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Full Length Research Paper

Frequency of red blood cells Allo-Antibodies among Sudanese multi-parous females in Khartoum State

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Abstract

Maternal allo-immunization, also known as iso-immunization, occurs when a women's immune system is sensitized to foreign erythrocyte surface antigens, stimulating the production of immunoglobulin G (IgG) antibodies. The most common routes of maternal sensitization are via blood transfusion or feto-maternal hemorrhage associated with delivery, trauma, spontaneous or induced abortion, ectopic pregnancy, or invasive obstetric procedures (Susan, *et al.,* 2006). The aim of this study is to detect the frequency and specificity of allo-antibodies against (RBCs) antigens among Sudanese multi-parous women in Khartoum state, and to associate between number of pregnancies, history of abortion and history of previous blood transfusion with allo-immunization. Eighty Sudanese multi-parous pregnant females were randomly selected for this study. Samples were collected and tested for ABO/Rh (D) groups; coomb's test was done using Coomb's technique for antibody screening and antibody identification using column agglutination method. Shows that 8(10.0%) of the pregnant females showed positive allo-antibodies, where as 72 (90.0%) were negative. Identification of antibodies showed anti-Kell 3(37.5%), anti-E 2(25.0%), anti-S1 (12.5%), anti-C^w 1(12.5%) and anti-Le^a 1(12.5%). Among the 8 pregnant females with positive antibody screening only 2 females (25.0%) were previously transfused, while 6 females (75.0%) were not exposed to blood transfusion. The relationship between abortion and allo-immunization was found to be significant (P value < 0.05) and the relationship between number of pregnancies and allo-immunization was found to be significant (P value < 0.05). This study concluded that the most common identified antibodies were anti-Kell. Allo-antibodies detected among the pregnant females were due to pregnancy and not blood transfusion. There was a relationship between abortion and allo-immunization and Increase number of pregnancies gives a chance for allo-immunization to occur.

Keywords: Red blood cells, Iso-immunization, Allo-antibodies, Sudanese, Khartoum State

INTRODUCTION

The immune response is initiated by the presentation of an antigen or immunogen (can initiate an immune response) to the immune system which determining that the antigen is non self. Antibody, is a complex protein produced by plasma cells, with specificity to antigens (or immunogens), that stimulates their production. An Ig is a

specific host self protein produced in response to a specific foreign non self protein (Denise, 2005).

According to differences in their heavy chain constant domains, immunoglobulins are grouped into five classes, or isotypes that includes IgG, IgA, IgM, IgD, and IgE. (Barbara et al., 1988; Pier et al., 2004).

The red cell is a convenient marker for serological reactions, for its surface antigens that interact with specific antibodies. Agglutination or lysis is a visible indication of an antigen-antibody reaction. The classification of the blood groups was based on the realization that agglutination had occurred because the red cells possessed an antigen and the corresponding specific antibody was present in the serum. When agglutination had not occurred, either the antigen or the antibody was missing from the mixture (Lewis et al., 2006).

Allo-immunization is a condition in which the body gains immunity, from another individual of the same species. Allo-immunity should not be confused with autoimmunity in which the body's immune system attacks its own cells without being provoked or influenced by substances or cells from another member of the same species. An alloantigen is an antigen that is a part of an animal's self-recognition system. When injected into another animal, they trigger an immune response aimed at eliminating them (Abbas and Lichtman, 2007).

Blood group antibodies are called allo-antibodies if they react with allo-antigens on the red blood cells (RBCs) of other individuals. Immune antibodies are produced following an exposure to foreign erythrocyte antigens through pregnancy or transfusion (Ernest et al., 2001).

Maternal all-immunization, also known as iso-immunization, occurs when a women's immune system is sensitized to foreign erythrocyte surface antigens, stimulating the production of immunoglobulin G (IgG) antibodies. The most common routes of maternal sensitization are via blood transfusion or feto-maternal hemorrhage (i.e. transplacental passage of fetal erythrocytes) associated with delivery, trauma, spontaneous or induced abortion, ectopic pregnancy, or invasive obstetric procedures. These antibodies can cross the placenta during pregnancies in allo-immunized woman and, if the fetus is positive for the erythrocyte surface antigens, result in IgG antibody-mediated hemolysis of fetal erythrocyte and anemia (Susan, 2006).

Antibodies detected by coombs test also known as anti-globulin test, (AGT). These are the Direct Coombs test and Indirect Coombs test. Direct Coombs test is used to detect these antibodies or complement proteins that are bound to the surface of red blood cells while indirect Coombs test is used in prenatal testing of pregnant women, and in testing blood prior to a blood transfusion. It detects antibodies against RBCs that are present unbound in the patient's serum.

Blood groups

The blood group, also called the blood type, is a classification of blood based on the presence or absence of inherited antigenic substance on the surface of red blood cells (RBCs).These antigens may be proteins, carbohydrates, glycoproteins, or glycolipids, depending on the blood group system, and some of these antigens are also present on the surface of other types of cells of various tissues. Several of these red blood cell surface antigens that stem from one allel (or very closely linked genes), collectively form a blood group system (International Society of Blood Transfusion, 2006).

Today, 29 human blood group systems are recognized by the international society of blood transfusion (ISBT). However, complete blood type would describe a full set of 29 substances on the surface of RBCs, thus an individual's blood type is one of the many possible combinations of blood group antigens. Across the 29 blood groups, over 600 different blood group antigens have been found, but many of these are very rare or are mainly found in certain ethnic groups (International Society of Blood Transfusion, 2006).

ABO blood group system

The ABO blood group system is the most important blood type system (or blood group system) in human blood transfusion. Two separated antigens were recognized, known as 'A' antigen and 'B' antigen. The antibody that reacted with 'A' antigen was known as 'anti-A' and the antibody that reacted with 'B' antigen as' anti-B. The associated anti-A antibodies and anti-B antibodies are usually IgM antibodies, which are usually produced in the first years of life by sensitization to ABH antigen –like substances in the diet or the environment such as food, bacteria and viruses (Neville and Bryant, 1982).

Hyper-immune anti-A and anti-B occur less frequently, usually in response to transfusion or pregnancy, but may also be formed following the injection of some toxoids and vaccines. They are predominately of IgG class and are usually produced by group-O and sometimes by group-A2 individuals. Hyper-immune IgG anti-A and/or anti-B from group-O or group-A2 mothers may cross the placenta and cause HDN (Dacie and Lewis, 1995).

Rhesus blood group system

The rhesus antigens are named C, D, E, c, d and e. The symbol "d" is used, however, to denote the absence of D antigen .All individuals who lack D antigen are said to be Rh-negative, regardless of whether the C or E antigens or both are present. The Rhesus system is the second most significant blood group system in human blood transfusion. The most significant Rhesus antigen is the

Rh-D antigen because it is the most immunogenic of the five main rhesus antigens. It is common for Rh-D negative individuals not to have any anti-Rh-D IgG or IgM antibodies. However, Rh-D negative individuals can produce IgG anti-Rh-D antibodies following a sensitization event. The majority of Rh antibodies are IgG (Landsteiner and Wiener, 1940).

Other blood groups

Lewis blood group system

The antigen either Le^a or Li^b the corresponding antibodies are anti-Le^a followed by anti-Le^b. these Antibodies are predominantly IgM and seem almost always to bind complement. This results in a kind of red cell destruction that is most often serious, namely intravascular hemolysis (Neville and Bryant, 1982).

Kell blood group system

The main antigens are K and k. Further Kell types are Kp^a, Kp^b, Js^a and Js^b. "Naturally occurring" antibodies in the Kell system are rare, although occasional examples of anti-K and anti-Kp^a have been identified in persons who have no history of exposure to the reactive antigen. Anti-K and (anti-k) are usually IgG. Both anti-K and anti-k can cause HDN (Neville and Bryant, 1982).

Duffy blood group system

The antigens are Fy^a and Fy^b and the antibodies are anti-Duffy (anti-Fy^a and Fy^b) is most often IgG, and usually reacts best by the indirect anti-globulin test, though a few examples agglutinate red cells suspended in saline. About 50% of anti-Fy antibodies bind complement (Neville and Bryant, 1982).

Kidd blood group system

Antibodies of the Kidd system (anti-Jk^a and anti-Jk^b) are usually IgG (but may be IgM); they are best detected by the indirect anti-globulin technique. The antibodies commonly bind complement. Antibodies in the Kidd system have been implicated in both hemolytic transfusion reaction (HTR) and in HDN (Neville and Bryant, 1982).

Lutheran blood group system

The antibody appeared after a transfusion of blood from a donor by the name of Lutheran. The antibodies of the Lutheran system are anti-Lu^a and anti-Lu^b, anti-Lu^a is usually occurs as a cold agglutinin that reacts best at $12c^o$. The antibody appears to be mainly IgG and may be "naturally occurring" (Neville and Bryant, 1982).

MNSs blood group system

The antigens are M, N, S, and s. Anti-M is a rare antibody occurring more commonly in infants than in adults it is usually IgG, though IgM anti-M is also frequently found. Anti-M may cause fairly rapid red cells destruction in patients who have not previously been transfused. HDN caused by anti-M is extremely rare, even when the antibody is known to be present in the mother. Anti-N is non- immune (i.e." naturally occurring) (Neville and Bryant, 1982). Anti-S is most commonly found as an immune antibody in patients having many transfusions, though "naturally occurring "examples have been reported. Most examples of immune anti-S are IgG. Anti-S has been implicated as a cause of HTR and is also known to have caused HDN. Anti-s is an extremely rare antibody. Most examples have been found to be IgG. The first example of the antibody was the cause of severe HDN (Neville and Bryant, 1982).

P blood group system

Anti-P_1 is usually a cold reacting IgM agglutinin. It sensitizes red cells to agglutination by anti-IgM and anti-complement, but not by anti-IgG. Anti-P_1 can cause severe adverse transfusion reaction but has not been implicated as the cause of HDN (Neville and Bryant, 1982).

Objectives

To detect the frequency and specificity of allo-antibodies against (RBCs) antigens among Sudanese multi-parous women in Khartoum state, and to associate between number of pregnancies, history of abortion and history of previous blood transfusion with allo-immunization.

METHODS

This study was a descriptive cross sectional study carried out in Khartoum State in pregnant females' attended Al-Turki Teaching Hospital (TTH). Eighty Sudanese multi-parous pregnant females were randomly selected for this study.

Samples were collected and tested for ABO/Rh (D) groups; coomb's test was done using Coomb's technique for antibody screening and antibody identification using column agglutination method.

Table 1. Frequency and percentages of ABO Rh D groups in study population

ABO Rh D Groups	Frequency	Percentage
A +ve	15	18.8%
B +ve	16	20.0%
AB +ve	3	3.8%
O +ve	35	43.8%
A –ve	4	5.0%
B –ve	1	1.2%
O – ve	6	7.5%
Total	80	100.0%

Table 2. Frequency and percentages of ABO, RhD groups in positive cases.

ABO Rh D Groups	Frequency	Percentage
A +ve	2	25%
B +ve	2	25%
O +ve	2	25%
O –ve	2	25%
Total	8	100.0%

Table 3. Frequency and percentages of antibody screening results among study population

Antibody Screening	Frequency	Percentage
-ve	72	90.0%
+ve	8	10.0%
Total	80	100.0%

Table 4. Frequency and Percentage of identified antibodies in positive cases

Identified Antibodies	Frequency	Percentage
Anti-C^w	1	12.5%
Anti-Kell	3	37.5%
Anti-Le^a	1	12.5%
Anti-E	2	25.0%
Anti-S	1	12.5%
Total	8	100.0%

Collected data and tests results were analyzed using the computer program SPSS16 (Statistical Package of Social Sciences).

RESULTS

According to their ages, most of pregnant female were between 15 and 50 years as seen in, those with positive antibody screening were between 36 and 40 years.

Frequency of ABO Rh (D) groups showed (43.0%) O-Rh positive, followed by B-Rh positive (20%), A-Rh positive (18.8%), O-Rh negative (7.5%), A-Rh negative (5.0%), AB-Rh positive (3.8%), and B-Rh negative (1.2%) as seen in (Table 1).

Table 2 shows the distribution of ABO Rh (D) among pregnant females with positive antibody screening. Two were A-Rh positive (25.0%), two were B-Rh positive (25.0%), two were O-Rh. positive (25.0%), and two females were O-Rh negative (25.0%).

Table 3 shows that 8(10.0%) of the pregnant females

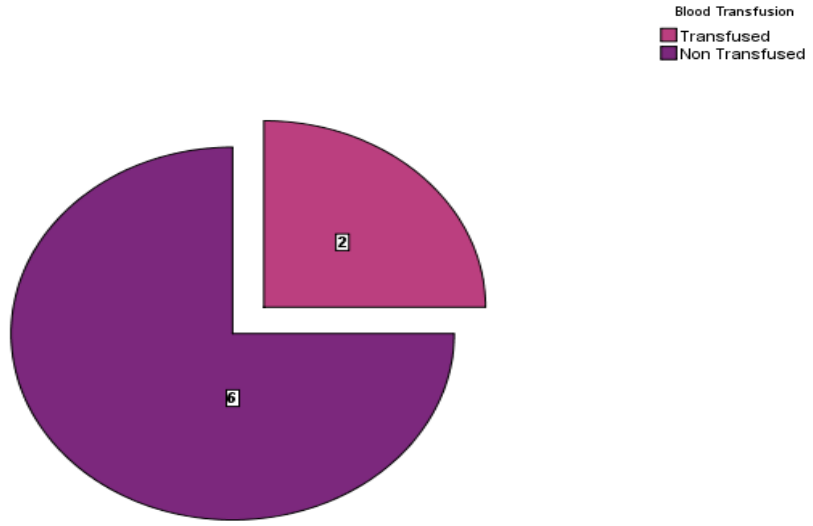

Figure 1. Frequency of blood transfused versus non-transfused in positive cases.

Table 5. Frequency of antibody screening according to blood transfusion:

Blood transfusion	Antibody Screening -ve	+ve	Total
Non transfused	60	6	66
Transfused	12	2	14
Total	72	8	80

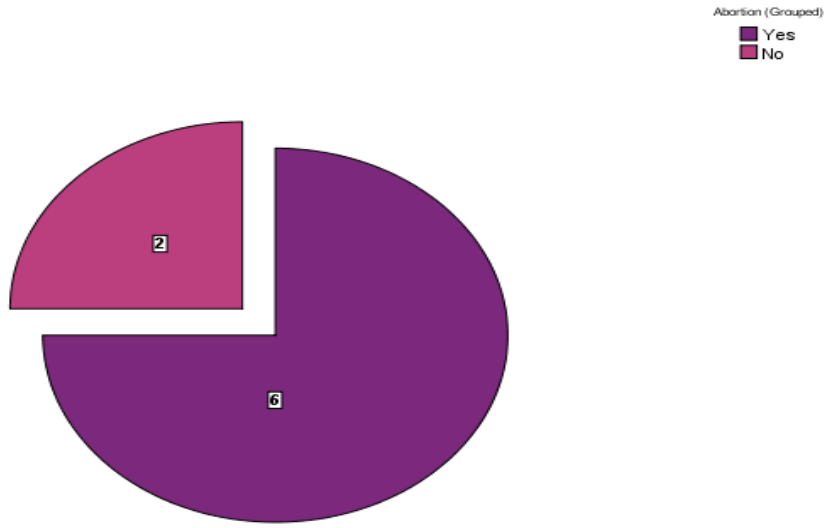

Figure 2. Frequency of abortion in positive cases.

showed positive allo-antibodies, where as 72 (90.0%) were negative.

Identification of antibodies showed anti-Kell 3(37.5%), anti-E 2(25.0%), anti-S1 (12.5%), anti-C^w 1(12.5%) and anti-Le^a 1(12.5%) as seen in (Table 4).

In blood transfusion, only 14 ladies (17.5%) were exposed to previous blood transfusion, and the number of units was ranging between one to two units while 66 women (82.5%) were not previously transfused.

Among the 8 pregnant females with positive antibody screening only 2 females (25.0%) were previously transfused, while 6 females (75.0%) were not exposed to blood transfusion as shown in (Figure 1).

Relationship between allo-immunization and history of

Table 6. Frequencies and percentages of number of abortions in positive cases

Number of abortions	Frequency	Percentage
1.00	1	16.7%
2.00	4	50.0%
3.00	3	33.3%
Total	8	100.0%

Table 8. Frequency of antibody screening according to abortion

	Antibody Screening		
Abortion	-ve	+ve	Total
No	47	2	49
Yes	25	6	31
Total	72	8	80

P value= 0.027 < 0.05 (significant relation).

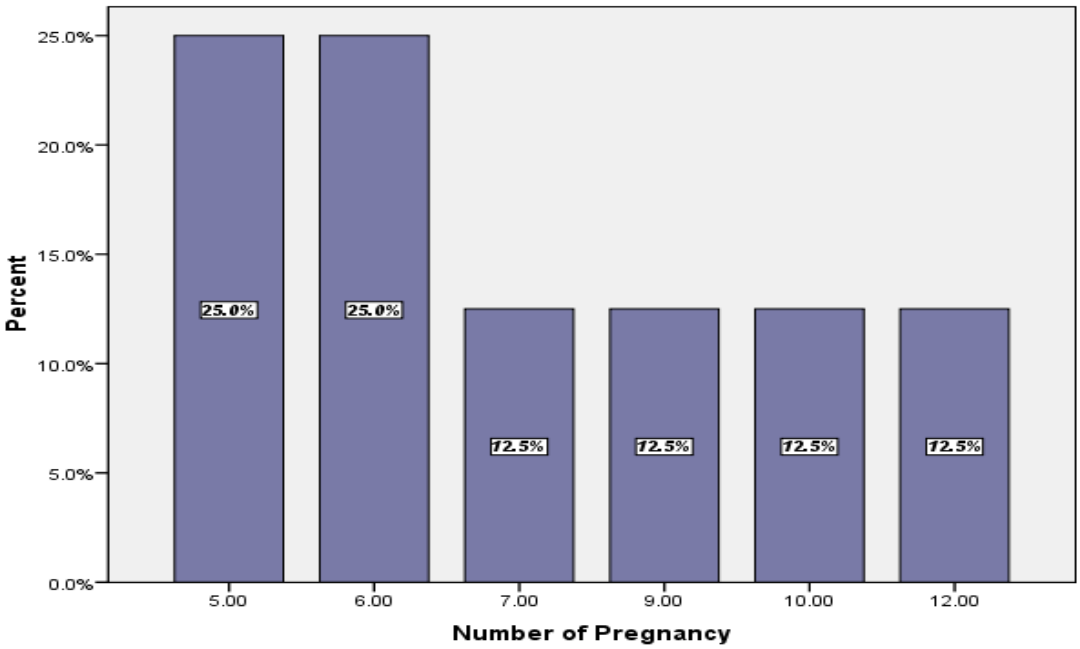

Figure 3. Percentages of number of pregnancies in positive case

transfusion was found to be insignificant (P value > 0.05). This means that, allo-antibodies detected among women were due to pregnancy and not blood transfusion (Table 5).

According to the history of abortion, 31 females (38.8%) with a history of abortion while 49 females (61.2%) with no history of abortion.

Among the eight positive cases, females with a history of abortion represent (75%), while others with no history represent (25.0%) seen in (Figure 2). Those with a history of abortion once were (16.7%), with abortion twice (50.0%), and those aborted three times were (33.3%) as seen in (Table 6). The relationship between abortion and allo-immunization was found to be significant (P value < 0.05). Among positive cases, females get pregnant for 5 times represent were (25.0%), females get pregnant for 6 times was (25.0%), females get pregnant for 7 times were (12.5%), females get pregnant for 9 times were (12.5%), females get pregnant for 10 times were (12.5%) and females get pregnant for 12 times were (12.5%) as seen in (Figure 3).

The relationship between number of pregnancies and allo-immunization was found to be significant (P value < 0.05).

Table 9. Frequency of antibody screening according to Number of pregnancies:

Number of pregnancies	Antibody Screening -ve	+ve	Total
1	6	0	6
2	14	0	14
3	12	0	12
4	16	0	16
5	15	2	17
6	4	2	6
7	3	1	4
9	1	1	2
10	0	1	1
12	1	1	2
Total	72	8	80

DISCUSSION

Antibody identification was performed for cases with positive antibody screening and the identification revealed that the most frequent antibody was anti-Kell and this is agreement with study of Pinon *et al*. (Pinon et al., 1981) who found anti-Kell was the most frequent antibody.

Gargouri *et al.,* detected that, the immunization of women according to the number of gestation shows an increasing rate ranging from 0.27%, for one gestation to 7.44% for 4 gestations or more. These findings agreed with the current results which revealed that significant relationship between number of pregnancies and allo-immunization.

Howard *et al.,* [14] and Lee et al., [15] studied the distribution of clinically important red cell antibodies in pregnancy, th/ey concluded that anti-D remains the most common clinically important antibody in pregnancy, so only Rh-D negative women need antibody screening for red cell antibodies. Their findings do not match with this study; this may be because of the small number of Rh D negative cases in this study (11 cases).

CONCLUSION

This study concluded that the percentage of alla-immunzation was highest in relation to all percentages found in literature review, and the most common identified antibodies were anti-Kell. Allo-antibodies detected among the pregnant females were due to pregnancy and not blood transfusion. There was a relationship between abortion and allo-immunization and Increase number of pregnancies gives a chance for allo-immunization to occur.

The results of the present study recommended that antibody screening should be performed to all pregnant women.

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