

Red Cell Antibodies Against High Frequency Antigens, A Two Year Experience At A Regional Transfusion Institute

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ABSTRACT

Objective: To assess the clinicohaematological presentation and outcome of patients with antibodies to high frequency antigen (HFA) in our setup.

Study Design: Cross sectional study.

Place and Duration of Study: Armed Forces Institute of Transfusion, Rawalpindi, from Jan 2016 to Jan 2018.

Methodology: We diagnosed 29 cases of uncommon red cell antibodies including antibodies to HFA, in a span of two years (2016-2018). Information regarding patient's age, diagnosis, transfusion history, incompatible cross match and history of pregnancy was obtained. Venous blood sample of 3ml was taken in EDTA for grouping, red cell phenotyping and Direct Anti-Globulin Test (DAT) and 5 ml venous blood was taken for Indirect Antiglobulin Test (IAT), antibody screening and identification. Tube method was used for forward and reverse blood grouping, DAT and IAT. Column Agglutination Technique (CAT) was used for antibody screening, identification and red cell phenotyping.

Results: Out of a total of 29 cases, 24 (83%) were of anti H antibody (Bombay blood group). Rest of 5 (17%) included one each of anti Rh 17, anti Rh 29 (Rh null), anti P1Pk, anti-ENA/WRB and anti P antibodies. Anti H Lectin (Ulexeuropaeus) was used for confirmation of H antigen absence, while rest of antibodies were confirmed by IBGRL Bristol, UK.

Conclusion: The sources of antigen negative blood are family members, rare donor registry, autologous donations and frozen blood banks. Finding compatible blood for patients with an antibody to HFA may be a challenge.

Keywords: Alloantibodies, Antibodies to high frequency antigens, Bombay blood group, Rh blood group.

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INTRODUCTION

The importance of red cell transfusions in diverse clinical situations cannot be overemphasized. Provision of safe compatible blood is preliminary requirement for the transfusion services. The clinical significance of blood group antigens expressed on red cell membrane is proportional to the risk of Hemolytic Transfusion Reactions (HTR) and Hemolytic Disease of Fetus and Newborn (HDFN). Therefore, it is imperative to screen the prospective red cell recipient as a part of pre transfusion testing. In case the clinically significant red cell alloantibody is detected, the next important step is the antigen negative red cell selection, for transfusion, to the corresponding antibody. The chances of availability of antigen negative red cells poses real dilemma in case of high frequency antigens (HFA). HFA by definition are those with the incidence of more than 90% but majority of these have more than 99% of incidence. More than 190 antigens are classified as HFA by

International Society of Blood Transfusion (ISBT). To diagnose the antibodies to HFA, the detailed knowledge of routine phenotypes is necessary. The mode and strength of reactivity with different red cell panels, is also of utmost importance. Due to lack of resources and the experience due to infrequent presentations, the routine transfusion laboratories cannot diagnose the antibodies against HFA and the help of reference labs have to be sought. The next step, after identification of one of these antibodies against HFA, is the huge task of finding the compatible red cells. In some of these instances, the help of rare group inventory is taken, in few others family donors have to be sought and quite rarely the appropriate antigen negative blood cannot be provided. With this background, we conducted this study to determine the frequency of red cell antibodies to high frequency antigens at a regional transfusion institute.

METHODOLOGY

This study was carried out in the department of Immunohaematology, Armed Forces Institute of Transfusion (AFIT), Rawalpindi Pakistan from January

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2016 to January 2018. This cross sectional study was conducted after the approval from the Institutional Ethics Committee vide letter number, Cons-HEM-4/READ-IRB/18/1448. Informed consent from all patients was taken.

Inclusion Criteria: Patients of any age and gender having ABO discrepancy and cross-match incompatibility were included in the study.

Exclusion Criteria: Patients having autoimmune haemolytic anaemia with positive auto control were excluded from the study.

Twenty-nine cases of antibodies to HFA were studied in the span of two years. Information regarding patient's age, diagnosis, transfusion history, incompatible cross match and pregnancy was obtained. Venous blood sample of 3ml was taken in EDTA tubes for grouping, red cell phenotyping and Direct Antiglobulin Test (DAT) and 5ml venous blood was taken for Indirect Antiglobulin Test (IAT), red cell antibody screening and identification. Tube technique was used for grouping, DAT, IAT, antibody screening and identification, while Column Agglutination Testing (CAT) was used for red cell phenotyping. Samples were collected by consecutive sampling technique.

Data were analyzed using SPSS-22. Numerical variables; age and blood group were presented as mean \pm SD. Categorical variables; gender, type of antibody were presented as frequency and percentages.

RESULTS

Total 29 cases of red cell antibodies to HFA were diagnosed during the study period. There were 15 (52%) males and 14 (48%) females. The age of patients ranged from 3 years to 62 years with the mean age of 30.0 ± 12 years. Out of these cases, 24 (83%) cases were of anti H antibody (Bombay blood group). Other five (17%) cases included, one each of anti Rh 17, anti Rh 29 (Rh null), anti PP1Pk, anti Ena/Wrb, anti P antibodies. Bombay group was confirmed by anti H Lectin (*Ulex europaeus*) and rest of antibodies to HFA were confirmed by The International Blood Group Reference Laboratory (IBGRL), Bristol, UK.

The challenge posed by red cell antibodies to HFA was double fold, first the difficulty in the determination of their specificity and secondly to find the compatible red cells. Our study revealed that the most common antibody to HFA was anti H antibody (Bombay group) with five other antibodies.

Out of 29, twenty-four patients were of Bombay group. These patients were referred to AFIT with

history of ABO discrepancies and cross match incompatibilities. Bombay group was finally confirmed by anti H Lectin. Family screening of these patients revealed that most of the family members have Bombay phenotype. Among the Bombay group, age ranged from 3-52 years, with a mean age of 30 ± 11 years. Twelve patients were males and 12 were females. Five (21%) patients of Bombay group were RhD negative while nineteen (79%) were RhD positive.

Single case of anti Rh 29 antibody was identified in a 28 year old pregnant female, presented with anaemia in pregnancy. She developed acute haemolytic transfusion reaction with renal failure and IUD after transfusion of RCC. The serological tests revealed that there was an IgG alloantibody, directed against some high frequency (public) red cell antigen. Red cell phenotype revealed the absence of D, C, c, E and e antigens (Table-I). Based on these findings anti Rh 29 antibody reactive at 37°C was suspected which was confirmed by IBGRL, Bristol.

Anti Rh 17 antibody was diagnosed in a 32 year old female who presented with history of progressive pallor, shortness of breath and lethargy of one-month duration. Her haemoglobin was 7g/dl. Request of two units RCC was received at our transfusion center. Serological tests revealed an IgG type alloantibody against some high frequency (public) red cell antigen. Red cell phenotyping revealed absence of C, c, E and e antigens (Table-I). Therefore, antibody suspected was anti Rh 17 reactive at 37°C, confirmed by IBGRL, Bristol.

Anti PP1Pk antibody was identified in a 62 year old male who presented with history of fever with rigors and chills and cervical lymphadenitis for one month. Biopsy of right cervical lymph node revealed metastatic squamous cell carcinoma. Request for issue of one unit RCC for transfusion was received. There was a pan reactive antibody. To find out the panreactive antibody, samples were sent to IBGRL, Bristol. They found the patient's cells to have a rare pp phenotype and his serum contained strongly hemolytic anti PP1Pk antibodies reacting at all temperatures and by all techniques including LISS IAT. Several examples of pp cells were compatible and no additional antibodies were detected. P1k cells were incompatible. However, the presence of anti K antibody could not be excluded (Table-II).

Anti Ena/Wrb antibody was identified in a male of 40 years being planned for cardiac bypass surgery, he was advised to arrange four units of RCC before

High Frequency Antigen

undergoing surgery. His work up at transfusion center was summarized in Table-III.

Further work up at IBGRL, Bristol revealed the presence of auto anti Ena/Wrb antibody in patient samples. The anti Ena/Wrb was found to be reacting strongly by LISS IAT and weekly at 18°C by direct agglutination with untreated cells. The auto control was also weak positive. However, the strength of reactivity varied between the use of plasma and serum. The antibody by IAT was much weaker when anti human IgG was used rather than polyspecific antihuman globulin. The patient's cells were found to have a consistently negative DAT. En (a-) rare cells were found to be compatible when tested untreated by LISS IAT and 18°C direct agglutination. All the papain treated cells tested,

lack of extremely rare Ena+/Wr (a+b-) cells that would be required to do so.

Our last case was anti P antibody identified in a 15 year old male referred to our transfusion center with history of cross match incompatibility at peripheral hospital blood bank. His work up was summarized in Table-IV. A panreactive antibody, reactive at room temperature and at 37°C by IAT was present.

To find out the specificity of panreactive antibody, clotted and EDTA samples were sent to IBGRL, Bristol, where it was found that the patient's red cells have rare P1K phenotype and his plasma contains anti P strongly reacting antibody by all techniques, including direct agglutination techniques at 18°C and 37°C, IAT and papain.

Table-I: Serological tests for Anti Rh 29 Antibody, Anti Rh 17 Antibody.

	Blood Group	Cross Match	Auto Control	DAT	Antibody Screening	Antibody Identification	Red Cell Phenotype
Anti Rh 29 Antibody	A Rh D Negative	Incompatible with all available RCC	Negative	Negative	Pan reactive	Pan reactive	D-C-c-E-e- (Rh Null) K-k+
Anti Rh 17 Antibody	B Rh D Positive	Incompatible with all available RCC	Negative	Negative	Pan reactive	Pan reactive	D+C-c-E-e- (Rh 17) K-k+

Table-II: Serological tests for Anti PP1Pk Antibody.

Blood Group	Cross Match	Auto Control	DAT	Antibody Screening	Antibody Identification	Red Cell Phenotype
B Rh D Positive	Incompatible with all available RCC	Negative	Negative	Panreactive	Panreactive	D+C+c-E-e+ K-k+

Table-III: Serological tests for Anti Ena /Wrb Antibody.

Blood Group	Crossmatch	Auto Control	DAT	Antibody Screening	Antibody Identification	Red Cell Phenotype
A Rh D Positive	Incompatible with all available RCC	Weak Positive	Negative	Panreactive	Panreactive	D+C+c+E- e+ K-k+

Table-IV: Serological tests for Anti P Antibody.

Blood Group	Crossmatch	Auto Control	DAT	Antibody Screening	Antibody Identification	Red Cell Phenotype
O Rh D Positive	Incompatible with all available RCC	Negative	Negative	Panreactive	Panreactive	D+C+c+E-e+ K-k+

Table-V: Antibodies to high frequency antigens, comparison with international studies.

Authors	Year	Place of Study	Total patients with antibodies	Antibodies To ABO Group	Antibodies to Rh Group	Other Antibodies	Other Antibodies
AFIT Pakistan (2013-2015)	2016-2018	AFIT Rawalpindi	29	Anti H Antibody 24 (83%)	Anti Rh 17, Anti Rh 29, 2 (7%)	Anti PP1 Pk, Anti P, 2 (7%)	Anti Era/Wrb 1 (3%)
Seltsam <i>et al.</i> (2003)	2003	Germany	52	Anti Kp b	Anti Vel	Anti Leuthran b	Anti Yta
Sanmukh <i>et al.</i> (2012)	2012	India	175	Anti H Antibody	Anti Rh 17	Anti colton	-

including rare cells and the patient's own cells were found to be incompatible with patient serum and plasma. All the antibodies were completely removed from patient plasma by auto adsorption. The further specificity of auto anti Ena/Wrb cannot be defined due to

Two examples of PK cells were compatible with patients plasma and three examples of pp phenotype cells were weakly incompatible (reacting preferentially with papain treated cells). The weak reactivity observed with pp phenotype cells is presumed to be due to

the presence of anti-PX2 in the patient's plasma. The PK cells found to be compatible with patients plasma, enabled the exclusion of anti- E, however the presence of anti-K could not be excluded.

DISCUSSION

In transfusion, a rare blood is the one, which lacks a high-frequency antigen and such blood donations help in transfusion to those recipients having alloantibodies to corresponding antigens.¹ A rare blood is the one that is found in a frequency of 1:1000 random samples in a given population.² Transfusion support was unsatisfactory in about one-third of the hospitalized patients with antibodies to high-frequency antigens.³

Our study revealed that the most common antibody to HFA was anti H antibody (Bombay group) with five other antibodies.

We have compared our data of antibodies to high frequency antigens with two international studies shown in Table-V. Our data were similar to that of India 1 with most frequent antibody is of ABO group (anti H antibody) & Rh group (anti Rh 17), however other antibodies are less common. In Indian study, they detected 10 cases of Bombay group, every year at their reference center. This number is in comparison with our study as we also received 24 cases of Bombay phenotype in approximate 2 years. Our data were different from German study 3 as they had different antibodies to high frequency antigens.

Transfusing blood group O red cells to Bombay blood group can cause a fatal hemolytic transfusion reaction. This highlights the importance of both forward and reverse typing in ABO blood grouping.⁴ Individuals with extremely rare Bombay phenotype (hh genotype) fail to express H transferase. The ABH antigens are absent from their red cells, regardless of their ABO blood group genotype. Their plasma contains anti-A, anti-B, and strong anti-H that can be hemolytic and is reactive with all blood types except the Bombay phenotype.⁵

Anti Rh 29 antibody (Rhnull phenotype) is a rare blood group characterized by the lack of expression of all Rh antigens (D, C, c, E and e) on the red cells.⁶ This condition was first described in 1961 by Vos and his colleagues.⁷ Then the term "Rhnull" was discovered.⁸ The clinical significance of its recognition is that such patients suffer from Rhnull syndrome associated with stomatocytes and varying degree of chronic haemolytic

anaemia.⁹ The first case of Rh Null phenotype was described in Pakistan in 2010.¹⁰

Anti Rh17 is also a rare alloantibody produced after immune stimulus, by individuals who lack C/c and E/e antigens of Rh blood group on their red cells. This rare blood group is designated as D,¹¹ and was first described by Race and Sanger in 1950. The clinical relevance of the -D- phenotype has been predominantly described in pregnant women, causing mild to fatal hemolytic disease of the newborn. Salamat *et al*, reported a case of anti Rh 17 (anti-Hr0) in 2004.¹²

Anti-PP1Pk is composed of a mixture of anti-P, anti-P1 and antiPk antibodies in the serum of p individuals. These are naturally occurring antibodies and mostly of IgM subtype.^{13,14} These antibodies can also cause hemolytic transfusion reactions and hemolytic disease of fetus and newborn, if antibody is of IgG type. There is a relationship between anti-PP1Pk and early spontaneous abortion (the placenta is rich in Pk and P antigens which are targeted by IgG isotype antibodies).¹⁵

Subjects who lack Glycophorin A (En a-), may make a spectrum of antibodies "anti En a", which are directed against different portions of Glycophorin A molecule. "Anti En a" has only once been found as naturally occurring antibody, it is also found following pregnancy and transfusion. Auto Ena antibody is found in certain patients with warm type of autoimmune hemolytic anaemia as in our case. In the previous literature, there was a case report of AIHA due to anti Ena, which was treated successfully.¹⁶

Anti Wr b was first described in a Wr (a+ b-) subjects. The antibody has been found as a separable specificity, together with "anti Ena", in the serum of En (a-) subjects who were also Wr (a-b-). Anti Wr b occurs commonly as auto antibody in autoimmune haemolytic anaemia.¹⁷

Allo anti-P is seen in the sera of P1k and P2k individuals. It is a naturally occurring antibody and is predominantly of IgM isotype (but may also be a mixture of IgM and IgG).¹⁸ This antibody is capable of causing hemolytic transfusion reactions and hemolytic disease of the fetus and newborn (if IgG isotype, therefore can cross the placenta).

Red cell antibodies to high frequency antigens occur less frequently but many of these antibodies, as in our study, have the potential of causing haemolytic transfusion reactions and haemolytic disease of fetus and newborn. The arrangement of RCC in these cases

poses real problem. Limited options may include the family donors, rare blood groups registry or frozen red cell concentrates, if available. Autologous transfusions can also be considered in planned surgeries.

CONCLUSION

The sources of antigen negative blood are family members, rare donor registry, autologous donations and frozen blood banks. Finding compatible blood for patients with an antibody to HFA may be a challenge.

Conflict of Interest: None.

Authors' Contribution

AK: Direct contribution to the conception, design, analysis & data interpretation, MA: Intellectual contribution to analysis & data interpretation, AM: Intellectual contribution to analysis & data interpretation, AK: Manuscript Preparation, NK: Literature Review, SY: Data Collection.

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