Assessment and correlation of Anti-sperm antibodies to sperm parameters in normozoospermic men

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Abstract:

Background:
The immune infertility caused by anti-sperm antibodies (ASAs) represented about 10- 20% of infertility among the couples, the ASAs interfere with sperm parameters such as the sperm motility and sperm ability to penetrate cervical mucus, sperm-oocyte binding, and fertilization and embryo developments.

Objectives:
The present study designed to assess semen analysis, presence of ASAs and DNA fragmentation index as well as correlation within these parameters in normzoospermic Iraqi subjects

Patients, Materials and Methods:
A total number of Iraqi subjects (116) with range of age (20 -51) years and their mean duration of infertility (4.70 ± 2.77). Seminal fluid for macroscopic and microscopic assessments done according to WHO 2010 criteria. The mixed agglutination reaction (MAR) test used to assess their ASAs in semen (direct method), seminal plasma and serum (indirect method) ; for the both IgG and IgA classes of antibodies and their distribution among different parts of spermatozoa as percentage.

Results:
The mean of IgG using direct method was (23.88± 1.75) and for indirect detection method the mean was (27.41± 2.41) with no significant difference ($P>0.05$) between the two methods. While the direct method for IgA detection mean (14.46 ±1.76) and the mean for indirect method of detection (6.86± 0.39) and there was significant difference ($P<0.05$) between the two methods. In addition to that, the distribution of IgG and IgA detected by direct method on sperm parts showed no significance difference except for the sperm tail; while the indirect method for IgA and IgG showed significant difference in distribution on sperm mid-piece.

Conclusion:
The correlation among sperm parameters and Immunoglobulin using direct and indirect methods showing variations in significance also in the correlation between the direct and indirect methods according to distributions on sperm surface.

Keywords: anti sperm antibodies, sperm parameters, normzoospermic, male infertility.

Introduction:

The tight junctions of Sertoli cells forming the blood-testis barrier provide immunologic protection from sperm antigens. The immune response that spermatozoon evokes when exposed to the systemic immune defense system once the barrier is disrupted, leading to the formation of anti-sperm antibodies (ASA)(1). However, the presence of naturally occurring ASA is a well-known cause of infertility in men and women, but the antigens for these antibodies are poorly characterized(2).

Sperm binding to with antibodies, impeding their motility, stopping recognition(3) ; and entry into the ovum, or targeting sperm for destruction when they reach the female reproductive tract(4). In addition, to that the levels of ASAs detected within semen samples from infertile men and have been associated with specific male genital tract pathology (e. g. , testicular trauma, surgery, torsion). Sperm ASAs believed to have an adverse impact on male fertility through two mechanism of action: 1.) Directly interfering with sperm surface interactions (e. g. , fertilization) and 2.) Indirectly by mediating the release of cytokines that can impair sperm function, possibly including DNA integrity(5,6). there fore, the aims of the present study were to: 1. Identify the correlations between sperm parameters and frequency of anti-sperm antibodies, and 2. The correlation of ASA and sperm parameters according to the distributions on spermatozoa

Materials and Methods:

Subjects: semen samples were obtained from one hundred sixteen Iraqi subjects attended to the Infertility Clinic at High Institute of Infertility Diagnosis and Assisted Reproductive Technologies/Al-Nahrain University.

Serum: The blood serum obtained from each participating subjects for the determining of IgG in serum by venipuncture and wait until the complete clotting of the blood for about ten minutes then centrifugation 2500 rpm for ten minutes.

Seminal fluid analysis:

According to the World Health organization WHO recommendations(7) ; and after labeling each sample. The collected samples using masturbation in wide mouthed Petri dishes that labeled with name laboratory numbers. Semen analysis should begin with a simple inspection soon after liquefaction, preferably at (30 minutes), but no longer than 1 hour after ejaculation, to prevent dehydration or changes in temperature from affecting semen quality

Anti-sperm Antibodies Test:

This test depends on physical mode of action between coated latex particles with anti-antibodies they will be monoclonal. SpermMar Latex particles is a suspension of polystyrene latex particles of approximately 2.0µm in diameter coated with monoclonal antihuman anti-IgA or IgG serum. The test is done by direct and indirect method for both immunoglobulins IgA and IgG (Ferti Pro® Kit, Belgium).

Direct SpermMAR-test for IgA

After reagents and specimens allowed became into room temperature. Then place on microscopic slide 10 µL of fresh liquefied semen drop 10 µL from reagent of SpermMar Latex particles using micropipette (Slamid, Germany). Then mix the sample and the Latex reagent 5 times with the edge of a cover glass (Sail brand, China) ; after that cover glass was put on the mixture and the mixture is observed under the power 40X to 60X magnification of light microscope using (ProWay®, Hb. Japan) ; reading after 3- 10 minutes.

Indirect SpermMAR-test for IgA

The reagent and seminal plasma specimens allowed becoming with in room the temperature. Inactivate the specimens by heating them using water bath (Memmert, Germany) 56°C up to 30 minutes. Then dilute inactivated specimens 1/4 with Earle's medium (FertiPro, Belgium). Thenafter wash, the motile sperm by letting them swim up in Earle's medium. Afterwards adjust the sperm concentration to $20X10^6$ spermatozoa/ml with Earle's medium.

Incubation of 100 µL from the suspension of motile spermatozoa with 100 µL of inactivated seminal plasma. Then 2 ml Earle's medium added, mix well and centrifuge for 10 minutes at 400 g. Decant or aspirate the supernatant. The pellet resuspends with 50 µL of Earle's medium; on a microscopic slide add the 10 µL of the sperm suspension and 10 µL from the SpermMar Latex particles reagent. Sample and reagent 5 times mixed in circular manner with the edge of the cover glass then after that gentle put of cover glass to spread mixture and the mixture is examined by light microscope using a 40x to 60x magnification. The result was as described for the direct SpermMar test.

Direct SpermMAR-Test for IgG

The sample and reagent with antiserum well allowed to room temperature, then on microscopic slide puttiedting of 10 µL of fresh untreated semen drop and beside it another 10 µL of SpermMar Latex particles and 10 µL of SpermMar Antiserum using different tips with micropipette (Slamid, Germany).

The semen 10 µL drop mixed first with reagent 10 µL drop for 5 times in circular manner then the mixture was mixed with 10 µL drop of antiserum that came with kit also by the same cover slip.

The cover glass is puttied on the mixture and the mixture is observed under a light microscope using a 40xor a 60x magnification.

The result taking after 2- 3 minutes. Observe for latex particles attached to motile sperm or not by counting of 100 spermatozoa determined the percentage reactive sperm. If no attachment of beads to sperm observed, also read again after 10 minutes.

Indirect SpermMAR-test for IgG

L*et al*l reagents and specimens to come to room temperature then deactivate the serum specimens that belong to same patient by heating them with water bath at 56°C for 30 minutes if glass test tubes used, 45 minutes. Wash the motile spermatozoa by letting them swim up in the pH adjusted medium (pH = 7.4-7.5). Swim up done in 5 ml glass or sterile plastic test tubes with round bottom at 37°C for 45 minutes.

Serially inactivated serum specimen was diluted by 1/16 with medium in a titer plate (Vulcan, USA). Mix 50 µL of diluted inactivated serum specimen from above with 50 microlitres of the washed motile donor sperm in a free well on the titer plate. Incubate for 60 minutes at 37°C.

10µL of sperm-serum mixture mixed with 10µL reagent firstly and then with the 10µL antiserum by the same manner with indirect method; gently cover glass was puttied on the mixture and observed by microscope.

The count 100 spermatozoa for determined the percentage reactive sperm. If no attachment of particles to sperm observed, read again after 10 minutes.

Statistical analyses:

The data were statistically analyzed using SPSS/ PC version 19 software (SPSS, Chicago). Sperm parameters were analyzed using one way ANOVA complete randomized design (CRD)(8). Differences among values of means were considered statistically significant at (P<0.05) and the correlation between the percentage of IgG and IgA.

Results:

Semen analysis for all subjects recorded and main sperm parameters appeared in the table (1), in this table the sperm concentration, progressive motility, non-progressive, total progressive sperm motility/ ejaculate and normal morphology were within normal criteria of WHO (2010).

The mean (23.88%) for positive cases of IgG of (no. 97) detected using direct method were no significant difference (P>0.05) found with the mean (27.41%) positive cases for IgG cases (no. 71) that detected using indirect method. The percentage of IgA in the direct and indirect methods for subjects showed significant correlation (P<0.05) in the IgA percent as in the figure (1).

Table (1): Sperm parameters compared to lower references value of WHO (2010)

Sperm Parameters	Subjects	WHO (2010) reference values
Sperm concentration (million/mL)	46.80 ± 0.6	≥ 15
Progressive sperm motility (%)	35.70 ± 0.83	≥ 32%
Non-progressive sperm motility (%)	28.30 ± 0.654	
Immotile sperm (%)	36. 0 ± 0.92	
Total progressive motility/ Ejaculate	39.91 ± 1.93	≥ 8.2
Normal sperm morphology (%)	31.291 ± 0..86	≥ 30%

Data are Mean ±S. E.
*Subjects No. 116

In the table (2), there was significant difference (P<0.05) between percentage of IgG and IgA detected using direct methods according to their distribution on the sperm tail, elsewhere non-significance difference (P>0.05) was found by the same method in the distribution around sperm head, and mid-piece. The distribution of IgG and IgA detected using indirect method within sperm mid-piece show significant difference (P<0.05) ; while non-significance difference (P>0.05) was noticed the same methods in the distributions on the sperm head and tail as shown in the table (2) The sperm concentration highly significant and negative correlated with IgA detected using the direct method (r=−0.421; P=0.001) and highly negative and significant with IgA detected by indirect method (r= -0.660; P=0.001) as in the table (3).

From the same table, the total sperm progressive motility negatively correlated and highly significant with IgA detected using indirect method (r=-0.663; P=0.001), and with direct method negatively significant (r= -0.320; P=0.002). The sperm progressive motility and IgA detected indirect method correlated negative and highly significant (r=-582; P=0.001).

Discussion

In the male, the presence of anti-sperm antibodies (ASAs) is an autoimmune disease causing immune infertility. ASAs have involved virtually all components of sperm and diminished sperm–oocyte binding and faulty zona pellucida penetration cervical mucus penetration and sperm survival(9). Further-

more, the ASAs linked to abnormal embryo development by retarding the cleavage process(10), and blocking the initiation of embryo(11) ; most significantly affected fertilization rates when localized both at the head and at the tail tip level of sperm. In the present study, the direct and indirect methods for detection IgG show no significant correlation between each one of them in all subjects and for the primary and secondary type of infertility for each of groups separately. That agreed with recent studies suggested that less than 1% of the serum IgG detected within male genital tract(10).

The origin of IgA in semen and pre-ejaculate has not been clearly determined. However, based on the molecular properties of Igs in these fluids, it appears that both the local synthesis, mainly in the penile urethra, and the circulation contribute to the Ig pool in these fluids(11). In female IgA formed due to allergy to sperms, infections of genital tract, coital trauma, and unexplained etiology(12).

Different results for Igs in several studies, as well as the present study may be due to different subclasses of Igs. The genital tract secretions that included IgA have various molecular properties from those of IgA in other body fluids(13). Contain of polymeric (p), dimeric, and tetrameric IgA reflect the dominant of S-IgA, with J chain and other secretory component (SC) essential during the selective transepithelial transport of pIgA; mIgA present in low quantities(14). The male and female genital secretions contain relatively similar properties of typical S-IgA, pIgA and mIgA(15). While the IgA subclasses (IgA1 and IgA2) are in proportions that differ from other body fluids present in genital secretions. The IgA2 more dominant in cervical mucus that secreted by cells in the endocervix(16), while in semen, IgA1 dominates, and the percentage of this subclass detected in semen is similar to the levels found in serum(17). Thus, this diversity of molecular forms of IgA in genital secretions reflects their origin(18).

The significant correlation between IgA detected using direct and indirect methods due to that there is IgA of secretory and local production in seminal plasma(19). In the present study, the antibodies IgG and IgA detected by the direct method according to their bound with the sperm head, there was no significant difference. The ASA directed against the sperm tail were involved in poor motility(20). Significant difference between the IgG and IgA detected using direct method distribution on the sperm tail bound, this result disagreed with another study whom investigated the impact of different Ig class according to their bounded site(21). While the antibodies directed against antigens in mid-piece detected by direct method reveal no significant difference between IgG and IgA for all subjects and those of secondary type infertility, which agreed with other study(22). In addition, the indirect method for both Ig classes according to the distributions on sperm parts for sperm head in the show no significant difference between the two methods for both classes that confirmed with previous studies(23). Moreover, the antibodies directed on sperm mid-piece showed significance difference that disagreed with previous study(24). It found that presence of ASA has negative effects on sperm-oocyte binding, penetration, fertilization and post fertilization events. IgA isotype of ASA demonstrated the lowest pregnancy rate. When majority of sperms coated with ASA on the sperms (heads, mid-piece and tail), cleavage rates were often impaired. Intra-cytoplasmic sperm injection recommended for such couples to achieve a successful pregnancy(25).

There were contradictory data on the relationship between the presence of ASAs and semen parameters, such as sperm concentration, motility and morphology(26). Most published results showed that there was no definite relationship between the presence of ASAs and sperm concentration, motility and morphology(27).

The concentrations of IgA and IgG in semen (detected by direct method) were not correlated; the results in present study disagree in that for the IgA may be because its secretory origin. The other studies found that prostate and seminal vesicle infection and subclinical reproductive tract infection might lead to dysfunction of sperm and changes in semen parameters, and the latter lead to infertility(26). Some possible mechanisms of the development of infertility are linked either for inhibition of spermatogenesis resulting from testicular damage or an autoimmune process(27). In this study, results revealing the total sperm progressive motility/ejaculate and progressive sperm motility significantly affected by IgG and IgA according to the sperm parts in both direct and indirect detection that well explained by the previous study (22). Possibly the ASAs could inactivate human sperm motility in the presence of complement, showing that complement dependent inactivation of sperm motility might be the biological mechanism of female infertility surface antigens of the acrosome and sperm tail principal piece appear to recognized by circulating sperm-immobilizing and sperm-agglutinizing antibodies(28).

The IgG detected by indirect method that is bound to the mid-piece and tail showed significant differ-

ence with normal sperm morphology, but with direct method showed no significant difference. While IgA detected by indirect method bound to the sperm head and mid-piece had significant difference compared with normal sperm morphology. In the other hand, no significant difference with the direct method of detecting IgA that is bound to different sites of the sperm.

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