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Original Article

Evaluation of Serum Antisperm Antibodies in Infertility

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Abstract

Aims and Objective: To evaluate the role of serum antisperm antibody (ASA) in infertility. *Method and Material:* This study was conducted in the Department of Obstetrics and Gynecology, Pt. J.N.M. Medical College, Raipur (C.G.), India, from December 2006 to July 2008 over 105 selected couples with primary and secondary infertility attending the infertility clinic. Their detailed clinical history was taken. Physical examination and routine as well as special investigations like pelvic USG, follicular study, and hysterosalpingography were done in the female. Complete physical examination and semen analysis of male partners were done. Couples were subjected to post coital test (PCT) 2-6 hours after intercourse to rule out cervical factor. Serum ASA titer in both partners was detected by ELISA. Results were interpreted for qualitative evaluation. ASA-positive cases were treated with low-dose daily oral prednisolone for 3 months and evaluated in terms of ASA titer, semen analysis, PCT result, and conception rate. The results were analyzed by statistical methods. *Results:* Out of 105 couples, serum ASA-positive males were 38 (39.19%), of which definite serum ASA positive were 9 (8.57%), borderline (equivocal) were 29 (27.61%), and negative were 67 (63.08%). Among females serum ASA positive were 42 (40%), in which definite ASA positive were 19 (18.09%), borderline 23 (21.9%), and negative 63 (60%). Asthenospermia was found more common in ASA-positive men (55.56%, p=0.0001). Poor PCT was most commonly associated in husband ASA negative and wife ASA positive. Treatment with low-dose oral prednisolone resulted in significant increase in motility of sperms in male partners and decrease in ASA titer in both the patients. Pregnancy was achieved in 45.23% ASA-positive females, while among couples with ASA-positive husbands, 31.57% of wives conceived. *Conclusion:* Serum ASA are considered to be cause of unexplained infertility and unexplained abnormal PCT. Antibodies against sperm prevent their motility through female reproductive tract and hamper the process of fertilization. Low-dose prednisolone was useful in infertility associated with ASA by improving sperm quality and giving rise to pregnancies.

Keyword: serum antisperm antibody, post coital test, ELISA.

Introduction

Infertility is defined as inability of couples to produce a living child. Inability may be the result of failure to conceive or failure to carry on conceptus to a viable state. If a couple fails to achieve pregnancy after 1 year of unprotected and regular intercourse, it is an indication for investigation.

Overall factors responsible for infertility comprise 30-40% in the male, 40-55% in the female, and ~10% in both partners. In ~10% cases the causes of infertility remain unexplained.

Cervical factors of infertility were present in 5-10% of infertile couples[1]. Abnormal cervix and its secretion are responsible for infertility in ~5-10% of women[2].

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Classical test for evaluation of the potential role of cervical factors in infertility is postcoital test (PCT). The PCT is designated to assess the quality of cervical mucus, the presence and number of motile sperms in the female reproductive tract after coitus, and the interaction between cervical mucus and sperms.

Immunological incompatibility may be the etiology of the infertility in some of the patients with unexplained infertility. In addition to these cases of unexplained infertility, a known cause of infertility may also be due to an immune reaction. This group of patients has an abnormal sperm cervical mucus interaction diagnosed using the PCT.

Immunological infertility is assumed to be the cause of infertility in 9-36% of the concerned couples. The main cause of immunological infertility is the formation of antisperm antibodies (ASA), which affect the capability of fertilization of spermatozoa[3,4].

ASA can be detected in male using spermatozoa, seminal plasma, and semen, and in female using serum and cervical mucus[5].

ASA were present in sera of 30% women with unexplained infertility. In this study ASA have been identified in 10-15% of men experiencing infertility and 15-20% of women with unexplained infertility[6].

The frequency of ASA in fertile population of women and men is <2%. In infertile men and women ASA are present in 5-25% of individuals[7].

Various factors are responsible for formation of ASA in male: obstruction of epididymis or vas including vasectomy, testicular trauma, biopsy or malignancy of testis, epididymo-orchitis, prostatitis, sexually transmitted diseases, homosexual rectal intercourse, and idiopathic.

In female ASA are formed due to allergy to sperm, infection of genital tract, coital trauma, and unexplained etiology.

Antisperm Antibody Effect

1. Reduced sperm output, sperm motility, and agglutination of sperm[8].
2. ASA impairing cervical mucus penetrations have been provided by donor sperm suppression exposed to semen containing ASA against the same sperm exposed to control semen without ASA[9].
3. ASA interference with fertility may be due to sperm injury caused by complement and/or phagocytic cell in the female genital tract[10].
4. ASA in men impair sperm-egg interaction, interfere in acrosome reaction, and binding to zona pellucida.

ASA level <75 IU was considered negative while levels >75 IU were considered positive[11,12].

ASA can identify immunological infertility and may be an additional cofactor associated with the more familiar reason for lack of conception. Therefore, all infertile couples should be subjected to immunological examination for the presence of ASA. The assessment of ASA in case of unexplained infertility is particularly important. ASA may be responsible for poor result in PCT, pathological spermiogram, and sperm agglutination.

Various methods are available for assessment of immunity to sperm: sperm micro agglutination test, sperm immbolization test, gelatin agglutination test, sperm cervical mucus contact test, immunofluroscence test, PCT, sperm cervical mucus penetration test, and newer methods like ELISA and indirect radio immunoassay. ELISA has provided a relatively simple, reliable, and highly reproducible method for detection of ASA.

Aims and Objective

To evaluate the role of serum ASA in infertility.

Method and Material

The study was conducted in 105 couples who attended the infertility clinic. Both primary and secondary infertility cases were considered. After obtaining detailed clinical history, general examination, and bimanual examination, routine and special investigations of both the partners were done. Type of infertility, duration, and other associated complaints were noted.

Detailed menstrual history was ascertained including age at menarche, regularity and duration of menses, as well as quantity of blood loss. Symptoms of ovulation including midcycle ovulatory pain, intermenstrual spotting, and dysmenorrhea were noted.

Detailed obstetric history was taken regarding married life, year of marriage, period of cohabitation, history of previous pregnancy, childbirth or pregnancy loss, number of abortions, whether spontaneous or induced in first or second trimester, and duration of secondary infertility. Past medical history of tuberculosis, diabetes, hypertension, thyroid disease, and polycystic ovarian syndrome was noted. Past surgical history like appendectomy, ovarian cystectomy, myomectomy, etc., in females, and herniorrpaphy, hydrocele operation, etc., in males was noted.

Personal history regarding exposure to heat, chemical, addiction like tobacco use, alcohol intake, frequency of coitus, any complaints during coitus, and knowledge regarding fertile period was taken.

Complete general as well as systemic examination of both the partners was done.

Internal examination of female genitalia consisted of per speculum examination for vaginal cervical conditions. Bimanual examination size, position, and mobility of uterus to rule out any congenital anomalies of uterus, tenderness in fornices, on movement of cervix, undue elongation, etc., were noted.

Routine investigation for infertility as well as special investigations like blood, TB, IgM, thyroid profile, prolactin, FSH, LH were done in selected cases. Ultrasonography of lower abdomen was carried out to rule out pelvic pathology. From 8th day of menstrual cycle evaluation of folliculogenesis to rule out anovulation, and hysterosalpingography to rule out genital tract abnormalities and patency of both tubes was performed.

Semen analysis after 3 days of abstinence was carried out for count, motility, morphology, and presence of pus cells.

PCT was done in preovulatory period after 2-6 hours after intercourse to rule out cervical factor. Serum ASA were studied in both partners to rule out formation of ASA and its titer. Patients positive for antisperm antibodies were subjected to low-dose corticosteroid (oral prednisolone) treatment for 3 months. Follow-up at 3 and 6 months was done after treatment. When pregnant, serum ASA titer, PCT, and semen analysis were noted. Data were analyzed using various methods like mean, t-test, chi-square test. The ratio of <1.0 was negative, 1.0-1.4 equivocal (borderline), and >1.4 positive.

OD cut off OD positive control X factor. The ratio for a particular patient sample was calculated. OD patients sample divided by OD cut-off.

Observations

The incidence of primary infertility was 76 (72.38%) and secondary infertility was 29 (27.62%) in the present study. Mean age at primary infertility was 25.76±4.72 and secondary infertility was 27.90±4.28 years, respectively.

Among males, 9 (8.57%) were ASA positive (ratio >1.4), 29 (27.6%) were borderline (ratio 1.0-1.4), and 67 (63.8%) were ASA negative (ratio <1.0). In females, serum ASA was found positive in 19 (21.9%), borderline in 23 (21.9%), and negative in 63 (60.0%). Among primary infertility cases ASA positivity was more preponderant in females (21.36%), while among secondary infertility cases male (10.34%) preponderance was more common (Table 1).

PCT is affected by serum ASA. Poor PCT was found more commonly in wife ASA positive and husband ASA negative. Negative PCT was found in 6 (6.52%), poor in 27 (28.42%), fair in 48 (50.52%), and good in 14 (11.02%) cases. Normal PCT was found in 65.26% and abnormal in 34.73% cases, (Table 2).

Asthenospermia/decreased sperm motility was found in 59 (56.19%) cases. In ASA-positive cases, >50% actively motile sperms were found in 44.44%, 30-50% actively motile sperms in 22.22%, and <30% actively motile sperms in 33.33% cases. Asthenospermia was found commonly in ASA-positive males. On statistical analysis the p-value was significant at 0.02 and <0.05 for positive versus borderline and negative cases, respectively (Table 3).

ASA-positive cases were subjected to treatment with low-dose oral prednisolone and response was predicted by conception rate, serum ASA titer, semen analysis, and PCT. Of nine couples with ASA-positive males, two wives conceived during treatment (22.22%), in three cases (33.34%) the ASA titer was found to decrease, and in one case (11.12%) ASA remained unchanged. Of 19 ASA-positive females, pregnancy occurred in eight (42.10%) during treatment, ASA titer remained positive in four cases (21.05%), and decreased in two (10.52%) (Table 4).

Table 1
Distribution of cases according to status of serum ASA

	Positive		Borderline ASA		Negative		Positive		Borderline ASA		Negative	
	No.	(%)	No.	(%)	No.	(%)	No.	(%)	No.	(%)	No.	(%)
Male	9	(8.57)	29	(27.61)	67	(63.8)	6	(7.89)	21	(27.61)	49	(64.47)
Female	19	(18.09)	23	(21.9)	63	(60)	17	(21.36)	14	(18.42)	45	(59.22)
Total	28		52		130		23		35		94	
	3	(10.34)	8	(27.58)	18	(62.0)						
	2	(6.89)	9	(31.0)	18	(62.0)						
	5		17		36							

Table 3
Distribution of cases according to ASA status and semen analysis

	ASA Status		Semen analysis			Motility			Morphology	
			Count million per ml			Actively motile sperms			Normal	Abnormal
	No.	(%)	>50	20-50	<20	>50	30-50	<30	>50%	<50%
Positive	9	(8.58)	5	3	1	4	2	3	7	2
BL	29	(27.62)	12	15	6	13	12	4	26	3
Negative	67	(63.80)	23	30	10	29	17	21	57	10
Total	105	(100)	40	48	17	46	31	28	90	15

Table 2
Distribution of cases according to ASA status and PCT result

S.No	ASA status			PCT n = 95							
				Negative		Poor		Fair		Good	
		No.	(%)	No.	(%)	No.	(%)	No.	(%)	No.	(%)
1	Husband positive wife negative	7	(7.36)	0	(0)	0	(0)	6	(6.31)	1	(1.05)
2	Husband positive wife BL	2	(2.10)	0	(0)	2	(2.10)	0	(0)	0	(0)
3	Both positive	0	(0)	0	(0)	0	(0)	0	(0)	0	(0)
4	Husband negative wife positive	13	(13.68)	1	(1.05)	6	(6.31)	6	(6.31)	0	(0)
5	Both BL	7	(7.36)	0	(0)	1	(1.05)	5	(5.26)	1	(1.05)
6	Husband negative wife borderline	14	(14.73)	1	(1.05)	8	(8.42)	3	(3.19)	2	(2.10)
7	Husband borderline wife negative	14	(14.73)	1	(1.05)	4	(4.21)	6	(6.31)	3	(3.15)
8	Both Negative	32	(33.68)	3	(3.15)	2	(2.10)	21	(22.10)	6	(6.31)
9	Wife positive husband borderline	6	(6.31)	0	(0)	4	(4.21)	1	(1.05)	1	(1.05)
	Total	95	(100)	6	(6.30)	27	(28.4)	48	(50.52)	14	(14.73)

BL borderline
Poor PCT was most commonly found in husband negative and wife positive serum ASA status.

Serum ASA positive and borderline couples were subjected to serum ASA titer, PCT, and male partner semen analysis was done after completion of therapy for 3 months. Out of 33 cases, PCT was found to be negative in 2 (6.06%), poor in 15 (45.45%), fair in 13 (39.33%), and good in 3 cases (9.09%), respectively. Normal PCT was found in 16 (48.48%) and abnormal in 17 (51.51%) cases. PCT was improved after treatment (Table 5).

The semen analysis of 14 men who underwent complete treatment showed an improvement in sperm count and motility (Table 6).

Discussion

Primary infertility is defined as lack of pregnancy after one year of unprotected regular intercourse, while secondary infertility is failure to conceive after having achieved a previous conception. The incidence of primary infertility in the present study was 76/105 (72.8%) and that of secondary infertility 29/105 (27.62%). Other studies also found similar incidences of primary and secondary infertilities[8,9,10].

Incidence of ASA in infertile population varied, depending upon the reporting center and method used for detection. Our study found it more or less similar to that observed in other studies[11,12].

Infertility can result from antibodies either directly binding to sperms or affecting the spermatogenesis due to allergic orchitis. ASA can interfere with sperm motility by immobilizing the sperm or interfering with sperm mucus interaction or disturbing sperm transport.

Table 4
Distribution of cases according to treatment response on ASA (husband)

									ASA titer after 3 months treatment								p-value
S. No.		Serum ASA titer									Positive		BL		Negtive		P
		No. (%)	%	Conception during treatment (month)			No. of drop out	No. of Treatment for 3 months	Conception after treatment in month			No.	%	No	%	No	%	%
				1	2	3			1	2	3						
1	>1.4	9	(23.68)	1	0	1	3	4	0	0	0	1	(25.0)	3	(75.0)	0	(0)	
2	1.0-1.4	29	(76.31)	3	4	3	9	10	0	0	0	0	(0)	8	(80.0)	2	(20.0)	<0.05
	Total	38	(100)	4	4	4	12	14	0	0	0	1	(7.14)	11	(78.57)	2	(14.28)	
									Female								
1	>1.4	19	(45.23)	2	2	4	4	7	0	0	0	4	(57.14)	2	(28.57)	1	(5.26)	<0.05
2	1.0-1.4	23	(54.76)	4	2	3	3	12	0	2	0	0	(0)	9	(75.0)	3	(25.0)	
	Total	42	(100)	6	4	7	7	19	0	2	0	4	(21.05)	11	(57.89)	4	(21.05)	<0.05

P positive vs. borderline
In female ASA titer decreased after treatment in 6 cases.

Table 5
Distribution of cases according to ASA and PCT result before and after treatment

Sr.no	ASA status				PCT							
					Negative		Poor		Fair		Good	
		No.	(%)		No.	(%)	No.	(%)	No.	(%)	No.	(%)
1	Husband positive wife negative	2	(6.06)	Before	0	(0)	0	(0)	2	(100)	0	(0)
				After	0	(0)	0	(0)	1	(50)	1	(50)
2	Husband positive wife BL	2	(6.06)	Before	0	(0)	2	(100)	0	(0)	0	(0)
				After	0	(0)	1	(50)	1	(50)	0	(0)
3	Both positive	0		Before	0	(0)	0	(0)	0	(0)	0	(0)
4	Husband negative wife positive	7	(21.21)	Before	1	(14.28)	5	(71.42)	1	(14.28)	0	(0)
				After	0	(0)	4	(56.96)	3	(42.84)	0	(0)
5	Both BL	2	(6.06)	Before	0	(0)	1	(50)	1	(50)	0	(0)
				After	0	(0)	0	(0)	2	(100)	0	(0)
6	Husband negative wife borderline	8	(24.24)	Before	1	(12.5)	5	(62.5)	2	(25)	0	(0)
				After	1	(12.5)	4	(50)	3	(37.5)	0	(0)
7	Husband borderline wife negative	9	(27.27)	Before	1	(11.12)	4	(44.48)	2	(22.24)	2	(22.24)
				After	1	(11.12)	3	(33.36)	3	(33.36)	2	(22.24)
8	Wife positive husband borderline	3	(9.09)	Before	0	(0)	3	(100)	0	(0)	0	(0)
				After	0	(0)	3	(100)	0	(0)	0	(0)
Total		33	(100)	Before	3	(9.09)	20	(60.60)	8	(24.24)	2	(6.06)
				After	2	(6.06)	15	(45.45)	13	(39.39)	3	(9.09)

Table 6
Distribution of cases according to ASA verses treatment response on semen analysis

Semen analysis		**Serum ASA status (n = 14 cases)**					
Semen parameter		**Positive**		**Border line**		**Negative**	
Count in million/ml		**No.**	**(%)**	**No.**	**(%)**	**No.**	**(%)**
>50	Before	1	(7.14)	10	(71.4)	0	(0)
	After	1	(7.14)	11	(78.5)	1	(7.14)
20-50	Before	2	(14.28)	0	(0)	0	(0)
	After	0	(0)	0	(0)	1	(7.14)
<20	Before	1	(7.14)	0	(0)	0	(0)
	After	0	(0)	0	(0)	0	(0)
Motility							
>50	Before	0	(0)	3	(22.33)	0	(0)
	After	1	(7.14)	4	(28.56)	1	(7.14)
30-50	Before	2	(14.28)	6	(42.84)	0	(0)
	After	0	(0)	6	(42.84)	1	(7.14)
<30	Before	2	(0)	1	(7.14)	0	(0)
	After	0	(0)	1	(7.14)	0	(0)
Morphology							
>50	Before	2	(14.28)	10	(71.40)	0	(0)
	After	2	(14.28)	11	(78.50)	2	(14.28)
<50	Before	2	(14.28)	0	(0)	0	(0)
	After	0	(0)	0	(0)	0	(0)

In the genital tract, sperm migration and motility can be detected by PCT.

PCT can be affected by various factors like infection in cervix, wrong timing and method, presence of ASA, and use of medicines like ovulation-inducing drugs. Sinha et al. (2005)[13] found that out of 68 infertile couples, 14 women had normal PCT and only 2 of them were IgA positive. Total 54 women had abnormal PCT with local ASA, cervical mucus contained nonmotile sperms in 42, and only weakly motile sperms in 12. None of them had ASA-positive serum.

Guzin et al. (1993)[7] found that in ASA-positive couples, PCT was positive in 27.2% cases (6/23) and negative in 72.8% (17/23) cases. Other studies also found that the PCT results were affected by serum ASA[3,4,11,14].

ASA may decrease the motility of spermatozoa through agglutination and immobilization, thus inhibiting sperm migration through the female genital tract. Decrease in sperm motility has been observed by other authors as well in ASA-positive males[15]. Treatment of serum ASA-positive couples with low corticosteroid (oral prednisolone) results in improvement of sperm count,

motility, PCT results, and conception, and decrease in titer of ASA. Some patients may not respond to treatment. Other studies also found that ASA titer reduced significantly after corticosteroid (oral prednisolone) treatment. Ulcova et al. (2000)[14] found that treatment with decreasing doses of oral prednisolone or dexamethasone for 3 months in case of serum or seminal antibodies, IgA sperm anticoagulating antibodies totally disappeared in 3 out of 11 men and 4 out of 11 women, but sperm IgM sperm antibodies persisted.

Omu et al. (1996)[16] found that 5 mg prednisolone given orally daily for 3-6 months resulted in significant improvement in ASA, pregnancy rate 20% was much greater than 5% in control group (p=0.01). After corticosteroid treatment with low-dose prednisolone the fertilization rate (34.9%) in the prednisolone group was similar to that of placebo group (38.7%).

Snick et al. (1997)[10] reported cumulative pregnancy rate of 31% during 9 months by prednisolone treatment which was significantly higher than the rate of 9.5% for placebo.

Philips and Ivar (2004)[17] reported that corticosteroid treatment resulted in increased pregnancy rate of 32% as compared to 17% in the control group.

Semen analysis done post treatment with prednisolone in those patients whose wives did not conceive showed improved sperm motility. Other studies showed treatment of male infertility with corticosteroid resulted in improved sperm motility[14,16].

Out of 33 PCTs after treatment with prednisolone normal PCT was seen in 14 cases and abnormal in 19 cases, result showed little improvement in PCT. Other studies also showed little improvement in PCT after prednisolone treatment[13,14,16].

Conclusion

Sperm antibodies are considered to be a cause of unexplained infertility and unexplained abnormal PCT. Low-dose prednisolone is useful in ASA associated infertility by improving sperm quality and giving rise to pregnancies.

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