Abstract

Recurrent pregnancy loss is a multifactorial, heterogeneous condition, with obscure etiology in about half of the cases. Immunological as well as genetic factors are reported to contribute significantly towards the maintenance of pregnancy. From an immunological point of view, the fetus is a foreign graft and immunomodulation is an initiating event for the acceptance of this semi-allogenic fetus by maternal immune cells. Following appropriate immunomodulation, it is essential that the vascular remodeling, xenobiotic metabolism and DNA repair pathways function normally and synergistically to enable the successful survival of the fetus in the maternal environment. Failure to control maternal immune response against the fetal tissues or alterations in any of the other pathways is believed to lead to adverse pregnancy outcome such as RPL. The increased incidence rates and unknown etiology of RPL stressed the need to identify genetic markers for predisposition. Hence the present study aimed at identifying the role of genetic polymorphism in the immunomodulatory genes (HLA G, KIR) as well as genes regulating the vascular remodelling (VEGF, ACE and MTHFR), xenobiotic metabolism (CYP1A1, GSTM1, GSTT1 and GSTP1) and DNA repair (XRCC1 and XPD).

The study comprised of hundred and four couples with unexplained RPL. The controls for the study were eighty couples and an additional of forty women with at least two children and no history of RPL. High molecular weight genomic DNA was isolated from the peripheral blood samples collected from the study subjects and the polymorphisms were analyzed by PCR based methods.

Immunomodulatory gene analysis revealed a significantly increased frequency of HLA G*0105N and HLA G I/D genotype in RPL women compared to the controls. In the partners of RPL and control women there was no significant difference in the HLA G alleles. However, sharing of G*0104 allele between the couples contributed significantly to RPL. Combined analysis of HLA G *0105N and I/D polymorphism did not reveal any significant association. Further, the analysis of the APCA revealed a significant relation between the absence of APCA and RPL. With respect to the KIR gene polymorphism, a significant difference was observed in the 2DL1, 2DL5, 2DP1 and 2DS4 genes in partners of RPL women and controls, while in RPL women no major difference was noted. When the number of activating and inhibitory KIR genes
was assessed, a significant difference was observed in the presence of all 5 inhibitory KIR genes in partners of RPL women and controls. In addition, sharing of 2DL1, 3DS1, 2DS1 and 2DS4 KIR genes was found to significantly increase the risk for RPL. Furthermore, a significantly increased risk for RPL was seen when the wife lacked the inhibitory receptor possessed by the husband and a significantly decreased risk for RPL was noted when the husband lacked the inhibitory receptor possessed by the wife. Analysis of the KIR haplotypes and sharing of haplotypes did not reveal any significant association with RPL. Stratified analysis based on the age and number of pregnancy loss for HLA G as well as KIR genes did not reveal any significant association.

The study on the vascular remodeling genes revealed a significant association between VEGF C/A genotype and RPL. However ACE and MTHFR genes did not reveal any significant association. With respect to the xenobiotic metabolism, CYP1A1 w1/m1 genotype and GSTM1 deletion genotype revealed a significant association with RPL. In contrast, GSTT1 and GSTP1 genes indicated a lack of association with RPL. The analysis of DNA repair genes indicated XPD Lys/Gln genotype to be associated with an increased risk for RPL. The gene-gene interaction within each of these pathways as well as among all the pathways combined revealed an increased risk when the high risk genotypes of the various genes occurred in combination.

The present study is thus the first of its kind in assessing the role of genes regulating both immunologic and genetic factors and gene-gene interactions in the south Indian population. The outcome of the study suggests a multigenic etiology for RPL which would enable identification of genetically predisposed individuals for RPL.