Original Article

# Alloimmunization in Transfusion Dependent Thalassaemic Patients.

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***Summary:***

**Background:** Life-long red blood cells (RBCs) transfusion remains the main treatment for severe cases of thalassaemia. The development of anti-RBC antibodies (alloantibodies and for autoantibodies) can significantly complicate transfusion therapy. Some alloantibodies are hemolytic and may cause, though not invariably, hemolytic transfusion reactions and limit the availability of further safe transfusion. Erythrocyte autoantibodies appear less frequently in blood cross match.

**Patients and methods:** This is a descriptive study ducted at Al-Karama Thalassaemia Center in Baghdad .The sampling was done from September 2005 to April 2006 and all patients were diagnosed as Thalassaemia Major according to the hemoglobin electrophoresis results were included in the study (60 patients). Antibodies identification was carried out on serum employing commercial two cell panel, using standardized blood bank methods. If the patients were found to have irregular red cell alloantibodies, then the antibodies identification was performed by indirect coombs test using 18 panel cells.

**Results:** Sixty thalassaemic patients were included in the study, 35 patients were males and 25 females. The age of patients ranged from 18 months to 33 years (median 25.2 7). Irregular red cell antibodies were found in 9 patients (15%). Mean age of patients who developed red cell antibodies was 25.2±7.0 years. Two patients developed autoantibodies (3.3%) and seven patients developed alloantibodies (11.7%).Six patients developed single antibodies (10%) while 3 patients developed multiple antibodies (5.0%). Total anti-k was found in 4 patients (6.7%), two patients had anti-k 1 and two patients had anti-k2. The higher rate of alloimmunization was in the rhesus Rh system, which was detected in (8.3%) 5 patients (one patient developed anti-D, one patient developed anti-c and 3 patients developed total anti-e). while total anti-M presented in 3 patients(5.09%)while one patient developed anti-Lea (1.7%).

**Conclusion:** We concluded that there is a relatively high rate of alloimmunization in our set of patients when compared to data from Iraq geographic region. However, more data required from various other large centers in Iraq. It is recommended that red cell alloimmunization should not be overlooked in patients with B- thalassaemia major receiving regular blood transfusion. Those patients with Thalassaemia Major repeatedly suffer from hemolytic transfusion reaction or not being able to maintain hemoglobin at desired level in spite of regular transfusion due to the presence of irregular alloantibodies in their circulation. Proper blood cross matching, regular screening, detection & identification of the red blood cell alloantibodies would add towards the better management of these patients & reduce the chance of development of these irregular antibodies & other possible additional alloantibodies.

**Key words:** Alloimmunization, Transfusion, Thalassaemia.

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Introduction:

Thalassaemias are the commonest monogenic disease in man. ?-thalassaemia is a common haemoglobinopathy in Iraq. Thalassaemias is a heterogeneous group of hereditary disorders Characterized by heterogeneous genetic deficiency in the synthesis of globin chain.

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The homozygous state (B- thalassaemia major) causes severe transfusion dependent anemia. (1, 2). Appropriate and regular red cell transfusion remains the main treatment choice for a large number of patients with severe B-thalassaemia major .Alloimmunization will develop in all patients who receive frequent blood transfusion even if they were not on hyperactive transfusion regimen.

As blood is routinely matched with respect to major-blood group antigens i.e. ABO and Rh D antigen, there is high probability that the donor will have minor blood group antigens not present in the recipients, which will result in

alloimmunization. This study is to detect the frequency of RBC alloimmunization and autoimmunization in the transfusion dependent Iraqi thalassaemic patients. (3, 4, 5).

**Patients and Methods:**

This study conducted from September 2005 to April 2006.A total of 60 thalassaemic patients who were on regular blood transfusions every 2-4 weeks at the center of thalassaemia in Al-Karama teaching hospital were included in this study . The age of patients ranged from 18 months to 33 years (median 25.27).Those patients were diagnose based on complete blood picture and hemoglobin electrophoresis. The phenotyping and antibodies identification for those patients were carried out at the National Blood Transfusion Center in Baghdad / Iraq. Clinical and transfusion information were collected by questionnaire and from the patient's case sheets. Those patients were on hypertransfusion regimen receiving packed cells cross -matched for ABO and Rh antigen. Patients with any other haemoglobinopathy or hematological disorder receiving multiple transfusions were excluded from this study. Blood sampling: Venous blood was collected from patients & control groups for RBCs grouping & phenotyping; serum for antibodies screening, detection & identification purposes. A 5-10 ml of blood was taken in sterile plain plastic tube without anticoagulant as EDTA. The blood samples were centrifuged, serum and red blood cells were provided. First: The serum of the patients were used for screening, detection & identification of irregular (alloantibodies) with the use of the already prepared panel of cells to give antigen negative blood to the irregular antibodies that presented in the patients serum . Second: The patients RBCs suspensions preparation shared in proper RBCs phenotypic determination (e.g. for ABO system antigens but not for irregular antibodies detection which were presented in the serum of the patients) as a baseline data which were essential for preliminary selection of proper blood units to be transfused for the candidate B- thalassaemia major patients. The patient's serum & RBCs suspensions were prepared by the following procedure & stored for 5-6 days at 4C°. Cell washing & making red cell suspensions was done using a method with the following steps (6):

1. High speed centrifugation of the blood sample collected in a plastic plain tube without anticoagulant were done in a ordinary bench top laboratory centrifuge at 1200-1500 g force (g = Relative centrifugal force) for 5 minutes, so the serum was separated from RBCs. Serum was removed to a clean plastic tube (12 x 75 mm) with a pasteur pipette for antibodies detection & identification.
2. With a Pasteur pipette, 0.2-0.5ml of packed red blood cell was placed in a glass (kahn) tube.
3. Saline was added to a level of 1 cm from the top of the tube.
4. Another high-speed centrifugation at 1200 - 1500 g force (g = Relative centrifugal force) for 5 minutes in a centrifuge with a proper rotor adapted for khan tubes.
5. Normal saline (0.9%) was drawn with a pasteur pipette.
6. The kahn tube was placed in the centrifuge to resuspend the RBCs cells, this constitute the first wash.
7. Step 3-6 is repeated at least twice, the last wash should always have clear saline left Around 3-5% of cell suspension was prepared by addition of 30 volumes saline to 1 volume RBCs for the same purpose mentioned above.

Antibody screening: The antiglobulin methods can detect almost all clinically significant antibodies; it is acceptable to use an Indirect Antiglobulin Test (IAT) for pretransfusion antibody screening. The procedure of an antibody - screening test consists of the following steps (6):

1. Prepared panel of RBCs of a known specific phenotypes (See table No.3) were added to12 X 75 mm glass (kahn) tube),equivalent in amount to the number in 2 drops of a 3-5% suspension of RBCs.
2. Two drops of bovine albumin and 2 drops of patient's serum were added to the same glass tube mentioned in the step 1 above , spin it to facilitate & accelerate the antigen - antibody reaction and read the reaction (i.e.presence of RBCs agglutination) under microscope immediately (immediate spin reading).
3. After that, the glass tube at 37C°was incubated for 30 minutes & then centrifugated and the reaction (i.e. RBCs agglutination) was read under microscope.
4. Thereafter, RBCs were washed three to four times with 0.9% normal saline.
5. Then 2 drops of poly specific anti human globulin (coombs reagent) were added to the glass tube, centrifuged and the reaction was read under microscope.
6. Coombs control (antibody - coated red cells) was used if step 5 is negative & the reaction was read under microscope (7,8and9).

Antibody Identification: A positive result in the antibody screen should be followed by antibody identification.

The serum or plasma under investigation is normally tested against a panel of nine group O red cell samples of known antigen composition. The specificity of an antibody can only be assigned when it is reactive with at least two examples of reagent red cells carrying the antigen and non-reactive with at least two examples of red cells lacking the antigen. Additional antibodies lacking antigen may be present in a serum and it is essential that the presence of additional clinically significant antibodies be not overlooked. This can only be achieved by testing the serum against additional red blood cell samples negative for the apparent specificity but positive for other antigens to which clinically significant antibodies may arise. A positive direct antiglobulin test (DAT) will invalidate test results (7, 8 and 9).

Control group (panel): Sixty six healthy randomly selected volunteers with 14 having blood group A;12 having blood group B;3 having blood groupAB&37 having blood group O (See table 2).The identification & distribution of ABO blood groups were done for patients &control groups. The blood samples &their processing were described above in details.

**Statistical analysis:** Statistical analysis had been made using the available statistical package of SPSS-11.5 (Statistical packages for social science- version11.5).Data were presented in simple measures of frequencies ,percentages, mean, standard deviation & range (minimum-maximum values).Independent student- t- test was used for testing the significance of difference between two means(Thalassaemic Vs. Control & Thalassaemic immunized Vs. non-immunized) & the Chi-square test ($X^2$) was used for testing the significance if association (Between qualitative data) for the difference in percentage among two groups. Statistical significance was considered whenever the P value was equal or less than 0.05.

**Results:**

Patients group: Sixty patients with B-thalassaemia major were included in this study .A 35 (58.3%) were males and 25 (41.7%) were females. The age of patients ranged from 18 months to 33 years (median 25.2 7). Amongst them, Arabian formed 59 (98.3%) & muslim 59 (98.3%). Demographic features of those patients were tabulated (See table No. 1). The overall rate of alloimmunization in patients with B-thalassaemia major on blood transfusion regimen in this study (i.e. not to compare between the immunized&non-immunized patient with B-thalassaemia major) was demonstrated (Seven out of sixty) (See table1), while the idea of comparison between the immunized & non immunized thalassaemic patients will be the issue of our new project of a bigger & more representative thalassaemic patient's sample in Iraq is our project. The ABO blood grouping & phenotyping of (66) patients with B-thalassaemia major & of (37) control group who were included in this study were tabulated. (See table No.2 (A)). Positive RBCs phenotypic distribution (Other than ABO system) in control groups & in blood group O controls (Panel) were included. This phenotypic distribution was the keystone as they represented the prepared blood.

**Table No. (1): The demographic features of patients included in this study individuals in total thalassaemic and those positive alloimmunized patients:**

| Phenotypic results | Thalassaemic (n=60) | | Alloimmunized patients (n=7) | | P value |
|---|---|---|---|---|---|
| | No | % | No | % | |
| Age <3 years | 8 | 13.3 | 2 | 28.6 | 0.235 |
| 3--20 years | 15 | 25.0 | 3 | 42.8 | |
| >20 years | 37 | 61.7 | 2 | 28.6 | |
| Mean+SD (range) | 25.2+7.0 (3-31 years) | | | | |
| Sex Male | 35 | 58.3 | 3 | 42.8 | 0.434 |
| Female | 25 | 41.7 | 4 | 57.2 | |
| M:F ratio | 1.4:1 | | 0.8:1 | | |
| Religion Muslim | 59 | 98.3 | 7 | 100.0 | - |
| Christian | 1 | 1.7 | - | - | |
| Ethnicity Arab | 59 | 98.3 | 7 | 100.0 | - |
| Kurdish | 1 | 1.7 | - | - | |

**Table No. (2): RBCs phenotyping thalassaemic patients group & in control group:**

A. ABO blood group distribution in thalassaemic and control groups*.

| Blood group | Thalassaemic(n=60) | | Control (n=66) | |
|---|---|---|---|---|
| | No | % | No | % |
| A | 27 | 45.0 | 14 | 21.2 |
| B | 9 | 15.0 | 12 | 18.2 |
| AB | 4 | | 3 | 4.5 |
| O | 20 | 33.3 | 37 | 56.1 |
| Total | 60 | 100% | 66 | 100% |

* $x^2$=9.80, d.f = 3, P = 0.023* (Significant)

B: RBCs phenotypic distribution (Other than ABO system) in control groups and in Blood group O controls (Panel).

| Phenotypic results | Control(n=66) | % | Control O blood group(Panel) (n=37) | | P value |
|---|---|---|---|---|---|
| | No | | No | % | |
| D | 59 | 89.4 | 33 | 89.2 | 0.974 |
| E | 19 | 28.8 | 10 | 27.0 | 0.849 |
| E | 60 | 90.9 | 33 | 89.2 | 0.777 |
| C | 51 | 77.3 | 29 | 78.4 | 0.897 |
| C | 47 | 71.2 | 25 | 67.6 | 0.632 |
| $Jk^a$ | 17 | 25.8 | 2 | 5.4 | 0.011 |
| $Jk^b$ | 14 | 21.2 | 4 | 10.8 | 0.182 |
| P | 20 | 30.3 | 10 | 27.0 | 0.726 |
| $Le^a$ | 12 | 18.2 | 5 | 13.5 | 0.540 |
| $Le^b$ | 40 | 60.6 | 26 | 70.3 | 0.327 |
| M | 59 | 89.4 | 34 | 91.9 | 0.681 |
| N | 36 | 54.5 | 21 | 56.8 | 0.962 |
| $S_3$ | 36 | 54.5 | 21 | 56.8 | 0.829 |
| $S_4$ | 36 | 54.5 | 20 | 54.1 | 0.962 |
| $K_1$ | 5 | 7.6 | 4 | 10.8 | 0.577 |
| $K_2$ | 44 | 66.7 | 31 | 83.8 | **0.061** |
| $Fy^a$ | 28 | 42.4 | 10 | 27.0 | 0.120 |
| $Fy^b$ | 23 | 34.8 | 12 | 32.4 | 0.804 |
| $Lu^a$ | 2 | 3.0 | 2 | 5.4 | 0.549 |
| $Lu^b$ | 37 | 56.1 | 15 | 40.5 | 0.131 |

Group O RBCs panel which was derived from the total control group how had no antibodies (neither alloantibodies nor autoantibodies). It was the essential RBCs panel for detection & identification of irregular (alloantibodies) antibodies in the serum of patients with B-thalassaemia major that were included in this study. (See table No. 2 (B))

Forty- five of those patients started their blood transfusion before age of 3 years. Thirty-eight patients were transfused with filtered blood & the remaining 22 without filter as in table no. (3) Below. There was statistical significance between the thalassaemic patients group & those who were alloimmunized thalassaemic patients group in this study in relation to the time commencement of blood transfusion therapy before the age of 3 years with a P value ?0.05 (P = 0.011) , but no statistical significance was demonstrated between the two groups mentioned above in relation to

frequency of blood transfusion therapy & to the usage of filtered blood therapy with P values of ?0.05.Splenectomized patients had a higher immunization rate (85.7%) in this study &of statistical significance (P value = 0.027).(See table No.3).

**Table No. (3): Distribution of individuals in total thalassaemic and those positive alloimmunized patients:**

| Parameter | Thalassaemic (n = 60) | | Alloimmunized patients(n=7) | | P value |
|---|---|---|---|---|---|
| | No | % | No | % | |
| Exam Splenectomy | 25 | 41.7 | 6 | 85.7 | 0.027* |
| Splenomegaly | 35 | 58.3 | 1 | 14.3 | |
| Hepatomegaly | 15 | 25.0 | 3 | 42.6 | 0.313 |
| Filtered blood Yes | 38 | 63.3 | 6 | 85.7 | 0.238 |
| No | 22 | 36.7 | 1 | 14.3 | |
| Blood transfusion every : 1 W | 4 | 6.7 | - | - | 0.818 |
| 2 W | 33 | 55.0 | 5 | 71.4 | |
| 3 W | 15 | 25.0 | 1 | 14.3 | |
| 4 W | 8 | 13.3 | 1 | 14.3 | |
| Blood group A | 27 | 45.0 | 2 | 28.6 | 0.404 |
| B | 9 | 15.0 | - | - | |
| AB | 4 | 6.7 | 1 | 14.3 | |
| O | 20 | 3.3 | 4 | 57.1 | |
| Patients started on their blood transfusion Before 3 years of age | 45 | 75.0 | 2 | 28.6 | 0.011* |
| After 3 years of age | 15 | 25.0 | 5 | 71.4 | |

The RBCs alloantibodies were detected in the sera of 9 patients out of 60 with the following results: Alloimmunization rate of 15%. Seven patients (11.7 %) had developed alloantibodies & two patients (3.3%) had developed autoantibodies. The antibody results distribution in thalassaemic group were tabulated (See table 4). Transfusion of a selected unit of blood devoid of a specific red blood cell antigens against which specified antibody present in the serum of alloimmunized thalassaemic patients was resulted in an increment of the interval (i.e. the period between two consecutive blood transfusions) in which the patient was not in need for additional blood transfusion after such a highly selected qualified blood units. This was a consequence of a decrease in the hemolytic process in alloimmunized thalassaemic patients. The increment of such an interval in immunized thalassaemic patients were as follows: Six patients (10%) had increment by one week, two patients (3.3%) had an increment by 10 days & only one patient (1.7%) had no response.

**Table No. (4): The antibody results distribution in thalassaemic groups:**

| | Thalassaemic patients | |
|---|---|---|
| | No | % |
| Immunized patients | 9 | 15.0 |
| Alloantibody | 7 | 11.7 |
| Autoantibody (DAT + ve) | 2 | 3.3 |
| Single antibody | 6 | 10.0 |
| Multiple antibody | 3 | 5.0 |
| Anti-K, | 2 | 3.3 |
| Anti-e, M | 2 | 3.3 |
| Anti-c | 1 | 1.7 |
| Anti-$K_2$ | 1 | 1.7 |
| Anti-D | 1 | 1.7 |
| Anti-$Le^a$ | 1 | 1.7 |
| Anti-e,-M,-$K_2$ | 1 | 1.7 |
| Total Anti-K | 4 | 6.7 |
| Total Anti-$K_1$ | 2 | 3.3 |
| Total Anti-$K_2$ | 2 | 3.3 |
| Total Anti-e | 3 | 5.0 |
| Total Anti-M | 3 | 5.0 |
| Rhesus | 5 | 8.3 |

**Discussion:**

Thalassaemia major patients are prone to develop red cell antibodies due to repeated blood transfusions. There are several factors, which influence the rate of antibody formation in these patients. These include the red cell antigen disparity between blood donor, and recipients, number of blood units transfused, age of patients at which transfusions were started and the immunological factors related to blood transfusions. The overall rate of red cell immunization in our study was 15% (9 patients) with 11.7% (seven patients) showed presence of red cell alloantibodies. The red cell antibodies developed in this study were anti-K (6.7%), anti-e (5%), anti-M (5%), anti-c (1.7%), anti-D (1.7%) and anti-Lewis (1.7%), whereas the highest incidence was observed with Rhesus (8.3%) and Kell system (6.7%) (3, 14). Various studies all around the world have reported different frequencies of immunization. The majority of these reports have a high rate of alloimmunization (e.g A study by Singer et al, reported frequency of alloimmunization of (22%) in patients with thalassaemia major (3), but a few centers had reported low rates when compared to our data (3, 14) (e.g. Study in Italy among patients of thalassaemia major by Sirchia et al revealed a rate of alloimmunization of (5.2%) (3)).Other many comparative studies to this study will be discussed below. Most of this published data had similar types

of irregular red cell alloantibodies as ours. (3,10). The majority of patients in the present study had a long term exposure to the filtered blood and higher alloimmunization and autoimmunization rate (88.9%) than the patients exposed to all leuko-depleted blood. We postulate a similar activated immune system among our patients, thereby increasing the propensity to form antibodies. Splenectomized patients & patients had a higher immunization rate (85.7%) probably due to further enhancement of the immune response to the infused foreign blood antigens, which are not effectively filtered. The formation of red cell antibodies may be influenced by the patient's age at which the red cell transfusions have commenced. It had been shown earlier that immunization rate was lower in patients who received blood transfusions before 3 years of age (33.3%), as compared to those who started later in life(66.6%).It is postulated that transfusions before 1-3 years of age may have an immunosuppressive effect, resulting in lower rate of red cell antibody formation (12,13). In the present study, red cell immunization was statistically significant between the patients group who started transfusions in the first three years of life (45 patients) & the patient group who required blood support at a later age (15 patients) with a P value 0.011(See table No.4). This effect may be due to the effect of the degree of red cell antigen homogenecity between the donor and recipient population. It is possible that the enhanced propensity of antibody formation is affected by degree of red cell antigen homogenecity between the blood donors and recipients, even though the transfusions are started after 1-3 years of age.The factors which lead to formation of acquired red cell autoantibodies in thalassaemia are unknown. One possible mechanism may be the altered RBC deformability in patients of thalassaemia leading to exposure of new antigens in the senescent red cells resulting in formation of autoantibodies (8). Other mechanisms like cell-mediated lysis may play a part in accelerated destruction of red cells, similar to that seen in other hematological disorders. The confirmation of cell-mediated lysis is difficult in vivo and in vitro as use of research methods are needed to verify them (14). The discrepancy in alloimmunization is expected to be related to the rate of homogenecity of red cell antigens between the blood donors and the recipients as among the patients of the same ethnic groups. Because of similar ethnicity, red cell antigens are likely to be more homogenous amongst the Blood donors and the patients in the setup. A Study in Italy among patients of thalassaemia major by Sirchia et al revealed a rate of alloimmunization of (5.2%), red cell alloantibodies were found in 74 out of 1432 patients. 136 different types of alloantibodies were found in 74 patients which were entirely confined to the common antigens of Rhesus, Kell, Duffy & MNS system. Twenty-one (28%) patients had two alloantibodies and seventeen (23%) had more than two alloantibodies. (3). Another Study conducted in Hong Kong among patients of Asian descent by HOR-Kung et al showed nine patients with alloantibodies in 68 patients (7.4%). The red cell alloantibodies found were anti-E, anti-M, but anti-K was not encountered. This is in contrast to our data where anti-K was seen in three patients. However, many studies have also reported a high rate of red cell alloimmunization. A study by Singer et al, reported frequency of alloimmunization of (22%) in patients with thalassaemia major. He reported that 19 red cell alloantibodies were seen in 14 out of 64 patients. Three antibodies were detected in one patient while two antibodies in three patients. Anti-Kell was most often identified. This report also interestingly states that patients who receive blood match for ABO system , Rhesus system and Kell system from their first transfusion, the rate of alloimmunization is found to be relatively low (3). Consequently, they inferred that transfusion of blood phenotypically matched for Rh and Kell systems compared to blood phenotypically matched for the standard ABO-Rh-D system could prove to be effective in preventing alloimmunization (3). Vasilikil Meehan et al found rate of alloimmunization to be (19.16%) in patients with thalassaemia major receiving regular blood transfusion. They reported alloimmunization in two groups of children. First group was those with a better blood cross-matched, which comprised of children who received blood compatible with ABO, Cc, Ee, D and K antigens as a better blood cross match method while the second group was those who received blood compatible with only ABO and Rh antigen as a usual blood cross match method. They also found that the overall frequency of alloimmunization between the usual match and better match group was not statistically significant. In this Study, the antibodies belonged to Rhesus, Kell, Duffy, Kidd, Lewis, and MNS system. In the usual match group, the distribution frequency of alloantibodies was RhD: 35.8%, Kell: 25.6%, Duffy: 10.2%, Kidd: 12.8%, MNS, and Lewis: 7.6% each. Spanos et al also found a high frequency of red cell alloimmunization in 22.6% thalassaemic patients receiving blood matched for ABO and RhD antigen. Alloantibodies belonged to the following systems: 34% to Rhesus, 29.8% to Kell, 7.9% to MNS, 8.1% to Kidd, 5.9% to Lewis, 4.1% to Duffy&1.32% to P. The results of the latter study were similar to the result of the this study regarding the Rh & Kell systems while in discrepancy with the other systems probably related to the difference in the frequency of these antigens of the other systems among the blood donors & the recipients. A low rate of alloimmunization may be expected when there is homogenecity of RBC antigens between the blood providers and recipients. Previous data on presumed homogenous populations in Greece and Italy showed an overall low rate (5% to 10%) of alloimmunization. A higher rate of approximately 20% was noted in 2 Greek studies when blood matched only for ABO& RhD antigens was used. A study conducted in Kuwait, a study by Ameen R. reported frequency of RBCs alloimmunization in 190 thalassaemia major patients was 30% patients which are in discrepancy with the current study in which 11.7% developed alloantibodies & this is probably related to the extent of the blood cross matching, phenotypically matched blood transfusion & the difference in the homogenecity among the

blood donors & recipients. The most common clinically significant alloantibodies were directed against antigen in Kell & Rh systems, which are similar to the results of the current study (14). In the same study conducted in Kuwait by Ameen R, Anti-K developed in 41 (72%) patients followed by anti-E in 26 (45.6%) patients. In addition to that, RBC autoantibodies developed in 21 (11%) patients with and without underlying RBC alloantibodies. Sixty six (49.6%) RBC alloantibodies developed between the age of 2 and 10 years. (14).

**Conclusion:**

The frequency of distribution of RBC alloantibodies and autoantibodies in transfusion dependent thalassaemic patients in the current study are as followings:

- Anti-K :( 6.7%); Anti-c : (5%); Anti-M: (5%); Anti-c: (1.7%); Anti-D : (1.7%) and Autoantibody (3.3%).

These antibodies are of clinical significance affecting the benefit from blood transfusion & normal growth of the patients. Extended pretransfusion compatibility testing in B-thalassaemia major should include blood group (ABO and RhD) of the recipient and donor, red cell antibody screening of patient using cell panels, and cross match of patient's serum with donor's red cells by performing indirect antiglobulin test (IAT) at 37C - Direct antiglobulin test (DAT) needs to be done on red cells of those recipients who give history of increase in the transfusion requirements. All these steps of tests are usually done at blood transfusion services centers. Moreover, ABO &RhD blood grouping tests of the recipient and donor are highly recommended to be repeated at bedside just in the pretransfusion period for further confirmation of preliminary blood compatibility & to exclusion of potentially fatal ABO incompatible blood transfusion for any reason including the common one, which are the clerical errors.

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