Advances in detection of antenatal alloimmunization and its management

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Establishment of Antenatal Serology Clinics

The sporadic reports of erythroblastosis fetalis were published since 1947[1-4] in India. Indian Council of Medical Research (ICMR) established Blood Group Reference Centre (BGRC) in the year 1957. BGRC started the first antenatal serology clinic in 1957 in "Nowrosjee Wadia Maternity Hospital" (NWMH), Bombay, which is one of the biggest maternity hospitals in Asia. ABO and Rh typing of all antenatal women was done by tube technique. They were screened for IgM and IgG atypical antibodies to detect suspected cases of hemolytic diseases of the newborn (HDN). Rh (D) negative women were regularly investigated for presence anti-D and follow up was done.

BGRC was requested to establish antenatal serology clinics in other maternity hospitals in Bombay. BGRC staff trained the hospital technicians in antenatal serology and also provided blood grouping and anti-human immunoglobulin reagents. In other parts of India also screening of pregnant women for alloantibodies started in early seventies.

Importance of other Rh specific antibodies was revealed in a paper published by Bhatia and Sanghvi[5] in the year 1959. Among 38 cases they detected nine women having anti-C+D, one anti-C, one anti-E and remaining cases with anti-D. Compound Rh antibodies like anti-Ce+e anti-cE were reported by Gupte *et al.*[6] Badakere and Bhatia[7] published a case of HDN in which mother had rare -D-/-D- genotype. She had multiple Rh antibodies.

1. Advances in antenatal detection of alloimmunization

During last decade there is an advancement in testing red cell antigen/antibodies using Gel or glass bead micro columns, microplate technique and EM technology which is a nanotechnology based on magnetization of red cells. The semi or fully automated systems are based on these techniques. Ready to use red cell panels are also available in the market. These techniques are used for antenatal detection of alloantibodies only in big institutions and often only in blood banks. Small maternity hospitals in India are sending antenatal samples to pathology laboratories and majority of them do only cell grouping on a slide and hence alloantibodies are not detected.

The most common specificities detected using these sensitive techniques are within Rh system. Devi *et al.*[8] detected one case of anti-M. A study by Varghese *et al.*[9] detected antibodies in 79 antenatal women using gel technique. Specificity was identified in 50 of them, majority (40.6%) were Rh specific. One woman had anti-E and anti-K.

2. Antenatal laboratory investigations for predication of severity

- DNA analysis for genotype of father or Rh typing of the fetus

Serological tests determine probable Rh genotype. Several techniques are available to carry out Rh genotype by DNA analysis. Rh typing of the fetus is possible using maternal blood, chorionic villus sample (CVS) or amniotic fluid.[10,11] The discovery of fetal DNA in maternal plasma has opened up new possibilities for noninvasive prenatal diagnosis. The fetal-derived *RHD* sequence in Rh D-negative pregnant woman's plasma indicates the presence of a rhesus D-positive fetus.[12]

- Rh antibody estimation

Manual Rh titration is universally accepted simple, cheap test and could be repeated frequently. However it is semi quantitative, visual test and the results show variation from person to person. Several methods are available for quantification of Anti-D.[13] A comparison between flowcytometry and auto-analyzer techniques has shown that validation is necessary before accepting flowcytometry.[14]

- Tests for determination of lytic potential of anti-D

Anti-D being a noncomplement binding antibody, destruction of sensitized fetal red cells occurs extravascularly. The adherence of sensitized erythrocytes (target cells) to Fc receptors on monocytes or macrophages (effectors cells) is the initial event that leads to erythrophagocytosis or cytolysis. Several bioassays have been developed to

measure lytic potential of anti-D using lymphocytes, monocytes or cultured macrophages. Among these cells monocytes are considered the best. Antibody dependant cell mediated cytotoxicity (ADCC) assay has more predictive value compared to microscopic phagocytic assay, Monocyte monolayer assay or Chemiluminescence assay.

- IgG subtypes of anti-D

The efficiency of transfer of IgG subclasses across placenta is IgG1 > IgG 3> IgG4 > IgG2. IgG_1 and IgG_3 are the most predominant subclasses and in severe cases they exist in combination.[15] It has been demonstrated that IgG_1 anti-D mediates red cell destruction by phagocytosis while IgG_3 by prolonged cytolysis.

- Spectrophotometric analysis of amniotic fluid

It is a time tested method of assessing severity but Liley's graph cannot predict the severity before 27 weeks gestation. Therefore modified graph has been recommended.[16] Considerable number of optical density values of the graph fall in zone II beginning or middle when the fetus is Rh (D) negative. Hence Intrauterine Transfusion (IUT) is recommended when results indicate end of zone II or zone III.

Intrauterine Transfusion

The main aim of IUT is to correct fetal anemia and prevent hydorps fetalis.

Criteria

Gupte *et al*.[17] used following laboratory criteria: 1. Amniotic fluid analysis suggesting the end of Liley's zone II or zone III. 2. Maternal anti-D titer ≥ 1:64 or anti-D concentration > 4 μg/ml. The IUT is performed when the fetal blood collected prior to IUT shows Hb concentration lower by >2 g/dl compared to the normal Hb level at the same gestation.

Frequency of IUT

IUT leads to suppression of fetal erythropoiesis. Therefore repeated IUTs are planned at the interval of one to three weeks till labor is induced.

Intraperitoneal IUT

Intraperitoneal IUT is given only when vascular access is not possible due to fetal position or if IUT is given prior to 20 or after 34 weeks of gestation.

The disadvantages are:

- Erratic and incomplete absorption of donor's RBC through the peritoneum.
- Risk of hyperbilirubinemia after birth due to hemolysis of donor's RBC trapped in the peritoneal cavity.
- In hydropic and severely compromised fetus this route is not effective.
- It is not possible to know degree of correction of anemia.

Intravascular IUT

Site of puncture: Generally the origin of the umbilical cord is the preferred target.[17] A more recent approach is transfusion via intrahepatic vein.[18] The other route which is practiced in emergency is direct transfusion into the fetal heart.

Indian experience with IUT: At Nowrosjee Wadia maternity hospital, an infrastructure has been created by the Institute of Immunohaematology (ICMR) to perform IUTs. We performed 79 IUTs for 35 Rh HDN cases.[19] The gestational age of the mothers ranged from 19 to 34 weeks. In most of the cases cordocentesis technique was followed. The pre IUT hemoglobin values of the fetuses ranged from 1.0 to 7.4 g/dl. The volume of blood transfused was calculated using a nomogram[20] based on hematocrit values of fetus, blood unit and desired value of 40%; gestational age and feto-placental blood volume.

Prophylaxis anti-D Immunoglobulin (Anti-D IgG)

After successful clinical trials Rh IgG was licensed in 1968.[21] In India Anti-D IgG is available since 1976. Anti-D IgG intramuscular injection is given to Rh (D) negative non-immunized mother of Rh(D) positive infant within 72 hours after birth. There is no uniformity in countries about dose of anti-D administered.

Dose of anti-D prophylaxis

The new British Committee for Standards in Haematology (BCSH) recently released following recommendations:[22]

1. Following potentially sensitizing events anti-D IgG should be administrated within 72 hours. The events include amniocentesis, CVS, cordocentesis, antepartum hemorrhage, abdominal trauma, ectopic pregnancy, molar pregnancy, therapeutic intervention like surgery, abortion, medical termination pregnancy, normal or instrumental delivery, caesarean section, intra-operative salvage etc.
2. Sensitizing events between 12 to 20 weeks pregnancy need minimum dose of 250 IU (50 μg), after 20 weeks 500 IU (100 μg).
3. For antenatal prophylaxis two doses (100 μg each) are given at 28 and 34 weeks (In many countries a single dose of 300 μg is administered).
4. Following birth atleast 500 IU (100 μg) is given guided by fetomaternal leak if delivery is complicated. (USA standards recommend 300 μg in after birth)

Monoclonal anti-D immunoglobulin

Production of polyclonal anti-D IgG needs human hyper Rh immunized donors. Therefore possibility of obtaining unlimited amounts anti-D monoclonal antibodies (MAb) by hybridoma technology is attractive. Despite success of production of murine MAb for human blood group antigens, no MAbs were produced for Rh antigen because rodents did not respond to D antigen. The effective MAb anti-D must be capable of binding RhD antigen on RBC and also interact with effector cells. Two antibodies (BRAD-3 and BRAD-5) have been studied for functional tests. At present many MAb anti-D are under trial for therapeutic use.[23] One Indian company since last 15 years is supplying MAb anti-D prophylaxis. Its authenticity is questionable.

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