REPORT

Seroprevalence of unexpected red blood cell antibodies among pregnant women in Uganda

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We conducted a population-based, cross-sectional study among pregnant women in Kampala, Uganda, to determine ABO and D blood types and to determine the percentage who have unexpected red blood cell (RBC) antibodies and their specificities. De-identified blood samples from routine testing of 1001 pregnant women at the Mulago Hospital antenatal clinics in Kampala were typed for ABO and D and screened for the presence of unexpected RBC antibodies with confirmation and subsequent antibody identification. Of the 1001 blood samples tested, 48.9 percent, 26.4 percent, 21.0 percent, and 3.8 percent tested positive for blood groups O, A, B, and AB, respectively. Of these samples, 23 (2.3%) were negative for D, and 55 (5.5%) showed initial reactivity with at least one screening RBC. The RBC antibody screen was repeated on these 55 samples, and antibody identification was performed at the Johns Hopkins Hospital Blood Bank in Baltimore, Maryland. Twenty-one of the 55 samples were confirmed to have evidence of agglutination. Nine of the 21 samples demonstrated the presence of clinically significant RBC antibodies with anti-S being the most common, 8 samples demonstrated the presence of benign or naturally occurring antibodies, and 4 had only inconclusive reactivity. This study revealed a relatively high frequency of D and a low frequency of demonstrable clinically significant alloantibodies that may cause hemolytic disease of the newborn or hemolytic transfusion reactions among pregnant women in Kampala, with anti-S being the most frequent antibody specificity. ***Immunohematology* 2012;28:115–7.**

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Alloimmunization is the development of antibodies to an antigen detected on the cells of another member of the same species. Red blood cell (RBC) alloimmunization among pregnant women is not infrequent given maternal exposure to fetal RBCs having different paternal antigens. The presence of these alloantibodies puts the fetus and newborn at risk of hemolytic disease[1] and places the mother and infant at risk for hemolysis if transfusion is needed. D has been found to be the Rh system antigen most commonly associated with hemolytic disease of the fetus and newborn (HDFN).[1] Currently, the prevalence of D has not been well studied, nor has the prevalence of other unexpected RBC antibodies in pregnant women in Kampala, Uganda. One recent study in southwestern Uganda found a maternal alloimmunization rate of 2.2 percent,[2] which is comparable to that found in a similar study conducted in Zimbabwe, which indicated a maternal alloimmunization rate of 1.7 percent.[3] Given the varying prevalence of different RBC antigens throughout the world,[4] further examination of this part of the world is important for clinical care. Therefore, we conducted a population-based, cross-sectional study to determine the ABO and D blood types among pregnant women in Kampala and to determine the percentage who have unexpected RBC antibodies and their specificities.

Materials and Methods

Study samples were drawn from 1009 de-identified blood samples collected in EDTA anticoagulant left over from routine testing at two antenatal clinics at Mulago Hospital. Eight of the 1009 total samples were found to have insufficient volumes of plasma for all tests to be carried out and were therefore not included in the analysis. All remaining samples were tested for ABO and D and screened for the presence of unexpected RBC antibodies. All blood typing and initial antibody screening were performed at the Uganda Core Laboratory of the Makerere University-Johns Hopkins University Research Collaboration, which provides diagnostic testing for patient care and clinical research studies at the Infectious Diseases Institute in Kampala. The plasma from all samples showing a positive screen for the presence of unexpected antibodies was frozen and shipped from the Uganda Core Laboratory to the Johns Hopkins Hospital Blood Bank in Baltimore, Maryland, for identification of antibody specificities. Shipment of samples was done in compliance with local and international regulations, by IATA certified laboratory personnel, and under the terms of a Materials Transfer Agreement approved by the Uganda National Council for Science and Technology as required. Both local and Johns Hopkins University institutional review board approvals for the study were obtained.

All leftover EDTA samples were stored at 4°C for a maximum of 30 days after collection. Blood typing and antibody screening were performed using protocols adapted from the Johns Hopkins Hospital Blood Bank. Forward and reverse ABO and D typing were performed on all samples. Forward ABO and D typing was performed using patient

RBCs and commercial reagents according to manufacturer's instructions (anti-A, anti-B, anti-D, and Rh control; Immucor, Norcross, GA). Reverse ABO grouping was performed using sample plasma and commercial group A[1] cells and group B cells according to manufacturer's instructions (Referencell2, Immucor). Weak D screening was performed on all samples testing D negative on immediate spin using monoclonal anti-IgG and Coombs control cells according to manufacturer's instructions (Immucor). Antibody screening was performed on all samples, using a commercial low-ionic-strength saline solution (LISS; Ortho Clinical Diagnostics, Raritan, NJ), commercial Screening Cell I and Screening Cell II (Immucor), and commercial anti-IgG and Coombs control cells by manufacturer's instructions. The plasma of all samples that showed a positive reaction at any step during the antibody screening procedure was extracted and stored at –20°C for later shipment to the Johns Hopkins Hospital Blood Bank for confirmation of antibody specificities. At Johns Hopkins, samples were retested for unexpected RBC antibody identification by the Capture assay on an automated instrument (Galileo, Immucor) or by gel test (ID-MTS Gel, Ortho Clinical Diagnostics).

Results

A total of 1009 maternal blood samples were collected for ABO and D typing, and unexpected RBC antibody testing was performed between June and August 2011. Only sex and presumed pregnancy status were known, as all samples were de-identified before testing. Of the 1001 blood samples tested (after 8 had been excluded because they had insufficient plasma volume), 48.9 percent, 26.4 percent, 21.0 percent, and 3.8 percent tested positive for blood groups O, A, B, and AB, respectively, and 23 (2.3%) were negative for D (Table 1). Fifty-five (5.5%) samples showed initial reactivity with at least one screening RBC. The RBC antibody screen and identification on these 55 samples were performed at the Johns Hopkins Hospital Blood Bank. If sample volume was limited, only an antibody identification panel was performed.

Table 1. ABO and D blood groups among 1001 pregnant women at Mulago Hospital

	n (%)		
ABO Group	Total	D–	D+
O	489 (48.9)	14 (2.9)	475 (97.1)
A	264 (26.4)	4 (1.5)	260 (98.5)
B	210 (21.0)	5 (2.4)	205 (97.6)
AB	38 (3.8)	0 (0)	38 (100)

Twenty-one of the 55 samples were confirmed to have evidence of reactivity. Nine of the 21 samples demonstrated the presence of potentially clinically significant RBC antibodies with anti-S being the most frequent (see Table 2),

Table 2. Antibody specificities among 21 pregnant women at Mulago Hospital

Antibody specificity	n*
Anti-S†	7
Anti-D†	1
Anti-K†	1
Anti-Le[a]‡	3
Anti-M‡	2
Anti-N‡	1
Cold agglutinins‡	2
Inconclusive reactivity	9*

*Total greater than 21 because 5 samples had inconclusive reactivity in addition to a specific red blood cell antibody.

†Clinically significant red blood cell alloantibodies.

‡Clinically benign antibodies.

8 samples demonstrated the presence of benign or naturally occurring antibodies (3 anti-Le[a], 2 anti-M, 1 anti-N, and 2 cold agglutinins), and 4 had inconclusive reactivity.

Discussion

Our study revealed that approximately half of 1001 pregnant women tested at Mulago Hospital in Kampala were group O, and 26.4 percent, 21.0 percent, and 3.8 percent were groups A, B, and AB, respectively. Overall, 2.3 percent were D–, which is lower than the 7.1 percent found in black blood donor populations in the United States[5] but comparable to the 3.6 percent recently reported from southwestern Uganda.[2] The percentage of pregnant women with unexpected RBC antibodies was 2.1 percent, which is relatively low given the high fertility rate of 6.7 births per woman in Uganda,[6] and is similar to the 2.2 percent rate found in southwestern Uganda.[2] Pregnant women in Uganda are not routinely typed and screened, yet only 1 of 23 D– women demonstrated an anti-D. Overall 9 (0.9%) women demon-strated RBC antibodies thought to be potentially significant for HDFN. Anti-S was the most frequent RBC alloantibody, detectable in 7 (0.7%) women, followed by 1 each of anti-D and anti-K. Anti-S was also the RBC antibody with the highest prevalence in another study among pregnant women in southwestern Uganda at a rate of 0.6 percent.[2]

Maternal hemorrhage associated with childbirth is also significant in Uganda. A recent study in Uganda reported that of 55,803 live births, blood was requested for 185 of 229 women with severe maternal morbidity.[7] However, blood was not available for 34 percent of these women as a result of either lack of blood in the hospital blood bank, lack of transport to the national blood bank, or lack of blood in the national blood bank. Although morbidity was not known to be caused by difficulty in obtaining compatible blood, antenatal screening for RBC antibodies would likely allow timely availability and compatibility of blood for transfusion for those women with identified clinically significant RBC antibodies.

The implications of our findings are limited given the de-identified nature of the specimens tested and lack of follow-up data on the newborns of these women. Consequently, this study only showed the presence of demonstrable alloantibody as opposed to the percentage of pregnant women with a history of alloantibody and those currently demonstrating alloantibody. Nevertheless, this study does give the frequency and specificities of demonstrable clinically significant antibodies that may cause HDFN or require compatible blood for transfusion among pregnant women and their neonates in Kampala and it demonstrates the feasibility of establishing type and screen testing for RBC alloantibody in a health care setting.

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References

1. Rose NC. Obstetric and intrauterine transfusion. In: Hillyer CD, Silberstein LE, Ness PM, Anderson KC, eds. Blood banking and transfusion medicine: basic principles and practice. Philadelphia, PA: Churchill Livingstone, 2003:321–34.
2. Natukunda B, Mugyenyi G, Brand A, Schonewille H. Maternal red blood cell alloimmunization in south western Uganda. Transfus Med 2011;21:262–6.
3. Cakana AZ, Ngwenya L. Is antenatal antibody screening worthwhile in the Zimbabwean population? Cent Afr J Med 2000;46:38–41.
4. Dean L. Blood groups and red cell antigens. Bethesda, MD: National Center for Biotechnology Information (NCBI), National Library of Medicine, National Institutes of Health, 2005.
5. Garratty G, Glynn SA, McEntire R; Retrovirus Epidemiology Donor Study. ABO and Rh(D) phenotype frequencies of different racial/ethnic groups in the United States. Transfusion 2004;44:703–6.
6. Uganda. The World Factbook. Central Intelligence Agency. Available at: https://www.cia.gov/library/publications/the-world-factbook/geos/ug.html. Accessed October 16, 2012.
7. Okong P, Byamugisha J, Mirembe F, Byaruhanga R, Bergstrom S. Audit of severe maternal morbidity in Uganda—implications for quality of obstetric care. Acta Obstet Gynecol Scand 2006;85:797–804.

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