61.9)	0 (0.0)	<0.0001
Negative	39 (38.1)	80 (100.0)	
Total	63 (100.0)	80 (100.0)	

The result illustrated in the table (2) showed a significant difference between groups according to anti chlamydia IgG this result supports the possibility of *Chlamydia trachomatis* playing a crucial role in the etiology of male infertility. This result agreed with Ali & Al-Kazaz, (2018) where they demonstrated that the *C. trachomatis* infection seems to be spread in infertile males and could lead to a decrease in sperm concentration, motility and morphology. Therefore, this result supports the possibility of *C. trachomatis* playing a crucial role in the etiology of male infertility. Also, affecting male infertility, infections of the genital tract constitute an important, hidden and disruptive factor, and it causes approximately about 8-35% of male infertility (Askienazy-Elbhar M, 2005).

The result of anti-sperm antibody showed a significant difference (P=0.00002) between control and patients (Table 3).

Table 3: Association between ASAbs results in studied groups.

Anti-sperm antibody	Infertile patients' frequency (%)	Control frequency (%)	P
Positive	13 (20.6)	0 (0.0)	0.00002
Negative	50 (79.4)	80 (100.0)	
Total	63 (100.0)	80 (100.0)	

The patients having a significant increasing of circulating immunoglobulin level (IgG) in their sera when compared with control group. The presence of Anti Sperm Antibodies could result from the damage in the blood-testis barrier (BTB). This immunoglobulin may transudate from circulation into reproductive tissue and, from the reproductive tissue back to the circulatory system (Yousif et al., 2015; Allow et al., 2017). Physiologically, the BTB is a tight junction between Sertoli cells and appears to play a major role in keeping the developing spermatozoa and immune system separate. It prevents those testicular cells expressing “foreign” antigens from coming into contact with lymphoid tissue and immunocompetent cells. However, the BTB is commonly breached by physiological leakage of normally sequestered sperm antigens. Autoimmunity to sperm may occur because sperm cell antigens are first expressed during sexual maturation, long after the prenatal period when immunological self-tolerance is induced (Allow et al., 2017; Xu et al., 2020). One of the main reasons for this phenomenon might be the presence of toxic agents in the environment (Swan et al., 1997). Work-related activities frequently involve exposure to toxic chemicals, most of which are damaging to reproductive health and cause infertility in humans (Pereira et al., 2003). Occupational or accidental exposure to endocrine disruptors – chemical substances that interfere with synthesis, storage/release, transport, metabolism, binding, action or elimination of natural blood borne hormones responsible for the regulation of both homeostasis and developmental process can lead to compromised fertility (Pereira, 2003).

The result in table (4) showed a significant difference according to anti chlamydia IgG antibody and presence of anti-sperm antibodies in infertile group ($p \leq 0.05$). The results of the current study disagreed with the result obtained by Eggert et al., (1997) who concluded that the clinical data fail to detect an association between chlamydial infections and the presence of ASAs in seminal plasma.

Table 4: Anti-chlamydia IgG antibody and presence of anti-sperm antibody in infertile group.

Anti-chlamydia IgG antibody	Infertile patients' frequency (%)			P
	Positive	Negative	Total	
Group1	5 (38.5)	8 (61.5)	13 (100.0)	0.97
Group2	19 (38.0)	31 (62.0)	50 (100.0)	

Conclusion

The results of the present study demonstrate that the *C. trachomatis* infection seems to be spread in infertile males. Therefore, this result supports the possibility of *C. trachomatis* playing a crucial role in the etiology of male infertility. Also, the presence of antisperm antibodies in the group of infertile men plays the same role in pathogenesis and development of disease

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