



Detection of antisperm antibodies in patients with unexplained infertility

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Abstract

Objective: To detect Antisperm antibodies in Patient's with unexplained infertility.

Method: This study was undertaken in 50 patient of unexplained infertility who were admitted in the indoor ward and those attending outpatient Department of obs and Gynae of M.L.N Medical College, Prayagraj.

fifty couples with no organic cause for infertility were selected for this study. 25.58% of couples with primary unexplained infertility showed positive ASA while only 14.42% of couples with secondary unexplained infertility showed positive results. No correlation was present between the presence of ASA and duration of infertility, socio economic status and ethnic group.

Result: Antisperm antibody has a high predictive value for the patient with unexplained infertility. Presence of antisperm antibody was a more accurate predictor of Unexplained infertility than other historical risk factors. The serological test is rapid and non-invasive, and our data suggests that it would be useful in investigation of infertility.

Conclusion: Antisperm antibody is a Sensitive, specific, easy, non-invasive and reliable test for diagnosis of Unexplained infertility.

Keywords: antisperm antibody, infertility, post coital test, unexplained infertility, immune infertility, agglutination test, corticosteroid therapy

Introduction

Infertility is defined by the failure to achieve pregnancy after 12 months or more of regular unprotected sexual intercourse. Infertility affects 15% of couples. Primary infertility is infertility in a couple who have never had a child. Secondary infertility is failure to conceive following a previous pregnancy^[1-4]. Male infertility is responsible for 20–30% of infertility cases, while 20–35% are due to female infertility, and 25–40% are due to combined problems in both parts. In 10–20% of cases, no cause is found. The most common cause of female infertility is ovulatory problems, which generally manifest themselves by sparse or absent menstrual periods. Male infertility is most commonly due to deficiencies in the semen.⁴ Infertility status of the couples was evaluated by history from both partners, clinical examination and different routine and specific test for infertility, so that the cause of their infertility could be detected and only those couples were taken for the study in whom even after this evaluation the cause of infertility could not be detected and remained unexplained.^[5, 6] The term unexplained infertility is applied to that couple who has failed to establish a pregnancy despite an extensive evaluation that uncovers no obvious reasons for infertility or after correction of factors identified as responsible for infertility.⁶ The importance of immunogenic mechanism as the cause of unexplained infertility has been receiving increasing attention in the clinical practice during recent years. It has been amply demonstrated that materials from the reproductive tract contain antigens capable of inducing the formation of antibodies which can be detected by

various serological tests^[7, 8]. Sperms are potentially immunogenic. In men the sperm antigens to which the adult organism is not tolerant are sequestered from the immune system by the large number of tight junctions between Sertoli cells that line the seminiferous tubules^[9]. Breach in this will trigger the formation of antisperm antibodies in man. Common events in men that have been noted to increase the risk of antisperm antibodies are vasectomy, trauma, torsion, cancer, varicocele, testicular biopsy, receptor rectal intercourse in homosexual and genetic predisposition. In men antisperm antibodies are found in three body compartments- blood serum, seminal plasma and on sperm surface^[9, 10]. In females antisperm antibodies have been demonstrated in cervical mucus and serum. Isoimmunization against sperm might be expected to be common in sexually active women as sperms are recognized as foreign antigens^[13]. Lack of immunity to sperm in majority of women maybe due to an immunosuppressive or anticomplementary effect of seminal plasma, naturally occurring blocking antibodies that hide spermatozoal antigens from immune recognition and rapid macrophage ingestion of majority of sperm that reach the intrauterine environment^[14]. Immune infertility is a challenging field of research fraught with difficulty and ASA are an important factor that can interfere with the various steps of fertilization. They must be screened for and carefully evaluated in order to identify the possible aetiology and provide treatment and as a last resort direct couples towards the most appropriate form of assisted reproduction^[14]. The present study is an attempt to determine the presence of

ASA among couples with unexplained infertility.

Materials and Methods

This study was undertaken in the Gayatri Hospital, Prayagraj over a period of 2 years from August 2015 to July 2017. The study population were selected from the couples attending the outpatient department of Obs and Gynae for their problem of infertility and those admitted in patient department for evaluation of their infertility status. A thorough and careful general and pelvic examination was made to detect any demonstrable cause for infertility in females. Similarly, a general physical examination of the husband was also carried out. Besides the routine hemogram and urine examination of the husband was also carried out. Besides the routine hemogram and urine examination the following investigations were carried out.

- Endometrial biopsy for confirmation of ovulation or for exclusion of any pathologic condition.
- Hysterosalpingography/ laparoscopy.
- Blood grouping and Rh typing of both partners.
- VDRL of both partners.
- Fasting blood sugar of both partners.
- Post coital test.
- Ultrasonography for serial folliculometry. (In some cases)
- Hormone estimation.

Other tests like x-ray chest, Mantoux test done where indicated. Seminal fluid analysis of husband in all cases were done for sperm count, sperm motility and presence of pus cells and also for detection of any spontaneous agglutination of sperms.

Criteria for selection of cases

- Both partners were in fertile age group
- Couples were living together without use of any contraceptive but conception failed to occur for one or more year
- Husband were normal with normal semen analysis.

Criteria for control study

- Couples with known fertility
- Pregnant female attending OPD for ANC
- Fertile patients admitted for other gynecological problems

Detailed history was taken and physical examination done, complete hemogram and routine examination done similar to the study group.

Methods for Antisperm antibody test

For antibody activity

Observation

Table 1: Amongst 43 couples of primary unexplained infertility 11 couples showed positive ASA. Of these 2 males and 4

Group of cases	Number of couples	Number of positive cases	Asa positive		
			Only males	Only females	Both partners
Primary infertile	43	11	2	4	5
Secondary infertile	07	1	0	1	0
Fertile control	10	0	0	0	0
Total	60	12	2	5	5

females were positive for ASA whereas in 5 couples both partners showed ASA. Amongst 7 couple of secondary unexplained infertility only one female showed positive ASA. None from fertile control group showed positive ASA

in semen microagglutination techniques TSAT (tube slide agglutination test) described by Franklin and Dukes (1964) and slightly modified by Gungah *et al* (1978) was adopted.

Collection of blood

5ml of blood was collected from both partners and serum was separated. The serum was then incubated at 56 degree for 30 min to destroy the complement.

Semen samples

The husband provided the semen after 3 days abstinence. Semen specimen was collected by masturbation in a clean, dry wide mouth glass container.

A complete semen analysis was performed by examining liquefaction time, colour, quantity total count, motility, presence of pus cells and WBC.

The amount of serum and semen taken for the test was 0.5 ml and 0.1 ml respectively. The serum agglutination test was carried out in serological tubes as follows

Tube 1- 0.5 ml of the wife's serum + 0.1 ml of husband semen

Tube 2- 0.5 ml of husband serum + 0.1 ml of husband semen

Tube 3- 0.5 ml of normal saline + 0.1 ml of husband semen

The tubes were incubated in the water bath at 37 degree Celsius for four hours. Samples were examined for agglutination after one hour, two hours and four hours. For the test one drop of sample was taken from the bottom of each of the serological tubes separately on separate slides and were covered with cover slip. They were then examined under high power of microscope for evidence of agglutination. Evidences of positive agglutination was considered to be the aggregation of two or more motile spermatozoa per HPF within four hours period. Agglutination around cell debris were not taken into consideration. Normal saline was used to detect nonspecific agglutination.

Grading

Agglutination were graded as

1. + when there was clumping of 2 to 10 motile sperm/HPF.
2. + when there was clump of 10-20 motile sperm/HPF
3. + when there was clumping of more than 20 sperm/HPF

The cases in which the presence of antisperm antibody was established were subjected to following treatment

Husband of wives with positive ASA were advised to use condom. Low doses steroids to male partners. Antibiotics were advised if needed. Follow up was done during the treatment period.

Table 2: correlation between asa and primary and secondary unexplained infertility

Subject	Asa activity		Total	
	Positive	Negative		
Primary	11	32	43	
Secondary	01	06	07	
Total	12	38	50	P>0.05

Of the 50 cases of unexplained infertility positive ASA was observed in sera of 12 couples.11 couples with positive ASA were of primary unexplained infertility and only one couple belonged to secondary unexplained infertility.Results are not significant statistically.

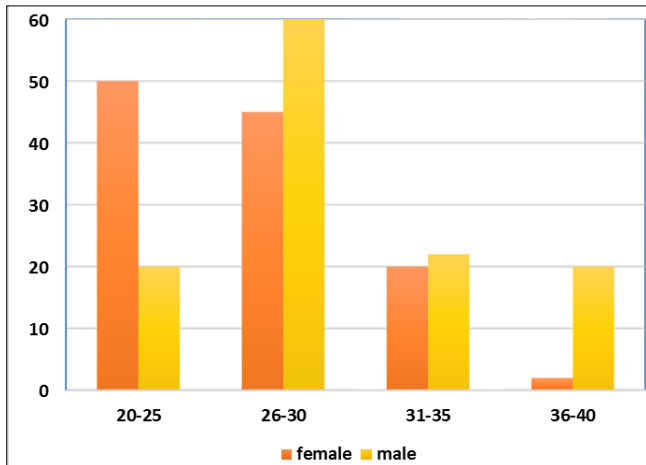


Fig 1: Age Distribution of Unexplained Infertility

From this table it is observed that 84% of the female partners and 70% of male partners were below 30years.

Table 4: Incidence of Asa in Infertile Husband and Wife

Subject	Asa activity		Total	P
	Positive	Negative		
Wife	10	40	50	
Husband	07	43	50	
Total	17	83	100	P>0.05

Amongst the partner of 50 unexplained infertile couples studied 10 wives and 7 husbands were positive for ASA. More wives showed positive ASA.The results are not significant statistically. (P>0.05)

Statistical analysis

Fisher exact was used to determine the significance of correlation between the ASA activity and primary and secondary type of unexplained infertility, post coital test results and primary and secondary type of unexplained infertility and ASA activity and fertile and infertile couple. McNemar's test was used to determine the significance of correlation between the presence of ASA in husbands and wives and their state of infertility. Modified chi square test was used to determine significance of the correlation between the ASA activity and post coital test results and students unpaired 't' test was used to determine the significance of correlation between age of husbands and wives with the presence of ASA in their sera.

Discussion

The present study was undertaken to detect the presence of antisperm antibody in cases of unexplained infertility and to provide appropriate treatment.

fifty couples with no organic cause for infertility were selected for this study.10 couples of known fertility were taken as controls. Sperm agglutinating antibodies were detected and Dukes technique with modification by Gungah. The cases were studied with respect to the age,duration of marriage,SES and ethnic group.Results of post coital test and ASA were also evaluated.positive ASA was detected in 12 couples with unexplained infertility. The percentage of positivity was higher in female partner(20%)than in male partners (14%)

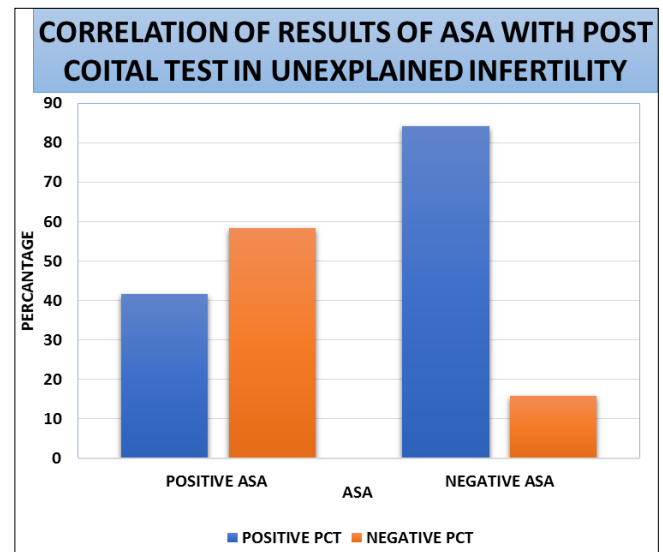


Fig 2: Correlation of Results of Asa with Post Coital Test in Unexplained Infertility

This table 5 shows that more patients with positive ASA unsatisfactory PCT (58.33%) than those with negative ASA (15.69%). The result is significant statistically. (P<0.05). 25.58% of couples with primary unexplained infertility showed positive ASA while only 14.42% of couples with secondary unexplained infertility showed positive results. Thus more ASA positivity was seen in couples with primary unexplained infertility. Among the couples of fertile group none showed positive ASA in their Sera Though more cases of positive ASA was seen among higher age group females there was no correlation between two. similarly no correlation was present between the presence of ASA and duration of infertility, socio economic status and ethnic group.More number of positive ASA had negative post coital test and less number of positive ASA had positive PCT whereas amongst negative ASA cases more had positive PCT (84.21) and lesser number (15.69) had negative PCT. It shows a significant correlation between the positive ASA and negative PCT.

On the other hand, amongst positive PCT 13.5% had positive ASA whereas among all negative PCT cases 53.84%had positive ASA.The seven male partners showing ASA were put on low dose corticosteroid therapy and for ten female their male partners were advised to use condoms

for 3 to 6 months. ASA estimation was done after a variable period 3 to 6 months. Most of the cases showed negative ASA on re-evaluation but no pregnancy was reported.

Antisperm antibodies have been shown to play an important role in unexplained infertility. Screening of serum samples for presence of ASA should be preferentially performed when results of routine examination do not render definite cause of infertility in a couple of suspected so-called unexplained infertility, poor post coital test or long-standing infertility without success of different therapeutic regimens. Testing interaction between serum and cervical mucus is clinically useful in determining whether antisperm antibodies are contributing to the patient's infertility. An effective treatment has not been established and few current treatments have been tested in controlled trials. Use of condoms may reduce antibody titre in woman but pregnancy rates are poor. Immunosuppression by corticosteroid has shown mixed success. Reduction in antibody titre have been reported but serious side effects can be ignored. Intra uterine insemination with washed sperm can produce pregnancies but since antibodies are difficult to clear from sperm by washing and since woman with antibody probably have them higher in the tract than simply in the cervix its values may be limited. The same problem limit the use of GIFT (gamete intrafallopian transfer) in female isoimmunisation but it may be of value in male auto immunisation. *in vitro* fertilization gives maximum control over interaction between the sperm and oocyte and antibody exposure.

Conclusion

Antisperm antibody is a sensitive, specific, easy, non-invasive and reliable test for diagnosis of Unexplained infertility.

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