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THE VALUE OF IMMUNOLOGICAL TESTS IN PREDICTING PREGNANCY OUTCOME

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ABSTRACT

In addition to ultrasonographic monitoring of folliculogenesis, urinary luteinizing hormone testing, luteal phase endometrial biopsy, serum antiphospholipid antibodies, etc., immunologic tests are recently being used to evaluate reproductive disorders. In this study, sharing of HLA-A, B, C and DR antigens and the presence of antipaternal cytotoxic antibody (APCA) was assessed using microcytotoxicity method.

Subjects of the study were 103 infertile couples, 47 fertile couples with 2-3 children and 123 couples with recurrent spontaneous abortions (RSA).

The results showed that the presence of antipaternal cytotoxic antibody is associated with fertility rather than infertility ($p<0.0001$), but sharing of HLA antigens failed to show any significant difference between infertile and fertile couples or couples who experienced spontaneous abortions compared with normal couples. Therefore it appears that HLA antigen sharing is not associated with pregnancy outcome and other antigen(s) may be involved in this process. Assessment of APCA could be useful in predicting the pregnancy outcome.

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INTRODUCTION

The process of reproduction in women is a highly complex issue involving many processes at the molecular and tissue level. There is claimed to be an immunological defect consisting of maternal antibody to trophoblasts in aborting women, and the common pattern is to abort all pregnancies.[1] It is believed that genetic, anatomic and hormonal factors are involved as minor causes; however, an immunological cause is suggested for more than 80% of pregnancy losses.[1,2] Women with RSA are subjected to treatment by husband lymphocytes or the third party.[2] It has been suggested that RSA and infertility are manifestations of a common situation. Immunological evaluations are assessed by tests such as HLA-typing, APCA and mixed lymphocyte culture (MLC).[1,3]

In this study the usefulness of HLA-typing and APCA tests were evaluated.

MATERIALS AND METHODS

Patients

Three groups of couples were chosen for the present study. 103 infertile couples who had married more than 5 years ago and without any contraception had not conceived; 123 couples with ≥3 abortions; and 47 normal pregnant women with 2-3 children participated in the study. All couples had no previous marriage and had no abnormality in semen analysis, hormone levels, antisperm antibody, etc.

Lymphocyte preparation

Lymphocytes were separated from peripheral blood

Table I. Clinical characteristics of the study couples.

Obstetric history	Total number	Age (years)		Gravidity	
		Mean	Range	Mean	Range
NPW	47	32	18-44	2.3	2-3
Infertile	103	34	24-39	-	-
Women with RSA	123	31	17-42	3.9	3-8

NPW: normal pregnant women.

samples. The blood was diluted in an equal volume of PBS. This diluted blood was layered over an equal volume of Ficoll-hypaque solution 1.077 (Lymphoprep special, Nycomid, Birmingham, UK) in round bottom tubes and centrifuged at 700g for 15 min. The thin layer of cells (lymphocytes) was separated and washed twice in Hank's balanced salt solution and resuspended in Hank's BSS to give 1×10^6 cells/mL.

Collection of sera

5 mL of blood was taken by venepuncture into sterile tubes from normal pregnant women, women with RSA, and infertile women. Blood was allowed to clot. The tubes were centrifuged at 500g for 5 min. The serum was pipetted off and transferred to two clean tubes and stored at -20°C until use.

APCA

Women's sera were tested for the presence of lymphocytotoxic antibody by the microdroplet assay described by Mittal et al. and standardized by the National Institute of Health.[4] A 64-well Terasaki microtitre plate (FloconLabTek, Naperville) was covered with light paraffin oil (BDH Chemicals, Poole, Dorset, England); 1 µL of lymphocyte suspension (1000 cells) obtained from the husband's blood was incubated with 1 µL of serum from his wife and plates were incubated at room temperature (RT) for 30 minutes. 5 µL of adult rabbit complement (Buxton Rabbit Co. LTD, East Grinstead, Surrey, UK) was then added to each well and plates were left for one hour at room temperature. To stain the cells, 1 µL of 5% eosin (BDH Limited, Poole, Dorset, UK) was added to each well after incubation. The plates were left for 5 min. at room temperature to equilibrate, and the cells were fixed using 1 µL of 36% formaldehyde (BDH, Poole, Dorset, UK) titrated to pH 7.4. The plates were read using an inverted microscope (Wilde, Switzerland). Positive samples were scored when the kill was 20% above background.

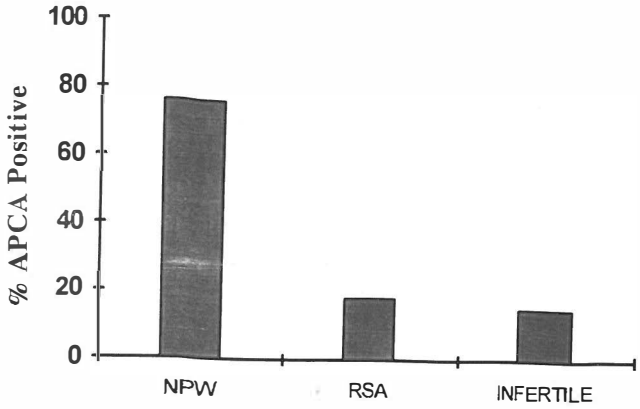

Fig. 1. Rate of APCA positivity in normal pregnant women (NPW), women with recurrent spontaneous abortions (RSA) and infertile couples.

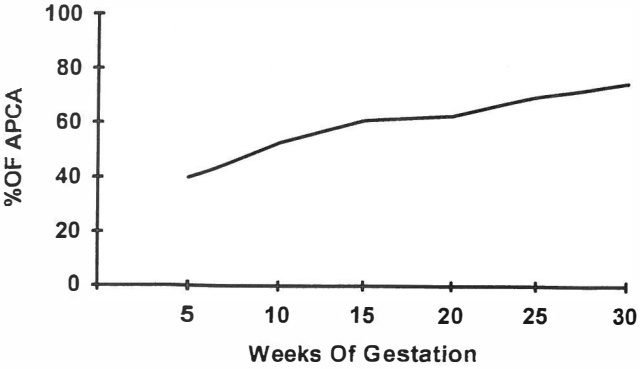

Fig. 2. Prevalence of APCA positivity during pregnancy.

HLA-typing

Human leukocyte antigen typing was performed for HLA-A, HLA-B, HLA-C and HLA-DR antigens on T and B-cells obtained from couples, using specific antisera from Behring and Biotest companies. B-cells for HLA-DR typing were obtained by passage of peripheral blood lymphocytes over nylon wool[5] and resuspended in 20% fetal calf serum. The main procedure was the same as APCA except all incubation times were increased to twice that described.

Statistical analysis

κ^2 and Fisher exact tests were used to analyze the differences.

RESULTS

Table I shows the clinical characteristics of the couples participating in the study. The mean age of infertile couples was 34 years (range 24 - 44 years). The mean age of fertile couples was 32 years (range 18-44 years), and none of them had a history of pregnancy loss. The mean age of aborter women (RSA) was 31 years (range 17-42 years) and the mean number of previous abortions was 4 (3-8).

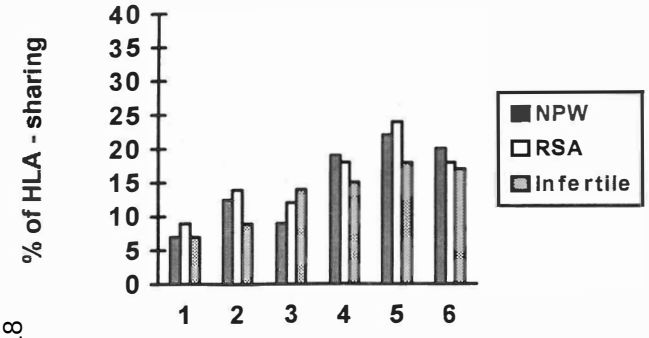

Fig. 3. Sharing of ≥2 HLA antigens between couples with recurrent spontaneous abortions (RSA) compared to normal

APCA

Fig. 1 shows the incidence of APCA in women with RSA, normal pregnant women (NPW) and infertile women. Paternal lymphocyte antibodies were detected in the serum of 76% of normal pregnant women (36/43), 18% (22/123) of women with RSA and 9% (32/123) of infertile women. A significant difference was observed when RSA and infertile women were compared with NPW ($p<0.0001$).

The frequency of APCA during pregnancy was documented in 15 fertile women. Fig. 2 shows that the prevalence of APCA positivity increased during pregnancy. All women in this group had completed 2 previous successful pregnancies.

However, concerning women with RSA who had had ≥3 abortions, the prevalence of APCA did not show any difference at any time (data not shown).

HLA sharing

Fig. 3 shows that the rate of HLA sharing of ≥2 antigens between couples with RSA is similar to fertile couples.

Although only 6 types of specific antigens are illustrated in Fig. 3, the situation was similar for all antigens which were typed. It should be mentioned that the percentage sharing shown concerns the woman's husband and then results are compared with the control group. κ^2 test was done for studying the percentage of HLA sharing, which revealed that there was no significant difference between the 3 groups ($p\geq0.05$).

The frequency of HLA-antigen sharing for ≥2 HLA-A, B, C and DR loci between different couples is illustrated in Table II. Comparing the frequency of HLA-antigen sharing between the different groups failed to show any significant differences.

From among 47 fertile couples studied 25% (12/47) shared ≥2 HLA-A, B, C or DR loci. 26% (24/103) of infertile couples and 26% (32/123) of RSA couples shared ≥2 HLA loci.

DISCUSSION

The results presented here show that the frequency of HLA-sharing between fertile, infertile and RSA couples is not associated with reproductive performance. Therefore, HLA antigen typing seems not to be able to predict pregnancy outcome. However, lymphocyte therapy is a common method of treatment of women with RSA and some laboratories perform such investigations which appear to be unnecessary. It should be noted that the results of other investigators are the same as the present study, i.e. they failed to show any significant differences between couples with various obstetric histories.[6,7]

Table II. Frequency of sharing ≥2 HLA antigens.

Obstetric history	Total number	Sharing of ≥2 HLA-Ag	
		Number	%
Fertile	47	12	25
Infertile	103	27	26
RSA	123	32	26

Other studies showed that antigens other than HLA antigens may be involved in pregnancy.[8,9] It is important to note that the Syrian hamster has been inbred without the advantage of MHC antigen polymorphism. This study also suggested that antigens other than HLA may be important.

Analysis of these results indicated that the presence of APCA in mothers in response to allostimulation through pregnancy may be a useful way of predicting the pregnancy outcome. It can be seen that similar to other studies,[6,7] a significant difference is present between fertile and infertile couples (Table II). APCA between pregnancies were evaluated in the present study; some investigators[10] failed to show any significant differences for APCA between women with RSA and fertile women. Concerning the method of APCA detection, Gilman Saches et al. analyzed the anti-lymphocyte antibody levels in women with RSA immunized with paternal leukocytes and concluded that two-color flowcytometric analyses seem more sensitive than other methods,[11] but this method is still not sensitive enough.

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