Perinatal Outreach Program of Southwestern Ontario

Partner

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RH ISOIMMUNIZATION

New DNA Technologies

Haemolytic Disease of the Newborn remains a devastating condition that affects, to some degree, 10.6/100 000 live births per year. A major cause of this condition continues to be anti-D antibodies in Rh-ve mothers which target the destruction of fetal red blood cells in utero in subsequent pregnancies. The history of Rh disease, with much groundbreaking work done in Canada, is a remarkable story. Despite the gains made in prevention, diagnosis and treatment of this condition, difficult situations still arise in the care of the isoimmunized patient which would be reduced if the Rh status of the fetus could be obtained prenatally. With this data, better informed decisions can be made with respect to invasive monitoring and timing of delivery. Recent work has been done in this area utilizing Polymerase Chain Reaction (PCR) technology which will allow for early and accurate determination of Rh status of the fetus.

Haemolytic disease of the newborn was first reported by a French midwife in the early 1600[s] in describing twins. One of the infants was grossly edematous and the other severely jaundiced. The cause of hydrops fetalis and icterus gravis were not known until the early 1900[s]. Diamond, in 1932, showed that a haemolytic process was responsible for the spectrum of disease. In 1938, Darrel correctly theorized that the passage of maternal antibodies into fetal circulation with resultant haemolysis was the basis of the disease. This antibody was later identified by Landstenier and Weiner in 1940 as Rh antigen specific. Much pioneering work in the diagnosis and treatment of Rh disease was done in Winnipeg by Dr. Bruce Chown. The Winnipeg Rhesus laboratory was first opened in 1944. This group was responsible for identifying many of the of the blood group antigens of the Rh system and others (Kell, Duffy, Diego). In 1964 the first intra-uterine intra peritoneal fetal transfusion in North America was performed in Winnipeg by Dr. Rhinehart Freisen. This followed Sir William Liley's amniotic fluid OD 450 work in 1961 and the first intra peritoneal transfusion in 1963. At this time, routine care of the isoimmunized pregnancy involved delivery at 32 weeks and carried a 25% mortality rate. The later introduction of Percutaneous Umbilical Blood Sampling (PUBS) and intra vascular transfusions in 1985 and '86 by Manning et. al. allowed for much improved decision making with respect to treatment and delivery of the affected fetus. Advances in care had resulted in 90% survival without the development of hydrops and 82% survival with hydrops fetalis. The area of Rh prophylaxis was also growing over this time as in 1970 Dr. Hans Hoppe of Germany developed an ion exchange chromatography method of isolating Rh immune globulin.

While the identification and treatment of the fetus at risk has greatly improved, and the ability to determine fetal Rh status using PUBS techniques has reduced the requirement for invasive monitoring in Rh positive fetuses, this procedure is invasive and carries a 1-3% fetal loss and much higher fetomaternal haemorrhage rate that can worsen an already affected pregnancy. With the recent cloning of the RhD and RhCcEe genes on chromosome 1, the molecular biology is now able to determine fetal Rh status using PCR technology on cells retrieved via amniocentesis. (We would not do CVS as it increases sensitization!)

The clinical scenario where the Rh status of the fetus will impact greatly upon decision making is not uncommon. The Rh negative mother and Rh positive father have a 50% or 100% risk of producing Rh positive children dependent upon the zygosity or

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genotype of the father at the RhD locus. Knowledge of paternal homozygosity for RhD simplifies monitoring, as all fetuses will be Rh positive and at risk if mother becomes Rh sensitized. The heterozygous father (56% of Caucasian Rh positive males) will result in 50% Rh negativity and can make invasive monitoring in an Rh sensitized mother unnecessary and defer unwarranted risk to the fetus. It is in these situations that the Rh status of the fetus will guide management to a great extent.

The technique of PCR is simple. but requires detailed knowledge of the nucleotide sequence of the target gene. Once a gene has been cloned (ie. its sequence is known), its presence or absence can be determined using an extremely small quantity of DNA from the subject. It is for this reason that amniocentesis obtained fetal cells are now adequate for fetal Rh determination. The basis of PCR amplification and identification of a specific gene lies in the use of a special DNA polymerase (Taq) which is able to function at the higher than normal temperatures required for denaturation of DNA, allowing targeted short sequence DNA primers to attach to the known gene sequence. This results in multiple copies of the target gene which are identified by their expected base pair length on an electrophoresis gel. The presence of the RhD gene as demonstrated by PCR amplification of DNA obtained by amniocentesis indicates that the fetus is RhD positive. Contamination by maternal cells is not a concern as the clinical situation dictates that the mother be RhD negative. The RhD gene is unique in that there is no recessive 'd' gene, but a deletion of the RhD gene sequence in RhD negative individuals. RhD and RhCcEe genes are in close proximity and share 93% sequence homology. Investigators have developed two PCR techniques which exploit the differences in these genes in order to identify the RhD gene.

Bennet et. al. reported their RhD determination of 30 fetuses using PCR technique and compared their findings to later serologic testing for determination of accuracy. Their protocol involved amplification of a portion of the RhCcEe gene, present in all subjects, as an internal control. Using a second set of primers on the same samples, a unique segment of the RhD gene was amplified. In those samples demonstrating two bands of different base pair lengths, the diagnosis of RhD positivity was made. Those displaying only one band (RhCcEe) must be negative for the RhD gene. This study involved small numbers but was conducted in two centres simultaneously (London and Paris) with 100% accuracy and correlation between centres. It was suggested by the authors that this technique could be performed by any laboratory with molecular biology capability and that the results could be available within 24 hours. This study also included RhD positive mothers with RhD negative infants. This is not clinically relevant, but does indicate sensitivity of the technique.

A year later the same group reported the application o f their procedure to 6 pregnancies which resulted in no further invasive monitoring in two pregnancies (Rh neg) and confidence in decision making with the other four. The requirement of only 2 ml of amniotic fluid, and its decreased risk of haemorrhage over CVS make it the method of choice for obtaining fetal cells thus far.

A group from Baltimore reported their technique in Obs Gyn (1994;171:1047-51) which differed from the English method by requiring only one set of DNA primers. This method also results in two bands in RhD positive and one in RhD negative subjects. Later, Yankowitz et. al. (Obs Gyn 1995;86:214-7) evaluated the accuracy of the Baltimore technique on 765 cases. The false negative rate was 2/632 and the false positive rate was 7/133. This gives a sensitivity of 99.7% and specificity of 94%. The implication of a false positive is that the monitoring continue, with its risks. These cases would not go on to PUBS and transfusion as the fetus would not be affected.

An even more exciting frontier in this area is the ability to determine RhD status of the fetus by PCR amplification of DNA from the fetal cells recovered from maternal serum. Yo et. al. (Lancet 1993;341:1147-48) applied PCR amplification to 21 cases using 10 ml of maternal peripheral blood. Their results are promising (80% sensitivity and 73% specificity) but other studies have not duplicated their numbers and no large scale study has been done. Until the sensitivity of the PCR technique improves so that the extremely small numbers of fetal cells in maternal circulation are adequate for very accurate diagnosis of fetal Rh status, this method is not applicable to clinical situations.

The determination of fetal RhD status by small volume amniotic fluid is available in our laboratory now. As more genes responsible for haemolytic disease are sequenced and PCR becomes more sensitive, the possibility of typing many fetal genes from fetal cells in amniotic fluid and maternal peripheral blood will become a reality.

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Project Profile

Breastfeeding Support Program Outcome Evaluation

Evaluation of the first year of the "Breastfeeding Buddies Program" indicates that the program had a positive impact:

- Women reported feeling that having a Buddy helped them breastfeed longer.
- The participants matched the breastfeeding duration of women in a comparison group who had other sources of support for breastfeeding.
- The duration of breastfeeding significantly increased for participants relative to their past experience

Background

The Postpartum Parent Support Program of South Bruce/Grey launched the "Breastfeeding Buddies Program" in March 1996. The goal of the Program is to increase the length that infants are breastfed to at least six months. Support to the mother is the strategy used by this program to obtain this goal. An evaluation of the Program was undertaken to assess:

- impact of the Buddies' support in terms of participants' perceptions
- impact of the Program in terms of length of breastfeeding duration

The Program facilitates support to mothers over the telephone by vulunteer "Buddies". Every effort is made to link mothers even if they do not have a telephone. "Buddies" are mothers from the surrounding community who have breastfed at least one baby successfully and are willing to be matched to a mother for the duration of her breastfeeding. Mothers are recruited for the program through public health prenatal classes, local hospitals in Hanover and Walkerton, newspapers, advertisements in physicians' offices and self-referral.

Method

All women who gave birth at the two hospitals between March 1, 1996 and February 28, 1997 were offered the program. There was 288 hospital deliveries recorded at the two hospitals during the study period. An estimated 80% of women who give birth intended to breastfeed when they left the hospital. Of those intending to breastfeed, 39 became participants in the Program and a further 125 agreed to participate in follow-up for comparison reasons. Both the "linked" mothers and the "comparison" mothers were followed up through a telephone call six months after their baby's birth.

Results

Nearly all (97%) of the linked mothers reported feeling that their Buddy had helped them breastfeed longer.

Forty-six percent of the linked mothers and 49% of the comparison mothers breasfed for six months or more. The data indicates that a greater percentage of linked mothers continued to breastfeed for the first six weeks than did comparison mothers. Linked mothers continued to do better than the comparison mothers until the four month point.

There were no statistically significant differences in the duration of breastfeeding between the "linked" mothers and the "comparison" mothers. The participants matched the breastfeeding duration of women in the comparison group who had indicated that they had other sources of support for breastfeeding.

When the previous and current breastfeeding experiences were considered, 71% of the linked mothers improved their breastfeeding duration, whereas only 37% of the comparison mothers breasfed longer than their previous experience. This represents a statisfically signifcant difference.

Discussion

The success of the Program appears to be in the Breastfeeding Buddies' abilities to provide support to mothers who might be at a greater risk of unsuccessful breastfeeding so tht they are as successful as the comparison mothers at breastfeeding.

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You Asked Us...

QUESTION:
Currently at our hospital there is no standard antibiotic regime for women who have undergone a Cesarean birth. What does the literature currently recommend?

ANSWER:
Reports suggest that at least one in five women suffer from febrile illness following Cesarean section. It is stated that the incidence of serious infections for example pelvic abscess, septic shock and septic pelvic vein thrombophlebitis is not rare. (1) Labour, ruptured membranes and obesity are three of the most significant risk factors associated with post cesarean section infection. The benefits of prophylactic antibiotics use has now been systematically reviewed and unequivocally proven. (2)

The use of antibiotics prophylactically has been proven to reduce the risk of both endometritis and wound infection equally in women having had an elective cesarean section as compared with those who underwent emergency surgery. (3)

Broad spectrum penicillin such as ampicillin, cephalosporins and metronidazole all have shown comparable effectiveness in reducing febrile illnesses post cesarean section. Evidence has not suggested that the use of second or third general cephalosporins or the addition of aminoglycosides to broad spectrum antibiotics offers any better prophylaxis. (4) Systemic administration appears to be as effective as intra-operative irrigation. (5)

Although most trials have not systematically reviewed the drug effect on infants, it would seem prudent to avoid infant exposure by administering the antibiotics after the umbilical cord had been clamped. Drug levels received by the infant through breast milk seem to be relatively low particularly if the duration of the antibiotic therapy has been short. (6)

"Withholding prophylactic antibiotics from women having cesarean section will increase the changes that they will experience serious morbidity." (7)

One useful antibiotic regime is as follows: Ampicillin 1 - 2 gm IV. ADC during cesarean section after clamping the cord and handing off the baby. Repeat two doses, six hours apart. If the patient is allergic to Penicillin, substitute Clindamycin 600 mgm IV ADC for Ampicillin.

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Upcoming Events:

ALARM
(Advances in Labour and Risk Management)
Course will be offered by the Society of Obstetricians and Gynecologists of Canada (SOGC) throughout 1998 at various times across Canada. The proposed schedule for Ontario courses is listed as follows:

London: Oct. 16 - 17 Edmonton: Nov. 7 - 8
Toronto: December 5 - 6
December 7 (Instructor Program)

For further information contact: SOGC, 774 Echo Drive, Ottawa, Ontario, K1S 5N8 tel: (613) 730-4192, fax: (613) 730-4314, Email: spaquette@sogc.com

National Launch and Conference Breastfeeding...Stepping Into Baby Friendly Initiative

Nov. 19 - 21, 1998 Westin Bayshore, Vancouver, BC
For more information please contact:
BC Reproductive Care Program
Tel: (604) 875-3737, Fax: (604) 875-3747

Breastfeeding Challenges for the new Millenium

May 6-7, 1999, London, Ontario
Call for abstracts (poster sessions) due Jan. 30th, 1999
Contact: Gwen Peterek, Middlesex-London Breastfeeding Committee (519) 646-6100, ext. 5901

Workshops with Penny Simkin

Two Day Doula Training Workshop
May 18 - 20, 1999, London, Ontario
Contact: Nancy Dodman, Perinatal Outreach Program of Southwestern Ontario (519) 646-6100, ext. 5900

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This newsletter is a publication of the Perinatal Outreach Program of Southwestern Ontario.

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