

1 **Association of Malaria Parasite with ABO/Rhesus Blood Group Among Out-Patients of**
2 **Township Clinic Gwagwalada Abuja, Nigeria**

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4 **ABSTRACT**

5 **Aim:** This study was carried out to determine association of malaria *parasitaemia* with
6 ABO/Rhesus blood group.

7 **Study Design:** A total of 150 blood samples were randomly selected and examined for the
8 presence of *Plasmodium falciparum* using microscopy, blood group was determined using
9 agglutination technique.

10 **Results:** A total 92 (61.3%) were found to be infected with *P. falciparum*, the prevalence was
11 highest among under five (0-10) than older groups, and higher among males 55 (63.2%) than
12 female 37 (58.7%). Majority of the patients were rhesus positive 90(64.3%) while 2(20.0%) were
13 rhesus negative. High percentage of blood group O 70 (46.7%) was observed, followed by A
14 39(26.0%), B 34 (22.7%) and AB 7 (4.6%). Among the males, blood group O 42(48.3%) were
15 highest, followed A 22 (25.3%), B 18 (20.6%) and AB 5 (5.8%) of the 63 females blood group O
16 were also higher 70 (44.4%), followed by A 17 (27.0%), B 34 (22.7%) and AB 7 (4.7%). All
17 ABO blood groups showed varied presence of *P. falciparum* 51(72.8%), 22(56.4%), 17(50.0%)
18 and 2(28.5%) for O, A, B and AB, respectively. Parasite density was also higher in blood group
19 O 70 (41.69%), followed by B 34 (30.67%), and A 39 (28.09%) then AB 7 (16.84%).

20 **Conclusion:** Blood group O were most infected with malaria and AB were the least infected.
21 Malaria paracetaemia is higher in males than females and the younger ages than the older ones.
22 However further investigation is needed to clearly establish the association ABO/Rhesus blood
23 groups and *P. falciparum* infection.

24 **Key Words:** Malaria, Paracetaemia, ABO/Rhesus Blood group, *Plasmodium falciparum*, Abuja,
25 Nigeria

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28 **Introduction**

29 Malaria is a very important disease in sub-Saharan Africa with high morbidity and mortality [1].
30 It is caused by *plasmodium* species transmitted by *Anopheles* mosquito, associated with the high
31 morbidity and mortality through anaemia, cerebral complications and other mechanisms [2].
32 About 694million people in Africa are estimated to be at risk of malaria, which reserve 21% of
33 the global population at risk according to the September 2015 WHO weekly epidemiological
34 record, there were about 214 million cases of malaria and 3438,000 deaths in that year [3]. The
35 most common individuals at high risk of malaria infection in endemic areas are people of low
36 immunity, for instance, foreigners, pregnant women, children [4].

37 Despite the high morbidity and mortality associated with malaria attacks, certain individuals
38 develop resistance owing to the development of an immune response by the host and to a varying
39 extent, on a certain innate characteristic possessing protective value against infection [5] The
40 factors include sickle cell trait (HbAs) and sickle cell disease Hbss [6]. The ABO blood group
41 types [7] and the level of G-6-p- dehydrogenase [8]. There are currently 30 known human blood
42 group types [9]. but the ABO and Rhesus Blood system are clinically the most important. The
43 ABO Blood groups consists of A, B and H carbohydrate Antigen which can regulate protein
44 activities during infection and against these infection [10]. The Rhesus system blood groups
45 consist of Rhesus- positive and Rhesus- negative on the basis of the presence or absence of
46 Rhesus antigens on the red blood cell surface. The link between ABO Blood groups and the
47 incidence of malaria parasitemia or immunity to malaria is still unclear [11]. This is probably
48 because the relations between the blood group and malaria have not been well studies [12].
49 Understanding the nature of relationship between ABO Blood groups and malaria parasitemia
50 should provide a significant knowledge on whether ABO Blood groups have an impact on
51 infection status of the individuals possessing a particular ABO Blood group [13]. In view of that
52 an investigation has been conducted to find out whether or not ABO/Rh blood groups antigens
53 are associated with susceptibility or severity of *P. falciparum* malaria among patients visiting
54 the-out-patient unit of Township clinic Gwagwalada, FCT, Abuja, Nigeria.

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Comment [C1]: But

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57 **MATERIALS AND METHODS**

58 **Study Area**

59 The township clinic microbiology Laboratory in Gwagwalada was selected for this study. The
60 clinic is located in Gwagwalada along park road of Abuja the FCT.

61 **Study participants**

62 A total number of 150 subjects (87 males and 63 females) took part in the exercise. These febrile
63 subjects were recruited while visiting the out-patient unit of the township clinic in Gwagwalada
64 Abuja. The subjects were between the ages of zero (0) to fifty (50) years.

65 **Ethical Clearance/ Patients Consent**

66 The study protocol was approved by the ethical committee of the township clinic. Informed
67 consent was sought for and obtained from all adult participants while consent for children was
68 provided by their parents/guardians. Only patients who tested positive for *Plasmodium*
69 *falciparum* malaria were retained for further studies.

70 **Sample Collection, Staining and Examination of Slides**

71 Capillary blood was collected by finger pricking using 70% isopropanol and sterile disposable
72 lancet. HCCI puncture was used for infants. Immediately thin and thick films were prepared on a
73 grease free slide. The thick film was fixed with methanol and allowed to dry. The dry films were
74 stained in 10% Geimsa for 10 minutes finally, the films were examined under an oil immersion
75 microscope objective (100x). According to Cheesbrough [14].

76 **Determination of Parasite Density**

77 Parasite Density was determined for febrile patients who tested positive for *P. falciparum* by
78 counting the number of parasites (asexual forms only) against 200 white blood cells (WBC). The
79 counting was done by using hand tally counters. The number of parasites counted was expressed
80 per microlitre of blood [15].

81 Number of parasites X 8000
82 _____ _____
83 Number of leucocytes 1

84 **ABO/ Rhesus Grouping**

85 ABO/Rhesus blood grouping was done using commercially prepared monoclonal anti-A, anti-B
86 and anti-D, (anti sera + Agappe Diagnostics TD, India). One drop of each grouping antiserum was
87 added to each of the three test tubes labelled anti-A, anti-bodies and anti-AB respectively. The
88 tubes were placed on wooden rag. One drop of the test blood sample was added to each tube and
89 the suspension properly mixed by tapping the tube. They were left undisturbed for one hour at
90 room temperature. After that the tubes were examined for agglutinations [14].

91 **Statistical Analysis**

92 Data were entered in Microsoft Excel, checked for its correctness, and exported to and analyzed
93 using SPSS version 13 (SPSS Inc, Chicago, IL). Chi-square test was used to assess the difference
94 between frequencies (the associations between blood groups and *P. falciparum* malaria cases).
95 ANOVA was used to test the difference between parasite densities means. Observed difference
96 was considered to be significant at $P < 0.05$.

97 **Results**

98 **Prevalence of Malaria**

99 Out of the total of 150 blood samples collected from febrile patients who visited township clinic
100 Gwagwalada for medical attention 92 (61.3%) were found to be infected with *Plasmodium*
101 *falciparum*. The prevalence was found to be highest among under five children (0-10) as
102 compared with order groups but the difference was not significant ($P > 0.05$). Similarly, the
103 prevalence was higher among males 55 (63.2%) than female 37 (58.7%) but there was no
104 significance difference (Table 1)

105 **Distribution of ABO Blood group among subjects.**

106 Majority of the patients were rhesus positive 90(64.3%) while 2(20.0%) rhesus negative. High
107 percentage of blood group O 70 (46.7%) phenotype was observed among the study participants
108 followed by A 39(26.0%), B 34 (22.7%) and AB 7 (4.6%). There was however no significant
109 difference between the various ABO Blood group and prevalence of *P. falciparum* by age of
110 subjects ($P > 0.05$) (Table 2).

111 **Distribution of ABO Blood group based on gender**

112 Among the 87 males that took part in the study, subjects with blood group O 42(48.3%) were
113 observed to be the highest in number followed A 22 (25.3%), B 18 (20.6%) and AB 5 (5.8%) of
114 the 63 females subjects with blood group O were higher 70 (44.4%), followed by A 17 (27.0%),
115 B 34 (22.7%) and AB 7 (4.7%). But Statistical Analysis showed no significant difference
116 ($P>0.05$) (table 3).

117 **Malaria prevalence based on the ABO Blood groups**

118 All ABO blood groups showed the presence of *P. falciparum* to a certain level, 51(72.8%),
119 22(56.4%), 17(50.0%) and 2(28.5%) for O, A, B and AB, respectively. There was also
120 significant difference in the distribution of malaria infection among the various blood groups
121 ($P<0.05$) (table 4).

122 **Malaria Parasite density By ABO Blood Groups of Subjects**

123 Parasite density was found to be higher in blood group O 70 (41.69%), followed by B 34
124 (30.67%), and A 39 (28.09%) then AB 7 (16.84%). The associations were also found to be
125 statistically different ($P<0.05$). (Table 5).

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136 Table 1- Prevalence of Malaria Parasite by Age, Sex and Rhesus Factor.

137	Age	No examined	No (%) Infected
138	0 – 10	22	20 (9.1)
139	11 – 20	39	25 (6.4)
140	21 – 30	37	21 (5.7)
141	31 – 40	28	16 (5.7)
142	41 – 50	24	10 (4.2)
143	Sex		
144	Male	87	55 (63.2)
145	Female	63	37 (58.7)
146	Rhesus factor		
147	Rhesus positive	140	90 (64.3)
148	Rhesus negative	10	2 (20.0)
149	Total	150	92 (61.3)

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157 Table 2- Prevalence of Malaria based on the ABO Blood Grouping in the study population

158	Age of subject	No Examined	No (%) with Blood Groups			
159	Examined		A	B	AB	O
160	0 – 10	22	6 (27.3)	5 (22.7)	1 (4.5)	10 (45.5)
161	11 – 20	39	9 (23.0)	10 (25.6)	2 (5.1)	18 (46.1)
162	21 – 30	37	11 (29.7)	9 (24.3)	1 (2.7)	16 (43.1)
163	31 – 40	28	7 (25.0)	6(21.4)	2 (7.1)	13 (46.4)
164	41 – 50	24	5 (25.0)	4 (16.6)	1 (4.2)	13 (54.2)
165	Total	150	39 (26.0)	34 (22.7)	7 (4.6)	70 (46.7)

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179 Table 3- Gender Distribution of ABO Blood Groups

180 Gender	181 No Examined	180 No (%) with Blood Groups			
		181 A	181 B	181 AB	181 O
182 Males	87	22 (25.3)	18 (20.6)	5 (5.8)	42 (48.3)
183 Females	63	17 (27.0)	16 (25.4)	2 (3.2)	28 (44.4)
184 Total	150	39 (26.0)	34 (22.7)	7 (4.7)	70 (46.6)

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201 Table 4- Prevalence of Malaria Parasite among the various Blood Groups

202 Blood	No	No (%) positive for
203 Groups	Examined	<i>P. falciparum</i>
204 A	39	22 (56.4)
205 B	34	17 (50.0)
206 AB	7	2 (28.5)
207 O	70	51 (72.8)
208 Total	150	92 (61.3)

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223 **Table 5- Malaria Parasite Density by ABO Blood Groups of Subjects**

224 Blood	No	Mean Parasite
225 Groups	Positive	Density \pm S.D
226 A	39	2809 \pm 1.6
227 B	34	3067 \pm 1.4
228 AB	7	1684 \pm 0.2
229 O	70	4169 \pm 1.6
230 Total	92	

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232 **Discussion**

233 The malaria prevalence of 92(61.3%) obtained in the study is a reflection of high rate of the
 234 infection in the area and suggesting hyperendemicity [16]. The prevalence was higher than
 235 54.6% obtained in the Northwest [17]. prevalence of 36.1% and 36.6% were observed in Abia
 236 State (southeast) and Plateau (north central) states respectively [18]. The higher prevalence of
 237 malaria in subjects 0-10years (9.1%) is an indication of their low immunity, and is in line with
 238 finding from other studies done within sub-Saharan Africa [19]. Males (63.2%) were more
 239 infected than to females (58.7%), the reason for this has not been established scientifically but
 240 may be due to the fact that males within the study area engage more in outdoor activities
 241 (occupation) that bring them in contact with mosquitoes during the early hours of the day and at
 242 dusk while women in this part of Nigeria are usually not exposed to the public owing to religion
 243 and cultural believes Portilo and Sullivan [20]. Apart from exposure, stress (physically and
 244 mentally) due to their responsibility, may also be the predisposing factor [21], however some
 245 suggested that genetic factors could play a role by endorsing female with immuno-regulatory
 246 potentials to cope better with some disease. This is also in agreement with studies by Agbonlahor
 247 *et al.*, [7].

Comment [C3]: State the biochemical reasons relating the stress and malaria survival

248 ABO Blood grouping is based on the presence or absence of A and B antigens in the surface of
249 red blood cells (RBCs) and Rh grouping is based on the D antigen presence or absence on the
250 RBC surface [22]. A higher percentage of blood group O 70(46.6%) phenotype was observed,
251 although [5] showed that a distribution pattern of blood group O followed by A is characteristic
252 for African countries endemic of malaria, stating that the distribution of blood groups is
253 geographically and ethnically dependent. The distribution frequency of ABO was similar to the
254 findings of other scientists [23; 24]. Also, out of 150 patients that were examined only 10 (%)
255 patients were Rhesus negative, the remaining 140 (%) patients were Rhesus positive, this is in
256 agreement to previous findings [25;26]. Individuals with blood group O were found to be more
257 susceptible to malaria infection O (72.8%) compared with other Blood groups. This could be as a
258 result of the fact that both ABO and Rh blood group have attracted enormous attention regarding
259 their association with genetic and infectious diseases [27], previous studies on patients of cancer
260 and tumor [28], heart disease [29] and parasitic and viral infections [30] indicated associations of
261 ABO and Rh blood groups. More so Malaria parasites are more common and severe in group O
262 individuals compared with other Blood groups. Blood group A, B, and AB has their
263 corresponding antigens whereas O has none. Malaria parasite find it hard to invade the red cells
264 of individuals with the A, B and AB groups and required to digest the surface Antigen through
265 enzymatic activity. There is however evidence that other Blood groups were almost at the same
266 level of morbidity, and thus there is need for assessment of relation between ABO and Malaria
267 severity. Wolofsky *et al.*, [31], showed that there was no significant relationship between the
268 prevalence of malaria and ABO blood groups and *P. falciparum* sporozoites invade and mature
269 irrespective of the different ABO blood groups [32].

270 **Conclusion**

271 Blood groups O are the most susceptible to malaria infection and AB are the least infected. It can
272 be concluded that malaria paracetemia is higher in males than female and the younger ages than
273 the older ones.

Comment [C4]: Recast your conclusion

274 **Recommendation**

275 Based on the findings of this study in-depth studies are required to clearly establish the
276 association, parameters such as Hbs, Hbc, and CR and iron status, place of residence should be
277 explored. Also due to the high prevalence rate obtained in the study there is need for an

278 intensified control methodology of malaria. Due to the implication of Rhesus negative in
279 abortion and haemolytic disease of the newborn there is need to educate the populace about its
280 effect.

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Comment [C6]: References are not uniform please