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Original Article

STUDIES ON SEMEN AND ANTISPERM ANTIBODIES IN MALE INFERTILITY

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ABSTRACT

Objective: To study the physical and biochemical parameters of human semen and observe its correlation with serum Antispermantibodies (ASA) in infertile males.
Setting: This study was conducted in the department of pathology and Mirobiology, Ziauddin Hospital North Nazimabad Karachi between Jan 1998 – Aug 1999. This was a cross – sectional study
Patients & Methods: Oligospermic infertile males aged 21-50 yrs normospermic infertile males aged 21-50 were included in the study. Patients who have undergone pelvic surgery, hernia repair, with diabetic neuropathy or taking steroids, cimetidine, sulfasalazine, nitrofurantion for the last 74 days were excluded.
Physical, microscopic and chemical properties of semen were studied in detail. Serum Antisperm Antibodies were determined by enzyme linked immunosrbent essay (ASA-ELISA)
Results: Out of 132 infertile males screened for Antisperm antibodies, 8 were found positive i.e. 6% of the infertile males were suffering from immunological problems. A correlation was found between older patients and high ASA titres. A correlation was also found between oligospermics and high ASA titres. Similarly we also found high ASA titres in infertile males with poor motility. High ASA titres were also found in oligospermic patients and patients with sperm morphology less than 50%. We also found significant difference ($P<0.05$) between serum ASA and semen white blood cells.
Conclusion: It is evident from the study that antisperm antibodies are one of the causes of unexplained infertility. They do not only decrease sperm motility but also decrease sperm counts and normal sperm morphology.

Key words: Antisperm antibodies (ASA), Male infertility.

INTRODUCTION

Although spermatozoa can be very antigenic, they are normally protected from entering the seminiferous tubules by the blood testis barrier. If this barrier sustains insult due to infection, trauma or surgery, sperm proteins may come in contact with the immune system, resulting in antisperm antibodies. Because a significant percentage of male infertility is believed to be immunologic, the specific role that antisperm antibodies play in infertility may become crucial in certain patient population. Antibodies have been shown to interfere with sperm motility and with the sperm's peneteration of the cervical mucus and the zonapellucida. In vitro fertilization has been used as a mean of overcoming these problems, however, antisperm antibodies can interfere with sperm – oocyte interaction in In vitro fertilization as well[1].

The Presence of antisperm antibodies in the male reproductive tract affects sperm function by possibly causing premature acrosome reaction and sperm immobilization

(agglutination) as well as by decreasing membrane integrity and opsonizing sperm for phagocytosis. Antisperm antibodies may bind to the sperm surface within the testis or epididymis (before ejaculation) or during the mixing of the sperm and seminal plasma at ejaculation. The presence of antisperm antibodies in the female reproductive tract may/impair sperm – egg interaction by interfering with the dispersion of cumulus mass and sperm binding penetration of the sperm into the zonapelucida and sperm egg fusion[2].

The purpose of present study was to determine serum antispermabntibodies by ELISA in infertile males. To observe the overall status of infertility semen analysis, viz, sperm count, normal sperm morphology, sperm motility, semen quantity, semen pH, semen fructose and semen white blood cells was also assessed. Furthermore, an attempt is made in this study, to establish a correlation between the different parameters of semen analysis and serum antisperm antibodies. This study further includes a comparison of these parameters among oligospermic and normospermic infertile males.

MATERIAL & METHODS

Male subjects with infertility

According to the following criterias, infertile males coming to Ziaudidn Hospital for semen analysis were included in the study.

Inclusion Criteria

Oligospermic infertile males aged 21 to 50 yrs and normospermic infertile males aged 21 to 50 yrs were included in the study.

Exclusion criteria

The subjects who have undergone pelvic surgery or hernia repair were not included in the study. Those patients with diabetic neurpathy or those taking non-steroidal anti-inflammatory drugs, steroids, cimetidine, sulfasalazine, nitrofurantion therapy for the last 74 days or with psychiatric disorders were also excluded. Out of these, 45 were oligospermics and 87 were normospermics. Infertile males were divided into two groups according to the sperm count.

Oligospermics are those who have sperm count less than 20 million/ml and normospermics have sperm count greater than 20 million / ml according to WHO guidelines.

Infertile males coming to the Ziauddin Hospital, Karachi for treatment or investigations were studied. In about 40 weeks of study, 160 infertile males were seen, 28 were excluded and 132 were the target cases. The history of the infertile males subjects included prior testicular insults torsoin, cryptorchism, trauma, infections (mumps, orchitis, epididymitis), environmental factors (excessive heat, radiation, chemotherapy) medications (anabolic steroids, cimetidine and spironolactone which may affect spermatogenesis; sulfasalazine and nitrofurantion that effect sperm motility). Loss of libido or visual disturbances may indicate a pituitary tumor. Past history also reveals thyroid or liver disease (abnormal spermatogensis, diabetic neuropathy retrograde ejaculation), pelvic or retroperitoneal surgery (absent seminal emission necessary to sympathetic nerve injury), or hernia repair (damage to the vas deferens or testicular blood supply).

RESULTS

Apart from detecting 6% antisperm antibodies in infertile males, following significant values were also found on semen analysis.

1. A positive correlation was found between the age of patients and antisperm antibodies greater than 70iu/ml.
2. A positive correlation was also found between antisperm antibodies greater than 70iu/ml and sperm count less than 20 million/ml and ?, 50% sperm morphology & sperm as shown in table 1 and 2.
3. A positive correlation was found between antisperm antibodies great than 70iu/ml and active sperm of patients as shown in table 3.
4. A positive correlation was found between antisperm antibodies greater than 70iu/ml and sluggish or non-motile sperms as shown in table 4.
5. Inverse correlation was found between ASA and normal sperm morphology as shown in Flg. 1.
6. Significant difference ($P<0.05$) between serum antisperm antibodies and semen white blood cells count of patients were found as shown in table 5.
7. A clear transparency and thin consistency of semen in oligospermic patients, whereas, turbid transparency and thick consistency innormospermic patient

DISCUSSION

Antisperm antibodies are detected in men suspected of infertility in 8% to 21% of the cases[3]. In the present study, out of 132 infertile males screened for antisperm antibodies, 8 were found positive for antisperm antibodies. This means that 6% antisperm antibodies were detected in infertile males of the cases studied. Our result is close to the result of Vazquez Levin (1997) study

FIGURE

Correlation between antisperm antibodies (ASA) and normal sperm morphology of infertile males

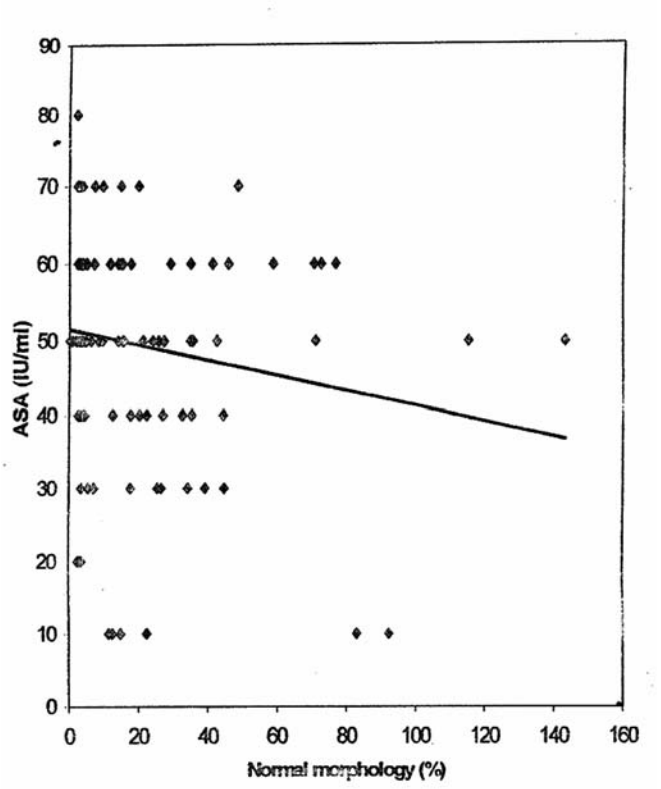


TABLE-1

COMPARISON BETWEEN > 50% NORMAL SPERM MORPHOLOGY AND SPERM COUNT IN INFERTILE MALES

The values are expressed as mean +standard error of mean. The number of cases or units are given in parentheses.

	NORMAL MORPHOLOGY (%)	SPERM COUNT (million/ml)
OLIGOSPERMICS: (19)	51.58* +0.86	10.79 +1.14
NORMOSPERMICS: (74)	59.59 +0.85	80.92 +6.61

*(P-value < 0.01)

TABLE – 2

CORRELATION BETWEEN SERUM ANTISPERM ANTIBODIES – ASA (IU/ML) AND SPERM COUNT (MILLION/ML) OF INFERTILE MALES

r is regression coefficient and r^2 is coefficient of determination

Variables	Correlation Coefficient	
	r	r^2
ASA/Count	-0.24	0.06
ASA/Count < 20	-0.14	0.02
ASA > 70 / Count < 20	0.62*	0.38*
ASA / Count > 20	-0.19	0.04
ASA > 70 / Count	-0.30	0.09
ASA < 70 / Count	-0.22	0.05

(* P Value <0.005)

TABLE – 3
CORRELATION COEFFICIENT OF SERUM ANTISPERM ANTIBODIES – ASA (IU/ML) AND ACTIVE SPERMS (%) OF INFERTILE MALES

r is regression coefficient and r^2 is coefficient of determination

Variable	Correlation Coefficient	
	r	r^2
ASA / Active Sperms	-0.13	0.02
ASA > 70 / Active Sperms	-0.64*	0.41*
ASA < 70 / Active Sperms	-0.08	0.01

(P-Value<0.05)

TABLE – 4
CORRELATION COEFFICIENT OF SERUM ANTISPERM ANTIBODIES – ASA (IU/ML) AND SLUGGISH SPERMS (%) OF INFERTILE MALES

r is regression coefficient and r^2 is coefficient of determination

Variables	Correlation coefficient	
	r	r^2
ASA / Sluggish Sperms	-0.05	0.00
ASA > 70 / Sluggish Sperms	0.46*	0.21*
ASA < 70 / Sluggish Sperms	-0.10	0.01

*(P-value < 0.05)

TABLE – 5

T – TEST FOR DIFFERENCE BETWEEN SERUM ANTISPERM ANTIBODIES (ASA) SEMEN WHITE BLOOD CELL (WBC) OF INFERTILE MALES

The values are expressed as mean +standard deviation.

#. Of Patients	WBC hpf	/Serum ASA IU/ml		P Value
		Mean	+19.6 S.Dev	
86	< 3	13.5	19.6	0.016*
46	> 3	21.4	29.0	

done at Centro de SaludReproductiva (CER), Buenos Aires, Argentina who found 8-21% case positive for ASA. Rooney et al 1992 in University of Wales, Cardiff, U.K. examined the effects of membrane attack complex (MAC) spermatotoxic effects are dependent on this assembly of MAC, they also examined decay accelerating[9] factor (DAF), MAC inhibiting protein (MIP) and CD59 antigen were present on membranes. Bohring and cruise 2003, Marburg, Germany also found that Antisperm Antibodies (ASA) in the male cause an autoimmune disease “Immune Infertility”. Apart from trauma and infection difference in culture between east and west and concepts regarding sex are probably the reasons behinds increased prevalence of immunological infertility in the Western Society. Intercourse during menstrual cycles, which is highly practiced in the west can be one of the reason of higher number of immunological infertility in Europe and America. Shedding of epithelium and blood products form female partner can be highly antigenic, they can cause development of antisperm antibodies in male partner during sexual contact. The male partner can be even

more susceptible of developing antisperm antibodies, if he has some abrasions or cuts in his genital organs. Apart from that, the shedding debris of menstrual cycle has high amount of bacteria, which can behave as antigens and develop antisperm antibodies in the male partner. A group of workers[4,5] suggested the use of semen manipulation techniques such as competitive in vitro immunsorption and rapid semen dilution to reduce the concentration of ASA in seminal plasma of men with low binding of immunolglobulins to sperm surface. However, in most cases the affinity of ASA for their corresponding antigens are high and the ASA – antigen complex binding occurs rapidly after ejaculation. ASA have a high affinity for the sperm membrane and consequently it is extremely difficult to either prevent the formation of ASA-antigen complexes or remove these complexes by traditional sperm processing methods. Sperm washing alone or high speed centrifugation methods do not seem to remove the ASA once the high affinity binding occurs. It is difficult to prepare sperm specimens for in utero insemination or assisted reproductive techniques when ASA are present because of agglutination, immobilization and reduction in membrane integrity. Attempts at dissociation the ASA – antigen complexes by reducing the pH or increasing the ionic concentration of the sperm's immediate environment result in irreversible loss of motility.[4,5].

It is believed that egg yolk components stabilize the sperm membrane surface by coating it with cholesterol, phospholipids, and lipoproteins. This type of interaction may also result in the removal of sperm surface component such as antigen sites or ASA – antigen complexes[6].

Assisted methods of conception including intra uterine insemination with washed sperm, gamete intrafallopian transfer and in vitro fertiizatin – embryo transfer have been shown to be of benefit in couples with immune infertility[7].Given recent advances in vitro fertilization technology that use intra cytoplasmic injection, a pregnancy can now be established with a single sperm[8]. Considering fertilization and implantation rates, intracytoplasmic sperm injection has proven to be an invaluable tool for the alleviation of male factor infertility. The technique involves the insertion of a single immobilized spermatozoon into the cytoplasm of metaphase II oocyte with the help of a fine drawn glasspipette. In case of regular fertilization the occyte will extrude the second polar body and two (i.e. male and female) pronuclei will be formed[9].

CONCLUSION

Therefore it is observed from this study that antisperm antibodies are one of the causes of unexplained male infertility. They not only impair motility of sperm but are also involved in decreased and abnormal spermatogenesis. It is concluded form the study that quantitative measurement of serum antisperm antibodies should be introduced routinely in all infertile males. Quantitative semen fructose test should also be introduced as directed by WHO guidelines so that we would be able to screen infertile males with seminal vesicle pathology effectively.

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