Original Article

Frequency of Antisperm Antibodies in Infertile Women

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Abstract

Background: Infertility is one of the common problems seen in couples of reproductive age. Presence of antisperm antibodies in semen and serum are amongst the causes of immunoinfertility. This study was performed to determine antisperm antibodies in cervicovaginal secretions and serum of infertile women and also measure serum levels of immunoglobulins (IgG, IgM and IgA).

Methods: The study consisted of 45 infertile women consulting the Kammal El-Sammrari Hospital for infertility from 2008 to 2009 and the control group consisted of 30 fertile women. Serum levels of immunoglobulins (IgG, IgA and IgM) were measured in the participants using single radial immune diffusion. Antisperm antibodies (ASAs) were detected in the serum of both infertile and control groups using indirect immune fluorescence test. ASAs were also detected in cervicovaginal secretion using direct sperm agglutination test in both infertile and control groups.

Results: Antisperm antibodies were found in the cervicovaginal secretions (62.2%) and sera (64.4%) of infertile women which were significantly higher ($p < 0.001$) than those of the control group (3.3% and 3.3% respectively). There was a significant increase ($p < 0.001$) in serum levels of IgG and IgA of infertile women (16.2 and 3.25 *g/L* respectively) compared with the healthy control group (7 and 1.2 *g/L*).

Conclusion: Humoral immune response and antisperm antibodies may contribute to reproductive failure in couples of reproductive age.

Keywords: Antisperm antibody, Cervicovaginal, Immunoflourescence, Immunoglobulin, Infertility.
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Introduction

Infertility is a common problem of couples of reproductive age and it is seen in one out of five infertile couples (1). In about 10-20% of the cases, no definitive cause could be identified, but in 9-36% of these cases, antisperm antibodies (ASA) have been regarded as the cause of infertility (2). In 1922, Samel R. Meaker was the first to document presence of ASA in women (3).

ASAs are detected in the blood, semen and follicular fluid as well as cervicovaginal secretions. ASAs act by blocking sperm movement, capacitation, fertilization and inhibition of embryo implantation (4). It has been proved that sexual practices do not affect ASAs development (5).

These autoantibodies develop against specific sperm antigens like nuclear autoantigenic sperm protein (NASP), (a histone-binding protein that affects fertility rate) (6). According to previous studies, ASAs are produced against fertilization antigen (FA-1) and dodecamer peptide sequence (YLPVGGLRRIGG designated as YLP12) are important in infertility (7). Acrosin antibodies present in the sera of infertile women inhibit sperm-zona pellucida binding through protease activity (8).

There are several methods for the diagnosis of immunological infertility and detection of ASAs including sperm immobilization test, immunobead

binding test (IBT), indirect immunofluorescence (IIF) and tissue engineering-based assay (9, 10).

Immunological reactions that occur between sperm and female genital secretions due to the presence of ASAs in serum and vaginal secretions are effective enough to inhibit pregnancy in rabbits (11). ASAs have been found in 25.6% of sera and 20.5% of cervicovaginal secretions of infertile women (12). The function of immune system is identifying exogenous antigens (sperm antigens) via antibody synthesis (IgG, IgM and IgA) (13). Thus, quantitation of immunoglobulins in the serum and cervical mucus of infertile women is of significance (14, 15).

Due to the importance of ASAs in reproduction, this study was done to detect them in the serum and pelvic discharges (cervicovaginal secretions) and measure immunoglobulin (IgG, IgM and IgA) levels of infertile women in comparison with the fertile control group.

Methods

The case group consisted of 45 infertile women with infertility for at least one year with no anatomical or endocrine problems, consulting Kammal El-Sammrari Hospital for infertility and in vitro fertilization in Baghdad, Iraq from Jun 2008 to June 2009. The clients' husbands were all with normal semen criteria (Normozoospermic). Clients with other causes of infertility (ovarian, tubal, galactorrhea, hormonal, infection, or women whose partners were infertile) were excluded from the study. This study included only infertile women with unknown cause of infertility.

The control group consisted of thirty fertile healthy multiparous women, with a normal ovulatory menstrual cycle duration who had delivered a healthy baby within the last two years.

The permission of medical ethics committee was obtained for both infertile and fertile groups.

Peripheral blood (5 *ml*) was collected from each partiapant. Cervicovaginal secretions were also collected via a syringe (1 *ml* insulin syringes). Meanwhile, seminal fluid was obtained from the male partrers by masturbation after 3-4 days of abstinence. The male participants were told to urinate, and wash their hands and penis before collecting the semen in sterile cups. Indirect agglutination test was done between cervicovaginal secretions and sperm of the participats' husbands for the detection of antibodies against sperm in cervicovaginal secretions. If 40% or more of the spermatozoa were involved, the condition was considered as immunological infertility (17).

Serum antisperm antibodies were detected by indirect immunofluorescence test (EURO IMMUNE, Germany). The fluorescence patterns of the indirect tests were recorded and the titers and isotypes of the antibodies were determined. Samples that were positive for ASAs directed against the head of the sperm were also tested for anti-nuclear antibodies using another substrate, rat hepatic tissue (EURO IMMUNE, Germany). This process was performed to get rid of cross-reaction between nuclear and head of sperm. All slides were evaluated in a blind testing in order to obtain correct results.

Sera of the infertile women were tested for immunoglobulins (IgG, IgA and IgM) using single radial immune diffusion kits (BINDARID^{tm} Kit Birmingham, UK).

Statistical analysis: The data were analyzed using descriptive statistics (mean and standard deviation). Inferential statistics (Fisher's exact test) were also used. Considering the scattering of the collected data, the non-parameteric Mann-Whitney test was employed. All the tests were done by using Minitab Statistical Software 13.20. A p-value smaller or equal to 0.05 was considered significant.

Results

The case group consisted of 45 female clients (22-45 years) with a mean age of 32.2±6.1 years.

The control group aged 17-39 years, mean= 31.57±6.08 years. There were no significant differences between the age distribution of the infertile and the control groups.

Table 1. Antisperm antibodies (Titer 1:10) in the cervicovaginal secretions of the infertile and the control groups

Groups	Parameters				
	Number of participants	Indirect agglutination test N (%)	Head to head N (%)	Head to tail N (%)	Tail tip to tail tip N (%)
Infertile	45	28 (62.2%)	12 (42.8%)	8 (28.5%)	8 (28.5%)
Fertile control	30	1 (3.3%)	1 (3.3%)	--	--
P-value*	p <0.001				

* Fisher's Exact Test was used.

Table 2. Indirect immunoflorescence test for the detection of antisperm antibodies in the serum of infertile women and the control

Groups	Titer	N (%)	N (%)	Indirect Immunoflorescent Results					
				Head N (%)	Neck N (%)	Tail N (%)	Head+Neck N (%)	Neck+tail N (%)	Head+neck+tail N (%)
Infertile (n=45)	1:10 1:100	15 (51.7%) 14 (48.2%)	29 (64.4%)	4 (13.7%)	9 (31.03%)	0 (0%)	7 (24.1%)	7 (24.1%)	2 (6.8%)
Fertile control (n=30)	1:10	1 (3.3%)	1 (3.3%)	1 (3.3%)	--	--	--	--	--
P-value *		p <0.001							

* Fisher's Exact test was used

Antisperm antibodies were detected in 62.2% of infertile women using indirect sperm agglutination test between sera and cervicovaginal secretions of these women where 42.8% of the cases had head-to-head agglutination (Table 1).

None of them had antinuclear antibodies. It was not possible to predict the class of antibodies by using direct agglutination tests.

Using indirect immunofluorescence test antisperm antibodies were detected in the serum of 64.4% of the infertile women (64.4%). The highest percentage (31.3%) of antibodies were directed towards sperm neck as shown in Table 2. In addition, no antinuclear antibodies were detected.

Using specific anti IgG, IgM and IgA labeled with fluorescence material to determine isotypes of ASAs (IgG, IgM and IgA), no significant differences in immunoglobulin levels (IgG, IgM and IgA) were observed in the serum of infertile women with ASAs and fertile women while significant increases (p <0.001) in IgG and IgA were observed in the sera of both infertile women with those of the control fertile group, as shown in tables 3 and 4.

Discussion

Immunoinfertility is one of the major causes of infertility in humans which entails production of specific autoantibodies against sperm (18). In this study, the percentages of ASAs were similar in cervicovaginal secretions (62.2%) and serum (64.4%) of infertile women using two indirect agglutination and indirect immunofluorescence (IIF) tests. This similarity between the two tests arises from the fact that indirect agglutination detects autoantibodies directed against surface sperm antigens while in indirect immunofluorescence method the internal sperm antigens are exposed after damage to the plasma membrane or use of methanol during fixation process. All these may lead to false positive results. In addition, cross-reactive antibodies may develop against exogenous antigens that react with sperm antigens. Furthermore, polyclonal B cell activation leads to formation of autoantibodies. All the above reasons may lead to false positive reactions similar to the results seen in indirect agglutination method. Therefore, we consider indirect agglutination method of more clinical importance and specificity than indirect immunefluorescence method.

Other authors like Kapoor et al. (19) found that 58.4% of ASAs in the sera of infertile women

Table 3. Serum immunoglobulin levels in infertile women and the fertile control group

Immunoglobulin levels (*g/L*)	Groups		
	Infertile women (n=45)	Fertile control women (n=30)	P-values
IgG			
Minimum	11.0	5.0	
Maximum	22.5	20.0	P <0.001
Median	16.2	7.0	
IgM			
Minimum	0.3	0.53	
Maximum	2.5	2.45	0.689
Median	1.89	0.855	
IgA			
Minimum	1.72	1.1	
Maximum	5.45	4.8	p <0.001
Median	3.25	1.20	

Table 4. Serum immunoglobulin levels in infertile women with positive antisperm antibodies and the fertile control group

Immunoglobulin levels (*g/L*)	Groups		
	Infertile women* (n=29)	Fertile control (n=30)	P-values
IgG			
Minimum	11.9	5.0	
Maximum	22.5	20.0	p <0.001
Median	16.2	7.0	
IgM			
Minimum	0.35	0.53	
Maximum	2.5	2.45	0.499
Median	1.89	0.855	
IgA			
Minimum	1.72	1.1	
Maximum	5.45	4.8	p <0.001
Median	3.25	1.20	

*Infertile women with positive antisperm antibodies in serum.

were comparable to their presence in the cervicovaginal mucus. Others found that 25% of infertile women had ASAs in cervicovaginal mucus and 12.7% in serum (20). These differences in results may be due to the types of methods used to detect ASAs. Another study found that ASAs in vaginal secretions were not important because pregnant women had also ASAs in body fluids and secretions and this might have been related to the hormonal influence on antibody activity (21).

ASAs directed against sperm tail may be responsible for the decrease in sperm motility, but antibodies directed against sperm head will affect sperm penetration of cervical mucus and the sperm-egg interaction (20). Antibody coated sperms may be more vulnerable to phagocytosis in the female reproductive tract (22). In our study, we found higher percentages of antibodies against sperm neck (31.03%), head and neck (24.1%) and neck and tail (24.1%). Therefore, sperm neck seems to be important in infertility.

Production of antisperm antibodies is due to mechanical or chemical disruption of the mucosal layer in female genital tract permitting exposure to foreign sperm antigens. In addition, sperms within peritoneal cavity which are found after transtubal passage following semen deposition in vagina could induce ASA formation through macrophage phagocytosis and presentation to T-cells (23). Clarke (24) proposed that cross-reactivity with microbial antigens could induce interferon-gamma production and possiblely generate anti-idiotype antibodies.

ASA isotypes found in the serum of infertile women were mixed (IgG, IgM and IgA) and in high amounts (1:100) because these different isotypes were directed against different sperm antigens (25). When the level of ASAs increases, sperm function will impair and the chance of spontaneous pregnancy will decline (22).

Measurement of serum immunoglobulins (IgG, IgM and IgA) showed a significant increase in IgG and IgA while IgM showed no significant difference. IgM is produced in the early stages of the immune system's exposure to sperm antigens and it gradually decreases and subsequently switches to other isotypes (IgG, IgA, *etc*) by B-lymphocytes, while IgG titer rises and remains in serum for a long period. In case of IgA, infertile couples had more IgA than the controls. This was in agreement with Lu et al. (20) who found that IgA was responsible for 'shaking phenomenon' (jittering in situ). While others found that total serum IgG, IgM and IgA levels in infertile women were within normal levels (26).

Conclusion

Immune response and antisperm antibodies may had an effect on reproduction.

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