

# Role of anti-human lymphocyte culture cytotoxic antibodies in recurrent spontaneous pregnancy loss women

Shankarkumar Umapathy,  
Aruna Shankarkumar,  
Vanita Ramrakhiani,  
Kanjaksha Ghosh

National Institute of  
Immunohaematology, KEM  
Hospital Campus, Mumbai,  
Maharashtra, India

#### Address for correspondence:

Dr. Umapathy Shankarkumar,  
National Institute of  
Immunohaematology,  
13<sup>th</sup> Floor, KEM Hospital,  
Parel, Mumbai - 400012,  
Maharashtra, India  
E-mail: shankar2kumar@  
rediffmail.com

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## ABSTRACT

**BACKGROUND:** Recurrent spontaneous pregnancy (RSA) is defined as a sequence of three or more consecutive spontaneous abortions. One of the major causes of RSA is immunological where alloimmune antibodies develop towards human leucocyte antigen (HLA) antigens. Earlier research had suggested that anti-HLA antibodies are produced in normal women; studies have been reported that normal pregnant women develop anti-HLA antibodies, mostly after 20–28 weeks of gestation. **AIM:** To evaluate the role of anti-HLA antibodies in RSA patients **MATERIALS AND METHODS:** A total of 80 randomly selected couples with unexplained three or more RSA and control group of 50 normal pregnant women were screened for anti-HLA A and B antibodies. The anti-HLA antibodies were analyzed following the standard two-stage NIH microlymphocytotoxicity assay. **RESULTS:** In our study group a high frequency of anti-HLA antibodies among women with RSA (26.25%) was detected compared to normal pregnant women (8.0%). Most of the sera showed HLA-A and HLA-B antibodies which had high titer, up to a dilution of 1: 4096. **CONCLUSION:** This incidence of high anti-HLA antibodies in RSA women during early weeks of gestation may explain the recurrent pregnancy loss.

**KEY WORDS:** Anti-HLA antibodies, incidence, RSA

## INTRODUCTION

Recurrent spontaneous abortion (RSA) is defined as a sequence of three or more consecutive spontaneous abortion. RSA is a heterogeneous condition which may have many possible causes; more than one contributory factor can lead to recurrent pregnancy losses. The major causes of RSA are genetic, endocrinological, immunological, anatomical factors and yet can be unexplained with all these in some cases. In unexplained RSA, immunotherapy (allogenic leukocyte immunization from the partner) has been used to treat the couples.

Antibody to human leucocyte antigen (HLA) was first identified in the serum of a polytransfused patient;<sup>[1]</sup> subsequently materno-fetal alloimmunization was also shown to produce anti-HLA antibodies in pregnant women.<sup>[2,3]</sup> Since then, pregnant women have been the most common source of these HLA antisera for routine HLA

serological typing (though monoclonal antibodies have also been raised recently).<sup>[4]</sup>

The aim of this study is to identify the role of anti-HLA antibodies in RSA patients.

## MATERIALS AND METHODS

A total of 80 randomly selected couples with unexplained three or more recurrent spontaneous abortion and control group of 50 pregnant women (age group 20 to 40 years) who had no previous abortions but had previous 1 or 2 pregnancies recruited in the 20-22 week of pregnancy.

All the women with RSA were found to be negative for cytogenetic and autoimmune abnormalities such as anti-phospholipid antibodies (APAs), antithyroid antibodies (ATAs), anti-nuclear antibodies (ANAs), antineutrophil cytoplasmic antibodies (ANCAs) and lupus antibodies (LA). Five to 10 ml of peripheral blood was obtained from

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**Table 1: HLA anti-bodies among the RSA patients on various serial dilutions**

HLA antibody in RSA samples																
Cell no.	Age	Neat	1:1	1:2	1:4	1:8	1:16	1:32	1:64	1:128	1:256	1:512	1:1024	1:2048	1:4096	
8764	23	+	+		+	+	+	+	+	+	+	+	+	+	+	+
8837	23				+	+		+		+	+	+	+	+	+	+
8886	26	+			+	+	+	+	+	+	+	+	+	+	+	+
8879	24					+	+	+		+	+	+	+	+	+	+
8721	20				+		+	+	+	+	+	+	+	+	+	+
8847	25						+				+	+	+	+	+	+
9081	32									+	+		+	+	+	+
8801	31		+	+	+		+		+	+	+	+	+			+
8787	25	+	+	+	+				+	+		+	+			+
8855	37				+	+	+	+	+	+	+	+	+	+		
9088	20	+			+			+	+	+	+	+	+	+	+	
8770	24		+	+	+	+		+	+				+	+		
8772	30				+	+	+		+	+		+			+	
8724	23	+			+	+	+		+	+			+	+		
8796	25				+	+		+			+	+				
9042	25	+			+			+	+		+					
8816	25	+	+				+									
8947	26	+	+	+	+											
8803	27	+	+													
9036	22	+														
8820	26															
HLA antibody in normal sample																
Cell no.	Age	Neat	1:1	1:2	1:4	1:8	1:16	1:32	1:64	1:128	1:256	1:512	1:1024	1:2048	1:4096	
8857	28						+		+	+	+	+	+	+	+	+
8849	23					+	+	+		+		+	+	+		
8843	25							+	+		+	+				
9044	24				+	+										

each individual. The sera of women were investigated for the anti-HLA antibodies against their husband's lymphocyte. The anti-HLA antibodies were analyzed following the standard two stage NIH microlymphocytotoxicity assay.<sup>[5]</sup> These RSA patients on follow-up showed that they aborted the foetus while the normal females delivered a normal alive when they were followed up during their pregnancy.

## RESULT

The anti-HLA antibodies were tested in 80 RSA and 50 control couples [Table 1]. In RSA case, 21 sera (26.25%) versus 4 sera (8.0%) were found positive for HLA antibodies against husband's lymphocytes. Most of the sera showed HLA-A and HLA-B antibodies which had high titer. For screening, sixty well tissue typing tray consisted of one positive, one negative control, 21 positive sera of RSA women and 4 antenatal control sera. The antisera were serially diluted up to 1 : 4096 and tested for anti-HLA antibodies. A distinctive pattern of antibodies was obtained as tabulated below. The results indicated that 9 out of 21 (42.85%) (OR=2.25; etiological fraction=0.23) positive sera showed reaction at dilution 1 : 4096 and 5 sera (23.80%) showed reaction at dilution 1 : 2048. One each of positive

sera showed no reaction at all the dilutions including neat samples used and positivity at neat sample, respectively. Few of the sera showed reaction at highest dilution only, but the same was not observed in the corresponding neat sample which indicates presence of very low amount of antibodies against HLA antigen. It is also observed that maximum positivity was for 1 : 8 dilution of all the sera used. In the case of normal controls, it was observed that only 1 out of 4 sera showed presence of antibodies at highest dilution while rest 3 sera tested showed variable results.

## DISCUSSION

Initially many studies have been done to determine whether or not there is a connection between RSA and couples who share HLA alleles. This alleles sharing hypothesis concerning RSA was first supported by Komlos and his associates.<sup>[6]</sup> Excess sharing of HLA antigens between spouses has been considered by some to be mechanism leading to maternal hyporesponsiveness to paternal antigens encountered in pregnancy and therefore subsequent miscarriage.<sup>[7]</sup> This hyporesponsiveness was considered to be shown by a lower incidence of anti-paternal antibody in RSA.<sup>[8]</sup>

Wide variation in the incidence of anti-HLA antibodies

has been reported in the sera of normal pregnant women. These are due to variations in immunization and the resultant outcomes. Values range from 7.3% to 36% (7.3%, Swedish women;<sup>[9]</sup> 18.7%, Caucasian women;<sup>[10]</sup> 21.6%, American women;<sup>[11]</sup> 29%, Mestizo women<sup>[11]</sup> and 9.6%, Warao women;<sup>[11]</sup> Venezulean women;<sup>[12]</sup> 36% USA women<sup>[13]</sup> and south Indian women 10.6%.<sup>[14]</sup> Another study detected anti-HLA antibodies in only 5% of women with successful pregnancies during the first trimester, compared with an antibody prevalence of 10% in women who miscarried.<sup>[15]</sup> In a prospective immunization study, Christiansen *et al.*<sup>[16]</sup> found higher frequencies of anti-HLA antibodies in women with RM who miscarried (29%) compared with 18% of women who successfully delivered.

Due to variation in immunization and the resultant outcome, a wide variation in the incidence of anti-HLA antibodies in the sera of normal pregnant women have been reported in literature. The values ranged from 7.3% to 36%.<sup>[17]</sup> It has been reported earlier that 27.8% of RSA women had anti-HLA antibodies from northern India.<sup>[18]</sup> In our study we have found an incidence of 26.25% anti-HLA antibodies, which suggests that the blocking factors as well as immunity towards the husband's lymphocytes could be different among western Indian RSA couples.

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