Prevalence of Anti-Rh (D) Antibody in Karbala

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Background: Neither Rh(D) positive individuals nor Rh (D) negative individuals normally have anti-Rh(D) antibody in their blood; however, Rh (D) negative individuals have the ability to form such antibody when they are transfused with Rh(D) positive blood.

Objective: To determine the prevalence of anti-Rh (D) antibody in Karbala.

Patients and methods: From January 2012 to March 2013, 226 patients (127 males and 99 females with age range of 20-49 years) were studied after their consent. For each patient, 4 ml of venous blood sample was obtained and investigated as follows:

Two ml of blood were transferred to EDTA tube for ABO and Rh(D) grouping using the commercially available kit (monoclonal anti-A, anti-B and anti-D) by doing the ordinary tile method (equal volumes of blood and reagent were mixed on tile looking visually for the agglutination for positive reaction and positive result. Absence of agglutination means negative reaction and negative result). The other 2 ml of blood were transferred to plain tube, centrifuged and serum is investigated for anti-Rh(D) antibody in Rh(D) negative individuals by double dilutions of each serum and antibody titration method.

Results: Out of 226 individuals, 186 (82.3%) were Rh(D) positive, 40 (17.7%) were Rh(D) negative, and 2 were found to be positive for anti-Rh(D) antibody. The prevalence of anti-Rh(D) antibody is 0.88%.

Conclusion: The study showed that the prevalence of anti-Rh(D) antibody in Karbala is 0.88%.

Key words: anti-Rh(D) antibody, ABO blood grouping, antibody titration.

الملخص

الخلفية:في الحالات الطبيعية لا يتواجد الجسم المضاد للريسس نوع دي في الدم بغض النظر عن فصيلة الدم (ريسس دي موجبة او سالبة) مع ذلك فان الاشخاص الذين يحملون فصيلة ريسس دي سالبة لهم القدرة على تكوين هذا الجسم في الدم عندما يتم نقل دم نوع ريسس دي موجبة لهم.

الهدف : لمعرفة نسبة وجود الجسم المضاد للريسس نوع دي في الدم.

الطرق: تمت دراسة 226 شخصا (99 من الاناث؛ 127 من الذكور) للفترة ما بين كانون الثاني سنة2012 لغاية آذار سنة 2013 بعد اخذ الموافقة منهم. تم سحب 4 ملليليتر من الدم الوريدي من كل شخص وتم اجراء الفحوصات المختبرية الخاصة بفصيلة الدم باضافة 2 مل من الدم الى انبوبة الاختبار نوع اي دي تي اي لمنع التخثر (اي بي او وكذلك ريسس نوع دي باستخدام الطريقةالشائعة ومشاهدة التفاعل الحاصل بين الكميات المتساوية من الدم والجسم المضاد في قطرة الاختبار بالعين المجردة.وجود تجمعات الخلايا يعني ايجابية التفاعل وايجابية النتيجة والعكس بالعكس) وتم وضع 2 مل من الدم في انبوبة اختبار بدون مادة التخثر ليتم فصل مصل الدم واجراء اختبار لوجود الجسم المضاد للريسس نوع دي في الفصائل التي تكون من نوع ريسس دي سالبة.

النتائج: تبين ان 186 (82.3%) من مجموع 226 شخصا يحملون فصيلة الدم ريسس دي الموجبة و 40 (17.7%) يحملون فصيلة الدم السالبة ويحمل 2 (0.88%) شخصا للجسم المضاد للريسس دي.

الاستنتاج: نسبة وجود الجسم المضاد للريسس نوع دي في الدم في كربلاء هو 0.88%.

Introduction

Many systems of red cell surface antigens are detected; however, some of them are clinically important like ABO grouping system because of the presence of naturally occurring potent IgM antibodies against A and B antigens. On the other hand, Rh grouping system is very important clinically despite the absence of naturally occurring anti-D antibodies. Anti-D antibodies are usually formed when Rh(D) negative person is transfused with Rh(D) positive blood or

transplacental passage of few milliliters of blood from Rh(D)positive baby to Rh(D) negative pregnant woman after normal delivery or following abortion with future risk of hemolytic disease of newborn (HDN)[1]. Other systems of antigens include Lewis system, MNS system, Lutheran system, Kell system, Kidd system, Duffy system and others. Rh system antigens include C, D, E, c and e antigens. Rh(D) antigen is the most important clinically because it is immunogenic and the normal immune system can develop antibodies against it[2]. Other antibodies like anti-C antibodies are rare cause of HDN and very rarely reported in literature[3]. Individuals who develop weak reaction to anti-D during Rh(D) grouping should be regarded as Rh(D) negative to prevent future Rh(D) positive blood transfusion and development of anti-D antibodies[4]. The isolation of chemical characterization and direct measurement of anti-D antigen of red cells has not yet been accomplished; however, relative differences in red cell D antigen reactivity have been serologically estimated by hemagglutination method[5]; however, autoradiography using ^{125}I-anti-D provides a mean of assessing the uniformity of D antigen content of individual red cells[6].

In clinical practice anti-D antibody has a variety of uses like prevention of sensitization of the immune system of the mother after normal delivery or abortion. It has been shown that 20 µg (100 iu) of anti-D immunoglobulin given intramuscularly will give complete protection from 1 ml of concentrated Rh(D) positive red cells (approximately 2 ml of blood). This figure is the basis for standard dose of 100 µg (500 iu) anti-D given postpartum in United Kingdom, as it will cover the vast majority of transplacental hemorrages. A dose of 3.2-4.0 mg of anti-D intravenously will protect the Rh(D) negative recipient against the consequences of transfusion of one unit of Rh(D) positive blood (200 ml of red cells)[4]. Another clinical use is intravenous anti-D therapy in immune thrombocytopenic purpura[7] and fetal intraperitoneal injection of immunoglobulins to treat alloimmune hemolysis[8].

Patients and methods

From January 2012 to March 2013, 226 patients (127 males and 99 females with age range of 20-49 years) who attend a private legal laboratory specialized for hematological investigations (Zaid Bin Ali medical laboratory/karbala city/Iraq) were studied after their consent. For each patient, 4 ml of venous blood sample was obtained and investigated as follows:

Two ml of blood were transferred to EDTA tube for ABO and Rh(D) grouping using the commercially available kit (monoclonal anti-A, anti-B and anti-D) by doing the ordinary tile method (equal volumes of blood and reagent were mixed on tile looking visually for the agglutination for positive reaction and positive result. Absence of agglutination means negative reaction and negative result). The other 2 ml of blood were transferred to plain tube, centrifuged and serum is investigated for anti-Rh(D) antibody in Rh(D) negative individuals according to recommended procedure for antiglobulin test[9] using the double dilution method of serum. A set of 12 glass tubes (75 X 10 mm) with numbering from 1 to 12. Two drops of serum for tube number 1 and 2 together with 2 drops of normal saline solution in tube number 2 to 12. Tube number one is undiluted and tubes from 2 to 12 are diluted serially by transferring 2 drops from tube number 2 after mixing to tube number 3, and so on. Blood group O Rh(D) positive red cell suspension is prepared (3% suspension) by four times vigorous injections (washing) and centrifugation of 0.5 ml of fresh O Rh(D) positive blood. After each centrifugation, the supernatant is discarded. Normal saline is added to the last concentrate to get 3% red cell suspension. One drop of red cell suspension is added to each tube starting

from tube number 1 to tube number 12. All tubes are mixed well and incubated at 37°c water bath for 45 minute. Tube number one is considered to be the neat (undiluted serum) while tube number 2 represents the 1:1 dilution, the third tube represents 1:2 dilution and serial dilutions end at tube number 12 which represents 1:1024 dilution. After incubation, four times washing is done for all tubes followed by adding 2 drops of antihuman globulin for each tube with thorough mixing and centrifugation at 500 g for 15 second followed by visual and microscopical detection of agglutination. Antibody titration was done for every anti-Rh(D) positive case.

Results

Results of ABO and Rh groups are shown in table 1 and 2, respectively. Out of 226 individuals, 186 (82.3%) were Rh(D) positive, 40 (17.7%) were Rh(D) negative, and 2 were found to be positive for anti-Rh(D) antibody (titers were 1:128 and 1:256) . The prevalence of anti-Rh(D) antibody is 0.88%. One of the two individuals who had anti-D antibody in a titer of 1:128 was thalassemic male patient with Rh(D) negative blood group and had been transfused with Rh(D) positive blood and the other one was female, Rh(D) negative blood group with anti-Rh (D) antibody titer of 1:256 and had previous abortion without suitable treatment or prevention of anti-D formation. Out of 226 individuals, 54 (23.89%) were group A, 58 (25.66%) were group B, 48 (21.24%) were group AB, and 66 (29.2%) were group O.

Table1. ABO blood groups.

ABO blood group	Number (%) out of 226
A	54 (23.89%)
B	58 (25.66%)
AB	48 (21.24%)
O	66 (29.2%)
Total	226 (100%)

Table 2. Rh (D) blood groups.

Rh(D) blood group	Number (%) out of 226
Rh (D) positive	186 (82.3%)
Rh (D) negative	40 (17.7%)
Total	226 (100%)

Discussion

The study showed that 54/226 (23.89%) had blood group A, 58/226 (25.66%) had blood group B, 48/226 (21.24%) had blood group AB, and 66/226 (29.2%) had blood group O. No similar studies were found regarding Karbala city to compare the results because such figures of blood groups are different in different countries and different cities. The study showed that 186/226 (82.3%) were Rh (D) positive while 40/226 (17.7%) were Rh(D) negative. Throughout the world the figure is well known to be 85% Rh(D) positive and 15% Rh (D) negative[1,2 &4]. The Rh system of antigens is a complex system and previous studies showed that the immunological reactivity of Rh (D) antigen depends on the different combination of such complex antigens, for example the agglutination of red cells of individuals who carry cDe/cDe gene is more than the agglutination of cells of individuals who carry CDe/CDe gene when cells react with anti-D antibody[10]. Similarly, ABO incompatible Rh system compatible red cell reactivity is less than ABO compatible Rh system compatible red cells when react with anti-D antibody [4&9]. A study showed that there is also a race difference in Rh (D) antigen reactivity as Rh (D) antigen reactivity is enhanced by the presence of E antigen in Nigro while Rh (D) antigen reactivity is significantly reduced by the presence of C antigen in Caucasian individuals[11].

It is possible that in utero exposure to anti-D antibody in cases of Rh(D)

incompatibility hightens the risk for structural brain changes which in turn increase the risk of developing schizophrenia as there is a preliminary evidence of significant volumetric differences between schizophrenia cases with or without maternal-fetal blood incompatibility[12]. Rh(D) antigenic sites are not only present on red cell surface, but the detection and quantification of such antigens on human leukocytes was present since more than 50 years[13].

Conclusion

The study showed that the prevalence of anti-Rh(D) antibody in Karbala is 0.88%. So that special attention is required during Rh (D) grouping together with careful management for each Rh (D) negative pregnant woman

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