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Alloantibodies response to Human platelet antigen and Leucocyte antigen class 1 in Multigravidas in Owerri, Nigeria

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Abstract

Alloantibodies pattern of response to Human Platelet Antigen and Leucocyte antigen class I were studied in multigravidas. Two hundred and fifty multigravidas within the age bracket of 18 and 65 years attending the obstetrics and gynecology clinic of Federal University Teaching Hospital Owerri (FUTHO) were recruited for the study. One hundred nulligravidas within the same age bracket served as control subjects. Ethical approval was duly obtained from the ethical committee of the hospital and the consent of the subjects were obtained. Valuable information was obtained by questionnaire. ELISA method was adopted for alloantibody determination. Data obtained were analyzed with IBM, SPSS: Chi-square test, student's t-test and 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 180(72%) out of 250 multigravidas tested were alloimmunized. There is statistically significant increase in the mean ± SD values of alloantibodies produced against gp IIb/IIIa (1.27 ± 0.48) and Ia/IIa (1.35 ± 0.54) in multigravidas compared with the values in nulligravidas (0.71 ± 0.17) and (0.69 ± 0.18) respectively. There is no difference statistically in the value of alloantibodies produced in gravidity 2 – 3 compared with the values produced in gravidity 4 – 5. There is statistically significant progressive increase from first trimester toward third trimester in the values of alloantibodies produced in multigravidas. Result indicates high frequency of alloimmunization in multigravidas (135(54%), with alloantibodies against Ia/IIa having highest frequency. These findings will help in proper antenatal and postnatal management of pregnant mothers in order to avoid conditions like foetal/neonatal alloimmune thrombocytopenia.

Keywords: alloantibodies, alloantigen, multigravidas, nulligravidas, 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. They express platelet specific antigen and other antigens like HLA class I antigens on their surface. These platelet antigens can be identified from the platelet membrane glycoproteins (gp IIb/IIIa, Ia/IIa, HLA and Ib/Ix) that carry them. When an individual blood platelet drops below 150 x 10^9/L, a condition called thrombocytopenia ensues. Pregnancy can expose an individual to allogenic human platelet antigen and elicit antibody response.[2] Alloantibodies against human platelet antigens can be responsible for clinical conditions like Fetal or neonatal alloimmune thrombocytopenia (FNAIT).[3]

Specific Objectives

1. To determine the prevalence of alloantibodies to HPAs and HLA Class I in multigravidas.
2. To investigate the relationship between platelet antibodies produced in different gravidity of pregnancy.
3. To access the prevalence of platelet alloantibodies in different trimesters of pregnancy.

Methods

A total of 350 subjects(250 multigravidas (test subjects) and 100 nulligravidas (control subjects)),within the age bracket of 18 – 50yrs were recruited for this study. The study was carried out at Federal University Teaching Hospital Owerri Imo State. Ethical clearance and informed consent were obtained and valuable information were obtained by questionnaire. Sample size was calculated using the formular proposed by Daniel in 1999.

Multigravidas whose gravidity ranges from 2 to 10 were included in the study. Those that have history of blood transfusion were excluded from the study. About 3milliliters of blood samples were collected from the subjects and dispensed into plain containers. On retraction of blood clot, the serum samples were separated into other plain containers for evaluation of alloantibodies response to human platelet antigen and human leucocyte antigen class 1 using Eliza method.

Laboratory Procedures

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

Determination of Human Platelet and Leucocyte Antibodies

The test was carried out by Enzyme Linked Immunosorbent Assay (ELISA) method using Monoclonal Antibody-Specific Immobilization of Platelet Antigen (MAIPA) Assay kit[4], as modified by apDia Belgium. The test principle is based on the capture of a 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 glycoprotein and analysis of bound human Immunogloblin G by an ELISA immuno–assay.

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 either test or 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 andemptied 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 was 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.

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 were presented in venn diagram and tables as follows:

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

Parameters	Positive N (%)	Negative N (%)	Total No	X^2	P-Value
IIb/IIIa	115 (46%)	135 (54%)	250	238	0.011
Ia/IIa	135 (54%)	115 (46%)	250	238	0.011
HLA	20 (8%)	230 (92%)	250	215	0.000
Ib/Ix	50 (20%)	200 (80%)	250	205	0.000

Data were presented as number and percentage; N = number

Table I reveals that there isstatistically significant difference between the number of test subjects alloimmunized against glycoproteins IIb/IIIa, Ia/IIa, HLA and Ib/Ix and the number not immunized ($P < 0.05$).

Table II: Mean ±SD Values of Alloantibodies Produced in Multigravidas Compared with Nulligravidas

Parameters	Multigravidas	Nulligravidas Control	t-test	P – value
IIb/IIIa	1.27 ±0.48	0.71 ± 0.17	5.05	0.000
Ia/IIa	1.35 ± 0.54	0.69 ± 0.18	5.35	0.000
HLA	0.88 ± 0.37	0.78 ± 0.19	1.12	0.269
Ib/Ix	0.92 ± 0.48	0.75 ± 0.22	1.48	0.144

Table II reveals that there is statistically significant increase in the mean ±SD values of alloantibodies against gp IIb/IIIa and Ia/IIa produced in multigravidas compared with nulligravidas ($P = 0.000$).

There is statistically no significant difference in the mean ± SD values of alloantibodies produced against gpHLA and Ib/Ix in multigravidas compared with nulligravidas ($P = 0.269$ and 0.144) respectively.

Table III: Mean ± SD Values of Alloantibodies Produced in Multigravidas (Gravidity 2 -3) Compared with Multigravidas (Gravidity 4 – 5).

Parameters	Gravidity 2-3 (n = 185)	Gravidity 4-5 (n = 65)	t-test	P-value
IIb/IIIa	1.25 ± 0.45	1.39 ± 0.55	0.92	0.360
Ia/IIa	1.32 ± 0.53	1.38 ± 0.56	0.34	0.738
HLA	0.95 ± 0.43	0.73 ± 0.16	1.86	0.069
Ib/Ix	0.91 ± 0.51	0.97 ± 0.46	0.32	0.751

Result shows that there is statistically no significant difference in the level of all the alloantibodies produced in multigravidas (gravidity 2-3) compared with multigravidas (gravidity 4 – 5). Women whose gravidity is greater than or equal to 6 were not represented.

Table IV: Mean ± SD Values of Alloantibodies Produced in Multigravidas in Relation to Trimester

Parameters (Glyproteins)	1st Trimester N = 30	2nd Trimester N = 80	3rd Trimester N = 140	F-value	P-value
IIb/IIIa	0.95 ± 0.01	1.128 ± 0.19	1.441 ± 0.53	4.24	0.036
Ia/IIa	0.805± 0.01	1.197 ± 0.58	1.487 ± 0.46	4.34	0.034
HLA	0.653 ± 0.01	0.817 ± 0.12	0.939 ± 0.19	2.75	0.112
Ib/Ix	0.815 ± 0.02	0.845 ± 0.22	0.943 ± 0.40	1.59	0.250

Key: SD = Standard deviation, HLA = Human leucocyte antigen

Table IV reveals that there is statistically significant progressive increase in the mean ± SD value of alloantibodies produced against glycoprotein IIb/IIIa and Ia/IIa from first trimester toward third trimester. (P = 0.036 and 0.034). There is statistically no significant difference in the mean ± SD value of alloantibodies produced against glycoprotein HLA and Ibix in relation to trimester (P = 0.112 and 0.250).

Figure 1: Venn Diagram Illustrating Prevalence of Alloantibodies against HPAin Multigravidas.

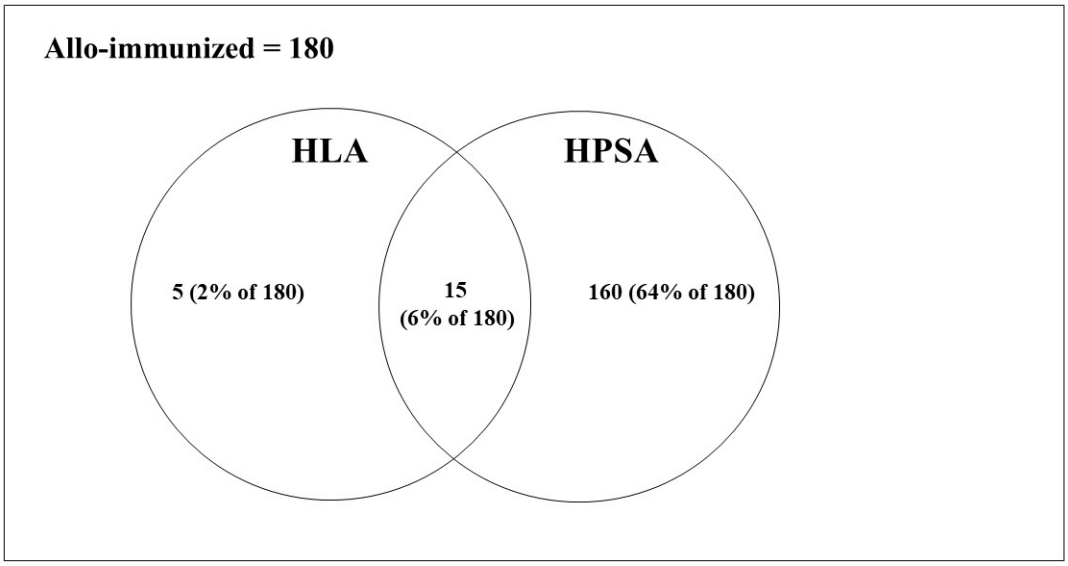

Figure 2: Venn Diagram Illustrating Prevalence of Alloantibodies against HPSA and HLA Class I in Multigravidas.

Discussion

There is high prevalence of alloantibodies against human platelet specific antigen in multigravidas. Out of 250 multigravidas that were studied, 180(72%) were alloimmunized, 70(28%) were not immunized. The most frequent alloimmunization in this study was against gpIa/IIa which the gp location for HPA-5a[5], followed by gpIIb/IIIa. The value of alloantibodies produced in multigravidas was found to be increased when compared with the value in nulligravidas.

Gp Ia/IIa and IIb/IIIa are integrin complex found on the surface of platelets. They are receptors of fibrinogen and can bind adhesive glycoprotein like von Willebrand factors thereby aiding in platelet adhesion. The antagonist of these glycoproteins prevents these functions and can block platelet aggregation[6].

This may lead to bleeding disorder. There is statistically no difference in the level of alloantibodies produced in multigravidas (Gravidity 2-3) compared with multigravidas (gravidity 4 -5). The incidence of alloantibody production increase with exposure to foreign platelet irrespective of gravidity of pregnancy. The level of alloantibodies produced against gpIIb/IIIa in Table III increased with trimester[7] recorded that the instance of feto-maternal haemorrhage has been observed in 7%, 16% and 29% of mothers during their first, second and third trimester of pregnancy respectively. Maternal alloimmunization occurs when the foetal platelet antigen inherited from the father which is different from that of the mother combine with maternal blood due to rupture of placental maternal barrier which often happen during delivery[7]. Most delivery occur in third trimester[8]. This may account for the increased level of alloantibodies produced in third trimester.

Conclusion

There is high frequency of alloimmunization against human platelet specific antigen (gpIa/IIa – Hpa 5a and gpIIb/IIIa – Hpa 1a, 3a, 4a) in multigravidas in Imo state. The frequency did not increase with gravidity, but was found to increase with trimester of pregnancy. These findings will help in proper antenatal and postnatal management of pregnant mothers in order to avoid clinical conditions like foetal/neonatal alloimmune thrombocytopenia.

Recommendations

1. There should be introduction of platelet antibody screening programme on antepartum women in the obstetric unit of the hospitals in Imo state, Nigeria.
2. There should be advances towards production of a prophylactic product that would be platelet equivalent of Rhesus immune globulin (Rhogam) in order to help prevent human platelet antigen associated fetal neonatal alloimmune thrombocytopenia.
3. There should be screening for fetomaternalhaemorrhage. This can serve as a paved way for further clinical studies.

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