

Molecular organization of 'Rh' gene is likely to be heterogeneous across the world

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Rh antigen has an important place in the history of immunohematology. Understanding hemolytic disease of the newborn through Rh isoimmunization and finally the understanding of how the ABO incompatibility when present with RhD incompatibility reduced the incidence of RhD HDN paved the way for RhD immunoglobulin prophylaxis in RhD-negative mother carrying RhD-positive fetus. Development of anti-globulin test to detect RhD sensitization superseding Albumin-based and enzyme-based tests are also important advances in the field. The complexity of Rh antigen was understood by both statisticians and biologists in different way leading to two different notations of Rh system.^[1,2] Modern molecular biologists have shown both the concepts to be wrong, and Rh gene complex is inherited as two RhD and RhCE genes situated face to face on chromosome number 1.^[3]

Serological study of Rh antigen allowed us to test for five antigens i.e. D, C, E, c, and e. As d antigen is an amorph or non-existent because it's presence is considered when RhD gene is deleted. Therefore, anti-d anti-serum is not available. So, earlier it was not possible to say with confidence serologically, whether a particular person is homozygous or heterozygous for D antigen without studying the RhD phenotype of the family members or statistically arriving the status of RhD homozygosity.^[4]

In Caucasian race, presence of 15-20% RhD-negative population poses a significant challenge towards elimination of HDN due to RhD isoimmunization. Moreover, polyclonal antibody obtained from human volunteers or heavily sensitized mothers, which have been used to sort out the issue is becoming extremely rare and increasingly scarce due to ethical issues. Hence, even though a cocktail of monoclonal antibodies against RhD antigen has been offered as a substitute to polyclonal antibody, clinical trials of adequate size has not yet been done to prove beyond doubt that these products are as effective as polyclonal RhD immunoglobulin in preventing the HDN due to RhD isoimmunization.

Under such circumstances, knowing the D zygosity

of the father for the fetus becomes extremely important in the assessment of the couple's risk of carrying a RhD-positive offspring, and finally it also saves the precious Rh immunoglobulin.

Till recently, the only way to know that RhD-negative mother carrying the RhD-positive fetus was either appearance of fresh anti-D antibody in the serum of antenatal mother or increasing the titer of this antibody in already sensitized mother. Similarly, RhD status of the father was done by serology-based Most Probable Genotype (MPG) technique. With the advent of DNA technology, single sperm analysis^[5] or microsatellite analysis^[6] was developed to find father's RhD zygosity. Chiu et al.^[7] developed two robust assays viz. double Amplification Refractory Mutation System (ARMS) assay and multiplex real-time quantitative PCR assay for this purpose.

In the present issue of the journal, Moghaddam *et al.*^[8] demonstrated the use of Sequence-Specific Priming PCR (SSP-PCR) to predict the RhD zygosity of the husband of a RhD-negative alloimmunized pregnant woman from Iran. In this case report, it is also mentioned that the test is available in the form of a kit, which can be easily used across the globe.

In the same issue of the journal, Ouchari *et al.*^[9] have presented an extensive data on molecular typing of RhD gene from Tunisia involving 2000 blood donors (1777 RhD-positive and 223 RhD-negative). They have intelligently used six sets of RhD-specific primers covering exons 3-7 and exon 9. They have carefully excluded exons 1, 2, 8 and 10, as these exons have extensive similarities with Rh CE gene. They correlated their genetic data with serological data involving all the Rh antigens in all the individuals.

Tunisia is an African country, but from very early period, the country has seen lot of admixture with Caucasian race. Hence, finding RhD negativity of 11.15% is not surprising. Their common genotypes were found to be R_1R_1 and R_1r , which is similar to those seen in India. The authors could amplify RhD-specific exons in RhD-positive individuals. However, in 15 out of 2000 samples, aberrant serological results

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were seen, which could be correlated with deletions of various exons of the RhD gene.

In India, molecular studies of RhD variants have started only recently.^[10] At our Institute, we also used a multiplex PCR^[11] and an algorithmic approach. Initially, a population of 10,000 RhD-positive individuals from Western India was screened for partial D variants using a serology-based kit. Fifteen cases of partial D variants were identified giving an incidence of 0.15%, which was much less than that reported for Tunisian population. We found DFR to be the commonest partial D variant in our country. Our study also pointed out inferiority of the serological test to molecular test, particularly when DFR variant is concerned. While partial D variant kit picked up 9DFR variants, multiplex PCR picked up 18 samples. It was also noted that serologically we could not characterize 33% of the partial D samples while using multiplex PCR, we could not characterize 25% of the samples. This clearly shows that even molecular techniques to detect D variants have to evolve considerably to characterize maximum number of variants by providing adequate sensitivity. Today, with the help of molecular biology tools, structure of Rh gene has been studied, and it shows very complex picture. As mentioned earlier, the work on this aspect has just started in our country. So, there is a real need to study through molecular biology tools, the organization of the RhD gene in our extremely heterogeneous, multiethnic Indian population. Such a study will tell us whether the existing techniques can be adopted with equal efficiency to the entire Indian population or we have to modify the technique. Such a study by Transfusion Medicine scholars from India may unfold new knowledge in the evolution of the Rh gene. Study conducted in African continent also showed extreme heterogeneity in organization of Rh gene complex; it is likely to be same for the world, and particularly so where endogamous population in large groups remained genetically isolated even when they inhabited the same geographical area in India.

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