IOSR Journal of Dental and Medical Sciences (IOSR-JDMS)
e-ISSN: 2279-0853, p-ISSN: 2279-0861.Volume 20, Issue 5 Ser.2 (May. 2021), PP 41-47
www.iosrjournals.org

Comparative Evaluation of Complicated and Uncomplicated Malaria among ABO Grouping Types of Infected Paediatric at a Tertiary Hospital in Port Harcourt, Nigeria.

*Akwuebu, S. O.,[1]Mbeera, B. S.[1],Aaron, U.U.,[2]Ibeh, N.,[1] Eze E. M.,[1]Mirabeau, T.Y.[3]&Jeremiah, A. Z.[1]

1) Department of Medical Laboratory Science, Faculty of Science, Rivers State University.
2) Department of Medical Microbiology and Parasitology, University of Port Harcourt.
3) Department of Medical Laboratory Science, College of Health Sciences, Niger Delta University, Wilberforce Island, Amasoma, Bayelsa State.

Abstract
Introduction*: There is this saying that Malaria is most prevalent where there is poverty and where methods of disease identification, documentation and reporting are weakest.The antigens of the ABO system (*A, B, *and*H *determinants, respectively) consist of complex carbohydrate molecules that are traditionally regarded as red cell antigens found in most human tissues, like epithelium, sensory neurons, platelets and vascular endothelium. This study is aimed at evaluating the prevalence of complicated and uncomplicated malaria among ABO/Rh Blood grouping types of the study subjects.****Materials and Methods****: This is a cross-sectionalstudywith randomized sampling method that used in the selection of subjects, taking into consideration, the total number of patients registered in the pediatric clinic at University of Port Harcourt Teaching Hospital, Rivers State. This study was carried out using samples from 822 Pediatric subjects that met the study criteria.****Results****: This study involved 822 subjects, out of which, twenty-five (25) (3.04%) had complicated malaria while four hundred and twenty-six (426) (51.82%) had uncomplicated malaria, whereas three hundred and seventy-one (371) (45.13%) were uninfected subjects (control). The mean age of the study participant was 4.68±0.134 years. Majority of the pediatrics 474(57.66%) were male, of which 14(56%) were infected with complicated malaria parasite and 253(59.39%) were infected with uncomplicated malaria parasite. Bulk of the participants 383(46.59%) had blood group O rhesus D positive. There was observed a significant statistical difference (P<0.0010) in the prevalence of complicated and uncomplicated malaria among the different ABO/Rh blood group types in this study.*
Conclusion*: This study had shown that ABO/Rh blood group specific antigens possessed by an individual's cells could be a significant factor in the distribution of severity of malaria within a given population.*
Key Words*: Complicated Malaria, Uncomplicated Malaria, ABO/Rh Blood Group, Antigen, Pediatric, Port Harcourt.*

Date of Submission: 29-04-2021 Date of Acceptance: 13-05-2021

I. Introduction

Malaria is most prevalent where there is poverty and there is little or no adequate facility for the disease identification, documentation, reporting and treatment (Worrall *et al*., 2005). A large proportion of severe malaria illnesses and deaths occur in people's homes without coming to the attention of a formal health service: for children under 5 years of age, this proportion has been estimated at 90% in The Gambia (WHO 2014) and similar in most third world countries like Nigeria.*Plasmodium falciparum* malaria is a known cause of morbidity and mortality especially in children of Sub-Saharan African enclaves (Afoakwah*etal*., 2016).

In 2018, an estimated 405,000 people died of malaria, mostly young children in sub-Saharan Africa. Within the last decade, increasing numbers of partners and resources have rapidly increased malaria control efforts (CDC, 2019).According to the 2019 World Malaria Report, Nigeria had the highest number of global malaria cases (25 % of global malaria cases) in 2018 and accounted for the highest number of deaths (24 % of global malaria deaths)(Severe Malaria Observatory 2020; Liu *et al*., 2016). Case numbers have plateaued at between 292 and 296 per 1000 of the population at risk between 2015 and 2018. Deaths however fell by 21% from 0.62 to 0.49 per 1000 of the population at risk during that same period (Severe Malaria Observatory 2020).

Malaria is transmitted all over Nigeria; 76 % of the population live in high transmission areas while 24 % of the population live in low transmission areas. The transmission season can last all year round in the south and is about 3 months or less in the northern part of the country. Malaria is usually classified as asymptomatic,

uncomplicated or severe. Asymptomatic malaria can be caused by all *Plasmodium* species; the patient has circulating parasites but no symptoms (WHO 2014).

Uncomplicated malaria can be caused by all *Plasmodium* species. Symptoms generally occur 7-10 days after the initial mosquito bite. Symptoms are non-specific and can include fever, moderate to severe shaking chills, profuse sweating, headache, nausea, vomiting, diarrhoea and anaemia, with no clinical or laboratory findings of severe organ dysfunction (Cserti& Dzik, 2007). Complicated or severe malaria is often associated with hyperparasitaemia and is linked with increased mortality. Severe or complicated malaria is most frequentlyassociated with*Plasmodium falciparum* infectionand less frequently with*Plasmodium vivax* or *Plasmodium knowlesi* infections (Afoakwah*et al*., 2016;Worrall *et al*., 2005).

Consequently, complicated malaria could lead to one or a combination of anaemia, more convulsions within a 24-h period, haemoglobinuria, Acidosis with a plasma bicarbonate of <15 mM or venous plasma lactate>5 mM. clinical manifestation as respiratory distress, significant bleedings, hypoglycaemia (Blood or plasma glucose <2.2 mM or <40 mg/dl), acute kidney injury with plasma or serum creatinine >265 μM (3 mg/dl) or blood urea >20 mM/l, jaundice with plasma or serum bilirubin >50 μM (3 mg/dl), hyperparasitaemia (with a parasite count >100 000/μl), pulmonary oedema with oxygen saturation <92% on room air and a respiratory rate >30/min and could ultimately lead to shock. Complicated malaria causedanaemia is associated with a haemoglobin concentration <5 g/dl or a haematocrit of <15% in children <12 years of age and haemoglobin<7 g/dl, haematocrit<20% in adults. This occurs in addition to a *Plasmodium* parasite count >10,000/μl in all cases (WHO 2014).Parasitaemia> 20% is associated with a high risk in any epidemiological context (Worrall *et al*., 2005).

Also, the clinical outcome of *Plasmodium falciparum* malaria in endemic areas is, among other factors, associated with erythrocyte polymorphisms including the ABO blood group which is known to be a system of carbohydrate antigens expressed on human erythrocytes and other human cells (Cserti& Dzik, 2007). Nevertheless, with exception to rare Bombay phenotype, which has no ABO antigens blood group that havetrisaccharide Aand B antigens on their erythrocytes, every other erythrocyte, had an "H" disaccharide on their surface (Dean, 2005). Rosetting(which is defined as the agglutination of uninfected erythrocytes around parasitized erythrocytes contributes to the pathogenesis of severe malaria by obstructing microvascular blood flow leading to the rosetting of parasitized erythrocytes and cytoadherence). Thus, depending on whether the other genes are encoded, it will then be determined if they will remain a type O or change to type A, B, or AB. Type O blood is the most frequent, and type B and AB are the least. In rare cases, even the initial precursor H antigen is not genetically encoded. These individuals are known as Bombay and are able to receive only Bombay type blood, because they make antibodies to not only type A, B, or AB donor RBCs but also to type O donor red cells (anti-H), causing hemolysis of the transfused donor RBCs (Dean *et al*., 2012).However, this study was aimed at determining the relationship between malaria parasite density and ABO/Rh blood groups.

II. Materials and Methods

The study was a cross sectional study carried out on randomly selected 822 pediatric patients with age range between 0-16 years, suspected to have malaria infection. Children registered in the pediatric ward of University of Port Harcourt Teaching Hospital, UPTH with suspected *P. falciparum*parasitemia presenting with complicated and uncomplicated febrile illness and children without febrile illness for immunization attending accident and emergency unit and the clinic were recruited for this study after obtaining a written consent from the parents or guardians from each participant. Ethical approval was obtained from the ethical committee of University of Port Harcourt Teaching Hospital and Informed consent of the participants involved was also obtained. Blood sample (9ml) was withdrawn with minimum stasis under aseptic conditions from the dorsum of the hand or ante-cubital vein. The sample was rocked gently to mix and kept at room temperature and then analyzed within 4 hours of samples collection. The sample was rocked gently to mix, then kept at room temperature and analyzed within 4 hours of samples collection. This study was divided into three groups, namely, uncomplicated Malaria group, complicated malaria group and control group (those with no malaria parasite seen).Theuncomplicated malaria group was defined with parasitemia<100000/μL while the complicated severe malaria (cerebral malaria) group was defined as one with parasitaemia≥100,000 parasites/μL (WHO, 2014). Diagnosis of *Plasmodium* species and estimation of parasitemia was carried out by the use of Giemsa stained thick and thin smear made from a well-mixed anticoagulated whole blood sampleon a clean grease free slide and labelled appropriately.Each labelled Giemsastained, dried slide was examined microscopically using the X100 objective lens thick film and parasite density estimated using the assumed 8000WBC count/uL. All results were collated in excel spread sheet ant transferred onto statistical package for social sciences (SPSS) IBM version 21 for statistical analysis using Chi-square and measures of dispersion.

III. Results

From this study, it was also observed that the overall prevalence of complicated and uncomplicated malaria was 3.04% (25/822) and 51.82% (426/822) respectively while those without malaria that served as our study control stood at a prevalence of 45.13% (371/822). However, the children population (514) (62.53%) in this study were more and had the highest prevalence of complicated malaria infection 60% (15/25) compared to the neonate (28%) (7/25) and adolescent (12%) (3/25) age groups. The mean age of the study participants was 4.68±0.134 years (Table 1). Majority of the pediatrics 474(57.66%) were male, of which 14(56%) had complicated malaria parasite whereas 253(59.39%) were diagnosed to have uncomplicated malaria (Table 2).

Table 1: Age Distribution of Pediatrics with Complicated and Uncomplicated Malaria, and Non-Malaria Infected Control

Age Group (Years)	N (%)	Subjects/Malaria Types						Test Statistics	
		Complicated		Uncomplicated		Non-Malaria Infected (Control)			
		N	%	N	%	N	%	X^2 value	*p-value*
Total	822 (100)	25	3.04	426	51.82	371	45.13	344.94	<0.0001****
Neonate/Infant	252 (30.66)	7	28.00	133	31.22	112	30.19		
Children	514 (62.53)	15	60.00	266	62.44	233	62.80		
Adolescent	56 (6.81)	3	12.00	27	6.34	26	7.01	1.31	0.8600^{ns}
Age Mean ±SEM)	4.68±0.134	5.23±0.936		4.70±0.188		4.63±0.198			

*Note: within characteristics, percentages may not add up to 100 due to rounding.Significance Levels: ***=p<0.001, ****=p<0.0001, ns=not significant (p>0.05).*

Table 2: Gender Distribution of Pediatrics with Complicated and Uncomplicated Malaria, and Non-Malaria Infected Control

Gender	N (%)	Subjects/Malaria Types			N (%)	Subjects/Malaria Types		Size	N (%)
		Complicated	Uncomplicated			Complicated	Uncomplicated		
Female	348 (42.34)	11	44.00	173	40.61	164	44.20		
Male	474 (57.66)	14	56.00	253	59.39	207	55.80	1.08	0.5831^{ns}

*Note: Within gender, percentages may not add up to 100 due to rounding. Significance Levels: ***=p<0.001, ****=p<0.0001, ns=not significant (p>0.05).*

This study's parasite density probability distribution curve showed an exponential distribution where the relative probability of the randomized *Plasmodium falciparum* parasite density reoccurring at any point in the research, thus, the malaria parasite densities of subjects recruited into this study for both the complicated and uncomplicated malaria groups are events that occur continuously and independently at a constant average rate in the Poisson point processes it is found in various other contexts (Fig. 2). This study witnessed a meantotal parasite density of 112083.68 parasites/µL and for different age groups neonates/infants, children and adolescents showed a mean parasite density of 115749.14 parasites/µL, 110994.67 parasites/µL and 112083.63 parasites/µL respectively (Fig. 1).

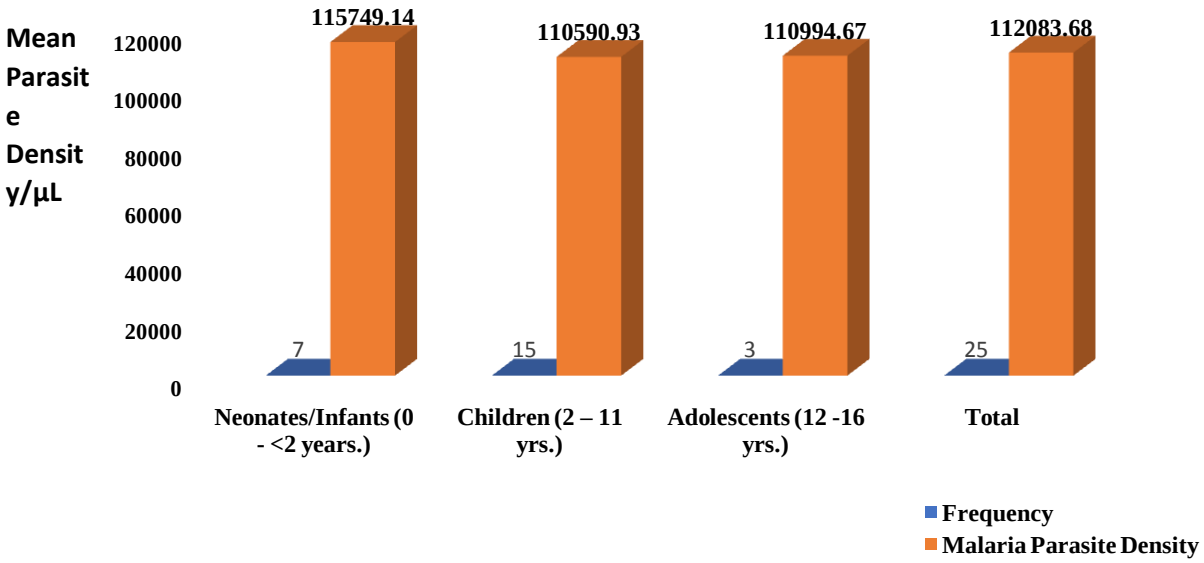

Fig. 1: Comparison of *Plasmodium*Parasites Density/ul of Blood of Pediatric Subjects with Complicated Malaria

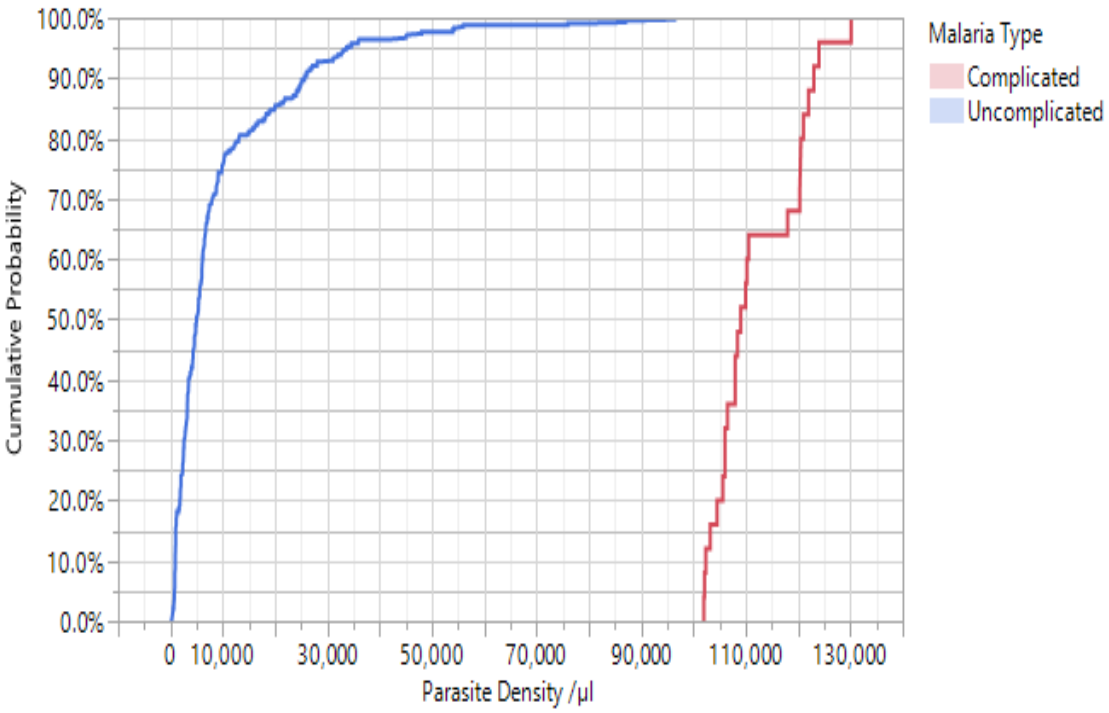

Fig. 2: Cumulative Distribution Function (CDF) Plot of Parasite Density in Pediatric Subjects with Complicated and Uncomplicated Malaria

Among this present study population, it was observed that blood group O Rh'D' positive had the highest prevalence 46.59% (383/822), Blood group B Rh'D' positive followed with a prevalence of 23.19%(191/822). Nevertheless, blood group B Rh'D' negative (1.34%)(11/822) followed by blood group AB Rh'D' negative (1.46%)(12/822) and O Rh'D'negative (8.2%)(68/822) (Fig. 3). The samples in this present study showed the highest mean plasmodium parasite density among those of blood group A^- (117953.80±6505.19 parasites/uL) and B+(12708.80±1321.00) for complicated and uncomplicated malaria respectively. However, there was found no significant difference in the mean parasite density of the sample population was observed among the various blood groups in this study (Table 3).

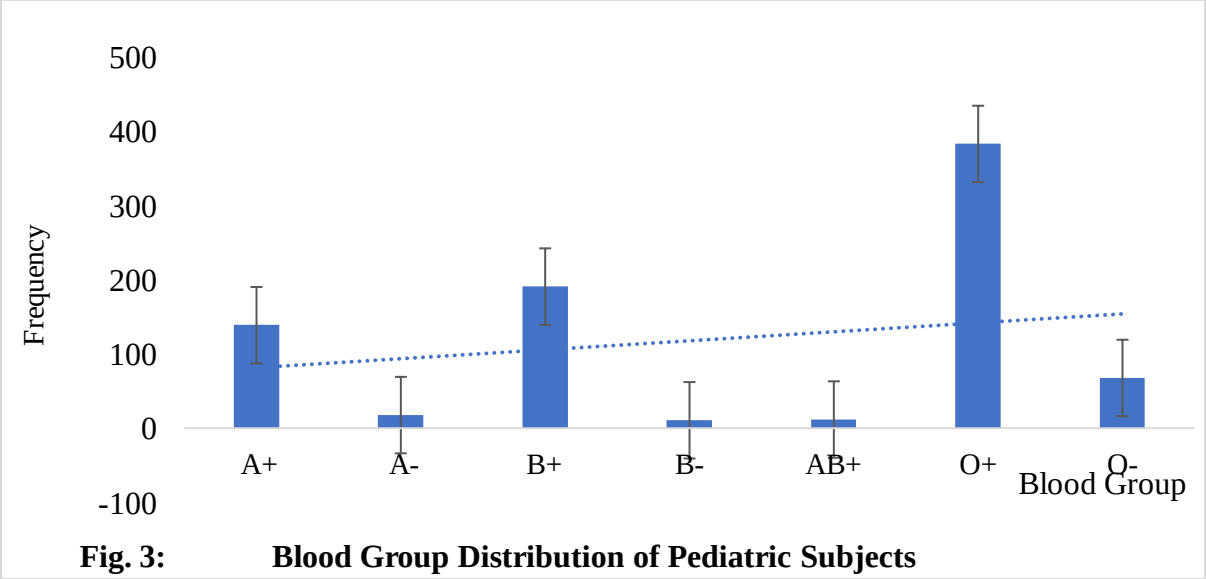

Fig. 3: Blood Group Distribution of Pediatric Subjects

Table 3: Distribution of Parasites Density/ul of Blood of Pediatric Subjects in this Study

Blood Group	Complicated Malaria Mean ± SEM	F/t-	Uncomplicated Malaria Mean ± SEM	Ratio	Prob> F/|t|
A+	113439.90±4917.46		8846.24±1533.29		
A-	117953.80±6505.19		9297.67±5311.47		
B+	108687.50±4599.87		12708.80±1321.00		
B-	------		3428.33±5311.47		
AB+	111116.33±5311.47		11084.33±5311.47		
O+	------		9470.76±924.61		
O-			4445.59±2031.88	0.8053	0.4914^{ns}

*SEM: Standard Error of Mean. Significance Levels: ***=p<0.001, ****=p<0.0001, ns=not significant (p>0.05).*

IV. Discussion

Each year approximately300–500 million malaria infections lead to over one million deaths, of which over 75 % occur in African children aged under five years infected with *Plasmodium falciparum.* About 40 % of the world's children live in malaria-endemic countries like Nigeria, according to WHO (Adu-Gyasi*et al*., 2012). This study witnessed a mean total parasite density of 112083.68 parasites/µL and for different age groups neonates/infants, children and adolescents showed a mean parasite density of 115749.14 parasites/µL, 110994.67 parasites/µL and 112083.63 parasites/µL respectively (Fig. 1). Thus, making this present study's parasitedensity probability distribution curve show an exponential distribution where the relative probability of the randomized *Plasmodiumfalciparum* parasite density reoccurring at any point in the research. Therefore, the malaria parasite densities of subjects recruited into this study for both the complicated and uncomplicated malaria groups are events that occur continuously and independently at a constant average rate in the Poisson point processes(Fig. 2).Comparatively, considering the research of Adu-Gyasi*et al*., (2012) where5,902 *Plasmodiumfalciparum* malaria positive samples were used, the geometric mean parasite density was 7,557 parasite/µL (95 % CI 7,144/µL to 7,994/µL) which was lower than that observed in any classified group of this study. Thus, this showed that malaria endemicity in Nigeria, and Port Harcourt in particular, is not a fluke. As such adequate enlightenment and control would be a solution in line with the aspirations and goals of the millennium development goals set by the world health organization. As a matter of fact, the reduction in the mortality estimates from malaria from985 000 in the year 2000 to 781 000 in2009 and 627 000 in 2010 (World Health Organization2014) adequately explains the role that malaria control actionscould play to reduce the global malaria burden of complicated malaria.

Therefore, the causal link between deployment of effective vector control measures (ITNs, insecticides) andeffective drugs (ACTs) and declining mortality as may have been used in this study could be practically relevant for further studies on actual relationship of this disease characteristics.Following this thought line, Steketee& Campbell (2010) said that causalityis likely when there are strong geographical associationsof control measures with improvements, absence ofchanges in some other causes of death and consistencywith predictions of the likely effects of malaria-specificinterventions.

Apparently, progression to complicated malaria and lethal disease may largely but notentirely confined to *P. falciparum* infections because this study revealed the exclusive prevalence of *P. falciparum* among the studied population. This notion was also corroborated by WHO (2014) which articulated that, even though *P. vivax* and *P. knowlesi*contribute much less than *P. falciparum* to the global burden ofcomplicated malaria, both do alsolead to fetal consequences like that of*Plasmodium falciparum*(Afoakwah,2016; WHO 2013, 2014).

That notwithstanding, some literatures support the idea that *P. falciparum*infection develop partial protection for severe symptoms at an early age under conditions of low and unstable malaria transmission, individuals of all age groups may present with acute or severe malaria disease as a consequence of low levels of naturally acquired immunity although also subclinical infections are detected (Alemu and Mama, 2016). Previously, Tadesse and Tadesse published a research article in Journal of Vector Borne Diseasesin 2013 showinga strong positive association that only being an individual of blood group O confers some protectionagainstcomplicated malaria (Tadesse and Tadesse, 2013). Nevertheless, adherence of *P. falciparum* parasitized erythrocytes to the endothelia of blood vessels is key to the pathogenesis of complicated disease. Antigens of blood groups A and B have been suggested to play important roles in cytoadherence. Due to the absence of A and B antigens on the surface of blood group O erythrocytes, cytoadherence, and hence rosetting and sequestration, is reduced in individuals with blood group O(Afoakwah,2016). It has been observed that blood group O individuals were less likely to suffer from complicated *P. falciparum* malaria as evidenced in this present study.Here we observed that only 18 (16.1%) of the 112 participants with complicated disease had blood group O, whereas as much as 74 (40.9%) of the 181 participants with uncomplicated disease had blood group O in a population with a high prevalence of Group O+ (87%) individuals compared to Group A (8 %), Group B (13%),and Non-A Non-B Group(11.0 %) according to Severe Malaria Observatory (2020)while Alemu& Mama (2016), showed that blood group O prevalence was 42.1%, blood group A 32.7%, blood group B 20.9%, and blood group AB4.3%.Although, this may still be argued on the contrary as some other published research articles have also shown great disagreement with this study(Oladeinde*et al*.,2014; Sirina and Clement 2013;Epidi*et al*., 2008),indicating that, there exists no significant relationship between ABO blood group and *Plasmodium* parasitemia.

V. Conclusion

Blood group O appeared to be the most prevalent blood group among the persons recruited into this study and as well the least prevalence of complicated and uncomplicated malaria: thus, suggesting that the blood group O confers some amount of protection against the disease severity of *Plasmodium falciparum* to individuals within the ABO variant. Therefore, this study has recognized that there exists a significant relationship between ABO blood group variants and their malaria parasite density.

Acknowledgement: We specially appreciate the efforts and assistance of the following Staff of the University of Port Harcourt Teaching Hospital, Porth Harcourt; Mr. Aleme G., Mrs. Ngowari Jeremiah, Mrs. WenahJustinah, and all the Staff at the Pediatrics Department of the Hospital. Not to forget the efforts of Mrs Ngozi for type setting this piece as well as the entire Staff and Students of the Department of Medical Laboratory Science, Rivers State University, Port Harcourt.

Author Contributions: Study Design (ASO, JAZ), Sample Collection and Practical (ASO), Article Write-Up (ASO, AUU, IN), Supervision of all stages (JAZ, EEM, IN), All authors approved the final manuscript.

References

[1]. Afoakwah,R.,Edmond, A.,James, P.,Ekene, K. N.& Johnson, N. B. (2016). Relative Susceptibilities of ABO Blood Groups to *Plasmodium falciparum* Malaria in Ghana. *Advances in Hematology*, ID 5368793, 1-4. http://dx.doi.org/10.1155/2016/5368793.

[2]. Epidi T. T., Nwani C. D., &Ugorji N. P. (2008) "Prevalence of malaria in blood donors in Abakaliki metropolis, Nigeria," Scientific Research and Essays,. 3(4), 162–164.

[3]. Sirina,M. &Clement,O., (2013). The prevalence of malaria parasitaemia and predisposition of ABO blood groups to Plasmodium falciparum malaria among blood donors at a Ghanaian Hospital.*AU Journal of Technology*, 16(4),255–260,.

[4]. Tadesse, H. & Tadesse,K.(2013). Assessing the association of severe malaria infection and ABO blood groups in northwestern Ethiopia. *Journal of Vector Borne Diseases*, 50(4), 292–296.

[5]. WHO (2014).Severe Malaria. *Tropical Medicine and International Health*, 19 (1), 7–131. John Wiley & Sons. Retrieved from https://www.who.int/malaria/publications/atoz/who-severe-malaria-tmih-supplement-2014.pdf. doi:10.1111/tmi.12313

[6]. Alemu,G.& Mama, M. (2016). Assessing ABO/Rh Blood Group Frequency and Association with Asymptomatic Malaria among Blood Donors Attending Arba Minch Blood Bank, South Ethiopia*Malaria Research and Treatment*, 8043768.https://doi.org/10.1155/2016/8043768.

[7]. WHO (2013).Management of Severe Malaria: A Practical Handbook (3rd ed, 2013), p8. John Wiley & Sons. Retrieved from https://www.who.int on 23/01/2021.

[8]. WHO (2015). Guidelines for the treatment of malaria. Retrieved from www.who.int on 23/01/21.

[9]. Worrall, E., Basu, S. & Hanson, K. (2005). Is malaria a disease of poverty? A review of the literature. *Tropical Medicine & International Health*, 10(10), 1047-1059. DOI: 10.1111/j.1365-3156.2005.01476.x

[10]. Cserti, C. M. & Dzik, W. H. (2007). The ABO blood group system and *Plasmodium falciparum* malaria. *Blood*, 110 (7), 2250–2258. https://doi.org/10.1182/blood-2007-03-077602.

[11]. B. H. Oladeinde, R. Omoregie, E. O. Osakue, and T. O. Onaiwu, (2014).“Asymptomatic malaria among blood donors in benin city Nigeria,” *Iranian Journal of Parasitology*, vol. 9, no. 3, pp. 415–422,.
[12]. D. E. Agbonlahor, F. I. Obi, O. Esumeh, A. A. Ajanaku, and E. O. Igumbor, “Association of ABO blood groups and malaria parasitaemia among students of Edo State University, Ekpoma, Nigeria,” Journal of Medical Laboratory Science, vol. 4, no. 2, pp. 12–19, 1993.
[13]. S. K. Singh, A. P. Singh, S. Pandey, S. S. Yazdani, C. E. Chitnis, and A. Sharma, “Definition of structural elements in Plasmodium vivax and P. knowlesi Duffy-binding domains necessary for erythrocyte invasion,” Biochemical Journal, vol. 374, no. 1, pp. 193–198, 2003.
[14]. Dean, L. (2005). Blood Groups and Red Cell Antigens Bethesda (MD): National Center for Biotechnology Information (US). https://www.ncbi.nlm.nih.gov/books/NBK2261/
[15]. Liu, L., Shefali, O., Dan, H., Yue C., Jamie, P., Jun Z. Lawn, J. E., Cousens, S., Mathers, C. & Black, R. E. (2016). Global, regional, and national causes of under-5 mortality in 2000–2015: an updated systematic analysis with implications for the Sustainable Development Goals. *Lancet*,388 (10063), 3027-3035. DOI: https://doi.org/10.1016/S0140-6736(16)31593-8.
[16]. Severe Malaria Observatory (SMO) (2020). Severe Malaria Facts. Retrieved from https://www.severemalaria.org/countries/nigeria on 27/01/2020.
[17]. Steketee, R.W.& Campbell, C.C. (2010). Impact of national malariacontrol scale-up programmes in Africa: magnitude and attribution of effects. *Malaria Journal*, 9, 299.
[18]. Chaparro-Narvaez, P. E., Lopez-Perez, M., Rengifo, L. M., Padilla, J., Herrera, S. and Arevalo-Herrera, M. (2016). Clinical and epidemiological aspects of complicated malaria in Colombia, 2007–2013. *Malaria Journal*, 15, 269.doi: 10.1186/s12936-016-1323-5.
[19]. Arevalo-Herrera, M., Lopez-Perez, M., Medina, L., Moreno, A., Gutierrez, J. B& Herrera S. (2015). Clinical profile of *Plasmodium falciparum* and *Plasmodium vivax* infections in low and unstable malaria transmission settings of Colombia. *Malaria Journal,* 14(4),154.
[20]. Carlson, J.(1993). Erythrocyte rosetting in *Plasmodium falciparum* malaria--with special reference to the pathogenesis of cerebral malaria.*Scandanavian Journal of Infectious Diseases*, 86, 1-79. PMID: 8493454.
[21]. Adu-Gyasi D., Mohammed Adams, Sabastina Amoako, Emmanuel Mahama, Maxwell Nsoh, SeebaAmenga-Etego, Frank Baiden, Kwaku Poku Asante, Sam Newton & Seth Owusu-Agyei (2012). Estimating malaria parasite density: assumed white blood cell count of 10,000/µl of blood is appropriate measure in Central Ghana. *Malaria Journal*, 11, 238.

Akwuebu, S. O, et. al. “Comparative Evaluation of Complicated and Uncomplicated Malaria among ABO Grouping Types of Infected Paediatric at a Tertiary Hospital in Port Harcourt, Nigeria.”*IOSR Journal of Dental and Medical Sciences (IOSR-JDMS)*, 20(05), 2021, pp. 41-47.