International Blood Research & Reviews

12(3): 23-31, 2021; Article no.IBRR.68552
ISSN: 2321–7219

Assessment of Gene Frequencies of Human Platelet Alloantigens in Rivers-State, Nigeria Based on ABO/Rhesus Blood Groups Distribution

J. E. Wenah-Emmanuel[1*], E. M. Eze[1], E. O. Nwachuku[1], E. Wenah[2] and Z. A. Jeremiah[1]

[1]Department of Medical Laboratory Science, Rivers State University, Port Harcourt, Nigeria.
[2]Department of Microbiology, University of Port Harcourt Teaching Hospital, Rivers State, Nigeria.

Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/IBRR/2021/v12i330152
Editor(s):
(1) Dr. Dharmesh Chandra Sharma, J. A. Groups of Hospital and G. R. Medical College, India.
Reviewers:
(1) Bruno Miguel Barbosa da Costa, Tâmeg,a e Sousa Hospitalar Center, Portugal.
(2) Dibyendu Banerjee, West Bengal University of Health Sciences. India.
(3) Mohammed Suleiman Obsa, Wolaita Sodo University, Ethiopia.
Complete Peer review History: http://www.sdiarticle4.com/review-history/68552

Original Research Article

Received 10 March 2021
Accepted 19 May 2021
Published 21 May 2021

ABSTRACT

Aim: The aim of this study was to assess the Gene Frequencies of Human Platelet Alloantigens in Rivers-State, Nigeria based on ABO/Rhesus blood groups distribution
Study Design: A randomized controlled trial.
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, 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 of Port Harcourt. 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

**Corresponding author: E-mail: justinawenaheemanuel@gmail.com;*

including HIV (RVS) screening, HBsag, HCV and VDRL were all as part of the inclusion criteria 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), while tile method also known as forward/cell grouping method which is based on haem-agglutination reaction was used for ABO/Rh blood grouping. The melt curve analysis was done using the MicPCR software while the frequency analysis was done using Number Cruncher Statistical Software (NCSS) Version 13. GraphPad 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 < 0.05 were considered to be statistically significant. Results were presented in percentages, mean+/- standard deviation and in tables

Results: The results showed that the A^+ blood group had highest frequencies of 19.2% and 17.7% for HPA-5 b/b and HPA-4 a/a, while the least was 0.8% each for HPA-3 a/a, HPA-4 b/b and HPA-5. For blood group B^+, the highest were 20.0% (HPA-5 b/b) and 16.7% (HPA-3 b/b), and the least were 5.0% each for HPA-1 b/b and HPA-4 a/b, while blood group B^- had highest frequencies for HPA-1 a/a, HPA-2 b/b, HPA-3 b/b, HPA-4 a/b and HPA-5 b/b (20.0% each). The blood group O^+ HPA gene patterns had their highest values at 19.7% (HPA-5 b/b), 16.5% (HPA-4 a/a) and 13.7% (HPA-3 b/b) and the least was 7.9% (HPA-1 a/b), while for the blood group O^-, the highest was observed for HPA-3 b/b and HPA-5 b/b (20.0% each) and the least for HPA-1 a/a and a/b, HPA-2 a/b and b/b, and HPA-4 a/b and b/b (10.0% each).

Conclusion: Based on the results, we conclude that A^+ blood group had highest HPA frequencies. Whilst, the highest for blood group B^+ were (HPA-5 b/b) and (HPA-3 b/b), and blood group B^- had highest frequencies for HPA-1 a/a, HPA-2 b/b, HPA-3 b/b, HPA-4 a/b and HPA-5 b/b. The blood group O^+ HPA gene patterns had their highest values (HPA-5 b/b), (HPA-4 a/a) and (HPA-3 b/b) and the least was (HPA-1 a/b), while for the blood group O^-, the highest was observed for HPA-3 b/b and HPA-5 b/b and the least for HPA-1 a/a and a/b, HPA-2 a/b and b/b, and HPA-4 a/b and b/b.

Keywords: Gene frequencies; human platelet alloantigens; Rivers-State; Nigeria; ABO/Rhesus blood groups distribution.

1. INTRODUCTION

Platelets express a variety of immunogenic markers on the cell surface. Some of these antigens are shared with other cell types as in the case of Human Leukocyte Alloantigens (HLA) or blood group (ABO) antigens whereas others are specific to platelets. Those antigens specific to platelets are the so called platelet specific alloantigens or human platelet alloantigens (HPA). Some of these platelet-specific markers can be recognized by autoantibodies or by antibodies induced by certain drugs and still others by antibodies made by pregnant women or recipient of blood transfusions [1-2].

These biconvex-shaped cells have a very small size, in both diameter and volume [3-5]. The inactivated platelet is biconvex and discoid in structure, about 3.0 X 0.5um in diameter with a mean volume of 7-11 fl [3-5]. Platelets contribute to haemostasis by producing a platelet plug and reinforcing the plug strength by the action of thrombin converting fibrinogen to fibrin strands. To execute their unique tasks, platelets have surface receptors that can bind adhesive glycoproteins; these include the GP1b/1X/V complex which support platelet adhesion by binding Von Willebrand factor, especially under conditions of high shear in the arterioles, and the GP11b/111a receptor, which is platelet specific and mediates platelet aggregation by binding fibrinogen and / or Von Willebrand factor [5]. There are other receptors that also contribute to platelet adhesion which are the integrin a2B1 (GP1a/11a) GPV1, collagen, fibrinogen and laminin [5]. Platelets can also act as store houses for a variety of molecules that affect platelet function, inflammation, innate immunity, cell proliferation, vascular tone, fibrinolysis and healing of wound: these agents are actively released upon platelet activation.

The ABO blood group system was developed in 1900 by the Austrian scientist, Karl Landsteiner as a consequence of the knowledge of the blood group antibodies in humans, specifically, and other mammals [6]. The system, in addition to its sister component, Rhesus blood group, has gained prominence in medical practice and human health. The ABO blood is relevant in almost all situations that require blood transfusion, such as in trauma, surgery and pregnancy, disease conditions like thalassemia

and other haemoglobinopathies, and all of these are crucial for survival and the reason blood and blood products require utmost attention ranging from matching to transfusion and monitoring of likely reactions. Components of the system vary among individuals, with some being prominent in some populations. For instance, proper matching of the ABO blood group among individuals have accounted for huge safety in situations of blood transfusion [7] and the fatality that may result from incompatibility.

A condition may also arise where a patient lack part or all of a particular platelet glycoprotein because of defective alleles of the GP– encoding genes. Such deficiency can result in bleeding disorders. For example lack of GP1b – V -1X (CD42 a – c) results in Bernard– Soulier Syndrome while the absence of GP11 (CD41) and GP 111a (CD61) causes Glanzman thrombasthenia.

Platelet glycoprotein GPIV (CD36) is expressed on different human cells including platelet macrophages, capillary endothelium, erythroblast and adipocytes. Some apparently normal individuals lack CD36 on their platelet (type 11 deficiency) or platelets and monocytes (type 1 deficiency). Some study on CD36 deficiency suggests that it may confer protection from malaria and has been shown to be a receptor for red cells infected with plasmodium falciparum. However, another report suggests CD36 deficiency may actually increase the risk for more severe forms of malaria infection [2]. The role of CD36 deficiency as either a protection or aggravating factor in malarial infection remains [8-9]. In type 1 CD36 –deficient individuals can become immunized via transfusion or pregnancy can make iso-antibodies against CD36 that have been implicated in cases of FNAIT, PTP and platelet transfusion refractoriness [2]. Other disease conditions that may result from platelet associated allo-immune abnormalities are passive allo-immune thrombocytopenia, drug-induced immune thrombocytopenia and transplantation-associated allo-immune thrombocytopenia [10-11]. Therefore, the aim of this study was to assess the Gene Frequencies of Human Platelet Alloantigens in Rivers-State, Nigeria based on ABO/Rhesus blood groups distribution

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.

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 region. 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 3ml into another EDTA sample container.

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 samples for PCR were 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 included;

- 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.

3.7.1.3 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.

2.7.2 ABO blood grouping

2.7.2.1 Method: Tile method

Principles

The ABO blood grouping system is based on haem-agglutination reaction. When red blood cells carrying antigens are exposed to the corresponding antibodies, they interact with each other to form visible agglutination or clumping.

2.7.3 Rh blood grouping

2.7.3.1 Method: Tile method

Principle

Is based on the principle of haem-agglutination, the red cells with Rh antigen (D antigen) will clump with anti-D serum at room temperature in presence of protein.

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 as well as the ABO / 'Rh' blood group. p-values of < 0.05 were considered to be statistically significant. Results were presented in percentages, mean+/- standard deviation and in tables.

3. RESULTS AND DISCUSSION

The ABO blood group distribution pattern among the five major ethnic groups in Rivers state as observed in this study was O & gt; A & gt; B & gt; AB: Bllod group O being the predominant ABO blood group type amongst the Rivers people and this order of distribution which agrees with Fang and colleagues [12] and Rhesus blood group of the participants reveal that 63 (60.6%), being the highest, are O^+, followed by A^+, while A^-, AB^+ and AB^- lacked representation respectively (Table 1). The high positivity of Rhesus, observed in this study is contrary to the finding of Lin-Chu and colleagues [13] cited in Apecu and colleagues [14] which reported high negativity among Africans, African-Americans and Asians, while reporting high positivity among Caucasians. The finding in this study, however, is in tandem with that of Iyiola and colleagues [15] which also reported the O blood group as the most prevalent, followed by AB, while this study observed blood group A as the second highest in prevalence. This variation may have been occasioned by difference in climate, altitude, culture and genetics between the different settings in which the studies were conducted.

On the basis of difference in blood group types in relation to the HPA types, this study noted the following observations. The frequencies for A^+ blood group in this study shows that the highest were 19.2%, 17.7%, 15.4% and 14.6% for HPA-5 b/b, HPA-4 a/a, HPA-2 b/b and HPA-3 b/b respectively, but HPA-1 a/b and a/a had 9.2% and 6.2% respectively, while the least 0.8% for HPA-3 a/a, HPA-4 b/b and HPA-5 a/b each (Table 2). This observation is in tandem with the findings of Abrahamson and colleagues [16] which mentioned the homozygosity of most HPA types among individuals with blood group A^+, which they described to be mainly consistent with the 'b' allele and that other blood group types are mostly skewed towards heterozygosity.

The HPA gene pattern for blood group B^+ was highest, 20.0%, for HPA-5 b/b, while HPA-3 b/b and HPA-4 a/a followed with 16.7% and 13.3% each (Table 3). Homozygosity, especially, of the 'b' allele is clearly visible here. Interestingly these observations are in concordance with the findings of Xu and colleagues [17] and Kupatawintu and colleagues [18].

These findings were also reported from Asian populations, where Willem and colleagues [1] studies of this kind mostly emanate from. This

does not in any way imply that other regions do not conduct similar study, but they may not have been properly documented for electronic usage by scholars, thus, restricting access to them. Other allo-antigen patterns observed in this study are 11.7% for HPA-2 b/b, 8.3% for HPA-1 a/a, 6.7% each for HPA-1 a/b and HPA-2 b/b, and 5.0% each for HPA-1 b/b and HPA-4 a/b. All of these show trends of homozygosity, but for HPA-1 a/b and HPA-4 a/b that are heterozygous, thus confirming the mostly homozygous nature of the HPA alleles, as stated by Willem and colleagues. [1].

With regard to blood group B^- and its association to the HPA types, this study observed that HPA-1 a/a, HPA-2 b/b, HPA-3 b/b, HPA-4 a/b and HPA-5 b/b had the highest frequencies, at 20.0% each (Table 4). Almost all of the HPA types were homozygous for the 'b' allele, except HPA-1, which was homozygous for the 'a' allele and HPA-4 that was heterozygous (a/b). This finding confirms the predominantly homozygous nature of the 'b' allele but also shows that not only the 'b' allele has the possibility of being homozygous, as heterozygosity was also observed. This, in part, agree with the findings of Willem and colleagues [1] but completely agree with that of Serrarens-Janssen et al. [19], which observed homozygosity of the 'a' allele in a Mexican study, but confirms that the fact that homozygosity is not peculiar to the 'b' allele and heterozygosity has the potential to surface, even in populations that are perceived to be predominantly homozygous.

Blood group O^+ is considered in relation to the HPA gene frequencies among population. The highest observed in this study was 19.7% for HPA-5 b/b, followed by 16.5% for HPA-4 a/a and 13.7% for HPA-3 b/b (Table 5). As can be observed, the homozygosity trend is skewed towards the 'b' allele, but for HPA-4, where the 'a' allele was visible. The predominant homozygous nature of the 'b' allele have been constantly mentioned by several investigators, including Li and colleagues [20] and is thus in tandem with the observation in this study. Other observations of association of HPA with the O^+ blood group are 9.8% each for HPA-2 a/b and b/b respectively and 6.0% each for HPA-1 a/b and HPA-1 b/b, while HPA-1 a/b had 7.9%. The observation was an even distribution between homozygosity and heterozygosity. Even with respect to homozygosity, there tends to be an equal distribution between the 'a' and 'b' alleles in relation to this blood group. The finding fairly agrees with that of Liu *et al*. [21] in a study conducted among the Korean population. The authours of the Korean study were of the opinion that, owing to closely knit cultural inclination that allows relatives to intermarry, existent HPA types tend to continue expressing itself in a recurring manner. This trend may also be overt in other societies like China, that shares similar cultural and environmental leaning.

Table 1. Frequency distribution of demographics

Variable	Category	Number	Percent (%)	Remark
Sex	Female	45	43.3	
	Male	59	56.7	
Blood Group (ABO Rh)	A+	26	25.0	
	A-	0	0	
	B+	12	11.5	
	B-	1	1.0	
	AB+	0	0	
	AB-	0	0	
	O+	63	60.6	Highest
	O-	2	1.9	

Table 2. Blood group A^+

HPA type	a/a	a/b	b/b
HPA-1 T>C	6.2%	9.2%	4.6%
HPA-2 C>T	0.0%	4.6%	15.4%
HPA-3 T>C	0.8%	4.6%	14.6%
HPA-4 G>A	17.7%	1.5%	0.8%
HPA-5 G>A	0.0%	0.8%	19.2%

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

Table 3. Blood group B^+

HPA type	a/a	a/b	b/b
HPA-1 T>C	8.3%	6.7%	5.0%
HPA-2 C>T	1.7%	6.7%	11.7%
HPA-3 T>C	0.0%	3.3%	16.7%
HPA-4 G>A	13.3%	5.0%	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 4. Blood group B^-

HPA type	a/a	a/b	b/b
HPA-1 T>C	20.0%	0.0%	0.0%
HPA-2 C>T	0.0%	0.0%	20.0%
HPA-3 T>C	0.0%	0.0%	20.0%
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 5. Blood group O^+

HPA type	a/a	a/b	b/b
HPA-1 T>C	6.0%	7.9%	6.0%
HPA-2 C>T	0.3%	9.8%	9.8%
HPA-3 T>C	0.6%	5.7%	13.7%
HPA-4 G>A	16.5%	1.3%	2.2%
HPA-5 G>A	0.0%	0.3%	19.7%

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

Table 6. Blood group O^-

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

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

The HPA gene pattern in association with blood group O^- was considered in this study and shows that HPA-3 b/b and HPA-5 b/b were the highest at 20.0% (Table 6). The observation agrees with the findings of Liu and colleagues [21] in respect to the dominance in homozygosity of the 'b' allele but differs in that of the 'a' allele. However, it agrees with findings from other investigators in Vietnam and Thailand [18, 21]. The least frequency in this study was observed as 10.0% and recorded for HPA-1 a/a and a/b, HPA-2 a/b and b/b, and HPA-4 a/b and b/b. Apart from the homozygous 'a' allele observed for HPA-1 a/a, This observation is in concordance with the finding of Halle and colleagues [21].

4. CONCLUSION

Based on the results, we conclude that A^+ blood group had highest HPA frequencies, with HPA-5 b/b and HPA-4 a/a, while the least was for HPA-3 a/a, HPA-4 b/b. Whilst, the highest for blood group B^+ were (HPA-5 b/b) and (HPA-3 b/b), and blood group B^- had highest frequencies for HPA-1 a/a, HPA-2 b/b, HPA-3 b/b, HPA-4 a/b and HPA-5 b/b. The blood group O^+ HPA gene patterns had their highest values (HPA-5 b/b), (HPA-4 a/a) and (HPA-3 b/b) and the least was (HPA-1 a/b), while for the blood group O^-, the highest was observed for HPA-3 b/b and HPA-5 b/b and the least for HPA-1 a/a and a/b, HPA-2 a/b and b/b, and HPA-4 a/b and b/b.

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.

ACKNOWLEDGEMENTS

Authors wish to acknowledge Mrs. Evelyn Tony-Enwin and Staff of Rivers State University Medical Centre for their efforts in sample collection and Akekua Tracy of Justcare Clinical Laboratory for assisting in sample sorting and serological testing. Authors also appreciate Dr. Emmanuel Nna, Director of Safety Molecular Pathology Institute for the PCR Assay and part of the data analysis and Dr. S.G. Christian for also assisting in data analysis.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Willem H, Michael FM, David LA. Human platelet and Neutrophil Antigens In: Practical Transfusion Medicine. Michael FM, Derwood F. (Eds) Blackwell Science Ltd Oxford USA. 2013;51-55.
2. Coppage M, Stroneck D, Mcfarland J, Blumberg W. Human Leukocyte and Platelet antigens In: Williams Haematology Kenneth K, Marshall AL, Josef TP, Marcel ML, Oliver WP, Lind JB, Michael AC. 9th Edition Mc Craw Hill Education New York. 2016;2359 – 2362.
3. Machls KR, Thn N, Itaia JE. Interpreting the developmental dance of the megakaryocyte: a review of the celllnd molecular processes mediating platelets formation. Br J Haem. 2014; 165(2): 227-36.
4. Hoffbrand AV, Moss PAH. (Eds). Platelets, blood coagulation and haemostasis in Hoffbrands Essential Haematology. 7th Ed. Blackwell Publishing USA. 2016;264-268.
5. Sussan S, Sidney W, Joseph E, Italiano J, Paul B, Barry SC. Platelet Morphology, Biochemistry and Function In: William Haematology Kenneth K, Marshall AL, Josef TP, Michael AC. 9th Edition McGraw Hill Education. New York. 2016;1829-1834.
6. Maatoghi JT, Paridar M, Shoushtari MM, Kiani B, Nori B, Shahjahani M, Khosravi A, Kelarijani NA, Ghalesardi OK, Far MAJ. Distribution of ABO blood groups and Rhesus factor in a large scale study of different cities and ethnicities in Khuzestan province, Iran. The Egy J Med Hum Gen. 2016;17:105-09.
7. Hosoi, E. Biological and clinical aspects of ABO blood group system. Journal of Medical Investigations, 2008;55(3, 4):174-82.
8. Chilongola J, Balthazary S, Mpina M. CD 36 (GPIV) Protects against malarial anaemia in children by reducing Plasmodium Falciparum-infected red blood cell adherence to vascular endothelium. J Tr Med intl He. 2009;14: 810-4.
9. Kajeguka D, Mwanziva C, Daou M. CD 36c. 1264 T>G null mutation impairs acquisition of IgG antibodies to plasmodium falciparum MSPI, a antigen and is associated with higher malaria incidences in Tanzania Children. Journal of Immunology, 2012;75:355-9.
10. Kupatawintu P, Nathalang O, O-Charoen R, Patmasiriwat P. Gene frequencies of the HPA-1 to 6 and Gov human platelet antigens in Thai blood donors. Immunohem. 2005;21: 5-9.
11. Curtis BR. Genotyping for human platelet alloantigen polymorphisms: applications in the diagnosis of alloimmune platelet disorders. Seminars on Thrombrin Hemostasis. 2008;34:539-48.
12. Fang C, Cohen HW, amp; Billett HH. Race, ABO blood group, and venous thromboembolism risk: Not black and white. Transfusion. 2013;53:187 - 92.
13. Lin-Chu M, Broadberry RE, Chang FJ. The distribution of blood group antigens and alloantibodies among Chinese in Taiwan. Transfusion and Clinical Biology. 1988;28: 350-2.
14. Apecu RO, Mulogo EM, Bagenda F, Byamungu A. ABO and Rhesus (D) blood group distribution among blood donors in rural south western Uganda: a retrospective study. BMC Research Notes, 2016;9:513-7.
15. Iyiola O, Igunnugbemi O, Bello O. Gene frequencies of ABO and Rh (D) blood group alleles in Lagos, South-West

Nigeria. Egypt Journal of Medicine and Human Genetics. 2012;13(2):147-53.
16. Abrahamson PE, Hall SA, Feudjo-Tepie M, Mitrani-Gold FS, Logie J. The incidence of idiopathic thrombocytopenic purpura among adults: a population-based study and literature review. European Journal of Haematology, 2009; 83:83-9.
17. Xu X, Zhu F, Ying Y, Tao S, Liu Y, Hong X, Yan L. Simultaneous genotyping of human platelet antigen-1 to 17w by polymerase chain reaction sequence-based typing. Vox Sang. 2009;97: 330-7.
18. Kupatawintu P, Nathalang O, O-Charoen R, Patmasiriwat P. Gene frequencies of the HPA-1 to 6 and Gov human platelet antigens in Thai blood donors. Immunohematology. 2005; 21: 5-9.
19. Serrarens-Janssen VM, Semmekrot BA, Novotny VM, Porcelijn L, Lotgering FK, Delemarre F M, Steegers EA. Fetal/neonatal allo-immune thrombocytopenia (FNAIT): past, present, and future. Obstetrics and Gynecology Survey. 2008; 63:239-52.
20. Li C, Piran S, Chen P. The maternal immune response to fetal platelet GP-Ib-alpha causes frequent miscarriage in mice that can be prevented by intravenous IgG and anti-FcRn therapies. J Clin Inv. 2011; 121:4537-47.
21. Halle L, Bigot A, Mulen-Imandy G, M'Bayo K, Jaeger G, Anani L, Martageix C, Bianchi F, Julien E, Kaplan C. HPA polymorphism in sub-Saharan African populations: Beninese, Cam-eroonians, Congolese, and Pygmies. Tissue Antigens. 2005;65:295-8.

© 2021 Wenah-Emmanuel et al.; This is an Open Access article distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/4.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sdiarticle4.com/review-history/68552