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Gene Frequencies of Human Platelet Alloantigens in Major Ethnic Groups in Rivers-State, Nigeria

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Authors' contributions

This work was carried out in collaboration among all authors. Authors contributed in different ways to the success of this study. All authors read and approved the final manuscript.

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ABSTRACT

Aim: The aim of this study was to evaluate gene frequencies of human platelet alloantigens among major ethnic groups in Rivers-State, Nigeria.
Study Design: A cross-sectional study.
Place and Duration of Study: Rivers State University Medical Centre, Port Harcourt, Safety Molecular Pathology Laboratory, Enugu State, Justcare clinical laboratory Port Harcourt Rivers State and University of Port Harcourt Teaching Hospital Port Harcoourt, between October 2019 and March 2020.
Methodology: The subjects consisted of apparently healthy individuals who were of Rivers State origin totaling 104 persons aged 17 to 42 years. They were under-graduate and post-graduate students of Rivers State University, Port Harcourt, Nigeria. Five major ethnic groups were considered which included Ikwerre, Ogoni, Ijaw, Etche and Ogba. Their demographic information was collected using a sample register and a questionnaire. Samples were collected from the antecubital vein. 10ml of blood was collected, 5ml was transferred into EDTA sample bottle (Ethylene diamine tetracetic acid) while 2ml was dispensed into plain bottle and labeled accordingly. Serological testing including HIV (RVS) screening, HBsag, HCV and VDRL were all

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done immediately after samples were collected. The remaining sample was analyzed using genotyping of Human Platelet Antigens by High Resolution Melting Curve Analysis Polymerase Chain Reaction (HRM-PCR). The melt curve analysis was done using the MicPCR software while the frequency analysis was done using Number Cruncher Statistical Software (NCSS) Version 13. Graph Pad Prism Version 8.0.2 was used to determine the statistical significance between the various HPA genotypes and the ethnic groups and p-values of *<.05* were considered to be statistically significant. Results were presented in percentages, mean+/- standard deviation and in tables

Results: The results showed that HPA-4 and HPA-5 a/a and b/b were the highest among Etche (20.0% each), followed by HPA-2 and HPA-3 at 16% each for b/b and HPA-1 a/a and a/b had 10.0% and 6.7% respectively, and HPA-1 b/b, HPA-2 a/b and HPA-3 a/a had 3.3% respectively. The HPA pattern for Ijaw was highest at HPA-4 a/a (15.7%), followed by HPA-5 b/b (15.3%) and the least was HPA-2 b/b (11.4%). The pattern for Ikwerre was highest for HPA-5 b/b (20.0%) and least for HPA-4 b/b and HPA-2 a/a (0.8% each). The HPA pattern for Ogba was highest for HPA-5 b/b (20.0%) and least for HPA-3 a/a and HPA-2 a/a (0.0% each), while the pattern for Ogoni is highest for HPA-5 b/b (19.1%) and least for HPA-5 a/b and HPA-3 a/b (0.9%). The HPA alleles showed that HPA-1 a/b was highest. 42 (40.4%), followed by HPA-1 a/a 34 (32.7%) and HPA-1 b/b 28 (26.9%), and the least was 3% for HPA-2 a/b. The highest for HPA-2 was b/b, 60 (57.7%) and the least is a/a, 2 (1.9%), while the highest for HPA-3 was also b/b, 75 (72.1%) and the least was a/a 3 (2.9%). Also, HPA-4 a/a allele is the highest in its category, 85 (81.5%) and the least was a/b 9 (8.7%), while b/b, 102 (98.1%) was the highest for HPA-5 and there was none for a/b and a/a alleles.

Conclusion: The HPA alleles showed that HPA-1 a/b was highest. The highest for HPA-2 and HPA-3 was b/b. HPA-4 a/a allele was the highest in its category, while b/b was the highest for HPA-5. HPA-4 and HPA-5 a/a and b/b were the highest among Etche. The HPA pattern for Ijaw was highest at HPA-4 a/a. The pattern for Ikwerre was highest for HPA-5 b/b. The HPA pattern for Ogba was highest for HPA-5 b/b, while the pattern for Ogoni was highest for HPA-5 b/b.

Keywords: Gene frequencies; human platelet alloantigens; ethnic groups; Rivers-State; Nigeria.

1. INTRODUCTION

Human platelets, also known as thrombocytes, are small blood cells that originate from the bone marrow along the megakaryocyte lineage. Their major function in circulation is to prevent blood loss, and this they do by adhering to blood vessel wall and initiate blood clotting process that ultimately reduces or prevent blood loss. In addition, platelets mediate immune response and this has associated them with some clinical diseases. These biconvex-shaped cells have a very small size, in both diameter and volume [1-3].

Through co-operative biochemical interactions, platelets can communicate with, and are affected by other blood cells and endothelial cells [3].

Despite the vital role of platelets in haemostasis, their adhesion and aggregation at inappropriate sites may lead to thrombosis and vessel occlusion. In both men and women, heart attack and stroke are the leading cause of morbidity and mortality worldwide and are caused by thrombosis in the coronary or cerebral arteries. Thrombotic events may also occur in the placenta which may lead to miscarriage. Thus, platelets can be said to be versatile cells that play critical roles in multiple human diseases [4-6].

Allo-antigens of platelets are associated with platelets, and have the capability of inducing antibody formation during pregnancy or when platelet is transfused [7]. They result from mutations in the normal sequencing of platelet origin and have their associated proteins, glycoproteins, and can elicit immune responses. It results from the allo-immunization of a mother to paternal allo-antigens inherited by the foetus from the father. Platelet allo-immunization is similar to allo-antibodies that cross the placenta of a mother to the foetus to destroy its red blood cells (RBCs), resulting in haemolytic disease of the new born, but in this case of platelet alloimmunization the mother has circulating anti-platelet antibodies directed against the platelet-specific antigens of the foetus. The main differences between haemolytic disease of the new born and allo-immune thrombocytopenia in the foetus or neonate is that the former usually

occurs in the first pregnancy and destroys RBCs while the later affects subsequent pregnancies and destroys platelets. However, they both share the characteristic of the severity increasing with the subsequent pregnancies [8].

Platelet allo-antigens are involved with polymorphism of platelet surface glycoprotein and can elicit production of alloantibodies when individuals lacking a particular polymorphism are exposed via pregnancy or transfusion [9]. Immune response to platelet allo-antigens are involved in the pathogenesis of several clinical syndromes, including fetal or neonatal allo-immune thrombocytopenia (FNAIT), post transfusion purpura (PTP) and occasionally in unresponsiveness to platelet transfusion [10]. Alloimmune thrombocytopenia can also be an unusual complication in which donor lymphocytes make alloantibodies specific for the platelets produced by the recipient of an organ allograft [11].

Formation of HPA allo-antibodies are among the non-infectious risk of blood transfusion as well as pregnancy and are implicated in the pathogenesis of clinical conditions like post transfusion purpura (PTP), fetal/neonatal alloimmune thrombocytopenia (FNAIT), and refractoriness to platelet transfusion. However, the development of the HPA system allows the alleles of the antigens to occur in pairs (a and b), and the frequency of the alleles varies in different populations, which is very important in diagnosing, investigating and managing disease conditions that arise from defects in the system.

Considering that a previous study has been done on platelet antibodies among multiparous women using ELISA methods, it was deemed necessary to investigate further on platelet antigens using molecular methods. Therefore, the aim of this study was to evaluate gene frequencies of human platelet alloantigens in major ethnic groups in Rivers-State, Nigeria.

2. MATERIALS AND METHODS

2.1 Study Design

The subjects consisted of apparently healthy individuals who were of Rivers State origin totaling 104 persons aged 17 to 42 years. They were under-graduate and post graduate students of Rivers State University of Port Harcourt recruited during their pre-admission medical examination into the University. Five major ethnic groups were considered which included Ikwerre, Ogoni, Ijaw, Etche and Ogba. In the study 25 of the subjects were of the Ikwerre origin, 22 from Ogoni, 24 from Ijaw, 6 from Etche and 22 from Ogba region.

2.2 Study Area

The study was conducted in Port Harcourt. Port Harcourt City lies on the geographical coordinates of 4^0 47' 21' N, and 7^0 59' 54' E. Port Harcourt features tropical wet climates and heavy Rainy Season for most part of the year and very short dry seasons which is usually between December and January. Also the harmattan season which climatically influences so many cities in West Africa and Nigeria in particular is less pronounced in Port Harcourt. Port Harcourt's heaviest precipitation occurs during September with an Average rainfall of 367mm while in December which on the average is the driest month of the year has an average rainfall of 20mm. The temperature throughout the year in PH city is relatively constant, showing little variation throughout the course of the year. Average temperature is typically between 25^0C-28^0C in the city.

2.3 Study Population

A total of 104 Rivers State indigenes were randomly selected. They were from the five major ethnic groups as intended in this study. Ikwerre, Ogoni, Ijaw, Etche and Ogba regions. The Ikwerre ethnic group was 25 in number while Ogoni totaled 22, Ijaw was 28, Etche had 6 and the Ogba group was 23 persons. These subjects whose ages ranged from 16 to 42years were apparently healthy individuals recruited from the Rivers State University during their pre-admission medical examination into the undergraduate and post-graduate programme of the university. Consent was obtained from each participant verbally prior to blood collection. Their demographic information was collected using a sample register and a questionnaire.

2.4 Sample Size

Convenient sampling method was employed for this study and a total of 104 subjects were recruited based on available resources.

2.5 Eligibility Criteria

Rivers State indigenes that were negative to HIV, HBSAg, HCV, and VDRL, within the ages of 16-

55 were included in the research after obtaining their informed consent. Non-Rivers indigenes as well as those with deformities and tatoos, also those that tested positive to any of the serological test were excluded as well as those who refused to partake in the study.

2.6 Sample Collection and Handling

Samples were collected from the antecubital vein. Swab (Cotton wool soaked in 70% alcohol) was used to clean the skin. Syringe and needle were then used to draw blood from the vein. Blood was collected without delay as soon as the tourniquet was tied so as to avoid fluid shift and haemo-concentration as a result of venous blood stagnation. This is particularly important in investigations requiring platelet count to avoid platelet aggregation. 10ml of Blood was collected, 5ml was transferred into EDTA sample bottle (Ethylene diamine tetracetic acid) while 2ml was dispensed into plain bottle and labeled.

Serological testing including HIV (RVS) screening, HBsag, HCV and VDRL were all done immediately after samples were collected, followed by full blood count. The remaining sample for PCR was stored at 2 to 6^0 C for a period of not more than 48hrs prior to PCR analysis.

2.7 Sample Analysis

2.7.1 Genotyping of human platelet antigenes by high resolution melting curve analysis polymerase chain reaction (Hrm-Pcr)

The analysis required three stages which includes;

- Processing of peripheral blood to GITC lysate.
- Genomic DNA Extraction
- HRM-PCR

2.7.1.1 Processing of peripheral blood to GITC lysate (White Cell Lysate)

Principle: Several molecular assays requires nucleic acids from white cells. Red cell lysis using ammonium chloride solution provides a faster means of harvesting white cells. The white cell pellet is then lysed in Guanidium Isothiocyanate (GITC) solutions. The GITC lysate is stable for long term storage and can be used for various techniques of nucleic acid extraction including DNA and RNA. GITC requires activation by ß- mercapto-ethanol (BME).White cell pellet can also be lysed in Trizol reagent for nucleic acid extraction if there are enough samples but for 5ml samples, process for GITC lysate only.

Quality Control (QC) for GITC Lysate: GITC is toxic and was carefully handled by wearing protective hand gloves and laboratory coat. BME has a pungent odour and face mask was used during the procedure. It was also ensured that the blunt needles were discarded into the sharp bin. Before the process of GITC lysate sample, it was ensured that enough 1x RCLB was prepared and the pH was at 7.4 when the pH was not 7.4, HCL was added when alkaline and NAOH when it was acidic. It was ensured that enough ice was made available during the assay.

2.7.1.2 Extraction of genomic DNA using GP spin

Principle of Extraction: This is based on a spin column method of selective adsorption of genomic DNA to silica membrane followed by micro centrifugation to remove waste and elution of DNA using alkaline buffer. The unique feature of this kit used is that it has two different spin column (RNA and DNA). DNA column is Green and RNA column is Red. But our interest is only on the DNA Green Column. This DNA/RNA GP spin kit was manufactured by Genetic PCR solution, Alicante Spain cat. NO: 0401.100

Quality Control for Extraction of Genomic DNA: The purity of DNA sample is paramount in all PCR analysis and as such all working materials, work bench, equipment, was disinfected with 70% ethanol. Pipette tips, pipette holders as well as the pipette were sterilized. All the DNA extraction operations were done in the UV- safety Cabinet and it was ensured that the UV-light and Visible light of the UV-Cabinet was on and allowed to sterilize for 20 minutes prior to extraction procedures. Protective gloves were worn. SOP for the extraction protocol was followed accordingly. Appropriate waste disposal was followed. Face masks were worn before analysis.

2.7.1.3 Genotyping of HPA by high resolution melting curve analysis PCR (HRM-PCR)

Principle: the high resolution melting curve PCR technique for genotyping HPAs uses intercalating dyes e.g. Eva Green, LC green in a real time PCR system using the HRM software as a tool

for the analysis and employing thermal cycling profile as indicated for all PCR procedure.

Quality Control for HRM-PCR: Extreme carefulness and concentration was employed during pipetting to avoid mix up. It is a delicate procedure, the standard operating procedure was followed accordingly. Personal protective equipment (PPE) was worn and work surfaces cleaned with 70% ethanol.

2.8 Data Analysis

The melt curve analysis was done using the MicPCR software while the frequency analysis was done using Number Cruncher Statistical Software (NCSS) Version 13. Graph - Pad Prism Version 8.0.2 was used to determine the statistical significance between the various HPA genotypes and the ethnic groups. p-values of *<.05* were considered to be statistically significant. Results were presented in percentages, mean+/- standard deviation and in tables.

3. RESULTS AND DISCUSSION

This study recruited one hundred and four (104) participants ranging 16-42 years of age, that were without any diagnosed health condition (apparently healthy) from the major ethnic groups in Rivers State. This comprised of 25 Ikwerres, 28 Ijaws (consisting of the riverine sub-ethnicities of Kalabari, Okrika, Bonny, Opobo and Andoni), 23 Ogbas, 22 Ogonis and 6 Etches that were randomly selected Table 1.

On the basis of gender, there were 45 females and 59 males. In regard to the gender distribution of the participants (104 participants), 45 (43.3%) were females and 59 (56.7%) were males. Majority of them, 28 (26.9%) are of Ijaw ethnicity, 25 (24.0%) are Ikwerre, 23 (22.1%) are Ogba, 22 (21.2%) are Ogoni and 6 are Etche (Table 1 & 2).

Table 1. Frequency distribution of demographics

Variable	Category	Number	(%)	Remark
Sex	Female	45	43.3	
	Male	59	56.7	
Ethnicity	Etche	6	5.8	
	Ijaw	28	26.9	Highest
	Ikwerre	25	24.0	
	Ogba	23	22.1	
	Ogoni	22	21.2	

Table 2. Overall frequency distribution of human platelet antigen genotypes

Variable	Category	Number	(%)	Remark
HPA-1 T>C	a/a	34	32.7	
	a/b	42	40.4	Highest
	b/b	28	26.9	
HPA-2 C>T	a/a	2	1.9	
	a/b	42	40.4	
	b/b	60	57.7	Highest
HPA-3 T>C	a/a	3	2.9	
	a/b	26	25.0	Highest
	b/b	75	72.1	
HPA-4 G > A	a/a	85	81.7	Highest
	a/b	9	8.7	
	b/b	10	9.6	
HPA-5 G>A	a/b	2	1.9	
	b/b	102	98.1	Highest

C=Cytosine, T=Thyamine, G=Guanine, A= Adenine, > = Substitution resulting to polymorphism

In this study, the HPA types of the major ethnic groups in Rivers state were also considered. In regards to the Etche ethnic group, the HPA-4 and HPA-5 a/a and b/b had the highest, 20.0% each, followed by HPA-2 and HPA-3 with 16% each for b/b and HPA-1 a/a and a/b had 10.0% and 6.7$% respectively, and HPA-1 b/b, HPA-2 a/b and HPA-3 a/a had 3.3% respectively (Table 3). The finding in this study for the Etche population is in agreement with the report of Liu et al. [12] which mentioned HPA-1a as the commonest allele in HPA-1. However, their study was conducted among Caucasians, which could indicate some degree of haematologic similarity between the Etche and some Caucasian populations.

The frequency of HPA pattern for the Ijaw ethnic group in our study revealed that the highest was HPA-4 a/a, 15.7%, followed by HPA-5 b/b, 19.3%, HPA-3 b/b, 15.0% and HPA-2 b/b, 11.4% (Table 4). The b/b allele was obviously dominant, in consonance with the finding of Willem et al. [13] among their Caucasian population. HPA-2 a/b is 8.6% and HPA-4 a/b 2.1%. This finding was generally in concordance with the finding of Davoren et al. [14] which mentioned that the heterozygous alleles were usually lower in frequency among the population, while the homozygous alleles are usually more, especially, the b/b allele.

The HPA pattern for Ikwerre ethnic group was also assayed, with HPA-5 b/b, 20.0%.was the highest, followed by HPA-4 a/a 16.8% and HPA-1 a/b 12.0% (Table 5). The finding of high homozygosity in HPA-4 a/a and HPA-5 b/b in this study concurs with that of Brouk and Quelaa [15], does not agree with the heterozygousity of the HPA-1 a/b. However, this study did not observe frequencies for HPA-5 a/a, a/b, and HPA-3 a/a respectively, while those of HPA-4 b/b and HPA-2 a/a had 0.8% each. As previously mentioned, not all alleles are found in all populations, with the reason being attributed to some factors that influence the occurrence of HPA alleles among population, while it may also be due to yet to be documented alleles within the population.

For the Ogba ethnic group, the HPA pattern revealed that 20.0% were HPA-5 b/b, 16.5% each for HPA-4 a/a and HPA-b/b were the highest in descending order (Table 6). This finding is consistent with homozygosity, which is in agreement with the observation of Willem et al. [13]. However, homozygosity was also observed in some types, such as 0.9% each for HPA-3 a/a and HPA-2 a.a, but these were the least in frequency. This finding agree with what Risson et al. [16] reported in their finding that some homozygosity is inevitable among some less commonly occurring forms of HPA, but heterozygosity predominates in these less common forms.

The HPA pattern of the Ogoni ethnic group was also considered in this study and showed that HPA-5 b/b with 19.1% was the highest, further thrusting the path of homozygosity of the 'b' allele (Table 7). This frequency was closely followed by 15.5% for HPA-4 a/a, HPA-3 b/b and HPA-2 b/b respectively' all depicting homozygosity, though, of different alleles, but mainly of the 'b'. All of these observations subscribe allegiance to the finding of Yurdakok [17], Bertrand et al. [8] and Willem et al. [13].

This study also observed that HPA-1 a/a, a/b and b/b had 7.3%, 6.4% and 6.4% respectively, while HPA-3 a/b and HPA-4 b/b had 3.6% each and HPA-5 a/b and HPA-3 a/b were the least at 0.9%. These findings are partially consistent with the findings of both Yurdakok et al. [17] and Willem et al. [13]. This inconsistency can be attributed to variation in the factors that influence HPA production and distribution among populations. Factors such as genetics and environment have been opined to play crucial roles in the varied distribution of HPA among different populations.

Table 3. Frequency of human platelet antigen genotypes in Etche

HPA type	a/a	a/b	b/b
HPA-1 T>C	10.0%	6.7%	3.3%
HPA-2 C>T	0.0%	3.3%	16.7%
HPA-3 T>C	3.3%	0.0%	16.7%
HPA-4 G>A	20.0%	0.0%	0.0%
HPA-5 G>A	0.0%	0.0%	20.0%

C=Cytosine, T=Thyamine, G=Guanine, A= Adenine, >= Substitution resulting to polymorphism

Table 4. Frequency of human platelet antigen genotypes in Ijaw

HPA type	a/a	a/b	b/b
HPA-1 T>C	7.9%	7.1%	5.0%
HPA-2 C>T	0.0%	8.6%	11.4%
HPA-3 T>C	0.0%	5.0%	15.0%
HPA-4 G>A	15.7%	2.1%	2.1%
HPA-5 G>A	0.0%	0.7%	19.3%

C=Cytosine, T=Thyamine, G=Guanine, A= Adenine, >= Substitution resulting to polymorphism

Table 5. Frequency of human platelet antigen genotypes in Ikwerre

HPA type	a/a	a/b	b/b
HPA-1 T>C	3.2%	12.0%	4.8%
HPA-2 C>T	0.8%	10.4%	8.8%
HPA-3 T>C	0.0%	9.6%	10.4%
HPA-4 G>A	16.8%	2.4%	0.8%
HPA-5 G>A	0.0%	0.0%	20.0%

C=Cytosine, T=Thyamine, G=Guanine, A= Adenine, >= Substitution resulting to polymorphism

Table 6. Frequency of human platelet antigen genotypes in Ogba

HPA type	a/a	a/b	b/b
HPA-1 T>C	7.0%	7.0%	6.1%
HPA-2 C>T	0.9%	9.6%	0.6%
HPA-3 T>C	0.9%	2.6%	6.5%
HPA-4 G>A	16.5%	1.7%	1.7%
HPA-5 G>A	0.0%	0.0%	20.0%

C=Cytosine, T=Thyamine, G=Guanine, A= Adenine, >= Substitution resulting to polymorphism

Table 7. Frequency of Human platelet antigen genotypes in Ogoni

HPA type	a/a	a/b	b/b
HPA-1 T>C	7.3%	6.4%	6.4%
HPA-2 C>T	0.0%	4.5%	15.5%
HPA-3 T>C	0.9%	3.6%	15.5%
HPA-4 G>A	15.5%	0.9%	3.6%
HPA-5 G>A	0.0%	0.9%	19.1%

C=Cytosine, T=Thyamine, G=Guanine, A= Adenine, >= Substitution resulting to polymorphism

4. CONCLUSION

The HPA alleles showed that HPA-1 a/b was highest, followed by HPA-1 a/a 34 and HPA-1 b/b, and the least was HPA-2 a/b. The highest for HPA-2 was b/b, and the least was a/a, while the highest for HPA-3 was also b/b, and the least was a/a. Also, HPA-4 a/a allele is the highest in its category, and the least was a/b, while b/b, was the highest for HPA-5 and there was none for a/b and a/a alleles. This HPA-4 and HPA-5 a/a and b/b were the highest among Etche, followed by HPA-2 and HPA-3. The HPA pattern for Ijaw was highest at HPA-4 a/a, followed by HPA-5 b/b and the least was HPA-2 b/b. The pattern for Ikwerre was highest for HPA-5 b/b and least for HPA-4 b/b and HPA-2 a/a. The HPA pattern for Ogba was highest for HPA-5 b/b and least for HPA-3 a/a and HPA-2 a/a, while the pattern for Ogoni is highest for HPA-5 b/b and least for HPA-5 a/b and HPA-3 a/b.

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CONSENT

All authors declare that 'written informed consent was obtained from the patient (or other approved parties) for publication of this case report and accompanying images. A copy of the written consent is available for review by the Editorial office/Chief Editor/Editorial Board members of this journal.

ETHICAL APPROVAL

All authors hereby declare that all experiments have been examined and approved by the appropriate ethics committee and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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