Commentary

Commercial anti-D reagents in Indian market & detection of partial D variants

The Rh blood group system is an important and complex blood group system of the human red blood cell. The demographic data of this antigen in different ethnic population are interesting. In Caucasian race, the prevalence of Rh negative individual is about 20 per cent. In the Middle East the prevalence comes to about 10 per cent but in South East Asia, including India, Rh negative population is about 5 per cent. However, if we observe it in Far East, the prevalence drops to about 0.1-0.5 per cent. Antigens of this blood group system are highly immunogenic and clinically significant. Naturally occurring anti-D is very rare but about 80 per cent of D negative (D-) individuals develop anti-D after transfusion of D+ RBC. Anti-D is formed usually in response to pregnancy, transfusion or deliberate injection.

The expression of D antigen depends upon simultaneous expression of E and C series of alleles on RBC surface. Homozygous D cells have about 1,00,000 to 2,00,000 sites on the cell which may go down 5-10 per cent depending upon the presence of other combination of Ce or Ee antigen. There is a definite quantitative variation in terms of depression. In this case, there is a weak expression of D, and D\(a\) is used to represent majority of weak expressions. It was first discovered by Stratton in 1946. However, other scientists subsequently discovered different grades of D\(a\). They are termed as ‘high grade’ D\(a\) where cells are agglutinated by some D agglutinins and also as ‘low grade’ D\(a\) which are detected only by anti-human globulin (AHG) test.

Anti-D reagents were initially manufactured from hyperimmune serum. Due to technical advancement, the detection level of anti-D has improved. When monoclonal antibody (Mab) was used for blood group reagents, detection level in terms of titre and avidity enhanced. There are no specific reagents to detect D\(a\) or other weaker variants of D. There are potentiating reagents which can enhance the reaction. Some of the D\(a\) and partial D are inherited. Other variety may be the trans effect of other component of D system. In case of D\(a\), AHG test is used for diagnosis. There are instances of very weak D\(a\) which may not be detected by AHG test. Absorption/elution test may be employed to come to a definitive diagnosis. However, detection of partial D and other variant is always tricky. Rh antigen may be of different quality and quantity in various ethnic population. It is always important to know the genetic make-up of Rh system in a given geographical location. For example, De1 type of very weak D\(a\) is found in Japanese population which is not detectable by AHG.

It is very important to avoid Rh immunization due to blood transfusion because of strong antigenicity of Rh system. In India, according to Drugs and Cosmetic Act (1945), direction has been given for correct detection of Rh system. It is recommended that two anti Rh (D) reagents from two different manufacturers should be used. Or, two reagents from the same manufacturers from different batches should be used. The Act also directs that the
Reagents should be blends of IgG and IgM Mab or blend of Mab IgM and polyclonal (human) should be used for AHG test for identification of weaker variant of D antigen\(^5\). These measures are taken to avoid mis-diagnosis and ultimately to prevent transfusion of weaker variant of D to D negative patients. This measure in the act seems to be adequate in general. However, are the reagents available in the market effective enough to detect all weaker variants? Can the present system prevent unwanted immunization of D negative individual because of transfusion with weaker variant? 

It is widely thought that patients typing can be safely achieved by the use of one high avidity or two very similar IgM monoclonal anti-D reagent that detect most variants. However, it may not be able to diagnose DVI category in simple tube or microplate saline tests. As mentioned earlier, there are many phenotype variants of Rh. Detection level of different Rh antigens varies with different reagents and typing techniques used by the laboratories. So, it is of utmost importance to select the correct typing reagents and techniques. It is observed that donor sample can also be safely typed by using potent monoclonal reagent in parallel with another potent polyclonal anti-D reagent\(^6\).

The article by Kulkarni et al\(^7\) in the current issue on usefulness of commercially available anti-D antisera available in Indian market is quite interesting. Authors have identified a real problem related to immunohaematology which ultimately has a direct impact on patient care. Authors have selected 42 confirmed partial D variants for testing with seven commercially available anti-D reagents in India. Reagents were a mixture of IgG and IgM, of which few were of blend type. In Mab variety, strong reactivity was observed in only in 59 per cent partial D variant cells. On the other hand, polyclonal anti-D showed weak reaction with 83 per cent of partial D cells. Authors have investigated reactivity of 21 combinations from six available anti-D reagents and tested with cell panel of partial anti-D. This was a right approach to investigate reactivity of two reagents as prescribed in the Drugs and Cosmetic Act (1945). However, only two combinations picked up all partial D. This is a matter of great concern that 90.5 per cent available combinations of reagents did not detect partial variants.

Similar observation was made by Judd et al\(^8\). They have also observed that individuals having partial D phenotype may develop anti-D if exposed to D+ RBC. However, testing donors for weak expression of anti-D continued to be a challenge. Though Rh immunization with a weak or partial D phenotype was uncommon, it was recommended that proper evaluation sera might be used. Judd et al\(^8\) studied anti-D from three manufacturers having combination of monoclonal and polyclonal anti-D reagents blended and human polyclonal diluted in high protein diluents. Direct AHG test was positive with partial D RBCs of type DII, DIIIa, DIIIb and DVI type 1 and DVI type 2 or DFR phenotype RBCs. They observed discrepant results with different Mab, polyclonal and high protein human anti D. In this study few reagent anti D could not detect weaker variant of D antigen.

In India, we have multiple studies on Rh typing in the population. However, there is no systematic study to understand variants of Rh antigen in different parts of the country in different ethnic populations. In the above mentioned study\(^7\), interesting result was observed from western India. There may be different information waiting to be explored from other parts of the country. If it is the real situation, there might have been regular unwanted Rh immunizations in partial D persons in India because of wrong diagnosis. This scientific information is not actually available to Indian blood banking community. There may be two ways of solving this problem. It might be at individual or collective level. At individual level, one might take corrective steps to select correct combination of anti-D reagents. However, it is difficult because most of the blood banks do not have infrastructure to have a confirmed panel of partial D cells. It is only possible at collective level, where government of India agencies like Indian Council of Medical Research (ICMR) or Department of Science and Technology (DST) may get involved to find out
Rh variants and gene frequencies in different ethnic populations. It will help us to understand the actual problem and their reactivity with available anti-D reagents. The most difficult part for blood bank services will be the selection of right combination of anti-D reagent. In India, all reagents are to be approved by the Central Drugs Standard Control Organization (CDSCO) for marketing. When any commercial firm wants to introduce new reagents, they apply permission for marketing to CDSCO and the sample is sent to National Institute of Biologicals (NIB) for evaluation. Only after receiving due technical approval, CDSCO allows the reagent for marketing. This is a well defined procedure by which these reagents come to the market. Despite that, how is it possible that 90 per cent combinations of anti-D reagents failed to detect partial D cell panel after passing through all these regulatory procedures. A systematic study is needed in Indian population to understand presence of partial D and variants present in the population. During preparation of a panel of red cells of partial D and weak D, various scientific institutes, like Institute of Immunohematology, Mumbai, having expertise on this subject should be involved. It would be probably right way to re-evaluate already available anti-D reagents in Indian market so that these reagents detect majority of probable D phenotypes including partial D.

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References


