

CLINICAL SIGNIFICANCE OF ANTISPERM ANTIBODY ANALYSIS IN EVALUATING MALE INFERTILITY OF SOUTH KARNATAKA

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ABSTRACT

The presence of anti-sperm antibodies (ASA) in semen or blood serum may impair sperm function leading to immunological infertility. Hence, the clinical significance of anti-sperm antibody (ASA) analysis is gaining momentum. ASA screening was done on 64 patients registered for infertility treatment. Out of 64 patients, 38 (59.3 %) members showed positive response for ASA in their blood serum. Among them maximum number of patients belong to age group between 31-40 years. In this study ejaculatory dysfunction showed 100% positive response for ASA followed by asthenospermic condition (83.33%). Response of oligospermic (57.14%) and azospermic (47.82%) were in accordance with the other studies. Poor semen parameters were observed, especially the alkaline nature of semen, increased liquefaction time and change in the colour which confirms the infection in the patient. More than 50% of infertile patient of all etiologies showed positive results for the presence of ASA, and the prevalence probably increases with increase in patient's age.

INTRODUCTION

Infertility is a major health disorder which causes intense mental agony and trauma that can only be best described by the infertile couples. It affects approximately 15% of couples of reproductive age. Globally 60–80 million couples suffer from infertility every year and 15–20 million are in India alone (WHO, 1996). A male related factor is solely responsible in about 50% of cases of infertility (Siddiqi *et al.*, 2009). The causes of male infertility may be due to various reasons and their management and treatment depends on proper diagnosis.

Antisperm antibodies (ASA) are one of the main causes of infertility and the prevalence is detected in 8% -21% of infertile males (Dana and Alan, 1996; Reza *et al.*, 2009). A remarkable percentage of infertile couples without any strong etiology for infertility have been shown to possess circulating antibodies capable of agglutinating spermatozoa (Runke and Hellingc, 1959; Jones, 1979; Soren, 1990). These antibodies are found in blood serum, seminal plasma, bound to sperm and also in cervical mucus (Mazumdar and Levine, 1998; Hadinedoushan and Ghafourzadeh, 2007). The patient suffering from the antisperm antibodies in their semen is quite prevalent in Indian populations (Gupta and Garg, 1975; Arora *et al.*, 1999; Kapoor *et al.*, 1999; Puneekar *et al.*, 2001; Rajeev and Reddy, 2004).

The common causes of ASA include previous genital tract infection, testicular biopsy, testicular trauma, testicular torsion, vasectomy, prolonged use of alcohol, smoking and environmental pollution (Broderick *et al.*, 1989; Koide *et al.*, 2000; Arap *et al.*, 2007). ASA is thought to impair fertility by inhibiting sperm motility (Caron and Saling, 1991), inability of the sperm to penetrate cervical mucus (D'Cruz *et al.*, 1991),

capacitation (Bronson *et al.*, 1982), acrosome reaction (Jaffe and Oates, 1994) or they may involve the complete cascade resulting in sperm lysis (Jaffe and Oates, 1994; Downie *et al.*, 1997) and can also prevent implantation, and arrest embryo development (Haas, 1986; Koide *et al.*, 2000). The real significance of ASA in infertile men is controversial and currently there is no standardized treatment available so far (Marshburn and Kutteh, 1994). In this view the present study was carried out to find out the prevalence and relatedness of antisperm antibody which interfere sperm function in infertile men.

MATERIALS AND METHODS

A total of 64 males who approached the Mediwave Fertility Research Centre and Semen Bank, in Mysore for the inability to have a child and for further evaluation and treatment were recruited in this study. 100 males with proven fertility were included as control group. Ethical clearance was approved by the institutional ethical clearance committee of the University of Mysore, concerned hospitals and IVF centers.

Semen collection

The semen samples were collected in a sterile plastic container from the patients as well as the control group through masturbation after 3-5 days of ejaculatory abstinence (WHO, 1992). Informed consent letters were taken from the participants before including them in this study.

The collected semen sample was allowed to liquefy at 37°C for 30 minutes and analyzed within one hour of collection. Physical examination such as liquefaction time, colour, pH and viscosity were recorded. Basic microscopic examination

was carried out to record the count, density and motility of the sperm according to WHO protocol (WHO, 1992).

Functional capacity of the sperms was examined by sperm function tests through Hypo-osmotic swelling test (HOS) (Misro and Chaki, 2008), Acrosome intactness test (AIT) and Nuclear chromatin decondensation test (NCD) was carried out (Gopalkrishnan, 1995). Values were recorded and subjected for statistical analysis.

Collection of Blood Serum

The peripherals blood samples were collected from the subjects and centrifuged at 4,000 rpm for 10 minutes. The serum was separated and used for antibody detection by using anti - sperm antibody ELISA kit (Bioserv Diagnostic, Germany).

RESULTS AND DISCUSSION

In the present study the prevalence of different infertile conditions with spermiogram is shown in the Table 1. Sperm motility and viability was not recorded in azoospermic and in cases of ejaculatory dysfunction. Sperm motility and viability was decreased in asthenozoospermic and oligospermic conditions where as, teratozoospermic and idiopathic infertility condition shows normal values as mentioned in Table 1. Seminal pH was high in all infertile conditions which indicates some extent of infection and is quiet evident in all the cases. Further this is also supported by the colour of semen which showed yellow and green tinge. Liquefaction time was observed to be longer in azoospermic and oligospermic condition which indicates prostatic dysfunction. The prevalence of antisperm antibodies in infertile men with different infertile conditions is shown in the Table 2. Positive response for Antisperm antibody test was 100% in ejaculatory dysfunction followed by asthenospermia (83.33%), idiopathic (66.66%) and least value is recorded in azoospermic condition. Prevalence of antisperm antibody in infertile men with different age group was shown in the (Fig 1). More of infertile cases were reported in the age group between 31- 40 years followed by 20-30 years. Response of sperm function tests to different infertile conditions with respect to presence of Antisperm antibody is shown in the Table 3. Except idiopathic infertile condition, all other conditions showed negative response for nuclear chromatin decondensation test, hypo osmotic swelling test and acrosomal intactness test. Response of ASA with respect to smoking habits is shown in Table 4, among infertile cases 42.1% of smokers have shown positive response and 30.8% have shown negative response for ASA in their blood serum.

Over the years, a large number of cases of infertility have been found to be strongly associated with sperm agglutinating antibodies *i.e.* antisperm antibodies. During initiation of spermatogenesis, a sperm-specific antigen first appears at the time of puberty. Since such sperm specific antigens are not present during development of immunological tolerance, these proteins are potential targets for an immune response and therefore generate Antisperm antibodies (Dana and Alan, 1996). In the present study a total of 64 infertile males were investigated out of which 38 (59.3 %) members showed positive result for ASA in their blood serum. The results are close to those reported by Husted and Hjort (1975), Wilkin (1998) and Kapoor *et al.*, (1999). Among the infertile cases maximum number of patients belongs to 31-40 years of age group in our study, which is not in accordance with other studies (Glass and Vadya, 1970; Kapoor *et al.*, 1999). From the present study, we can understand that the possible cause for variation could be due to late marriages. In the present study 57.14% were oligospermic, 47.82% were azoospermic in accordance with Mathur *et al.*, (1983) where he reported 68% of oligospermic males were autoimmune to spermatozoans. Jones (1979) has reported a strong association between sperm count and antibody occurrence, stated that auto-immunity to sperm antigens can be related to infertility in men by an association with disordered spermatogenesis resulting in oligospermia or azoospermia. The infertility condition could also be result of cellular immunity and cytotoxic antibodies. Our study does not show any significant variation of ASA with respect to smoking habits.

Taking into consideration the function of blood testis barrier and other microenvironmental immunomodulatory mechanisms that provide tolerance to sperm molecules, It is clear that every breakdown of the barrier and the protection immunomodulatory mechanisms may lead to infertility with

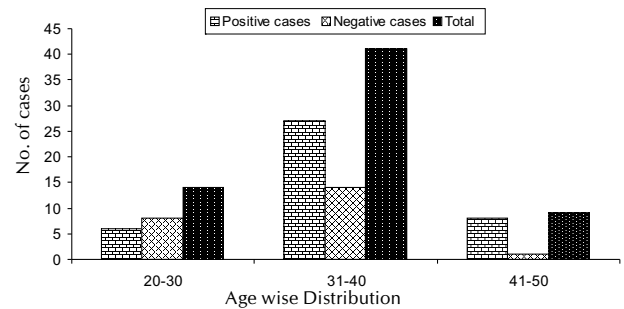


Figure 1: Prevalence of anti sperm antibodies (ASA) in infertile men with different age groups

Table 1: Prevalence of different infertile conditions with spermiogram

Condition	Sperm	Viability	pH*	Liquefactiontime		Colour	
	motility*50	*75	7.2 to 7.8	*(30 min)	> 45(min)	*Grayish white	Tinge green/ yellow
Azoospermia	-	-	8.1 ± 0.2	17	6	11	12
Asthenozoospermia	29.4 ± 16	54 ± 18	8.2 ± 0.3	4	2	4	2
Ejaculation dysfunction	-	-	-	-	-	-	-
Oligozoospermia	38.7 ± 21	55 ± 19	8.1 ± 0.2	9	5	11	3
Teratozoospermia	55 ± 14	63 ± 18	8.2 ± 0.2	4	4	6	2
Idiopathic	62 ± 11	76 ± 10	8.2 ± 0.2	6	3	8	1

* Indicates normal values

Table 2: Prevalence of anti sperm antibodies (ASA) in infertile men with different infertile conditions

Condition	Total No. cases	No. of cases with positive response	Positive response of cases in (%)	No. of cases with negative response	Negative response of cases in (%)
Azoospermia	23	11	47.82	12	52.17
Asthenozoospermia	6	5	83.33	1	16.66
Ejaculation dysfunction	4	4	100.00	-	00.00
Oligozoospermia	14	8	57.14	6	42.85
Teratozoospermia	8	4	50.00	4	50.00
Idiopathic	9	6	66.66	3	33.33

Table 3: Comparison of sperm function test with respect to Antisperm antibody analysis

Sl. No.	Conditions	Present of ASA (%)	HOS > 65% (normal)	NCD > 70 (normal)	AIT > 50 (normal)
1.	Azoospermia	28.9	-	-	-
2.	Asthenozoospermia	13.1	46.0 ± 27.0	61.0 ± 26.0	46.5 ± 14
3.	Ejaculation dysfunction	10.5	-	-	-
4.	Oligozoospermia	21.0	63.7 ± 17.0	60.0 ± 18.1	46.0 ± 22
5.	Teratozoospermia	10.5	51.0 ± 28.1	58.3 ± 28.0	39.0 ± 21
6.	Idiopathic	15.7	66.5 ± 15.6	65.0 ± 16.0	67.0 ± 16.

the autoimmune etiology. In most cases, the autoimmunity on testicular molecule resulting from trauma or infectious disease can generate ASA (Naz and Menge, 1994; Mc Donald, 2000; Sakamoto *et al.*, 1995). Mechanisms that can provide the autoimmunity and ASA production are microenvironmental acceleration of Th1 immunity, enhanced secretion of proinflammatory cytokines like IL-1, IFN- γ , TNF- α , reduced secretion of anti-inflammatory cytokines such as IL-10 and TGF- β . These mechanisms are associated with upregulation of major histocompatibility molecular expression and down-regulation of immune cells apoptotic mechanism (Naz and Menge, 1994; Sainio-Pollanen *et al.*, 1996; Mc. Donald 2000; Sakamoto *et al.*, 1995). In this study, we found that variation in semen parameters especially alkaline nature of semen, increased liquefaction time and change in the colour confirms the infections in the patient (Table 1).

Sperm immobilization (Shibahara *et al.*, 1995, 1996), inhibition of cervical mucus penetration (Kremer and Jager, 1992), and interference with events that lead to sperm-oocyte binding are some of the mechanisms by which anti-sperm antibodies impede fertilization (Clarke *et al.*, 1986, Shibahara *et al.*, 1993, Tasdemir *et al.*, 1995; Francavilla *et al.*, 1997). In the present study negative response of sperm function tests directly correlates with infertile cases where ASA are evident which inhibits the sperm motility, viability and penetrations (Table 1, 3). Review of numerous retrospective and prospective analyses of pregnancy rates for couples with circulating anti-sperm antibodies leads one to question, the prognostic value of anti-sperm antibody screening for diagnosis of infertility.

Table 4: Distribution of positive and negative response of antisperm antibody with respect to smoking habits

Conditions	No. of cases	Smokers (%)	Non Smokers (%)
No. of cases with Positive response	38	42.1	57.9
No. of cases with Negative response	26	30.8	69.2
Total	64		

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