Detection of Antisperm Antibodies in Sera of Iraqi Males and Females and Their Role in Fertilizing Capacity

Muhammad-Baqir M-R. Fakhrildin *Phd* **, Sundus Fadhil Hantoosh EL-Nahi** *MSc*

Abstract

Background: Antisperm antibodies (ASAs) have a main role in the immunological infertility. Antisperm antibodies negatively affect sperm movement and interfere with fertilization and may cause abortion .

Objective: to Investigate the occurrence of antisperm antibodies in sera of men and women and their role in fertilizing capacity.

Method: Sixty men and thirteen women were involved in this study . Indirect immunofluorescent test kit was used . As a counterstain , Evans blue pigment was used . The fluorescent microscope was used . For sixty males, seminal fluid analyses were performed. For thirteen females, direct microscopic vaginal tests were done.

Results: Forty five men (75%) and ten women (76.9%) showed positive reactions and antibody titres were either 1/10 or 1/32 .

Conclusions: Serum antisperm antibodies play a significant role in autoimmune infertility and should be treated.

Keywords: serum, antisperm antibodies, infertility, immunity.

IRAQI J MED SCI, 2009; VOL.7 (3):19-23

Introduction

Antisperm antibodies can be defined as immmunoglobulins of the IgG, IgA and / or IgM isotype that is directed to various parts of the spermatozoa (head, tail, midpiece or combination thereof)[1]. Antisperm antibodies can be detected in seminal fluid, cervical mucus, oviductal fluid or follicular fluid of women and blood serum of men and women [2]. The occurrence of antisperm antibodies give rise to immunological infertility [3]. In males, testicular trauma, infection, cancer, cryptorchidism and varicocele are involved in generation of antisperm antibodies [1].

In females, the contributing factors include: mechanical such as uterine cervix surgery or chemical disruption of the mucosal layer of the genital tract, foreign antigens gaining access to the female genital tract, lymphocytes in semen , sperm with surface bound antibodies abnormal, senescent or damage sperm, gastrointestinal exposure to sperm and sperm within the peritoneal cavity after transtubal passage[1].

The possible effects of immunologic reaction to fertility are disordered spermatogenesis, inhibiting the effective transport of spermatozoa in male reproductive tract, autoagglutination of ejaculated spermatozoa, sperm cytotoxicity, immobilizing of sperm in the female tract, enhancement of phagocytic clearance of spermatozoa by macrophages, inadequate spermatozoal traverse of cervical mucus, disordered acrosome reaction, blockage of sperm-ovum interaction, induction of sperm immunity in the female, and postfertilization reproductive failure and occult abortion[1,2,4]. Therefore, this study is designed to detect sASAs in both infertile men and women , their causes and role in immunological infertility .

Materials and Methods

Seventy–three infertile patients [sixty males (82.2%) and thirteen

Institute of Embryo Research and Infertility Treatment, AL-Nahrain University.
Adress Correspondence to:Dr Sundus Fadhil Hantoosh EL-Nahi .
E-mail: Sundus_study_update@yahoo.com.
Received: 26th April 2009, Accepted: 15th July 2009.

females (17.8%)] attending Institute of Embryo Research and Infertility Treatment at AL–Nahrain University were included in this study during the period from March 2007 to May 2008.

Ages of males ranged from twenty-four to fifty-seven years. Ages of females ranged from twenty-one to thirty-five years. Information concerning smoking, drinking alcohol, varicocele and varicocelectomy were collected from them. To detect antisperm antibodies in the sera of these patients indirect immunofluorescent test kit (Euroimmune,Germany) was used.

This kit contained BIOCHIP slides and each slide contained ten BIOCHIPs coated with smears of human spermatozoa. As a counterstainer, Evans Blue pigment was used. Once the blood sample was collected from each patient, it was centrifuged till the serum was obtained. This serum was collected into an eppendorf tube and was kept at 0 C until performing the test. To perform the test, four serial dilutions for each sample were prepared (1/10, 1/100, 1/1000, 1/10000).

To prepare 1/10 dilution, 11.1 microleters of the serum were added to 100 microleters of phosphate buffer saline-Tween (PBS–Tween). For the other dilutions, 11.1 microleters of each previous dilution were added to 100 microleters of PBS–Tween. Then 25 microleters of each dilution of each sample were applied to each reaction field of the reagent tray. The reaction started by fitting the BIOCHIP slide into the corresponding recesses of the reagent tray. Each sample contacted with its BIOCHIP. Then the slide was incubated for 30 minutes at room temperature.

After incubation, the BIOCHIP slide was rinsed with a flush of PBS–Tween and was immediately immersed in a dish containing PBS-Tween. After 5 minutes, 20 microleters of fluorescein–labeled anti–human globulin were added to each reaction field of a clean reagent tray and within 5 seconds, the BIOCHIP slide was removed from the dish and the slide was immediately put into the recesses of the reagent tray. The slide was protected from the direct sunlight and was incubated for 30 minutes at room temperature.

After 30 minutes, the BIOCHIP slide was rinsed with a flush of PBS-Tween and was put into a dish containing 150 millileters of phosphate buffer added to it 10 drops Evans Blue pigment as a counterstainer and was left for 5 minutes. Then, 10 microleters of glycerol per each reaction field was added onto a coverglass and within5 seconds the BIOCHIP slide was removed. The BIOCHIP slide with the BIOCHIPs facing downwards was put onto the prepared coverglass and it was now ready for checking by using fluorescent microscope at power 40X.

Under this power, any portion of spermatozoa with green colour indicated positive reaction and any portion of spermatozoa with red colour indicated negative reaction .

Of 60 males, seminal fluids were collected by masturbation after three days of abstinence and seminal fluid analyses were performed within two hours. It was estimated according to WHO guideline in year 1999. The following parameters were concerned in this study: sperm agglutination, sperm motility and presence of pus cells. Of 13 females included in this study, vaginal swabs were done and subjected to direct microscopic examination.

Results

In this research only the first dilution (1/10) showed positive reaction and the other dilutions (1/100, 1/1000, 1/10000) exhibited no positive reactions

Table 1: the reactivity exhibited by both males and females.

Reactivity and %	Sex	Male	Female
Negative reaction and %		15 (25%)	3 (23%)
Weak reaction and %		22 (36.7%)	4 (30.8%)
Moderate reaction and %		23 (38.3%)	6 (46.2%)
Total		60 (100%)	13 (100%)

In this test, weak reactions were given the antibody titer 1/10 and moderate reactions were given the antibody titer 1/32.

Table 2: the attachment of serum antisperm antibodies to various portions of spermatozoa .

Sex and nomber	Portion of spermatozoa	Only head	Only mid - piece	Only tail	Head and mid – piece	Head and tail	Mid – piece and tail	Head mid-piece and tail
Nomber of males		13	0	4	1	24	0	3
Nomber of females		1	0	1	0	8	0	0

Table 3: Descriptive data of some sperm parameters in infertile males.

Sperm parameters	Percentage of infertile patients	Normal value
Percentage of sperm activity grade A	1-Lower than 25% [No.= 54 (90%)] 2-Positive serum ASAs and sperm grade A activity = zero [No. = 34 (56.67%)] 3-Positive serum ASAs and normal sperm grade A activity [No.= 1 (1.67%)]	≥ 25%
Positive Serum ASAs	1- No.= 45 (75%)	Nil
Percentage of sperm agglutination	1-More than 10% sperm agglutination [No.= 26 (43.33%)] 2-Negative serum ASAs reaction in the normal range of sperm agglutination.	< 10%
Pus cells count	1-Patients with notable no. of pus cells [No.= 11 (18.33%)] 2-Patients with notable no. of pus cells and positive serum ASAs [No.=10 (16.67%)] 3-Patients with notable no. of pus cells and negative serum ASAs No.=1 (1.67%)	≤1 cell/HPF

Of thirteen females subjected to direct microscopic vaginal examination, notable pus cells were recognized in the smears of four females (30.77%).

Of sixty males, thirty–nine (65%) were suffering from primary infertility. Fifteen (25%) of them exerted weak reaction, sixteen (26.67%) exerted moderate reaction and eight (13.33%) exerted no reaction. Also of sixty males, twenty–one (35%) were suffering from secondary infertility. Of these twenty–one males, eight (13.33%) exhibited weak reaction, six (10%) exhibited moderate reaction and seven (11.67%) exhibited no reaction.

Of thirteen females, six (46.15%) were suffering from primary infertility. Of these six females, two (15.38%) showed weak reaction, three (23.08%) showed moderate reaction, and one (7.69%) showed negative reaction. Out of thirteen females, seven (53.85%) were suffering from secondary infertility. Of these seven females, two (15.38%) showed weak reaction, three (23.08%) showed moderate reaction, and two (15.38%) showed negative reaction.

In this study, none of males or females was alcoholic. In addition, none of females was smokers. Out of sixty males, thirty–eight (63.33%) were mild to heavy smokers. Of these thirty–eight, seventeen (28.33%) exerted moderate reaction, fourteen (23.33%) showed weak reaction, and seven (11.67%) exhibited no reaction.

In this study, of three primary infertile males (5%) subjected to varicocelectomy, one (1.67%) showed weak reaction and the other two (3.33%) gave no reaction. Varicocele was detected in six males (10%). Of these six males, four (6.67%) were primary infertile and of these four males, three (5%) exerted moderate reaction and one (1.67%) showed no reaction. The other two males (3.33%) were secondary infertile and one (1.67%) showed moderate reaction and the other (1.67%) exerted weak reaction.

Discussion

Infertility is defined as the inability of a couple to conceive after a period of twelve months of intercourse without the use of contraception[1]. Table 2 shows the attachment of serum antisperm antibodies to various portions of spermatozoa contained in BIOCHIP slides. Antisperm antibodies is an important cause of immunological infertility in humans and may result from the presence of antisperm antibodies in sera of individuals [5, 6]. Therefore, this study was designed to study antisperm antibodies in circulating blood of infertile patients.

The results revealed the prevalence of serum antisperm antibodies in both primary and secondary infertile males and females. These results agreed with Dorr *et al*. [7] who mentioned that antisperm antibodies were present in a high percentage of infertile patients.

The results indicated that there was a significant correlation between smoking and immune infertility. Moreira and Lipshultz[8] demonstrated that exogenous agents as nicotine which considered as a gonadotoxic agent, might interfere with male infertility. Autoimmune infertility may result from gonadotoxins[4].

Bes [3] stated that there was an association of varicocele with an autoimmune response against spermatozoa.

Antisperm antibodies cause clumping or agglutination of sperms [4]. Hijort[9] revealed that when notable antisperm antibodies titres were detected in serum the amount of antisperm antibodies on the spermatozoa would also be notable . Antisperm antibodies

had a significant negative effect on sperm motility and increase the proportion of motile sperms involved in agglutination [2, 10]. (Table 3) shows the negative effect of antisperm antibodies on sperm motility.

Jose [1] demonstrated that infections in the genital tract were associated with the generation of antisperm antibodies.

For the treatment, the immunosuppressive corticosteroid prednisolone was administered orally to the patients in dosages 5mg /three times a day for two weeks. This drug showed considerable good results. Khudher [11] demonstrated that prednisolone was administered to patients with antisperm antibodies and the patients had reduced serum antisperm antibodies after treatment and, in some cases, an apparent increased chance of pregnancy.

Conclusion

Serum antisperm antibodies play a considerable role in immune infertility and should be treated.

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