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Evaluation of Alloantibodies to human platelet antigen and Leucocyte antigen class 1 in Multitransfused patients in Owerri, Imo state

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Abstract

Alloantibodies to human platelet specific antigen and leucocyte antigen class 1 were evaluated in haemato-oncology multitransfused patients. Study subjects were 120 multi-transfused patients (96 males and 24 females) within the age bracket of 18 and 65 years who were on admission at Federal Medical Centre Owerri. Sixty apparently healthy individuals (40 males and 20 females) who have never received blood transfusion in life within the same age bracket were recruited as control subjects. Ethical approval was obtained from the ethical committee of Federal Medical Centre Owerri and the consent of the subjects were obtained. Valuable information were obtained by questionnaires.ELISA method (using monoclonal antibody immobilization of platelet antigen kit) was adopted for antibody determination. Data obtained were analysed with IBM SPSS. Chi-square test, student's t-testand fisher's analysis of variance were used to compute the relationship between variables. Statistical significance was set at a confidence limit of 95%. Results revealed that out of 120 multitransfused subjects tested, 107(89.17%) were alloimmunized, 13(10.83%) were not immunized. Those immunized against human platelet specific antigens and leucocyte antigens class 1 were 75(70%) and 4(3.78%) respectively. Those immunized against both human platelet specific antigens and leucocyte antigens were 28(26.1%). The prevalence of platelet specific glycoprotein antigens and leucocyte antigens were obtained as follows: Gp 11b/111a 97(80.83%), Gp1a/11a 46(38.33%), HLA class 1 32(26.67%), and 1b/ix 20(16.67%)

Result indicates high frequency of alloimmunization against human platelet specific antigens with alloantibodies against glycoprotein 11b/111a (HPA-1a,3a,4a) having the highest frequency in multitransfused subjects. Frequency of alloimmunization did not increase with the number of units of blood transfused. Finding will help ensure safety of transfusion therapy in other to avoid clinical conditions like Platelet refractoriness and post transfusion purpura.

Keywords: alloantibodies, alloantigens, multitransfused, thrombocytopenia

Introduction

Alloantibodies are antibodies produced by an individual against alloantigens produced by members of the same species. Alloantigens are proteins or other substances that are present in some members of a specie and so can stimulate alloantibody production in other members of the same specie who lack it[1].Platelets are cytoplasmic fragments of megakaryocytes produced in the bone marrow,that play important role in haemostasis. Platelets express both platelet specific antigen and other antigens like HLA class 1a on their surface. Platelet membrane glycoproteins(gp-11b/111a,1a/11a,hla,and 1b/ix) carry platelet antigen which can be identified by the glycoproteins that carry them. When an individual blood platelet level drops below 150 x 10^9/L, a condition called thrombocytopenia ensues. Transfusion of whole blood or other blood products can expose an individual to allogenic human platelet antigen and elicit human responses[2]. Alloantibodies against Human platelet specific antigens and leucocyte antigens class 1 in multi transfused patients can be responsible for clinical conditions like Post Transfusion Pupura or Platelet Refactoriness. Detection of these antibodies in the blood of Multitransfused Patients may serve as predictive indicator of these clinical conditions. Prevalence of human platelet and leucocyte antibodies in multitransfused patients was studied in this research work.

Methods

The study was carried out at Federal Medical Centre, Owerri, Imo State. Study subjects were made up of 120 multitransfused patients (96 males and 24 females), with 60 non transfused individuals (40 males and 20 females) who served as control subjects. Their age bracket was between 18–50 years. Ethical clearance and informed consent were duely obtained. Valuable information were obtained by questionnaires.

Subjects who received units of blood ranging between 2-30 units were included in the study. Female multitransfused patients with history of pregnancy were excluded from the study. About 4mls of blood samples were collected from each subject. 2mls of the blood sample were dispensed into EDTA anticoagulant containers for investigation of some haematological parameters (platelet count, haemoglobin estimation, total white blood cell count and packed cell volume determination) using automated method. 2mls were dispensed into plain containers. On retraction of blood clot, the serum were separated into other plain containers for evaluation of alloantibodies using ELIZA method. Tests were run along with control samples.

Laboratory procedure

Reagents were commercially purchased and the manufacturer's standard operating procedures were followed.

Determination of human platelet and leucocyte antibodies.

The tests was carried out by Enzyme Linked Immunosorbent Assay (ELISA) method using Monoclonal Antibody Specific Immobilization of Platelet Antigen (MAIPA) assay kit[3], as modified by apDia Belgium. Test principle is based on the capture of platelet antigen using a mouse monoclonal antibody that reacts specifically with a single human platelet membrane glycoprotein, followed by binding of human antibodies to antigens on this glycoproteins and analysis of bound human immunoglobulin G by an ELISA immuno-assay[3].

MAIPA procedure

The uncoated microplates were arranged accordingly in an empty frame for dispensing test and control samples, leaving one plate as blank. Platelet samples were prepared by adding 50ul of the platelet sample into each well except the blank well. The samples were washed two times using cell buffer. The platelet samples were incubated for 30 minutes with both test and control serum samples in each well, except the blank well, and washed two times. The mixture of platelet samples and serum samples were incubated with monoclonal antibodies for 30 minutes. The plates were washed four times to remove unbound monoclonal antibodies and

emptied by inversion. Platelet lyses buffer was added to lyse and remove platelet debris. 100ul of lysates containing the complex, monoclonal antibody, glycoprotein and antibody were transferred into the well arranged goat anti mouse IgG coated microplates and incubated for 30 minutes at 37°c. The plates were washed six times to remove lysate proteins. 100 ul of peroxidase labeled goat antihuman IgG was added in each well, incubated and washed six times to remove unbound peroxidase labeled goat antihuman IgG. 100ul of TMB substrate was added and incubated for 15mins. 100ul of acid solution was added to stop color development. The absorbance of the final solution in the wells were measured at wavelength of 450nm in a microplate reader and results were recorded. Assay validation was based on OD (absorbance) of controls. Positive values had absorbance equal or greater than 1.500 while negative values had absorbances less than 1.500.

Determination of platelet and total WBC count, haemoglobin level and packed cell volume

Values of some haematological parameters (platelet count, packed cell volume, haemoglobin level and total white blood cell count) were determined by automated method.

Statistical analysis

Data obtained were analyzed with IBM SPSS statistics for windows, version 19.0. Analysis of variance(Fisher's analysis of variance), chi-square test and student's t-test were employed to investigate the relationship between variables. Values were given in percentage and mean±SD. Results were presented in Venn diagram and tables. Statistical significance was set at a confidence limit of 95%.

Results

Results are presented in venn diagram and tables as follows:

Prevalence of Alloantibodies against HPA and HLA (class 1) in Multitransfused patients.

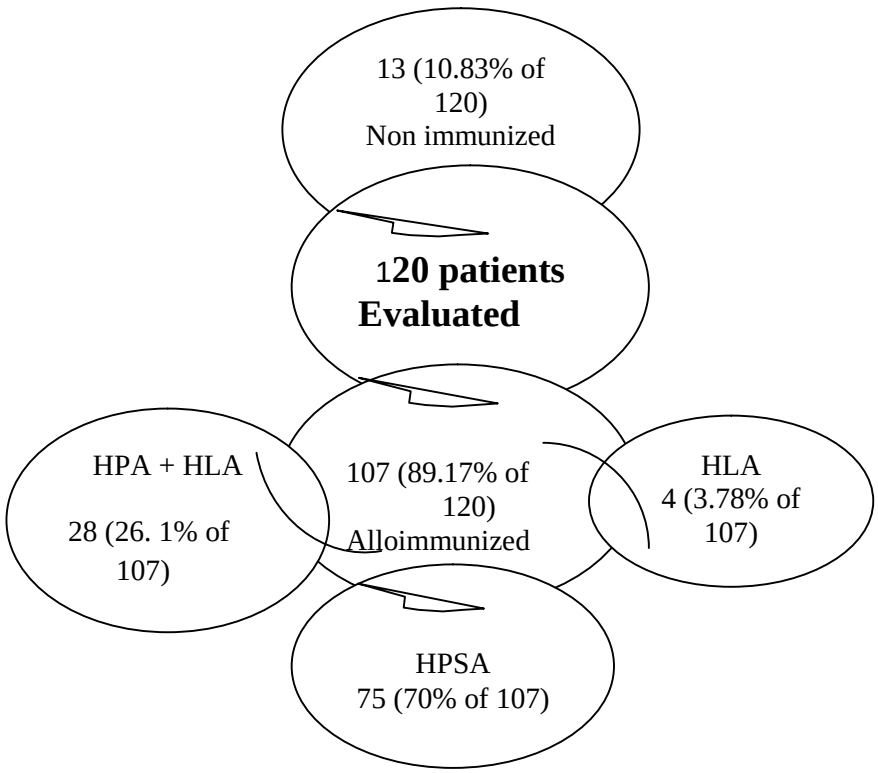

Figure 1: Venn diagram illustrating prevalence of alloantibodies against HPA and HLA Class I in Multitransfused patients.

Figure 1 reveals that out of 120 multitransfused subjects 107(89.17%) were alloimmunized, 13(10.83%) were not immunized. Out of 107 that were immunized, 75(70%) were alloimmunized against HPSA, 4(3,78%) were alloimmunized against HLA while 28(26.1%) were alloimmunized against both HPSA and HLA.

Table I: Prevalence of Alloantibodies against Glycoproteins IIb/IIIa, Ia/IIa, HLA and Ib/Ix in Multitransfused subjects.

Parameters Alloimmunization against	No Positive (%) alloimmunized	No Negative (%) Non-alloimmunized	Total No	X^2	P-Value
IIb/IIIa	97 (80.83%)	23 (19.17%)	120	248.00	0.000
Ia/IIa	46 (38.33%)	74 (61.67%)	120	232.25	0.000
HLA	32(26.67%)	88 (73.33%)	120	230.29	0.000
Ib/Ix	20 (16.67%)	100 (83.33%)	120	225.87	0.000

Table II: Mean ±SD value of alloantibodies against glycoproteins IIb/IIIa, Ia/IIa, HLA and Ib/Ix in Multi-transfused Subjects compared with non transfused Subjects.

Parameters Alloantibodies against GPs	Test (MTPs) n=120	Control (NTIs) n=60	t-value	p-value
IIb/IIIa	1.59±0.35	0.98± 0.21	7.4	0.000
Ia/IIa	1.35± 0.48	0.98± 0.27	3.4	0.001
HLA	1.26± 0.40	1.11± 0.27	1.6	0.123
IB/IX	1.15± 0.35	1.00±0.10	1.8	0.075

Key: GPs = Glycoproteins
MTPs = Multitransfused patients
NTIs = Non-transfused individuals

Table II reveals that there is a statistically significant difference mean ± SD level of alloantibodiesproduced against gpIIb/IIIa (1.59 ± 0.35) and gpIa/IIa (1.35 ± 0.48) in MTPs compared with the mean ± SD level of alloantibodies produced against gpIIb/IIIa (0.98 ± 0.21) and gpIa/IIa (0.98± 0.27) in non transfused individuals (P = 0.000 and 0.001).

There is no statistically significant difference between the mean ± SD level of alloantibodies produced against HLA class I (1.26 ± 0.40) and gpIb/Ix (1.15 ± 0.35) in MTP compared with mean ± SD level of alloantibodies produced against HLA class I (1.11 ± 0.27) and gp Ib/Ix (1.00 ± 0.075) in non-transfused individuals (P = 0.123 and 0.075).

Table III: Mean ± SDvalue of PCV, Hb, TWBC and Platelet in alloaimmunizedmultitransfused subjects compared with Non Immunized multitransfused Subjects

Parameters	Immunized Subjects n =107	Non Immunized Subjects n = 13	t-value	p-value
PCV (l/l)	0.27±0.05	0.27± 0.04	0.07	0.942
Hb (g/l)	91.86± 15.74	89.93± 15.66	0.52	0.602
TWBC ($x10^9$/l)	4.33± 0.97	4.64± 1.14	1.18	0.241
Platelet ($x10^9$/l)	103.43± 27.63	234.95±72.78	14.62	0.000

Key: PCV = Packed Cell Volume, Hb = Haemoglobin, TWBC = Total White Blood Cell

Table III reveals that there is no statistically significant difference between the mean and standard deviation of PCV (0.27±0.05l/l), Hb (91.86 ±15.74g/l), and TWBC (4.33 ±0.97 x10^9/l), in alloimmunized subjects and compared with mean ± SD value of PCV (0.27 ±0.04l/l), Hb (89.93± 15.66g/l), and TWBC (4.64±1.14x10^9/l), respectively in non immunized subjects (P = 0.942, 0.602 and 0.241). There is statistically significant difference between the mean ± SD value of platelet count (103.43 ± 27.63x10^9/l) in alloimmunized subjects compared with mean ± SD value of platelet count (234.95 ±72.78 x10^9/l) (P = 0.000).

Table IV: Mean ±SD value of alloantibodies against glycoprotein IIb/IIIa, Ia/IIa, HLA and IB/IX in Multi-transfused Patients in Relation to Units of Blood

Parameter	2-4 units n = 29	5-7 units n= 72	>7 n = 6	f-value	p-value
IIb/IIIa	1.7±0.30	1.64±0.22	1.74±00.86	0.75	0.481
Ia/IIa	1.48±0.48	1.51±0.48	1.2±0.44	0.47	0.631
HLA	1.50±0.42	1.43±0.25	1.51±0.67	0.13	0.881
IB/IX	1.17±0.42	1.27±0.38	1.22±0.44	0.21	0.810

Table IV reveals that there is statistically no significant difference in the mean ± SD value of alloantibodies against glycoprotein IIb/IIIa, Ia/IIa, HLA and Ib/Ix in multitransfused patients base on number of units of blood.

For IIb/IIIa = 2 – 4 units – 1.7±0.30, 5 – 7units – 1.64±0.22, > 7 units – 1.74±00.86 (P = 0.481)
For Ia/IIa = 2 – 4 units – 1.48±0.48, 5 – 7units – 1.51±0.48, > 7 units – 1.2±0.44 (P = 0.631)
For HLA = 2 – 4units – 1.50±0.42, 5 – 7units – 1.43±0.25, > 7units –1.51±0.67 (P = 0.881)
For Ib/Ix = 2 – 4units – 1.17±0.42, 5 – 7units – 1.27±0.38, > 7units – 1.22±0.44 (P = 0.8100).

Discussion

In this study, there is high prevalence of alloantibodies against human platelet specific antigens in multitransfused subjects. Out of 120 multitransfused subjects that were studied, 107(89.17%) were alloimmunized. 75(70%) were alloimmunized against human platelet specific antigen only, 4(3.78%) were against HLA Class 1 only while 28(26.16%) were against both HPSA and HLA Class 1 (Fig 1).

The most frequent alloimmunization in this study was against glycoprotein 11b/111a 79(80.8%) being the glycoprotein location for HPA 1a[4]; followed by glycoprotein 1a11a 46(38.33%) (table I, II).

Glycoprotein 11b/111a is an integrin complex found on platelets. It is a receptor for fibrinogen and von Willebrand factor and aid in platelet activation, also glycoprotein 1b/ix and 1a/11a are receptors on the surface of platelets that can bind adhesive glycoprotein like von willebrand factor thereby supporting platelet adhesion. The antagonist of all these glycoproteins prevent the binding of fibrinogen thereby blocking platelet aggregation. This may lead to bleeding disorder. This finding is in agreement with the reports given by Jeremiah *et al* 2011 and Kenneth *et al*, 2011[5,6].

In this study, the value of platelet count in alloimmunized subjects is statistically significantly low in this study when compared with the values in non immunized subjects (table III). Alloantibodies to human platelet specific antigen and leucocyte antigens do not occur naturally but are acquired by exposure to alloantigens through blood transfusion and pregnancy. The alloantibodies produced may have caused damage to the transfused platelets thereby causing reduction in the expected number of platelets in the patient's blood. This may bring about platelet refractoriness which may lead to bleeding disorder.

The number of units of blood transfused were considered in this study. The multitransfused subjects were grouped according to the number of units of blood they received (Table IV). There is statistically no significant difference in the level of alloantibodies produced against the different glycoproteins evaluated among the alloimmunized patients in relation to the number of units of blood transfused. Sarkar *et al* (2015)[7] gave a contrary report noting that there is statistically significant increase in the level of alloantibodies produced in relation to the number of units of blood transfused. Platelet antibodies are known to be produced in patients receiving large quantities of whole blood, platelet rich plasma or platelet concentrate. The incidence of alloantibodies increases with the length of exposure to only foreign platelets and not to the units of blood transfused[8].

It can therefore be concluded here that there is high frequency of alloimmunization against human platelet specific antigens, glycoprotein 11b/111a (Hpa-1a,3a,4a) and glycoprotein 1a/11a (HPA 5b) in multitransfused patients in Owerri, Imo state. The frequency did not increase with the number of units of blood transfused.
The platelet reactive antibodies may result in shortening the survival of donated platelets thereby rendering the patients refractory to platelet transfusion.

Also immune complexes made up of anti - HPA - 1a and transfused soluble HPA -1a may bind non specifically to autologous platelets leading to platelet clearance through macrophages in the reticuloendothelial system.

These findings will help in safeguarding effective transfusion therapy, prediction of the severity of thrombocytopenia as well it's management in multitransfused patients.

Recommendations

1. There is need to carry out platelet antibody screening on patients before transfusion.
2. There is need to do platelet crossmatching before transfusing whole blood or blood platelet in order to guarantee the safety and efficiency of platelet therapy.
3. Patients should receive only the required component or components during transfusion instead of transfusing whole blood all the time.

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