Meteroic Determination of Immunological Infertile Males

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Abstract
Background: *The inability to conceive within 1 year of unprotected intercourse is estimated to affect upto one in five couples of reproductive age and poses a significant health problem. Presence of antisperm antibodies in semen can lead to a condition called 'immunological infertility'. We envision the use of Mixed Agglutination Reaction for the detection of antisperm antibodies.*
Methods: *Patients referred to Department of Pathology for semen analysis from Cheluvamba hospital and K R Hospital is included in the study. After 3 days of abstinence, they were advised to collect semen by masturbation into a sterile wide mouthed container. Semen analysis is carried out where viability, motility and count of sperms are recorded followed by Mixed Agglutination Reaction*
Results: *Out of the 96 cases studied, majority of the patients were in 30-35years age group. Most common history mentioned was primary infertility. MAR positivity was detected in 37% of cases. Routine semen analysis parameters such as sperm count, viability, motility and pH was found inappropriate for detection of antisperm antibody.*
Conclusion: *Immunological infertility is important as it has got prognostic and therapeutic implications. As the principal treatment of immunological infertility is IVF, present study will be helpful to predict the successful outcome of IVF, hence in preventing financial burden on to the family.*
Keywords: *Immunological Infertility, Male, Meteroic, Mixed Agglutination Reaction.*

Introduction

The inability to conceive within 1 year of unprotected intercourse is estimated to affect upto one in five couples of reproductive age and poses a significant health problem. In 10-20% of the cases, no definitive cause can be identified, and the infertility remains unexplained. Presence of antisperm antibodies in semen can lead to a condition called 'immunological infertility'.[1]

As mature sperm cells appear at puberty after establishment of immune tolerance to self, sperm may be seen as antigenic by the adult immune system, eg following antigen exposure in the case of vasectomy, inflammation and trauma to epididymis and the vas deferns or congenital absence of the vas deferns.[2]

ASA have ability to disrupt normal interactions of sperm with the cervical mucus and to inhibit sperm penetration into cervical mucus. High levels of ASA have been shown to affect sperm motility, acrosome reaction, penetration of the cervical mucus by highly motile sperm, binding to

the zona pellucida and sperm-oocyte fusion, and also significantly reduce fertilization of human oocytes.[3]

The Mixed Agglutination Reaction test uses anti-Dand antihuman IgG on semen, a positive result is indicated by the formation of sperm agglutinates. This study was done to assess the usefulness of MAR test on diagnosis of Immunological infertility.[4]

Inclusion Criteria: Patients referred to Department of Pathology for semen analysis from Cheluvamba hospital and K R Hospital is included in the study.

Exclusive Criteria: Inadequate sample

Aims and objectives

1. To detect antisperm antibody
2. To study the correlation of antisperm antibody with semen analysis parameters in male infertile subjects
3. To estimate the frequency of immunological infertility in male infertile subjects.

Materials and Methods

Patients referred to Department of Pathology for semen analysis from Cheluvamba hospital and K R Hospital is included in the study. After 3 days of abstinence, they were advised to collect semen by masturbation into a sterile wide mouthed container. Semen analysis is carried out where viability, motility and count of sperms are recorded.

The direct mixed agglutination reaction was performed by placing three drops on a microscope slide: one drop of fresh semen, one drop of anti-D and one drop of antihuman IgG. The three drops were mixed well and a cover slip was placed on top of the mixture. After 2 or 3 minutes, and again after 10 minutes, the wet preparation was observed under microscope in bright light. In the absence of coating antibodies, the sperms are seen swimming free between the particles. If sperm antibodies are present on the sperm, agglutination was observed, in form of head to head, head to tail and tail to tail agglutination.>80%

agglutination were considered as positive MAR test.

Statistical Method

With the incidence margin from 30-40%, using confidence interval approach with 5% level of significance and 10% error, the estimated sample size was found to be around 96

Sampling Technique

Frequency, Percentage, Incidence rate and suitable graphical technique using R software

Result

Out of the 96cases studied, majority of the patients were in 30-35years age group.(Table 1) Most common history mentioned was primary infertility. Other history mentioned include secondary infertility, history of varicocoele, primary infertility with history of epididymo-orchitis and positive history in wife such as PCOD and multiple abortions.(Table 2)

Azoospermia was present in 7 cases. Oligozoospermia was detected in 18 cases. Remaining 71 cases showed normal sperm count. (Table 3) Excluding 7 cases of azoospermia, other 89 cases were subjected for MAR test.MAR positivity was detected in 37% of cases. All 13 cases with history of varicocoele did not show MAR positivity. 15 cases with primary infertility and 18 cases with secondary infertility showed positivity. (Table 4) Head to head, head to tail and tail to tail agglutination was observed, >80% agglutination were considered positive (Fig. 1,2). In negative cases, agglutination is absent. (Fig. 3)

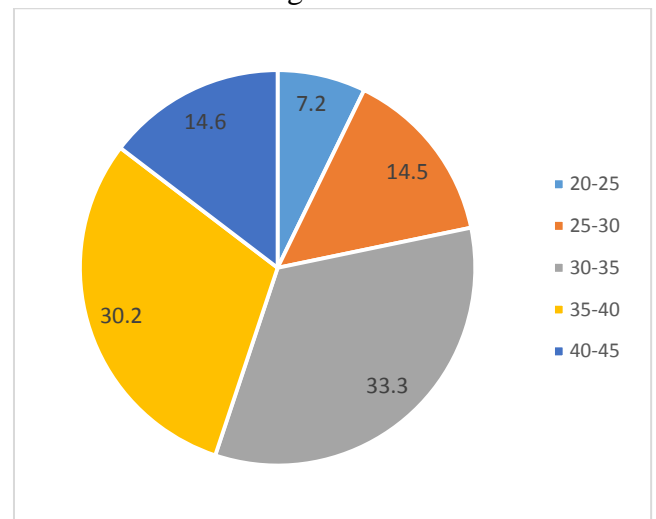**Table 1:** Pattern of Age Distribution

Table 2: Distribution of cases based on the history

SL No	HISTORY	No of cases
1	Primary infertility	32
2	Secondary infertility	23
3	History of varicocele	13
4	Positive history in wife	
	- PCOD	17
	- Multiple abortions	7
5	Primary infertility with epididymo-orchitis	4

Table 3: Distribution of cases based on sperm count

SL No	COUNT MILLION/ml	No of cases
1	0-20	25
2	20-40	8
3	40-60	20
4	60-80	18
5	80-100	15
6	100-120	2
7	120-140	8

Table 4: Results of Mixed Agglutination Reaction (MAR) test

Sl no	History	No agglutination	Agglutination
1.	Primary infertility	17	15
2.	Secondary infertility	5	18
3.	History of varicocoele	13	0
4.	Positive history in wife	24	0
5.	Primary infertility with epididymoorchitis	4	0

Table 5: Lower reference limits for semen analysis parameters

SEMEN CHARACTERISTIC	LOWER REFERENCE LIMIT
Volume , mL	1.5
Total sperm number , 10^6	15
Total motility (PR + NP) ,%	40
Progressive motility (PR) ,%	32
Vitality ,%	58
Sperm morphology(normal forms) ,%	4
pH	> Or = 7.2

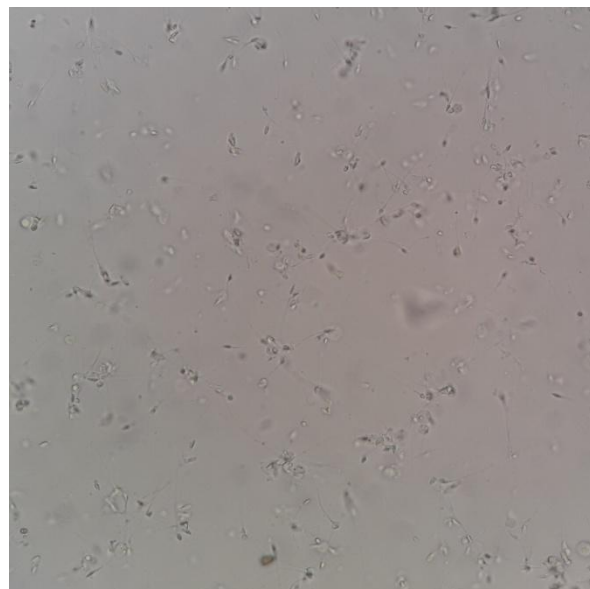(PR= Progressive motility, NP= Non-progressive motility)

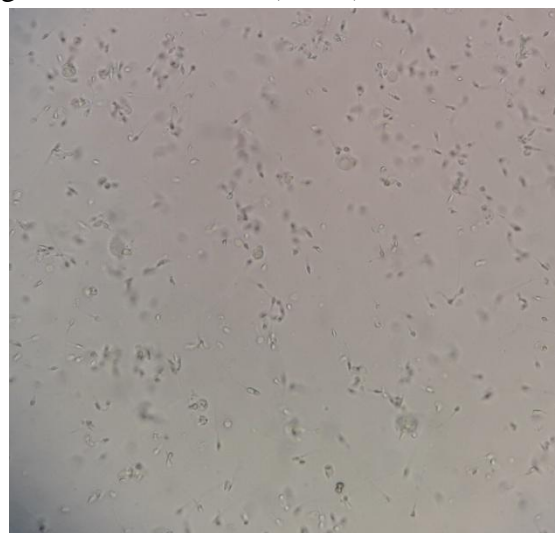**Fig. 1** Photograph showing positive Mixed Agglutination Reaction (MAR) test

Fig. 2: Photograph showing positive Mixed Agglutination Reaction (MAR) test
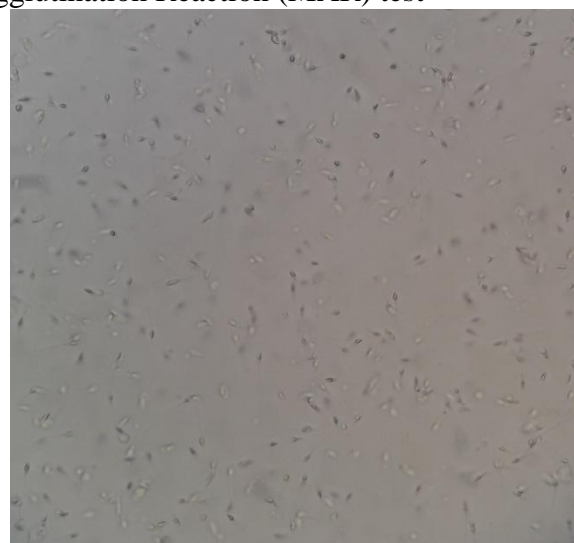

Fig. 3: Photograph showing negative Mixed Agglutination Reaction(MAR) test

Discussion

A male infertility factor is often defined by abnormal semen parameters but may be present even when the semen analysis is normal. Male infertility can be due to variety of conditions, which should be identified for further treatment. Antisperm antibodies have been considered as a cause of infertility, which is known as immunological infertility. Finding antisperm antibody in infertile male provides the clinician within formation which may be useful in their sub sequent management.[5] Therefore, the diagnosis of antisperm antibody is important, as it has got prognostic and therapeutic implications.

Antisperm antibodies can form when there is a breach in the blood-testis barrier and the immune system is exposed to large quantities of sperm antigens or after vasectomy. Trauma, torsion, biopsy, orchitis, testicular cancer, and vasectomy are the risk factors for ASA formation.[1]

Antisperm antibodies can be found in the circulation, in seminal plasma and on the sperm surface. Mixed agglutination Reaction (MAR) test is preferable as it can be performed rapidly, requires less cost, less skill and has good accuracy. Thus it can be used for rapid screening of infertile male patients.[6] Similar findings was observed Practice Committee opinion of the American society for Reproductive Medicine.

In our study MAR test was positive in 37% of cases whereas 40% of cases showed positivity in study done by Mahmoud et al.

In our study, semen analyses parameters were studied based on the lower reference limits defined by the WHO laboratory manual for the examination and processing of human semen in 2010 similar to study by Amir et al.(Table 5)

It was found that high levels of antisperm antibodies in semen was significantly affect sperm motility. Similar findings was observed by Ahangari et al.

MAR test is quick, less expensive and sensitive method. Similar findings was observed by Al-Dujaily S S et al and Mahmoud A et al. Therefore, MAR test can be included in routine semen analysis in screening infertile male patients.

Conclusion

Infertility is a growing burden in current couples. Health care cost will be extremely high for treatment of infertility. Immunological infertility is important as it has got prognostic and therapeutic implications. MAR test is a reliable and reproducible method able to detect antisperm antibodies. As the principal treatment of immunological infertility is IVF, present study will be helpful to predict the successful outcome of IVF, hence in preventing financial burden on to the family.

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