Incidence of Acute Haemolytic Transfusion Reaction in ABO Group-Compatible Compared with Group-Identical Blood Recipients in Ilorin, Ni!!geria

Abstract

Background: *Acute haemolytic transfusion reaction (AHTR) is a rare but fatal complication of blood transfusions especially in developing countries.*

Objectives: *To determine the relative frequencies of group-compatible and group-identical blood transfusions and to compare the incidences of acute haemolytic transfusion reactions in the two types.*

Methods: *A prospective study of 140 patients above 15 years who received one unit of either group-compatible or group-identical blood for correction of anemia was carried out. Five ml of pre- and post-transfusion blood samples were collected from every patient and analyzed for evidence of hemolysis by estimating: PCV, intravascular agglutinates, plasma hemoglobin and total bilirubin concentrations, Direct Coombs' test (DCT) and visual plasma inspection.*

Results: *All subjects had 1 unit of either group-compatible (42.9%) or group-identical (57.1%) blood transfusions each. None of the patients suffered AHTR, as none of the samples was positive for DCT. Group-identical transfusions however, were associated with higher increments in PCV (xx% vs. xx%; p=).*

Conclusion: *Group-identical transfusion was associated with a higher increament in PCV than group compatible one, but the latter can still be practiced in developing countries where there is no availability of blood of all groups.*

Keywords: Acute hemolytic transfusion reaction; Group compatible transfusion; Group identical transfusion; Ilorin.

Introduction

Ottenberg in 1911[1] first suggested the concept of universal donors. Despite the awareness of the presence of á- and â-hemolysins in the donor plasma, he still advocated the use of group O blood as universal donor because he felt the agglutinins would be so diluted in the recipients' plasma as to be insignificant.

Subsequently, the use of group O blood for transfusion to non-group O patients (i.e. group-compatible transfusions) spread rapidly. It was the main type used during the Second World War, in fact in most cases without any preliminary matching tests.

It was later found that transfusions of group O plasma to group A, B or AB recipients may

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cause severe red cell destruction. The first few cases were reported by Aubert *et al*[2] and Tisdall *et al*[3]. They observed varying degrees of hemoglobinemia, hyperbilirubinemia and intravascular agglutinations in all the cases. 23-40% of the group O donors were found to have these hemolysins with the mean titres associated with hemolysis ranging from 512 to 640 or higher. Intravascular agglutinations were found in almost all the recipients in both studies[2,3].

Ervin *et al*[4] showed that IgM anti-A and anti-B (i.e. naturally occurring anti-A and anti-B) are easily neutralized with the AB substances and prevented from causing hemolysis.

The frequencies of complications of blood transfusions vary inversely with the care exercised in the preparation for and the supervision of the transfusions[5]. The overall incidence of complications arising from blood transfusions is approximately 2-5%[5]

Inadequate supply of blood and blood products in blood banks of most hospitals in developing countries necessitated the practice of group-compatible blood transfusions. Tests for hemolysins and antibody screening which help to identity dangerous universal donors and recipients with atypical antibodies respectively are routinely performed in some blood banks in Nigeria[6]. However, others including University of Ilorin Teaching Hospital (UITH) do not, despite the reported high prevalence of hemolysins among group O donors[7].

This study was aimed at determining the frequency and severity of acute hemolytic transfusion reactions in group-compatible compared with group-identical adult blood recipients.

Materials and Methods

One hundred and forty patients above 15 years of age who received one unit of either group compatible or group identical whole blood for correction of anemia arising from any cause were selected randomly and recruited for the study.

Patients with haemolytic or auto-immune disorders either acquired or congenital as well as patients that had evidence of hemolysis in their pre-transfusion plasma were excluded from the study.

Verbal and written consent from patients and hospital ethical committee's approval were obtained for the study.

Specimen collection

5ml of venous blood was obtained from the antecubital vein after antiseptic cleansing before, 6 hours and 48 hours after transfusions. Each of the samples collected before and 6 hours after transfusion was dispensed into a specimen bottle containing heparin at 20 iu/ml of blood, while the ones collected 48 hours after transfusion were collected into EDTA bottles to prevent coagulation. Hemolysis during collection was avoided by using wide bored needles (SWG 20), clean venepuncture, slow and careful dispensation of samples into the specimen bottles after removing the needle.

Laboratory tests done and methodologies

1. *PCV:* A microhaematocrit method carried out on blood contained in capillary tube of about 75 mm in length and an internal diameter of 1 mm[8] was used.
2. *Intravascular agglutinates:* A gentle agitation tip-and-roll macroscopic method was employed[9].
   After performing 1and 2, each sample was centrifuged at 2000g for 5 minutes to separate the plasma and packed cells. Investigations 3, 4 and 5 were performed on the plasma.
3. *Visual inspection of the color of plasma:* Plasma was visually inspected under bright light for hemolysis as evidenced by pinkish colouration.

Hemolysis was graded as:

0: no pinkish coloration or normal straw-colour.
1: tinge of pinkish coloration.

2: deep pinkish coloration.
3: very deep pinkish coloration.
4. Plasma hemoglobin concentration: Peroxidase method involving the use of benzidine reagent, hydrogen peroxide, acetic acid and standard[10] was used.

Normal plasma hemoglobin concentration was taken to be 10-40mg/L.

5. Plasma total bilirubin concentration: Bilirubin Kit by Randox (Randox laboratories Ltd, Ardmore, Diamond road, Crumlin, co. Antrim, UK, BT29QT) was used.
   Normal plasma total bilirubin concentration was taken to be <17μmol/l (0-1mg/dl)[12].
6. Direct antihuman globulin (Coomb's) test: A spin tube technique[11] was used.

Packed cell volume (PVC) was performed on pre- and 48 hours post transfusion samples while other tests were performed on the pre- and 6 hours post-transfusion samples.

Statistical Analysis

Data was analyzed using EPI Info version 6 statistical software.

Continuous variables were analyzed as means while non-parametric variables were analyzed as percentages.

Protocol form was designed and used to document the clinical history and physical examination findings, before and immediately after transfusion. Information obtained was used to compare and contrast the clinical and laboratory findings of acute hemolytic transfusion reaction.

Results

One hundred and forty patients satisfied the criteria for the study within the study period. Sixty (42.9%) had one unit of group-compatible whole blood transfusions while 80 (57.1%) had one unit of group-identical whole blood transfusions. Of the recipients, 66 (47.1%) were males while 74 (52.8%) were females, Table 1. The age range was 18-80 years with the mean of 42±11 years. Clinical complaints/ symptoms related to adverse effects of blood transfusion during and after the transfusion were noticed in 42 (30%) subjects. These were fever, headache, chest tightness, and jaundice in 36 (25.7%), 4 (2.8%), 1 (0.7%) and 1 (0.7%) of all the subjects respectively, Table 1.

Seventy-four (52.9%), 30 (21.4%), 24 (17.1%) and 12 (8.6%) of the subjects had surgical, medical, gynaecological and haematological indications for transfusion respectively.

Post-operative transfusion, anaemia secondary to cancer of the prostate, anaemia secondary to cancer of the breast, upper gastro intestinal bleeding and chronic appendicitis constituted 40 (54%), 14 (18.9%), 10 (13.5%), 5(6.8%) and 5 (6.8%) of the surgical cases respectively while abdominal tuberculosis, HIV/AIDS, peptic ulcer disease, typhoid enteritis, diabete mellitus and oesophageal varices constituted 9 (30%), 8 (26.7%), 5, 4, 3 (10%) and1 (3.3%) of medical cases.

Anaemia in pregnancy, abruptio placenta, incomplete abortion and septic abortion constituted 10 (41.6%), 6 (25%), 4 (16.7%) and 4(16.7%) respectively of gynaecological cases, while sickle cell anaemia, chronic myeloid leukaemia, aplastic anaemia and multiple myeloma constituted 5 (41.7%), 4 (33.3%), 2 (16.7%) and 1 (8.3%) respectively, Table 2.

Table 3 shows the results of all the laboratory parameters measured before and after transfusions.

A stastical significance difference in increament in PCV above 3% and a drop in PCV was noticed between GC and GI blood recipients, p-value <0.05. No significant difference was noticed with an increment in PCV of 1-3% p-value >0.05. Normal post-transfusion plasma was seen in 86.7% and 92.5% of subjects with GC and GI blood recipients respectively, while

Table 1: Sex distribution of all subjects and the clinical complaints/symptoms seen in 42 subjects

Parameters		Number and Percentage of patients N (%)		
Sex		GC N (%)	GI N (%)	Total N (%)
	Males	18 (12.9)	48 (34.3)	66 (47.1)
	Females	42 (30)	32 (22.9)	74 (52.9)
	Total	60 (42.9)	80 (57.1)	140 (100)
Clinical presentations	Fever	20 (14.3)	16 (11.4)	36 (25.7)
	Headache	0 (0)	4 (2.9)	4 (2.9)
	Chest tightness	1 (0.7)	0 (0)	1 (0.7)
	Jaundice	1 (0.7)	0 (0)	1 (0.7)
	Nil	38 (27.1)	60 (42.9)	98 (0.7)
	Total	60 (42.9)	80 (57.1)	140 (100)

Table 2: Indications for blood transfusion in all subjects

INDICATIONS FOR TRANSFUSION	Number and Percentage of Patients N (%)
Surgical:	
1. Post operative	40 (54%)
2. Cancer of the prostate	14 (18.9%)
3. Cancer of the breast	10 (13.5%)
4. Bleeding Peptic Ulcer Disease	5 (6.8%)
5. Chronic appendicitis	5 (6.8%)
SUB TOTAL	74 (52.9%)
Medical:	
1. Abdominal tuberculosis	9 (30%)
2. HIV/AIDS	8 (26.7%)
3. Peptic Ulcer Disease	5 (16.7%)
4. Typhoid enteritis	4 (13.3%)
5. Diabetes Mellitus	3 (10%)
6. Oesophageal varices.	1 (3.3%)
SUB TOTAL	30 (21.4%)
Obstetric and Gynecologic	
1. Anaemia in pregnancy	10 (41.6%)
2. Abruptio placenta	6 (25%)
3. Incomplete abortion	4 (16.7%)
4. Septic abortion	4 (16.7%)
SUB TOTAL	24 (17.1%)
Hematological	
1. Sickle cell anaemia	5 (41.7%)
2. Chronic myeloid leukaemia	4 (33.3%)
3. Aplastic anaemia	2 (16.7%)
4. Multiple myeloma	1 (8.3%)
SUB TOTAL	12 (8.6%)
GRAND TOTAL	**140 (100)**

Table 3: Changes in laboratory parameters before and after transfusion in all subjects

Parameters		GC N (%)	GI N (%)	p-values
Changes in PCV	≥ 3% increament	14 (23.3%)	28(35%)	<0.05
	1-3% increament	44(73.4%)	44 (55%)	>0.05
	Drop in PCV	2 (3.3%)	8 (10%)	<0.05
	Total	60 (100%)	80 (100%)	
Intravascular agglutinate	Present	0 (0%)	0 (0%)	
	Absent	60 (100%)	80 (100%)	
	Total	60 (100%)	80 (100%)	
Color of Post transfusion plasma	Straw-coloured	52 (86.7%)	74 (92.5%)	>0.05
	Pinkish	8 (13.3%)	6 (7.5%)	>0.05
	Total	60 (100%)	80 (100%)	
Post transfusion plasma hemoglobin concentration	10-40mg/l	59 (98.3%)	79 (98.8%)	>0.05
	> 40mgll	1 (1.7%)	1 (1.2%)	>0.05
	Total	60 (100%)	80 (100%)	
Post transfusion plasma total bilirubin concentration	< 17µmol/l	58 (96.7%)	78 (97.5%)	>0.05
	> 17µmol/l	2 (3.3%)	2 (2.5%)	>0.05
	Total	60 (100%)	80 (100%)	
DCT	Positive	0 (0%)	0 (0%)	
	Negative	60 (100%)	80 (100%)	
	Total	60 (100%)	80 (100%)	

the rest in each group (13.3% and 7.5% respectively) had pinkish colouration of their post-transfusion plasma, p value >0.05. Post-transfusion plasma haemoglobin concentration was within normal range of 10-40mg/dl in 98.3% and 98.8% of GC and GI blood recipients, with 1.7% and 1.2% of them respectively having elevated levels, p value >0.05.

In 96.7% and 97.5% of subjects with GC and GI blood recipients respectively, post transfusion plasma total bilirubin concentration was <17ì mol/l while it was >17ì mol/l in the rest subjects in each group (3.3% and 2.5% respectively), p value >0.05. Direct antihuman globulin test was negative in all the subjects in both groups.

Discussion

Acute Haemolytic Transfusion Reaction (AHTR) is a rare but potentially fatal complication of blood transfusion. ABO incompatible transfusion remains the main cause of serious adverse events associated with transfusion[13]. This is commonly due to procedural errors within the transfusion

process but recently due to increased hemovigilance, the fatality is reducing[14].
In the UK and USA, approximately less than 1 in 30,000 units of red cells transfusions is ABO incompatible. In the UK the actual incidence of AHTR is 1 in 25,000 transfusions[13] while deaths due to ABO incompatibility were about 1 in 600-800,000 transfusions[15]. In the USA the incidence is 1 in 200,000 transfusion[16].

Current immuno- hematology practice recommends serological diagnosis of immune hemolytic transfusion reaction based on the findings of a positive Direct Coombs' tests in the post transfusion sample[17]. Base on this the incidence of AHTR is this study was zero because all the post transfusion samples were negative for Direct Coomb's test. This result is in keeping with that of Beamount[13] and Badami *et al*[16] quoted above, although the sample size in this study was rather small.
However one patient had clinical signs and symptoms suggestive of AHTR (fever, chest tightness and later jaundice) with elevated levels of plasma haemoglobin and bilirubin concentrations in the post transfusion plasma, but negative DCT and no demonstrable intravascular agglutinates. The patient was group A and had 1 unit of group O whole blood for correction of anemia in pregnancy. Her PCV remained unchanged from pre transfusions level of 15%. Her BP increased from 70/40mmHg (pre transfusions) to 120/70mmHg (post transfusion) and her heart rate also increased from 120beats/min (pre- transfusion) to 140beats/min (post transfusion). Her DCT result could have been a false negative one.

False negative DCT, frequently encountered, could be due to insufficient antibody on the RBC surface, early dissociable antibody or the use of antiserum that lack antibody against a subclass of immunoglobulins. 300-500 molecules of D-antigen per cell is necessary for a positive DCT[18].

In this study, the only significant difference between GC and G1 transfusion was changes in PCV. Group-identical transfusions were associated with higher increments in PCV than GC ones (p value <0.05), although the GI transfusion were also associated with drops in PCV than GC ones, p- value <0.05.

Two patients had post-transfusion plasma hemoglobin concentrations above the normal range of 10-40mg/l. One (1.7%) had GC while the other (1.2%) had GI transfusion, with no statistical significant difference, p value = 0.98. Hemoglobinaema in these two patients could be due to other reasons apart from blood transfusion reaction.

With non-availability of donor blood of all groups at all times in most health care centers in developing world like Nigeria, GC transfusions will still continue to play major roles in transfusion practice.

Limitation to the study

For a rare disorder like the one under study, a very large sample size is desirable, but in its absence a very sensitive method like spectrophotometry would have been appropriate to demonstrate the level of plasma haemoglobin and the colour of plasma. The use of spectrophotometer to determine the plasma haemoglobin concentrations and evidence of haemolysis in pre and post transfusion samples was not paramount because the gold standard for the diagnosis of immune haemolytic transfusion reaction is a serological evidence of positive Direct Coomb's test (DCT) and presence of intravascular agglutinates in the recipient. In this study plasma haemoglobins were measured using peroxidase method while the colour of plasma were assessed visually, but DCT and intravascular agglutinate were performed with the most sensitive techniques.

Conclusion

The incidence of AHTR was zero in this study based on the results of DCT and intravascular agglutinates. Group compatible transfusion was not proved to be more associated with AHTR than GI transfusion but group identical

transfusion was associated with higher increament in PCV than group compatible ones.

Recomendation

Although the sample size for this study is rather too small judging from the reported incidences of AHTR in various countries, the study can be a baseline one for a possible larger multicentre studies. GC blood can still be safely practiced for transfusion in developing countries where there is inadequate supply of blood in most blood banks.

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