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Abstract - The study was conducted to elucidate the correlation between direct antisperm antibody (ASA) test and some semen characters of infertile men at the Institute of Embryo Researches and Infertility Treatment at Al-Nahrain University from March 2009 to June 2010. Microscopic and macroscopic semen analysis, Direct Sperm MAR IgA test and Direct Sperm MAR IgG test were performed on semen samples. Forty-two men were included in the study, of whom, all underwent direct sperm MAR IgA test, but due to observer bias, only 36 Direct sperm MAR IgG tests were included in the study. There was a significant correlation between suspected IgA and viscosity (r=0.65, p=0.02); suspected IgA and pH (r=0.42, p=0.04); and suspected IgG and liquefaction time (r=0.44, p=0.04). A significant negative correlation was found between suspected IgG and progressive sperm motility (r=-0.44, p=0.04); suspected IgG and suspected IgA with morphologically normal sperm percentage (r=-0.65, p=0.03 and r=-0.43, p= 0.04, respectively); suspected IgG and sperm agglutination (r=- 0.54, p=0.03).

Keywords : *Direct antisperm antibody, IgA, IgG, immunological factor infertility*

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Direct Antisperm Antibody Examination of Infertile Men

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Abstract - The study was conducted to elucidate the correlation between direct antisperm antibody (ASA) test and some semen characters of infertile men at the Institute of Embryo Researches and Infertility Treatment at Al-Nahrain University from March 2009 to June 2010. Microscopic and macroscopic semen analysis, Direct Sperm MAR IgA test and Direct Sperm MAR IgG test were performed on semen samples. Forty-two men were included in the study, of whom, all underwent direct sperm MAR IgA test, but due to observer bias, only 36 Direct sperm MAR IgG tests were included in the study. There was a significant correlation between suspected IgA and viscosity (r=0.65, p=0.02); suspected IgA and pH (r=0.42, p=0.04); and suspected IgG and liquefaction time (r=0.44, p=0.04). A significant negative correlation was found between suspected IgG and progressive sperm motility (r=-0.44, p=0.04); suspected IgG and suspected IgA with morphologically normal sperm percentage (r=-0.65, p=0.03 and r=-0.43, p= 0.04, respectively); suspected IgG and sperm agglutination (r=- 0.54, p=0.03).

This study, recommends the use of Direct tests instead of the indirect assays in andrology laboratories in Iraq.

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I. Introduction

Infertility is a worldwide problem which affects approximately 15% of all couples, and is defined as, inability to conceive after twelve months of contraceptive-free unprotected intercourse (Irvine, 1998). Half of all the infertile couples remain untreated and/or unresolved. The main diagnostic groups for infertility are divided into those due to the male (25%), ovulation (25%), tubal, (20%), unexplained cause (25%) and endometriosis (5%) (Templeton, 1995).Among infertile couples, 40% are primary due to the infertility of the male partner, while in 20% of these cases, it is a combination of both male and female factors (Agarwal and Kulkarni, 2003).The common causes of male infertility are varicocele, accessory gland infection, immunological factors, congenital abnormalities, obstructive azoospermia; iatrogenic systemic and endocrine causes.

Irvine, (1998) reported that the occurrence of spontaneous antisperm antibodies (ASA) to human spermatozoa from infertile men was first described by Rumke and independently by Wilson in 1954. Antisperm antibodies (ASA) are the main cause of immunological infertility as they impair sperm function by binding to the sperm membrane (de Krester and Baker, 1999). ASA can be detected in seminal plasma, where they may be bound to the sperm surface or solubilized in the seminal plasma, or in cervical mucus, oviductal fluid or follicular fluid of women. Naturally occurring ASA in men can affect fertility by various mechanisms (Gardini et al., 1995). Some of them are mainly related to the extent of the sperm autoimmunization (e.g. sperm agglutination and impaired cervical mucus penetration); others are related to immunoglobulin (Ig)-isotype (e.g. complement-mediated sperm injury through the female genital tract); or to antigenic specificity of antisperm antibodies (e.g. interference with gametes interaction)(Gardini et al., 1995).

Antisperm antibodies can be defined as immunoglobulins of the IgG, IgA, and/or IgM isotype that are directed against various aspects of the spermatozoa (head, tail, midpiece or combination of them) (Templeton, 1995). Immunoglobulin subclasses IgA and IgG have been demonstrated in the ejaculates of men with antisperm autoimmunity. Though IgM seems to have no clinical impact, it is rarely detected alone or combined with IgA or IgG (Hijort, 1999). In Iraq, due to difficulties and high expenses in importing the kit of direct ASA test, the procedures that diagnose the antisperm antibodies in the andrology laboratories are indirect assays, using sera of men. It has been reported that anti-sperm IgG can be tested in serum but this is of little benefit as serum anti-sperm IgG neither correlates with anti-sperm Ig in semen, nor influences fertility prognosis (Esteves et al., 2007; Agostini and, Lucas, 2011). Globally, semen sample assays of men are used.

Antibodies can be measured in cervical mucus and seminal plasma as well as in serum or as an antigen-antibody complex on the surface of sperm. Direct assessment of sperm antibodies on the sperm surface is preferred to indirect measurement because capacitation of sperm might be useful in defining antibody reactivity (Esteves et al., 2007).This study is designed to find out the correlation between direct ASA test and certain semen characters of infertile men.

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II. Methods

Infertile males attending Institute of Embryo Researches and Infertility Treatment at AL-Nahrain University during the period from March 2009 to June 2010 were included in this study.

Seminal fluids were collected by masturbation after three to five days of abstinence and seminal fluid analyses were performed within one hour of sample collection. Seminal fluid analysis was performed according to WHO guide line (1999). For example, sperm concentration $\geq$ 20 million/ml, progressive sperm motility (a+b=50%), morphologically normal sperm ($\geq$ 30%), and round cells (<5 cells per high power field). All of the samples were subjected to Direct sperm MAR IgA, and sperm MAR IgG tests (Fertipro N.V. industriepark Noord 32.8730 Beemem, Belgium).

Direct Sperm MAR IgA and IgG tests are qualitative latex tests for detection of sperm antibodies of the IgA and IgG classes, respectively. For Direct Sperm MAR IgA test, the reagent used was Sperm MAR latex particles and for Direct Sperm MAR IgG test, the reagents used were Sperm MAR latex particles and Sperm MAR antiserum. For both tests, on a microslide 10 microliters of fresh semen and 10 microliters of Sperm MAR latex particles were added. The sample and the latex reagent were mixed five times with the edge of a cover glass. The cover glass was put on the mixture and the mixture was observed under a light microscope at power 40x magnification.

The result was read after 2-3 minutes. The latex particles attached to motile sperm were observed. One hundred spermatozoa were counted to determine the percentage of reactive sperms. If no attachment of beads to sperm was observed, the reading was again performed after ten minutes as described in the information booklet accompaniny with the kits (Jager et al., 1978 ; Hinting, 1988).

Samples with less than 10% reactivity were labeled as "Normal". The diagnosis of immunological infertility was "Suspected" when 10-39% of the motile spermatozoa were attached to latex particles. If 40% or more of the spermatozoa were attached, immunological infertility was "Highly Probable" as mentioned in the instructions accompanied with the kits (Jager et al., 1978). Based on these findings, the patients were divided into three groups for each, IgA and IgG, into normal, suspected, or highly probable immunological infertility.

III. Statistical Analysis

The data was expressed as mean $\pm$ standard error (SE). Further analysis was accomplished using vicariate Pearson's correlation coefficient. P-value with less than 0.05 was considered significant (Sorlie, 1995).

IV. Results

Forty-two patients were included in this study. Their age ranged between 22 to 45 years. Most of the patients had primary infertility (80.9%), whereas the remaining patients had secondary infertility. Forty-two men were subjected to Direct Sperm MAR IgA and Sperm MAR IgG tests. IgG test for thirty-six out of the total forty-two samples were included in the study; the remaining six were excluded because of bias in their results.

Table 1 shows no significant correlation between IgG and IgA with semen volume (r=0.27, p=0.22) and (r=-0.18, p=0.44) respectively. However, there was a significant negative correlation between normal IgA and semen volume (r=-0.54, p=0.03). A significant correlation was observed between suspected IgA and viscosity (r=0.65, p=0.02), and between suspected IgA and pH (r=0.42, p=0.04). There was a significant correlation between suspected IgG and liquefaction time (r=0.44, p=0.04).

Table 2 shows the correlation between both IgG and IgA with microscopic semen analysis. A significant correlation was noticed between high IgG and sperm concentration(r=0.46 p=0.04) with a significant negative correlation between suspected IgG and progressive motility (-0.44, p=0.04). There was also a significant correlation between suspected IgG and suspected IgA with morphologically normal sperm percentage (r=-0.65, p=0.03 and r =-0.43, p=0.04, respectively). The correlation coefficient for suspected IgG and sperm agglutination had a significant negative value (r=- 0.54, p=0.03).

V. Discussion

The results revealed no correlations of both IgG and IgA with semen volume, liquefaction time and pH, which are all normal in immunological infertility. However, there was a negative correlation between normal IgA and semen volume. This can be interpreted by the fact that in a sample with a low semen volume, normal IgA level is an indicator of accessory glands disorder rather than an immunological factor as the underlying cause of infertility. Several authors have stated that there is no relation between normal IgA antibodies value and abnormal semen parameters (Soffer et al., 1990). The other coefficient correlation was found between suspected IgG and liquefaction time, which means that for each semen sample taking a longer time for liquefaction, more IgG reactivity could be detected. A positive correlation of suspected IgA with viscosity and pH was also observed. This finding may be due to acute or chronic genitourinary tract infections, asymptomatic Chlamydia trachomatis infections, or an infection with human immunodeficiency virus (Naz et al., 1990; Soffer et al., 1990) .It is thought that locally

produced IgA is the most frequently implicated of humoral immune infertility (Francavilla et al., 2007).

In this study, we noticed a negative correlation between suspected IgG and sperm motility, sperm morphology and sperm agglutination .These correlations emphasized the cause of infertility. Antisperm antibodies that are located on the tail of sperm can cause the sperm to become immobilized or clumped together; and when antisperm antibodies are found on the head of sperm, they can prevent the sperm from being able to efficiently make its way through a woman's cervical mucus to the ovum (Agostini and Lucas, 2011). A significant increase in the proportion of motile sperms involved in agglutination has been reported in the presence of antisperm antibodies (Hijort, 1999).

Most of the men with antisperm antibodies had both, IgG and IgA on their spermatozoa but some had only IgG. Pure IgA reactions were not recorded and this is consistent with other studies (Bohring et al., 2001). An opsonizing effect is exerted by IgG antisperm antibodies which enhance sperm phagocytosis and lysis by peritoneal macrophages of the female and this effect is mediated by Fc-receptor for IgG (Agostini and Lucas, 2011). Cytotoxic effects due to complement activation can be produced by IgG but not IgA sperm bound antibodies (Hinting, 1988).

Aberration of the immune homeostasis may give rise to "immunological infertility". The majority of subjects with asthenozoospermia and/or oligozoospermia have a higher incidence of low complement-inhibiting activity and a reduced level of complement regulatory proteins in their seminal plasma, such that nonspecific activation of the alternative complement pathway occur, inflicting injury to sperm (Soffer et al.,1990).

In this study, six infertile patients showed significant high percentages of IgG with no agglutination in their semen. One explanation is that the autoimmune disease is at its initial stages. If those patients remained untreated for a certain period of time and were subsequently subjected to seminal fluid analysis test, notable agglutination might be noticed. However, there is variety of treatments available to help the couples struggling with ASA-mediated infertility, such as corticosteroids, intra-uterine insemination by sperm washing and *in-vitro* fertilization programs (Francavilla et al., 1997).

Direct sperm MAR IgG and Direct sperm Mar IgA tests are quick, easy, and sensitive, but require motile spermatozoa to ensure that the erythrocytes or immunobeads adhere to the sperm membrane (Jager et al., 1978). In several countries, Direct sperm Mar test kits are used to detect antisperm-IgG in semen. Positive and dubious samples are subsequently tested for antisperm IgA. IgA antibodies are more significant clinically, but very rarely occur without associated IgG; therefore, the test of antisperm IgG antibodies in semen is sufficient as the initial screening procedure (Naz et al., 1990).

Direct sperm MAR test is commercially available, cheap and quicker, and are generally considered as more suitable for routine screening of all semen analyses (Francavilla et al.,1997). Direct and indirect tests which do not detect the regional specificity of antibody link or which lack specificity for surface antigens because of concomitant exposure of internal antigens, are not suitable for delineating a diagnosis (Hinting et al., 1988).

One of the most rational ways to test for antisperm antibodies in males is by means of the direct mixed agglutination reaction (MAR) test (Bohring et al., 2001). This test is quick, easy, and sensitive, but requires motile spermatozoa to ensure that the erythrocytes or immunobeads adhere to the sperm membrane.

It is an excellent screening test if the reaction is negative or weakly positive (Bohring et al., 2001). In addition, sperm MAR is commercially available and requires no sperm processing (Esteves et al., 2007).

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Table 1 : Correlation of IgG and IgA with macroscopic seminal fluid parameters.

Kind of Ig(n)	Mean±SE In %	Volume In ml	r p	Liquefaction Time	r p	Viscosity	r p	pH	r p
IgG Normal 15	5.47±0.06	2.92±0.08	-0.22 0.31	36.0±0.63	0.28 0.33	0.20±0.03	0.27 0.40	7.76±-0.02	0.38 0.12
IgG Suspected 15	19.87±0.22	2.92±0.08	0.27 0.22	36.0±0.65	0.44* 0.04	0.2±0.04	-0.14 0.55	7.76±0.02	0.19 0.33
IgG High 6	78±0.47	2.92±0.04	------	36.0±0.33	-0.15 0.33	0.2±0.02	-0.08 0.88	7.76±0.01	0.02 0.90
IgA Normal 18	4.55±0.07	2.88±0.05	-0.54* 0.03	38.65±0.52	-0.05 0.91	0.38±0.04	-0.11 0.78	7.89±0.01	-0.03 0.88
IgA Suspected 21	16.48±0.18	2.88±0.06	-0.18 0.44	38.65±0.51	-0.08 0.66	0.38±0.04	0.65* 0.02	7.89±0.01	0.42* 0.04
IgA High 3	49.33±0.24	2.88±0.05	-----	38.65±0.53	-0.08 0.77	0.38±0.04	-0.07 0.67	7.89±0.01	0.02 0.89

Values are Mean±SE
r= Correlation coefficient
p= Probability

Table 2 : Correlation of IgG and IgA with microscopic seminal fluid parameters.

Kind of Ig Reactivity (n)	Mean ± SE	Count	r p	Progressive motility	r p	\ WBC+ germ cells	r p	Morphology	r p	Agglutination	r p	Round Cells	r p
IgG Normal 15	5.47±0.06	52.05±0.61	0.08 0.65	39.2±0.89	-0.34 0.22	1.2±0.18	0.27 0.32	35..95±0.70	-0.06 0.66	5.25±0.49	0.21 0.30	8..35±0.31	0.07 0.64
IgG Suspected 15	19.87±0.22	52.05±1.71	0.73 0.44	39.2±0.92	-0.44* 0.04	1.2±0.20	0.94 0.52	35..95±0.74	-0.65* 0.03	5.25±0.51	-0.54* 0.04	7.45±0.31	0.210 0.22
IgG High 6	78±0.47	52.05±0.76	* 0.46 0.04	39.2±0.42	0.003 0.99	1.2±0.09	0.15 0.44	35..95±0.34	-0.36 0.08	5.25±0.23	-0.10 0.57	8..35±0.31	0.10 0.66
IgA Normal 18	4.55±0.07	53.31±1.21	-0.27 0.21	41.08±0.61	-0.08 0.77	1.02±0.13	0.01 0.98	38.65±0.56	0.28 0.34	6.08±0.36	0.07 0.97	7.45±0.44	- 0.54* 0.03
IgA Suspected 21	16.47±0.18	53.31±1.21	0.20 0.88	41.08±0.61	0.09 0.90	1.02±0.13	-0.22 0.55	38.65±0.56	-0.43* 0.04	6.08±0.36	0.10 0.99	8..35±0.31	-0.13 0.88
IgA High 3	49.33±0.24	53.31±1.21	0.29 0.34	41.08±0.61	-0.02 0.87	1.02±0.13	0.15 0.67	38.65±0.56	-0.37 0.21	6.08±0.36	-0.13 0.33	7.45±0.21	-0.10 0.88

Values are Mean ± SE
r= Correlation coefficient
p= Probability

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