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Immunology

Identification of Auto and Alloantibodies in Patients with Hereditary Haemolyticanaemia (HHA)

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Abstract **Original Research Article**

Introduction: Hereditary hemolytic anemia (HHA) is a group of rare and varied illnesses caused by anomalies in the metabolism of plasma membranes and/or red blood cells (RBCs), which lead to the premature lysis or clearance of these cells. In Bangladesh, data regarding red cell antibodies (allo as well as autoantibodies) in multiple transfused patients is insufficient. Therefore, this study aimed to identify the auto and alloantibodies among transfused patients with HHA. ***Methods:*** This cross-sectional study was conducted in the Department of Immunology, BIRDEM and Armed Forces Institute of Pathology (AFIP), Dhaka Cantonment, Dhaka, Bangladesh for one year period. A total of 300 patients were included with HHA according to selection criteria. ***Results:*** Among 300 patients, the majority (35.67%) of them were in the age group 1-10 years. Of all patients 186(62%) were male, and 114(38%) were female. Rh was positive in 96.67% of patients and only 10(3.33%) patients were Rh-negative. In HHA patients, the age of the first transfusion was 3.09±4.89 years. The number of blood units transfused was 75.70±89.03. The antibody was detected in 12(4.0%) patients with HHA. Among HHA patients, only auto-antibody, only alloantibody, or both were detected in 2(0.67%), 6(2.0%), and 4(1.33%) patients respectively. ***Conclusion:*** In our study, we found that 4.00% of the subjects have antibodies. The majority of the alloantibodies were anti-E, anti-C, and anti-Kell, which are part of the Rh system. The majority of the autoantibodies were IgG.

Keywords: Autoantibody, Alloantibody, Hereditary HaemolyticAnaemia.

INTRODUCTION

HHA is a group of rare and varied illnesses caused by anomalies in the metabolism of plasma membranes and/or red blood cells (RBCs), which lead to the premature lysis or clearance of these cells.[1]. Representative laboratory findings include reticulocytosis, abnormal red blood cell shape, increased levels of unconjugated bilirubin and lactate dehydrogenase (LDH), decreased haptoglobin, and recurrent iron overload. Clinical manifestations include jaundice, gallstones, splenomegaly, and varied degrees of hemolytic anemia. The diagnosis of HHA is made using test data, clinical evaluation, family history, and persistent hemolytic anemia [1]. Overlaps in hematological and clinical characteristics exist among the different forms of HHAs, making differential diagnosis difficult, particularly for atypical and mild types. HHA has classically been categorized into three distinct types: haemoglobinopathies such as thalassemia and sickle cell anaemia (SCA), RBC membranopathies such as hereditary spherocytosis (HS) and RBC enzymopathies such as glucose-6-phosphate dehydrogenase (G6PD) deficiency. Patients with HHA typically present with anaemia, jaundice, increased reticulocyte count, and often splenomegaly [2-5]. However, RBC membranopathies were shown to be the most prevalent kind of HHA (the prevalence was 87.1% from 1997 to 2006 and 71.3% from 2007 to 2016) [6].

Combined data from 18 studies on 4005 sickle cell patients and 11 studies regarding 3394 thalassemia patients showed an overall alloimmunization risk of 22% and a total of 1606 RBC antibodies were reported in 675 sickle cell patients and 834 antibodies in 446

thalassemia patients [7]. Multiple antibody specificities were present in 35% of patients [8].

RBC antibodies, which can become undetectable over time, can cause delayed transfusion reactions after incompatible blood transfusions. Several authors advocated that transfusions given to patients who are likely to become transfusion-dependent over a longer period should be matched for antigens other than ABO and Rh (D antigen only) in an attempt to prevent alloimmunization [9]. The immunization rate, expressed as the number of antibodies per 100 transfusions, varied between 1.7 and 4.0 [10]. Red cell allo and autoantibodies should not be overlooked in repeated transfused patients. It should always be considered if the patient repeatedly suffers from haemolytic transfusion reactions or not being able to maintain haemoglobin at a desired level despite regular transfusions. In Bangladesh, data regarding red cell antibodies (allo as well as autoantibodies) in repeated transfused patients is scarce. A study regarding alloantibodies in repeatedly transfused patients done by Khatun A found that the frequency of alloimmunization is 6% in thalassemia patients and Rh and Kell's antibodies are alloantibodies commonly identified [11]. Since transfusion is essential for the treatment of patients with hematologic illnesses and cancers, individuals who undergo repeated transfusions may acquire clinically significant RBC alloantibodies and autoantibodies, which can cause serious issues if transfusion therapy is continued for an extended period. Due to the prevalence of transfusion responses and clinically significant RBC antibodies, allo and autoantibodies might make it challenging to obtain suitable RBC units [12].

Therefore, in this study, we aimed to identify the auto and alloantibodies among transfused patients with HHA.

METHODOLOGY & MATERIALS

This cross-sectional study was conducted in the Department of Immunology, BIRDEM, and Armed Forces Institute of Pathology (AFIP), Dhaka Cantonment, Dhaka, Bangladesh during the period of one year. In this study, we included 300 patients with hereditary HHA who attended the BIRDEM and AFIP outpatient departments.

These are the following criteria to be eligible for enrollment in this study: a) Patients aged up to 60 years irrespective of sex; b)Patients diagnosed with HHA; c) Patients who received at least five units of blood transfusion; d) Patients whose sample was taken at least four weeks of last transfusion; e) Patients who were willing to participate were included in the study and a) Patients with pregnancy; b) Patients with Auto Immune HaemolyticAnaemia (AIHA); c) Patients in whom antibody was previously detected; d) Patients with any history of acute illness (e.g., renal or pancreatic diseases, ischemic heart disease, asthma, COPD etc.) were excluded from our study.

Laboratory Method: About 5 ml of venous blood was collected from each patient. Blood was centrifuged at 100-120 rpm/min for 10 minutes to separate into cell and serum then 5% cell suspension was made. Blood grouping and Direct Anti Globulin (DAT) tests were performed with a cell suspension of all samples. A poly-specific coomb's reagent (Blend of IgG and C3d) was used. In cases of a positive DAT test, further investigation using specific monoclonal reagents to detect IgG or a complement (C3d) was carried out. Serum was used to detect red cell alloantibodies using standard blood bank methods (albumin, enzyme, and indirect Coombs test method). The serum was stored in a -20°C freezer. Antibody identification (anti-D, anti-C, anti-c, anti-E, anti-e, anti-K, anti-Jka, anti-JKb, anti-Lea, anti-Leb, anti-Fya, anti-Fyb, anti-M, anti-N, anti-S) was performed in antibody screening positive samples using red cell identicells.

Data Collection &Analysis: A signed informed written consent was taken from patients after explaining the nature & objective of the study. All data were recorded systematically in the preformed data collection form. Quantitative data was expressed as mean and standard deviation and qualitative data was expressed as frequency distribution and percentage. The study was approved by the Ethical Review Committee of the Bangladesh Diabetic Association.

RESULTS

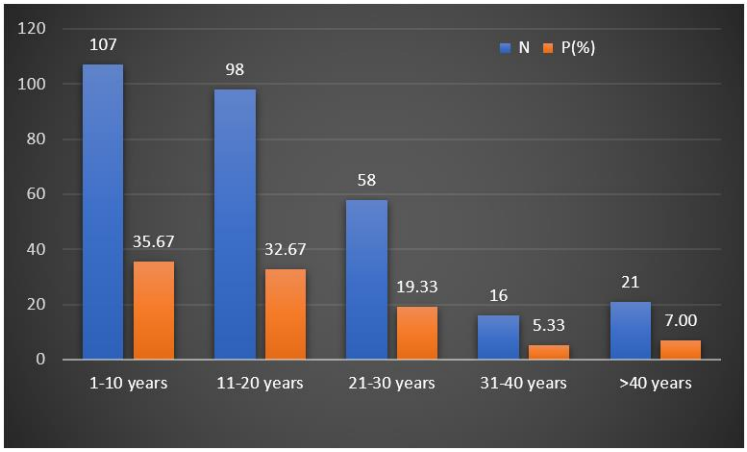

Figure 1: Age distribution of our study patients

Figure 1 shows that among 300 patients, 107(35.67%) of them were in the age group 1-10 years followed by 98(32.67%) in the age group 11-20 years, 19.33% were aged 21-30 years and 7% were more than 40 years old.

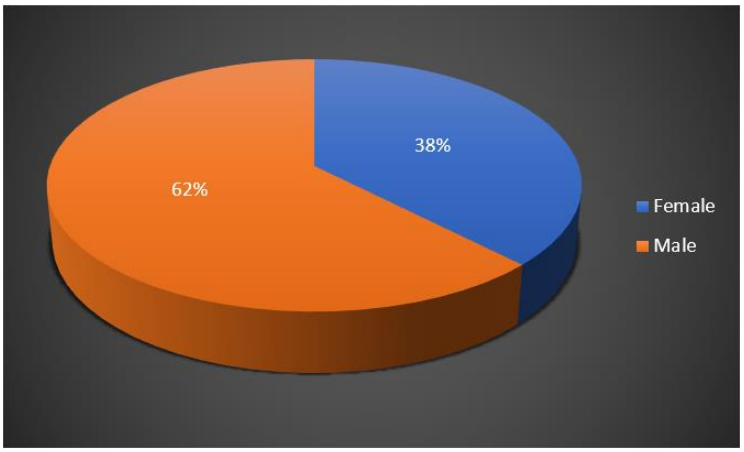

Figure 2: Gender distribution of our study patients

The pie chart shows that 186(62%) of the total patients were male, and 114(38%) were female, and the male-female ratio was1.6:1 in the study.

Table 1: Distribution of the study patients by blood group & transfusion history

Blood Group	**N**	**P(%)**
O^+	98	32.67
B^+	113	37.67
A^+	62	20.67
AB^+	17	5.67
Total	**290**	**96.67**
O^-	2	0.67
B^-	3	1.00
A^-	5	1.67
Total	**10**	**3.33**
Transfusion History		
Age of 1st transfusion (years)	3.09±4.89	
Total number of transfusions (units)	75.70±89.03	

The time interval between transfusion (weeks)	4.07±1.04

Table 1 shows that among all patients, Rh was positive in 96.67% of patients with the highest percentage of B positive (37.87%), followed by O positive (32.67%) and A positive (20.67%). Only 10(3.33%) patients were Rh-negative. In HHA patients, the age of the first transfusion was3.09±4.89 years. The number of blood units transfused was 75.70±89.03. However, the time interval between subsequent transfusions was 4.07±1.04 weeks.

Table 2: Distribution of our study patients by types of antibodies

Types of Antibodies	N	P(%)
Only Autoantibody	2	0.67
Only Alloantibody	6	2.00
Both Antibodies	4	1.33
Total	12	4.00

Table 2 shows that the antibody was detected in 12(4.0%) patients with HHA. Among HHA patients, only auto-antibody, only alloantibody, or both were detected in 2(0.67%), 6(2.0%), and 4(1.33%) patients respectively.

Table 3: Distribution of the study patients by types of anti-RBC antibodies

Types of Antibodies		N	P(%)
Autoantibodies	Anti IgG	5	62.5
	Anti C3d	3	37.5
	Total	8	100.0
Alloantibodies	Anti E	4	26.67
	Anti K	3	20.00
	Anti c	2	13.33
	Anti-JKa	1	6.67
	Anti-FYa	1	6.67
	Non-specific	4	26.67
	Total	15	100.00

Table 3 shows that out of 8 auto-antibodies, 5(62.5%) were anti-IgG followed by 3(37.5%) were anti-C3d. Out of 15 alloantibodies, 4(26.67%) were anti-E, 3(20.0%) were anti-K and in 4(26.67%) cases specificity of alloantibody was not detected.

DISCUSSION

The present study was conducted to identify the prevalence of red cell autoantibodies and alloantibodies in repeatedly transfused patients with HHA. In the current study, the patients were divided into 5 groups based on age. Among 300 patients maximum of 107(35.67%) were in the age group 1-10 years followed by 98(32.67%) in the age group 11-20 years, 19.33% were aged 21-30 years and 7% were more than 40 years old. Of all patients, 186(62%) of them were male, and 114(38%) were female. The male-female ratio was 1.6:1 in the study. Out of 300 patients, Rh was positive in 96.67% of patients, and blood group B was the most common blood group followed by group O. Rh negative group is seen in 3.33% of total patients. In this study, mean total no of transfusions was 75.70+89.03 among HHA patients. The mean age of starting transfusion was 3.09+4.89 years. Singer *et al.*, showed that starting of transfusion at an early age may offer some immune tolerance and protect against alloimmunization [13].

In this study, the frequency of development of red cell auto and alloantibody was 4.00% among patients with HHA who had undergone repeated transfusions. Sood *et al.*, showed the incidence of RBC alloimmunization ranges between 1-6% in occasionally transfused and up to 30% in poly-transfused patients [10]. A study done by Dhawan *et al.*, reported the frequency of alloimmunization in multi-transfused patients in India is comparatively low varying from approximately 3% to 10% [14]. In other studies, alloimmunization to RBC was positive in 7.4% of patients in Iran done by Natukunda *et al.*, 9.2%, in Pakistan done by Mohsin *et al.*, 22% in Saudi Arabia done by Shamsian *et al.*, 6.1% in Uganda done by Bilwani*et al.*, in patients who received multiple transfusions with different diseases [15-18].

Among 300 HHA patients, only auto-antibody, only alloantibody, or both were detected in 2(0.67%), 6(2.0%), and 4(1.33%) patients respectively. A study reported on 697 thalassaemic patients who had received transfusions. Allo and autoantibodies were reported in 115(16.5%) and 34(4.9%) subjects respectively [16]. In this study, out of 8 autoantibodies, 5 antibodies were

typed warm IgG antibodies and 3 were complement C3d. Autoimmunization was associated with alloimmunization. A similar result was shown in another study which showed a relation of autoimmunization with splenectomy [18]. In this study, out of 15 alloantibodies, 4(26.67%) were anti-E, 3(20.0%) were anti-K and in 4(26.67%) cases specificity of alloantibody was not detected.

In the present study, the antibodies that were found were from the Rh, Kell, Duffy, and Kidd groups. Out of fifteen positive patients, a maximum of them were positive for alloantibodies against the Rh system.

Another research carried out in Rawalpindi produced similar findings, showing that red cell alloantibodies found in individuals who had received many transfusions primarily belonged to the Rh system [19]. Twenty-two alloantibodies were found in 13 of the 306 multiple transfused patients in India; the most common was Anti-E, which was found in six of the instances, followed by Anti-C, Anti-JKa, and Anti-K [20]. Additional research conducted in India revealed that the prevalence of alloimmunization was 3.79% among 211 individuals with multiple transfused thalassaemia. Anti-E, anti-K, anti-D, anti-Kpa, anti-c, and anti-Jka were the alloantibodies that were found [21]. Anti-E and anti-K alloantibodies were found in 116 thalassaemic patients in another investigation carried out in India [22].

Most of the alloantibodies identified in this study had specificity against the Rh and Kell antigen systems because of their high immunogenicity, which is in line with prior alloimmunization investigations [23]. RBC alloantibodies may form against mismatched antigens, although transfusion of blood matched for Rh and K antigens may inhibit alloimmunization and result in a significant difference in alloimmunization rates [23].

This study has some limitations. The study was only two-centered. The study period was short and the sample size was small so it doesn't represent the whole population of the country.

CONCLUSION AND RECOMMENDATIONS

The results of this investigation indicate that 4.00% of the subjects have antibodies. The majority of the alloantibodies were anti-E, anti-c, and anti-Kell, which are part of the Rh system. The majority of the autoantibodies were IgG. For improved treatment outcomes, patients who need repeated transfusions might be advised to undergo red cell auto and alloantibody screening and identification.

So further prospective and longitudinal study design including a larger sample size needs to be done to validate the findings of our study.

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Ethical approval: The study was approved by the Ethics Committee

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