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Abo Blood Variants of Selected Babcock University Students and Their Link with Malaria Parasitaemia

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Abstract: Five milliliters (5ml) of venous whole blood was collected from one hundred and eighty three students made up of 93(50.8%) male and 90(49.2%) female students of Babcock University, Ilishan Remo randomly selected across various Departments. Whole blood samples were dispensed into sequestrinized (EDTA anticoagulated) blood containers, properly mixed and labeled. Malaria *Plasmodium falciparum* parasite screening was done semi-quantitatively by Field stain A and B staining. ABO blood phenotyping was carried out with monoclonal Antisera A, B and D. A total of 169 (92.3%) and 14(7.7%) students were rhesus positive and negative respectively of which 92(54.4%) and 77(45.6%) samples were rhesus positive male and female students respectively and of which 1(7.1%) and 13(92.9%) students were rhesus negative male and female students respectively. One hundred and thirty five (73.8%), 36(19.7%) and 12(6.5%) of the sampled student population belonged to 17-20, 21-24 and 25-30yr age brackets respectively. One hundred and ten (60.1%), 38(20.8%), 29(15.9%) and 6(3.3%) students were of O, A, B and AB blood phenotypes respectively. Out of the 183 blood samples obtained from 93 (50.8%) and 90(49.2%) male and female students respectively, 126(68.9%) students were infected with *P. falciparum* malaria parasites. More males were infected than females and were significantly associated with malaria infection ($X^2_{0.05,\ 1}$ =3.841, Cal. X^2 =25.253, P<0.05). Also, out of the 68.9% infected students, 47(37.3%) and 79(62.7%) had severe and non-severe forms of malaria infection respectively. Severe malaria frequency occurrences were 85.0%, 70.6%, 50.0% and 50.0% for blood types A, O, B and AB respectively while non-severe malaria frequency occurrences were 83.3%, 75.0%, 65.8% and 57.9% with respect to blood types A, AB O and B respectively. ABO blood types especially type A were significantly associated with severe form of *P. falciparum* malaria infection ($X^2_{0.05,\ 3}$=7.815, $X^2_{0.01,\ 3}$=11.350, Cal. X^2 =284.601 and P<0.05, P<0.01). ABO blood types were also significantly associated with non-severe form of malaria especially type A (Cal. X^2 =230.768 and hence, P<0.05, P<0.01). Implications of rising trend of rhesus negative factor in female students, population variations in association with ABO blood types and malaria parasitaemia are discussed.

Keywords: ABO Variants, Link, Malaria Infection, Students, University

1. Introduction

ABO blood types was the first human blood group discovered in 1901 by Landsteiner followed by Rh blood group in 1941 [1, 2]. About thirty major blood groups have been recognized by The International Society of Blood Transfusion (ISBT) and among these thirty are the ABO and Rh blood groups [3, 4]. The ABO system is the most investigated erythrocyte antigen system for all populations and due to the ease of identifying its phenotypes, it has been used as a genetic marker in studies of associations with infectious and non-infectious diseases [5, 6]. The ABO is the most clinically important antigen classification system to date. Its recognition is central to the practice of transfusion medicine, because of the immediate recognition and rejection of major incompatible non-self-cells.

Since the second World War, blood and blood component transfusion have been used to correct severe anaemia, deficiency of plasma clotting factors, thrombocytopenia, immunodeficiency states, hypoalbuminaemia and problems related to electrolytes [7, 8]. Transfusion of compatible blood at least for ABO and Rh antigens reduces transfusion reactions in recipients. The ABO and Rh blood groups are

also useful in clinical studies, population genetic studies and researching population migration patterns as well as resolving certain medicolegal issues particularly of disputed paternity cases [9]. Therefore, knowledge of the ABO and Rh blood group distribution in specific population has paramount importance in the context of transfusion medicine. Many previous studies in sub-Saharan Africa reported that blood group O and Rh positive are the most frequent ABO and Rh blood groups respectively but the proportion varies by location [10-13].

An antigen is any substance to which the immune system can respond. For example, components of the bacterial cell wall can trigger severe and immediate attacks by neutrophils. If the immune system encounters an antigen that is not found on the body's own cells, it will launch an attack against that antigen. Conversely, antigens that are found on the body's own cells are known as "self-antigens", and the immune system does not normally attack these. The membrane of each red blood cell contains millions of antigens that are ignored by the immune system. However, when patients receive blood transfusions, their immune systems will attack any donor red blood cells that contain antigens that differ from their self-antigens. Therefore, ensuring that the antigens of transfused red blood cells match those of the patient's red blood cells is essential for a safe blood transfusion [14].

Blood group antigens are either sugars or proteins, and they are attached to various components in the red blood cell membrane. The antigens of the ABO blood group are sugars. They are produced by a series of reactions in which enzymes catalyze the transfer of sugar units. A person's DNA determines the type of enzymes they have, and, therefore, the type of sugar antigens that end up on their red blood cells. In contrast, the antigens of the Rh blood group are proteins. A person's DNA holds the information for producing the protein antigens. The RhD gene encodes the D antigen, which is a large protein on the red blood cell membrane. Some people have a version of the gene that does not produce D antigen, and therefore the RhD protein is absent from their red blood cells. If this protein is present on a particular blood type, that blood type is called positive then if absent, it is called negative [15].

Malaria (which is caused by *Plasmodium* species transmitted by anopheles mosquitoes) is the second leading disease following Acquired Immunodeficiency Syndrome-AIDS [16] associated with high morbidity and mortality through anaemia, cerebral complications and other mechanisms [16].

Despite the high morbidity and mortality, certain individuals living in malaria endemic regions appear relatively protected compared to those who suffer frequent severe malaria attacks. Resistance to malaria infection is dependent on the development of an immune response by the host and to a varying extent, a certain innate characteristic possessing protective value against infection [17]. These factors include Sickle cell trait (HbAS) and Sickle cell disease –HbSS [18], ABO blood types [19] and the level of G-6-P- dehydrogenase [20].

Both ABO and Rh blood groups have attracted enormous attention regarding their association with genetic and infectious diseases [21]. Previous studies on cancer and tumor patients [22], heart diseases [23], parasitic and viral infections [24] indicated associations of ABO and Rh blood groups. In particular, The ABO antigens regulate cellular activities suggesting their impact on determining susceptibility and severity of certain diseases [25]. It has been more than four decades since association of ABO blood group and malaria was suggested. There is also a hypothesis that Plasmodium falciparum malaria has shaped the distribution of ABO blood groups in humans [26].

It is thought that an understanding of the nature of relationship (if any) between ABO blood groups and malaria parasitaemia should provide an invaluable window in the scourge and that studies of malaria parasitaemia from that stand point in populations of malaria endemic regions will be helpful in elucidating any such relationship [12].

A high percentage of severe malaria cases have been reported among blood group A individuals [27, 28]. Furthermore, Migot-Nabias [29] and Pathirana *et al*. [30] observed low paraitaemia and uncomplicated malaria cases among blood group O individuals.Similarly, a significant advantage of *Plasmodium falciparum* with ABO blood groups with respect to group O has been reported by some authors [31, 32]. Other studies have shown high frequency of malaria episodes among blood group A individuals as compared with other blood group individuals [33]. Akhigbe *et al*. [34] reported that blood group AB persons had the lowest malaria attack while blood group A persons had the highest attack.

Studies to investigate any possible association between ABO blood group system and some disease conditions have been carried out by some authors [35-40]. Some of these studies reported significant associations thereby suggesting that ABO blood groups have an impact on the infection status of the individuals possessing a particular ABO blood group [37, 38, 39, 40].

The absence of significant association between *P. falciparum* prevalence and ABO antigens has also been reported by some other studies [16, 41, 42, 32]. Other authors are less equivocal in relating malaria parasitaemia to ABO blood group [43, 44]. Otajevwo [9] in a study associating malaria parasitaemia with ABO blood types among residents of Warri, Nigeria, reported that 6.9%, 19.0%, 20.7% and 53.3% of a total of 174 whole blood samples processed belonged to blood types AB, B, A and O respectively and 138(79.3%) of total sample size were infected with malaria parasites of which *P. falciparum* was the predominant species. In the report, the highest malaria parasite load was observed among group O (52.2%) individuals while the least was noticed among blood group AB (8.7%) individuals. Malaria parasitaemia was higher among the males (83.3%) than females (75.0%). Otajevwo [45] also reported ABO blood types among students of Igbinedion University, Okada, Nigeria of which out of 104 samples analyzed, 44(3.9%), 16(15.4%), 32(30.8%) and 52(50.0%) students occurred in AB, A, B and O groups respectively. On the whole,

80(76.8%) of total samples processed, were positive for malaria parasitaemia.

Nkuo-Akenji [46] reported that blood group O individuals maybe more susceptible to malaria attack. A study conducted in Edo State University, Nigeria reported that blood groups O and B male individuals were the most and least susceptible to malaria attack respectively [19]. In a study conducted on inhabitants of Odakpu, Anambra State,Ilozumba and Uzozie [12] reported ABO blood group prevalence of 2.63%, 12.05%, 21.1% and 63.8% for groups AB,B,A, and O respectively. Zerihun *et al*. [47] reported that A, B and AB blood group individuals are more vulnerable to *P. falciparum* infection compared to blood group O individuals in a study they carried among 269 febrile Ethiopian out patients who visited a health care.

Despite the above researches, a consensus on possible association between ABO blood group genes and malaria infection is still lacking [48]. This might be due to limited data or unreported data on the cell antigens [46], peculiar demographic distributions and characteristics of study areas [41]. A definite statement on the apparent trend can be made therefore, if results of more studies on ABO blood groups associated with malaria infection in different parts of Nigeria and other neighboring countries are made available and accessible [12]. No known study has been done involving students of Babcock University of their ABO blood types were linked or associated with *Plasmodium falciparum* malaria infection and hence, the aim of this work is ABO blood variants of selected Babcock University students and their association with malaria infection with specific objectives as listed below:

1. ABO blood phenotypes and rhesus frequency distribution among students recruited for study with respect to sex.
2. Age distribution of ABO blood types of Babcock University students recruited for the study
3. Sex distribution of *Plasmodium falciparum* malaria infection among Babcock University students recruited for the study.
4. Association of severe and non-severe forms of *Plasmodium falciparum* malaria infection with ABO blood phenotypes of students recruited

2. Materials and Methods

2.1. Ethical Clearance

Ethical clearance was obtained from Babcock University Health Research Ethics Committee for the approval of the research proposal and other related materials after the necessary reviews and corrections. The students who volunteered their blood samples signed informed consent forms to show their approval before blood samples were collected from them.

2.2. Criteria for Selection of Subjects

Both symptomatic and asymptomatic subjects (students) were used for the study. Symptomatic subjects were those that were positive for malaria parasite test and showed obvious signs of illness. Asymptomatic subjects were those who were apparently healthy although positive for malaria parasite test. For clarity, symptomatic subjects were grouped as those having severe malaria parasitaemia while asymptomatic subjects were those with non-severe malaria parasitaemia.

2.3. Sampling

Five milliliters (5ml) of venous blood was collected from each of one hundred and eighty three students made up of 93 (50.8%) male and 90 (49.2%) female students of Babcock University Ilishan Remo, Ogun State randomly selected across various departments. Blood collection was done by venous puncture. With the aid of a tourniquet, a prominent vein was located on the fore arm and the vein area was sterilized with 70% ethanol soaked cotton wool swabs. Using sterile 5ml syringe and needle, five milliliters (5ml) of venous blood was withdrawn from subjects into appropriately labeled ethylene diamine tetra-acetic acid (ETDA) blood containers. All containers were properly mixed by standard method in order to sufficiently mix the anticoagulant with the blood to stop coagulation from taking place. Blood samples were collected by a qualified and licensed Medical Laboratory Scientist who is a Babcock University staff. Sterile Hand gloves and knee length laboratory coats were worn all through blood collection and disposal period. Specimens were properly labeled, packaged and kept in a functional refrigerator before and after use.

2.4. Duration/Venue of Study

This study was carried out between when ethical clearance was obtained and end of March, 2016. The venue of the research was the Microbiology laboratory of Bioscience Department of Babcock University, Ogun State, Nigeria.

2.5. Malaria parasite Staining

Thick blood films were made on grease free microscope slides (after appropriate labeling) and allowed to air dry on laboratory working bench. Slides were arranged on a staining rack and flooded with Field Stain A (containing methylene blue) and Field Stain B (containing eosin) analytical grade (Glanson Chemicals, Nigeria). Staining was done according to standard methods [49](Brooks *et al.,* 2004). The plus system was used for the determination of parasite density [50](WHO, 1990). Only positive results were recorded and used for ABO group typing.

2.6. Typing Blood Samples for Blood Group Antigens

The ABO blood typing of each subject was determined using cell grouping Antisera according to methods described by Rosenfield [51] and Cheesbrough [52]. Monoclonal Antisera A, B and D (Agape Diagnostics, Ltd, India) were used. Antisera A and B were for ABO blood typing while Antiserum D was for Rhesus factor typing.

2.7. Statistical Analysis of Data

Chi square (x^2) analysis using test of independence of two characters or associations using a 4 x 3 contingency table at 95% confidence interval was used. The software used was the statistical package for social sciences (SPSS) version 17.0. Confidence interval at 95% (0.05) was calculated using mean ± 0.05 S_X with degree of freedom calculated using (r-1) (c-1).

3. Results

The data on ABO blood groups and Rhesus factor frequency distribution among Babcock University students are shown in Table 1. A total of 183 whole blood samples obtained from 93 (50.8%) and 90 (49.2%) male and female students respectively were processed for ABO blood types. Out of this sample size, whereas 92 (54.4%) and 77 (45.6%) students were rhesus positive males and females respectively, 1 (7.1%) and 13 (92.9%) students were rhesus negative males and females respectively. On the whole, a total of 169 (92.3%) and 14 (7.7%) students were rhesus positive and negative respectively.

A total of 54 (58.1%), 24 (25.8%), 12 (12.9%) and 3 (3.2%) male students belonged to groups O, A, B and AB blood types respectively while a total of 56 (62.2%), 17 (18.9%), 14 (15.6%) and 3 (3.3%) female students were grouped into O, B, A and AB blood types respectively in that descending order. This showed that the most predominant blood group among Babcock University students is group O while the least is AB type. In the male students' population, the next highest occurring blood group was group A while it was group B in the female students' population.

Table 1. *Phenotypic ABO blood types and rhesus frequency distribution among students recruited for study with respect to sex.*

ABO blood types	Sex	Rhesus positive (%)	Rhesus Negative (%)	Total
A	M	24 (26.1)	0 (0.0)	24 (25.8)
	F	11 (14.3)	3 (23.1)	14 (15.6)
B	M	12 (13.0)	0 (0.0)	12 (12.9)
	F	12 (15.6)	5 (38.5)	17 (18.9)
AB	M	3 (3.3)	0 (0.0)	3 (3.2)
	F	3 (3.9)	0 (0.0)	3 (3.3)
O	M	53 (57.6)	1 (100)	54 (58.1)
	F	51 (66.2)	5 (38.5)	56 (62.2)
Total	M	92 (54.4)	1 (7.1)	93 (50.8)
	F	77 (45.6)	13 (92.9)	90 (49.2)
Overall total		169 (92.3)	14 (7.7)	183 (100)

The results of the age distribution of ABO blood types of Babcock University students recruited for the study are shown in Table 2. The subjects were grouped into 17-20, 21-24 and 25-30 age brackets of which 135 (73.8%), 36 (19.7%) and 12 (6.5%) students belonged to each group respectively. This suggested that the highest of subjects involved in the study belonged to the 17-20yr age bracket with an average age of 19yrs. This was distantly followed by 21-24 age bracket with an average age of 23yrs.

Table 2 clearly shows in decreasing order, that 110 (60.1%), 38 (20.8%), 29 (15.9%) and 6 (3.3%) students belonged to blood types O, A, B and AB respectively indicating that the highest and next highest occurring blood types in the studied population were groups O and A while the least occurring was clearly AB group.

In decreasing order, 77 (57.0%), 21 (58.3%) and 12 (100.0%) group O students were of 17-20, 21-24 and 25-30yr age brackets respectively. Twenty nine (21.5%), 9 (25.0%) and 0.0% group A students belonged to the same age groups respectively. Similarly, 23 (17.0%), 6 (16.7%) and 0.0% blood group B students were of the same age brackets respectively. Vertically, the distribution of ABO blood types in the 17-20 age group included 77 (57.0%), 29 (21.5%), 23 (17.0%) and 6 (4.4%) belonging to types O, A, B and AB respectively. In the 21-24 age group, 21 (58.3%), 9 (25.0%), 6 (16.7%) and 0.0% students were of O, A, B and AB groups respectively and lastly, in the 25-30 bracket, only 12 (100.0%) students were of group O blood type (Table 2).

Table 2. *Age distribution of ABO blood types of Babcock University students recruited for the study.*

ABO Blood Types	17-20yr (%)	21-24yr (%)	25-30yr (%)	Total (%)
A	29 (21.5)	9 (25.0)	0 (0.0)	38 (20.8)
B	23 (17.0)	6 (16.7)	0 (0.0)	29 (15.9)
AB	6 (4.4)	0 (0.0)	0 (0.0)	6 (3.3)
O	77 (57.0)	21 (58.3)	12 (100.0)	110 (60.1)
Total	135 (73.8)	36 (19.7)	12 (6.5)	183 (100.0)

The distribution of malaria parasitaemia among Babcock University students based on their sex is shown in Table 3. A total of 183 blood samples obtained from 93(50.8%) and 90(49.2%) male and female students respectively were processed to determine their malaria parasite status. Out of this sample size, 126(68.9%) students were infected or positive for various asexual forms of plasmodium falciparum in their peripheral blood circulation of which 66(52.4%) and 60(47.6%) were infected male and female students respectively. Malaria parasite load in terms of (+), (++), (+++) and (++++) semi-quantitatively estimated were all grouped as MP positive. A total of 57(31.1%) students made up of 27(47.4%) and 30(52.6%) male and female students respectively were un-infected or negative for malaria parasites in their blood system. Chi square (X^2) analysis carried out on data showed that sex (gender) was significantly associated with malaria parasitaemia at both 95% and 99% confidence intervals ($X^2_{0.05,\,1}$ =3.841, $X^2_{0.01,\,1}$ = 6.635, Cal. X^2 = 28.797 and hence, P<0.05, P<0.01).

Table 3. *Sex distribution of malaria parasitaemia among Babcock University students recruited for study.*

Sex	Malaria Parasite Status		TOTAL
	Positive malaria parasite Test (%)	Negative malaria parasite test (%)	
Males	66(52.4)	27(47.4)	93(50.8)
Females	60(47.6)	30(52.6)	90(49.2)
Total	126(68.9)	57(31.1)	183(100.0)

df = (r-1) (c-1) = 1 x 1 = 1
Critical (P-value) of $X^2_{0.05,\,1}$ = 3.841
Critical (P-value) of $X^2_{0.01,\,1}$ = 6.635
Calculated X^2 = 25.253
At 95%, 99% C.I, P<0.05, P<0.01).

Data on severe and non-severe malaria parasitaemia among ABO blood types of randomly selected Babcock University students are shown in Tables 4 and 5 respectively. Infected students whose stained thick films showed the presence of P. falciparum asexual forms such as schizonts, merozoites and who showed obvious signs of malaria infection were grouped as suffering from severe malaria parasitaemia. Out of a total sample size of 183, a total of 126(68.9%) students were infected with malaria parasites out of which 47(37.3%) and 79(62.7%) had severe and non-severe forms of malaria infection respectively. Blood type O recorded the second to the highest rate of severe malaria infection (70.6%) and the third highest rate of non-severe malaria infection (65.8%). The highest rates of severe and non-severe malaria infection which were 85% and 83.3% respectively were recorded by blood group A students. Whereas blood group A students recorded the second highest rate of severe malaria infection (70.6%), they recorded the third highest rate of non-severe malaria infection of 65.8%. Group AB students however, recorded the second highest rate of non-severe malaria parasitaemia. The lowest rate of severe and non-severe forms of malaria infection was recorded by group B students. A total of 19(28.8%) and 38(32.5%) students across all blood types were un-infected with severe and non-severe forms of P. falciparum malaria parasites respectively. Statistically, there was a significant association of the blood types with severe form of *P.falciparum* infection (X^2 $df(3)_{0.05}$ = 7.815, X^2 $df(3)_{0.01}$ = 11.350, Cal. X^2 = 284.601, P<0.05, P<0.01) and there was also a significant association of the blood groups with non-severe form of *P.falciparum* infection (X^2 $df(3)_{0.05}$ = 7.815, X^2 $df(3)_{0.01}$ = 11.350, Cal. X^2 = 230.768 and hence, P<0.05, P<0.01)

Table 4. *Distribution of severe malaria parasitaemia among ABO blood types of Babcock University students recruited for study.*

Malaria Status	ABO Blood Types				TOTAL
	A	B	AB	O	
Infected	17(85.0)	5(50.0)	1(50.0)	24(70.6)	47(71.2)
Un-infected	3(15.0)	5(50.0)	1(50.0)	10(29.4)	19(28.8)
Total	20(30.3)	10(15.2)	2(3.0)	34(51.5)	66(36.1)

df = (r-1)(c-1) = (2-1)(4-1) = 1x3 = 3
Critical (P-value) of $X^2_{0.05,\ 3}$ = 7.815
$X^2_{0.01,\ 3}$ = 11.350
Calculated X^2 = 284.601
At 95% C.I, P<0.05
At 99% C.I, P<0.01

Table 5. *Distribution of non-severe malaria parasitaemia among ABO blood types of Babcock University students recruited for study.*

Malaria Status	ABO Blood Types				TOTAL
	A	B	AB	O	
Infected	15(83.3)	11(57.9)	3(75.0)	50(65.8)	79(67.5)
Un-infected	3(16.7)	8(42.1)	1(25.0)	26(34.2)	38(32.5)
Total	18(15.4)	19(16.2)	4(3.4)	76(65.0)	117(63.9)

df = (r-1) (c-1) = (2-1) (4-1) = 1x3 = 3
Critical (P-value) of $X^2_{0.05,\ 3}$ = 7.815
$X^2_{0.01,\ 3}$ = 11.350
Calculated X^2 = 230.768
At 95% C.I, P<0.05
At 99% C.I, P<0.01

4. Discussion

This study was geared towards determining the ABO blood types/rhesus factor statuses of a sampled population of Babcock University students as well as probing into any possible link of these ABO phenotypes with *Plasmodium falciparum* malaria infection. This has immense significance in terms of transfusion medicine and malaria control [53]. This is because transfusion of compatible blood groups is useful in clinical studies, population genetic studies, researching population migration patterns and resolving certain medico-legal issues especially relating to disputed paternity cases [54]. Rhesus grouping is based on the presence or absence of the D antigen on red blood cells [10, 55].

In this study, 169 (92.3%) and 14 (7.7%) students were rhesus positive and rhesus negative respectively of which 92 (54.4%) and 77(45.6%) male students respectively were rhesus positive and of which 1(7.1%) and 13(92.9%) male and female students respectively were rhesus negative. This revealed that rhesus positive factor occurred by over 70% more than rhesus negative factor. This result is supported by the report of some previous authors [56, 57, 45, 58]. The overall rhesus negative frequency of 7.7% obtained in this study appears small compared to the sample size but however, has far reaching medical implication in the area of childbirth and stillbirth which may arise from haemolytic disease of newborn (HDN) or erythroblastosis fetalis which occur only in rhesus negative pregnant women at second delivery. This rhesus negative frequency rate when compared to 5.8% recorded by Otajevwo and Igoniwari [58] suggests that rhesus negative factor is occurring in increasing frequencies from population to population and hence, there is need for relevant healthcare providers as well as the Ministry of Health to track down people (women) with this factor through compulsory blood typing tests especially during pre-natal hospital visits. Many previous studies have however reported that rhesus positive population are much more frequent compared to that of rhesus negative although in varying proportions based on varied locations [59, 9, 11, 12].

The findings in this study also show that the frequency occurrence of the ABO blood types were 60.1%, 20.8%, 15.9% and 3.3% for groups O, A, B and AB respectively (Table 2). This further validates the reports of previous authors which stated that blood groups O and AB are the most and least prevalent in any population [13, 38, 45, 47, 60-66, 34, 12]. These findings are not however consistent with an earlier report which stated that groups O and A are the highest and least occurring groups [19]. These differences may be due to ethnic, racial and geographical disparities inherent in various populations.

ABO blood types as well as rhesus blood types have attracted enormous attention regarding their association with genetic and infectious diseases [55]. Previous studies on patients of cancer and tumor [67], heart disease [58], parasitic and viral infections [22] indicated associations of ABO and rhesus blood groups. In particular, the ABO antigens regulate cellular activities suggesting their impact

on determining susceptibility and severity of certain diseases [68]. In this study also, out of a total sample size of 183, 126(68.9%) students were infected with *P.falciparum* malaria parasites of which 47(37.3%) and 79(62.7%) had severe and non-severe forms of *Plasmodium falciparum* malaria infection respectively. This parasitaemia rate of 68.9% is significant and shows that Babcock University campus environment maybe endemic for malaria. This calls for the necessary or relevant University authorities to step up efforts to control the scourge. Students' hostel environments, hostel rooms and indeed, the entire University environment should be kept clean.

The parasitaemia rate obtained in this study however, appears to be low compared to 93.4% obtained in Odoakpu, Onitsha South [12] and 77.4% obtained in Owerri [69]. Parasitaemia rate obtained in this study (68.9%) appears high however when compared with prevalence rate of 66.9%, 58.3%, 43.2%, 10.0% and 6.0% obtained respectively for subjects in Ogbomosho [34]., children in Awka [70], coastal dwellers in Lagos [71], blood donors in Ibadan [72] and blood donors in Maiduguri [73]. These differences in parasitaemia rates may suggest the existence of regional differences in malaria infection rates in Nigeria with Western, Midwestern and Eastern areas (represented by IIishan Remo, Ogbomosho, Onitsha and Owerri) ranking highest in prevalence rating and the northern areas (represented by Maiduguri) occupying the lowest position while Lagos and Ibadan take a middle position. More studies however, should be done by other authors in other parts of Nigeria before a more definite statement on the apparent trends could be made.

At 95% and 99% confidence intervals, chi square statistical analysis carried out on data showed that male students were significantly more infected than the females. This finding is supported by the reports of some previous studies which stated that males were more vulnerable to malaria infection than females [74, 12, 69]. Finding is however, inconsistent with the report of some workers [34]. According to Portilo and Sullivan [75], genetic factors could play a role by endowing females with immune-regulatory potentials to cope better with some diseases.

In this study, attempt was made to link two forms of malaria (severe and non-severe) with ABO blood types. According to Beng-Ong and Hill [76], pathogenesis of severe malaria is due to the presence of of asexual forms such as schizonts, merozoites and trophozoites of *P.falciparum* in a stained thick blood film under microscope view with patient showing signs of malaria infection. The frequency occurrence of severe malaria therefore among the blood types were 85.0%, 70.6%, 50.0% and 50.0% for groups A, O, B and AB respectively (Table 4). This implied that group A students were most susceptible to severe malaria infection followed by group O subjects while the least vulnerable were groups B or AB. The finding by some authors stating that blood group O seems to confer a certain degree of protection against severe cases of malaria disease does not agree with the present finding in that regard [77, 78, 29, 79]. Also, according to Athreya and Coriell [24], malaria is a disease for which an association with ABO blood group distribution seems to exist and which may have played an important role in shaping the prevalence in favour of group O.

The finding in this study linking group A individuals as most prone to malaria infection does not agree with the reports of Singh *et al*. [80] which stated that group O subjects are more susceptible to malaria than other ABO blood groups. Other authors reported that group AB individuals were more affected than other groups by malaria [12, 81].

With respect to non-severe form of malaria and its association with the ABO blood types, the ABO blood type frequencies of non-severe infection were 83.3%, 75.0%, 65.8% and 57.9% with regard to blood types A, AB, O and B respectively. It is obvious that group A subjects (as in the case of severe malaria) were most vulnerable to non-severe form of *P.falciparum* malaria infection. Surprisingly, group AB subjects were the second most susceptible to non-severe form of malaria. Clearly, group B students were least vulnerable to severe and non-severe forms of *P.falciparum* malaria infection (Tables 4 and 5).

The report of a previous study stated that group O subjects were most prone to severe and non-severe forms of malaria and this contradicts findings in the present study. This study also shows that group B individuals were least prone to malaria and this is supported however by the report of Otajevwo and Igoniwari [57]. Also, in this study, group O individuals recorded the second highest and third highest frequencies of severe and non-severe forms of malaria infection respectively and this negates the finding by some authors stating that blood group O seems to confer a certain degree of protection against severe cases of malaria disease [77, 78].

The finding in this work that group O subjects are not most susceptible to malaria is not supported by the reports of some earlier authors [82, 29, 79, 34, 83, 84]. Present finding also disagrees with the reports of some previous authors which stated that blood group O individuals usually have low malaria attack because parasitized red blood cells have stronger tendency to form rosettes with un-infected erythrocytes of the A, B and AB blood groups than with those of blood group O [85, 86, 87, 88].

Both ABO and rhesus blood groups have attracted enormous attention regarding their association with genetic and infectious diseases [89]. Previous studies on patients of cancer and tumor [67], heart diseases [58], parasitic and viral infections [22]. indicated associations of ABO and rhesus blood groups. In particular, the ABO antigens regulate cellular activities suggesting their impact on determining susceptibility and severity of certain diseases 23]. There is a hypothesis that Plasmodium falciparum malaria has shaped the distribution of ABO blood groups in humans [24]. The pathophysiological plausibility of an interaction between malaria parasite and red blood cells as well as the potential role erythrocyte surface antigens may play in cytoadhesion of infected erythrocytes and parasite invasion may give credence to a possible association between ABO blood types

and malaria infection [82].

Numerous studies have been carried out to determine any possible association between ABO blood groups and malaria [16] and most findings from such study have been contradictory [16]. Herrera *et al.* [41] reported that there was no association between ABO blood types and malaria parasitaemia and that the controversial association of these variables previously found in other populations may be due to their peculiar demographic distributions and characteristics.

Chi square statistical analysis was carried out to associate severe and non-severe malaria parasitaemia with ABO blood groups. In the case of severe malaria infection and ABO blood types, there was a significant association existing between severe malaria blood infection and ABO blood types particularly with respect to group A and there was also a significant association between non-severe malaria infection and ABO types with emphasis on group A also. This established association between both variables in this study is in disagreement with some earlier studies and their reports which recorded no association [16, 46, 36, 89, 90, 13, 11, 66]. Present finding however, is supported by [47] who reported significant association between variables.

Many other co-existing factors made the study of relationship between malaria and ABO blood types difficult with conflicting findings reported. It is possible that complexity of the interaction between the parasites and host immune responses as well as the impact of other RBC polymorphisms may be responsible for such differences [25, 19, 91-94].

6. Conclusion

This work has revealed that there are more rhesus positive male Babcock University students than females but on the other hand, there are more rhesus negative female than male students. Rhesus negative frequency of 7.7% is high and shows a steady rise in its occurrence in populations. ABO blood types O and AB recorded the highest and lowest occurrence frequencies respectively. More male students than female students were significantly associated with malaria parasitaemia of which group A individuals were significantly associated with both severe and non-severe forms of P. falciparum malaria infection compared to the other groups. Hence, severe and non-severe forms of malaria were significantly associated with ABO blood types. Although findings suggested that group A subjects were most prone to malaria attack, malaria parasites don't discriminate between blood types and hence, all ABO blood groups are equally at risk and therefore, available prophylactic and therapeutic anti-malaria strategies by relevant health agencies should take into account persons of all groups. Rhesus negative frequency of 7.7% in female students in this study shows a steady rise compared to previous studies and this calls for the attention of prenatal and ante-natal Healthcare providers in order to prevent HDN from occurring.

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