

The Role of The Anti-Sperm Autoantibodies in the Management of Patients with Primary Infertility

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Abstract

The role of antibody mediated infertility in patients with primary/secondary unexplained infertility is the subject of current interest worldwide. A prospective study was conducted to study the role of anti sperm antibodies in Pakistani patients with infertility. Patients reporting in the outpatient clinic of a local gynaecology department with problem of infertility were subjected to a detailed scrutiny by history and clinical examination including post-coital test and hysterosalpingography. Hormonal profile consisting of serum FSH, LH, Prolactin and Progesterone was assessed. Most of the ladies also underwent a pelviscopic examination. A total of 117 patients were selected where post-coital test was abnormal, semen analysis was not satisfactory or who had unexplained infertility. Tests were performed on the serum specimen from the husbands, wives and in some cases on the seminal plasma as well for the measurement of the anti sperm agglutinating and anti-sperm immobilizing antibodies by the microagglutination technique. Fifteen of these patients were found positive for these antibodies, 7 were positive for the agglutinating antibody, 6 for the immobilizing antibody and two for both types. These results indicate that immunomodulation may be responsible for some cases of infertility in our population (JPMA 45:203,1995).

Introduction

Infertility is a global problem and the stress provoked by it, can subject both the spouses to general, physical and psychological dysfunction¹. An estimated 17% of the couples experience some sort of problem with their fertility². The most well equipped world clinics² report an incidence of 20-30% for the unexplained infertility. Immune mechanisms both humoral and cell mediated have been investigated for their possible involvement in otherwise unexplained infertility³. Up to 40% of the couples with this problem have been reported to be harboring anti sperm antibodies⁴. It has also been reported that there is a progressive decline in the chance of fertility, as the anti-sperm antibody titre rises. Pregnancy has not been recorded in women having anti-sperm antibodies above a certain limit⁵. A study was planned to analyse the possibility of the involvement of the immune mechanism in relation to infertility in our patients. The tests measuring anti-sperm antibodies, resulting in decreased mobility and agglutination were selected, as the serum antibody levels, thus picked up, have related comparatively closely with the underlying clinical problem. The study was conducted in the Armed Forces Institute of Pathology, Rawalpindi and Military Hospital Rawalpindi in collaboration with 4 other hospitals.

Patients and Methods

The couples were admitted to the study provided they had not conceived in the past two years. They were investigated according to the standardized investigation protocol of the infertile couple prepared by World Health Organization⁶. A thorough menstrual, obstetric, sexual, contraceptive and drug history was obtained and any symptoms relating to endocrine disorders were recorded.

Physical examination was performed in females with special emphasis on height, weight, any stigmata of endocrine diseases, secondary sexual characteristics and pelvic examination. The male partners underwent semen analysis twice at an interval of 14-90 days. No further investigations were carried out if the two results were within normal limits.

The female partners had a thorough evaluation of their menstrual and ovulatory function. Hormonal profile was obtained by the radioimmunoassay of serum for FSH and LH in the early follicular phase, serum progesterone in the mid luteal phase, serum TSH, Prolactin and testosterone levels were measured where indicated. Hysterosalpingography and pelviscopy were performed to exclude any mechanical hinderances to fertility. The post-coital test was performed by the microscope slide method in the stimulated pre-ovulatory period. Adequacy of coitus, cervical hostility⁷, quality and quantity of the cervical mucus, sperm viability and motility were assessed and any direct or indirect evidence of infection was actively sought.

Immunological screening was done in couples with unexplained infertility, when dead spermatozoa were detected in the cervical mucus on post coital test despite a normal semen analysis, on observation of the shaking phenomenon in spermatozoa on postcoital test, spontaneous sperm agglutination on semen analysis and an abnormal semen analysis e.g., Azoospermia and Oligospermia. The specimens analysed were sera of the husband and wife and seminal plasma (in some cases only). The test could not be extended to the examination of the other genital tract secretions because of the resource constraint. Four ml of the venous blood was collected from each selected patient. It was allowed to stand at room temperature for 2 hours: Serum was then separated and stored at -20°C. The test was carried out in batches at least once every month. Modified microtitre tray agglutination and the sperm immobilization tests (SIT), were used for the detection of the anti-sperm antibodies.

Two ml of RPMI 1640 and 5% fetal calf serum (FCS) were layered over semen and incubated at 37°C for 30 minutes. The upper 1.5 ml was then aspirated and examined for the motility of sperms under the microscope. The count was adjusted to 2000 sperms/ul with the motility and the morphology of the sperms were found to be satisfactory. The test was carried out in the "Terrasaki trays". The test and the control specimens were prepared by first inactivation of the complement by incubation at 56°C for 30 minutes. These specimens were then diluted in RPMI 1640+5% FCS to make up serial dilutions of, 1:2, 1:4, 1:8, 1:16 and 1:32. These specimens were then dispensed in the Terrasaki trays in volumes of 1 ul in each well. The test and the control specimens were treated in similar ways. One ul of the donor sperms (2000 sperms/ul) were added in each well and incubated in 37°C for 30 minutes in an atmosphere of high humidity. Two ul of the fresh rabbit complement was then added in the rows meant to check complement mediated immobilization. The plate was then incubated for 60 minutes at 37°C. The plate was then studied for the agglutination and the mobility of the donor sperms under the low power of the inverted phase contrast microscope.

Results

A total number of 117 patients, including 62 men and 55 women were subjected to anti-sperm antibody screening. Anti-sperm agglutinating antibodies were identified in 7 patients, anti-sperm immobilizing antibodies were detected in 6 patients and both were identified in 2 patients. So 15 patients (12.82%) were positive for anti-sperm antibodies in serum. Out of these 15 anti sperm auto-antibody positive patients, 8 were males and 7 females. Seminal plasma was screened for anti-sperm antibodies in 5 patients. One was positive for anti-sperm immobilizing antibody.

Discussion

For a couple to attempt to procreate has been one of the basic aims of the society through ages. About

17% of the couples², however, experience some problem in their fertility which remains undetected in 20-30% of the cases despite all the efforts³. Spermatozoa have been demonstrated to be potential auto antigens which could generate an anti-fertility response^{8,9}. Active immune suppression operates physiologically to prevent an autoimmune response^{10,11}. Therefore, antibodies against sperm antigens are not physiologically present in the sera of either male or female partners in a couple. If detected, they reflect an aberrant immune response which may lead to sperm dysfunction¹². These may be detectable in the sera of either partner or in the genital tract secretions¹³. Antisperm antibody screening when selectively applied to couples with unexplained infertility has given positive results in up to 40% of patients with unexplained infertility¹⁴. Other important risk groups include, patients with an abnormal semen analysis or post-coital test⁹ and patients with genital tract infection or trauma¹⁴. The analysis of our findings show that the infertile couples have an increased expression of anti-sperm antibodies as compared to the fertile couples: The percentage of our patients found positive for these antibodies corresponds well with the one reported in some of the current studies⁴. These preliminary findings suggest that a comprehensive work up of the couples with unexplained infertility, abnormal post-coital tests or abnormal semen analysis should include investigations for the presence of anti-sperm antibodies. It will help in investigating the possible immune mechanisms leading to infertility which may be amenable to treatment. The assay should be performed by a combination of different techniques, because these antibodies may have different mechanisms of action and may behave differently with individual in-vitro settings. Efforts should be directed to detect these antibody levels in genital tract secretions because antibodies in the genital tract are more likely to effect the physiological function. Their presence in the serum does not necessarily mean that these antibodies may also be found in the genital tract. The investigation suffers from the handicap of being rather poorly standardized. The target antigens remain largely unknown and a variety of assays employed for the detection of these antibodies results in detecting a heterogenous population of autoantibodies. These results then relate poorly and to a variable extent with the underlying problem. It is important to keep in mind that investigations using fixed spermatozoa may inadvertently result in detection of auto-antibodies directed against antigens which are only expressed after fixation and not available in vivo. Comparison of different modes of investigations points to the usefulness of the test involving the functional aspects of normal spermatozoa. The assays involving the use of microbeads coated with sperm antigens have the advantage of the ability to detect different isotypes of the auto antibodies. These assays relate comparatively better with the problem of infertility. We employed the tests looking indirectly into the functional aspects which may be affected by the autoantibodies to the spermatozoal antigens. However, we could not differentiate between different isotypes of the anti-sperm auto antibodies by these techniques. The results obtained with these assays have been reported to relate comparatively specifically with the underlying clinical problem. Our study has demonstrated the possibility of the involvement of the autoimmune effect or mechanisms in the causation of the infertility in our patient population. This observation may be utilized in further studies with the inclusion of the immunomodulation in well controlled clinical trials.

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