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Research Article

Rh Disease and Potential Implication of Fetal Microchimerism: A Case of Rising Rhesus "D" Antibodies Levels in a Woman with Rh "D" Negative Fetus

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Abstract

This is a classical case involving a woman with a history of giving birth to a child with Hemolytic Disease of the Newborn (HDN). The client was in her eleventh week of her third pregnancy when she reported to our facility for antibody detection, identification and titration. Consequently, we detected two alloimmune antibodies capable of crossing the placenta barrier; IgG B and Rh "D" antibodies. The Rh "D" antibodies were in alarming and increasing states from week 11 to week 25 when the woman traveled out of jurisdiction. The IgG anti-B was in a relative stable quantity throughout the monitoring period.

She was delivered on the 38th week of gestation and the baby was typed to be A Rh "D" Negative and was not carrying any of the antigens to the antibodies detected. We found this worth reporting because there was no fetal stimulus causing the rising levels of the Rh "D" antibody; we thus propose fetal microchimerism as the potential cause since the woman had a history of bearing children with the antigens to the antibodies detected. This is a phenomenon that has been postulated to be involved in the etiology of hemolytic disease of the newborn.

Introduction

Hemolytic Disease of the Newborn (HDN) or erythroblastosis fetalis is a grave hemolytic anemia in neonates that results from the development of anti-Rh or blood group antibody in an Rh-Negative or any clinically significant blood group antigen Negative mother, in response to the Rh antigen or that blood group antigen in the fetal blood. HDN could be as a result of hyperimmune ABO antibodies, Rh (D, C, c, E, e) antibodies and other minor blood group antibodies. These antibodies are IgG in nature and have the capacity to bypass the placenta barrier causing lysis of the fetal cells.

HDN is characterized by many erythroblasts in the circulation, and often generalized edema of the fetus with enlargement of the liver and spleen. Hemolysis due to alloimmune antibodies is seen with acute and delayed RBC transfusion reactions, following stem cell transplantation where there is an antigenic blood type difference between the donor and stem cell recipient, and during the neonatal period as a result of differences in maternal and fetal RBC antigens [1]. The quantum of clinical problems in hemolysis occurring in the fetus ranges from minimal hyperbilirubinemia to severe anemia with hydrops fetalis and/or kernicterus. HDN is further characterized by hemolysis as a consequence of maternal sensitization to fetal RBC antigens inherited from the father resulting in the presence of IgG antibodies in maternal circulation, which causes hemolysis in the fetus by crossing the placenta.

Early detection and treatment of neonatal hyperbilirubinemia is important in prevention of bilirubin-induced encephalopathy. Rh "D" incompatibility remains the commonest cause of HDN, although other RBC incompatibilities are increasing in incidence [2,3]. The mechanism through which blood cells from the fetus cross the placenta barrier to sensitize the mother is well understood. It is established that a lot of sensitization of Rh "D" Negative mothers occurs during delivery or through transfusion. The fetal cells in the mother may persist longer than 40 years through a mechanism called fetal microchimerism [4]. Cells of fetus that persist in the mother may trigger an immune response in later years. Though fairly understood, microchimerism is one phenomenon that could be implicated in causing HDN as observed in cases of autoimmune diseases and multiple sclerosis [5].

In this study, we report on the potential implication of fetal microchimerism in the development of Rh disease in a pregnant woman and the ABO and Rh antibody titer levels that can be detected. We clinically understudied a woman sensitized from her two previous pregnancies and were now

in her eleventh week of gestation with the third pregnancy. The husband is reportedly blood group B Rh "D" Positive whilst she is A Rh "D" Negative. Her sample was taken for antibody screening and identification as well as the levels of the antibodies detected until she left to give birth at a hospital in Lebanon. She returned to Ghana later with successful delivery of a child with blood group A Rh "D" Negative.

Materials and Methods

This is a clinical follow up study of a woman with Rh "D" Negative blood group (A Rh "D" Negative) and a husband of Rh "D" Positive antigen (B Rh "D" Positive). She has a history of two previous successive births with the latter baby having a complication of hemolytic anemia and jaundiced; placed under phototherapy upon delivery. The first born who has a rhesus D antigen was successfully delivered with minimal or no complications. However, the mother was not taken through the anti-IgG (RHOGRAM) recipe recommended for cases of this nature.

The client reported to the 37Military Hospital during her 11th week of gestation. She is a Lebanese aging 42 years with a body weight of 64 kg on her first visit. She was scared of delivering a stillborn child for her third pregnancy.

10 ml of venous blood sample was taken into a BD plain tube and she was asked to return a fortnight. Seven samples were taken within the period, at weeks 11, 13, 15, 17, 20, 22 and 25 of gestation. Various tests were run on the sample including blood group, antibody detection, identification and titration.

Blood Group Test

The standard tube grouping technique was used to determine the blood group. Both the forward and reverse grouping techniques were carried out. The serum was separated from the cells and a 5-10% cell suspension was made after the cells had been washed three times in warm saline (Saline at 37°C); this was used for the forward grouping using antisera from Rapid Diagnostics Ltd. Reverse grouping was done using a drop of 5-10% known A, B, O cells against two drops of the woman's serum.

Antibody detection, Identification and titration

With the antibody detection, identification and titration, the Ortho BioVue Gel Technique was used in the analysis of the sample. This technique makes use of a gel matrix impregnated with anti-human globulin/anti-C3d. The gel system is based on the principle that the matrix acts as a filter through which sensitized erythrocytes get entrapped. A Negative reaction is indicated by a clear pellet of red cells settling at the bottom of the microtube.

In the antibody detection, the indirect Coomb's test was performed. A drop each of 0.1% cell suspensions of known O Rh "D" Positive and O Rh "D" Negative (so as to prevent the interference of ABO antibodies) was used against two drops of the serum of the client. This was incubated at 37°C for 10 minutes according to the manufacturer's instruction. It was then allowed to stand at room temperature for 5 minutes and span to observe for agglutination. Commercially prepared monoclonal anti-D was used as the control. An auto-control was also set using the woman's serum and her washed cells.

Table 1: Detection of ABO blood antigens and antibodies in the serum of the mother.

ABO/Rh-D Phenotype	Antibody detection	Antibody Identified
A Rh "D" Negative	Positive	1. Anti-D 2. IgM anti-B 3. IgG anti-B

The antibody identification made use of commercially prepared eleven panel cells from Ortho Clinical Diagnostic Panel C. The results were matched against the Panel C from Ortho Clinical Diagnostic, a John & Johnson company product. A control was set using the anti-D monoclonal antibody and an auto-control.

In the antibody titration, a double serial dilution of twelve labeled tubes was made using the fresh serum. Again, the serum was pretreated with Dithiotretiol (DTT) to immobilize any IgM present by incubating equal volumes of 1M of DTT and the serum at 37°C for 2 hours. A double serial dilution of the DTT pretreated serum was also made. 0.1% of Known cell suspension of rhesus D antigen was used to titrate the serially diluted fresh serum for the anti-D antibodies whilst same percentage of B Rh "D" Negative was used to titrate both the serially diluted fresh serum and the DDT pretreated serially diluted serum for the total anti-B and IgG anti-B respectively.

The standard anti-IgG/C3d test tube technique was used in parallel to confirm the results of the gel technique described. However, in the tube technique, 5-10% cell suspension in all cases was used. A fresh sample was also always run parallel with the immediate previous sample (sample stored at 4°C-8°C) to compare the outcome.

Results

Two different alloimmune antibodies were detected. They include anti-D and IgG anti-B. The client's blood group was also confirmed to be A Rh "D" Negative as indicated in Table 1.

The level of anti-D fluctuated in increasing quantities. The highest of 8142 was recorded in week 25 with the lowest titer of 512 occurring in weeks 11, 13 and 15 of gestation. The alloimmune anti-B was in relatively stable levels with the highest titer of 32 recorded in weeks 11, 12 and 17 whilst the titer of 16 was recorded in the rest of the weeks as shown in Table 2.

There were steady and sharp rises in the level of anti-D detected from week 11 through to week 25. The alloimmune anti-B was however, relatively stable as shown in Figure 1.

Table 2: The titer levels of the antibodies detected at various weeks of gestation.

	Antibody Titers			Antibody Titers (after 2weeks storage at 4-8°C)		
	Anti-D	IgM+IgG Anti-B	IgG Anti-B	Anti-D	IgM+IgG Anti-B	IgG Anti-B
11	512	128	32	512	128	32
13	512	64	32	512	64	32
15	512	64	16	512	64	16
17	2048	64	32	2048	64	32
20	1024	64	16	1024	64	16
22	1024	64	16	1024	64	16
25	8142	64	16	-	-	-

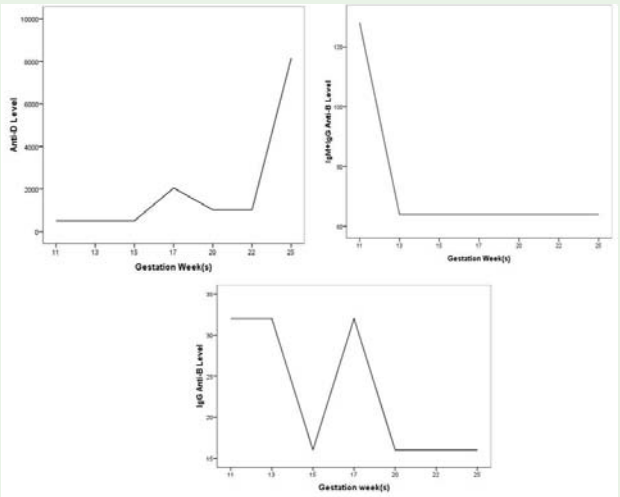

Figure 1: The levels of Anti-D, IgM+IgG Anti-B and IgG Anti-B detected at various gestation weeks.

Table 3: A comparison of the level of freshly run antibody titers with titer levels repeated after several weeks.

Sample (week)	Fresh Anti-D	Repeat Anti-D	Fresh IgM+IgG Anti-B	Repeat IgM+IgG Anti-B	Fresh IgG Anti-B	Repeat IgG Anti-B	P value
1 (11)	512	512	128	128	32	32	0.9999
2(13)	512	512	64	64	32	32	0.9999
3(15)	512	512	64	64	16	16	0.9999
4(17)	2048	2048	64	64	32	32	0.9999
5(20)	1024	1024	64	64	16	16	0.9999
6(22)	1024	1024	64	64	16	16	0.9999

There was not much difference in the titers of the antibodies after two weeks of storing sample at 4-8°C. When the titer levels were compared, there was no significant level variation shown in Table 3.

There were significant fluctuations in the anti-D antibodies until she travelled to Lebanon for delivery. The IgG anti-B antibody was however relatively stable. Before she travelled the anti-D level was 8142 IU/L with the IgG anti-B level at 16 IU/L.

Discussion

The titer level of alloimmune antibodies at which an immediate attention is needed is 16 IU/L [6]. The test results indicated that the anti-D level exceeded this value by a factor of 2^9 whilst the IgG anti-B was exactly at that level at week 25. This suggested a higher chance of a still birth and the life of the mother at risk. The results were reported periodically to the obstetrician in charge. We recommended a clinical psychologist be employed so as to aid in calming the nervousness of the woman which grew by the day. The anti-D levels rose steadily from 512 to 1024 in the final week of our testing whilst the IgG anti-B remained relatively stable.

For a classical situation of an Rh "D" Negative pregnant woman, continual production of the anti-D would only be as result of the presence or continual exposure of the woman to the antigen (Rh "D" antigen. However, our case revealed that the woman was carrying a baby with rhesus "D" Negative (A Rh "D" Negative). The woman had no history of transfusion and the only possible way she could have been sensitized was from her two previous births (both of whom were carrying the rhesus "D" antigen). For the sensitization from those previous pregnancies to persist and cause continual production of the antibody, it is postulated that "fetal microchimerism" is the most likely mechanism.

Generally, microchimerism is the persistent presence of a few genetically distinct cells in an organism. Though the mechanism is fairly understood various processes leads to the presence of genetically distinct cells in an organism. Most commonly, fetal microchimerism results from the exchange of cells across the placenta during pregnancy. Another increasing evidence of microchimerism indicates that cells may be transferred from mother to infant during nursing [7,8]. In addition to exchange between mother and fetus, there may be exchange of cells between twins *in utero*, and there is also the possibility that cells residing in the mother, obtained from an older sibling may find their way back across the placenta to a younger sibling during the latter's gestation. Women may have microchimeric cells both from their mother as well as from their own pregnancies, and there is even evidence for competition between cells from grandmother and infant within the mother.

Even though the immunogenic consequences are not well understood [9] there is evidence of the implication of microchimerism in multiple sclerosis, an autoimmune disease [5].

We hypothesized that the change in antibody levels was as a result of fetal microchimerism which might have occurred due to the previous pregnancies. However, we were unable to establish this hypothesis as we were limited in resources and material availability. We projected that since pregnancy is accompanied by low hemoglobin level due to increased utilization of iron, there is an increase hematopoiesis (erythropoiesis) in the bone marrow so as to compensate for the low hemoglobin. Therefore, during pregnancy, there is an increase synthesis of normal cells and fetal microchimeric cells causing the stimulus for anti-D production to increase if these microchimeric cells are in the erythropoietic tissues; hence the rising anti-D levels. The fetal antigen for anti-B production may also have increased, but the woman's naturally occurring IgM anti-B antibodies bind this antigen making it less available for more IgG anti-B production.

Conclusion

This postulate, if established, would mean that fetal microchimerism may be implicated in HDN and can aggravate the problem. In this case, there was successful delivery and the fetus was unharmed due to the lack of the Rh "D" antigen and B antigen on its red blood cell surface. It may not be so if in subsequent pregnancy, fetus carries the D or B-antigen from the father. There is a high risk of fetal death as chimeric cells can cause the production of potent antibodies (IgG).

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