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MALARIA IN CHILDREN, ITS ASSOCIATION WITH ABO BLOOD GROUP AND HAEMOGLOBIN GENOTYPE

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ABSTRACT

The prevalence of malaria among children and its association with ABO blood group and haemoglobin genotype among children attending Agbonchia Health Centre, Eleme was investigated. Blood samples were obtained from 250 children were examined for malaria parasitemia by Giemsa staining thin and thick blood film, and examining under the microscope. The prevalence of malaria among the children was 122(48.6%). The prevalence of malaria by age group showed the children between 0-4 yrs had the highest prevalence (43.2%). Prevalence of malaria by ABO blood group and haemoglobin genotype, blood group O had the highest prevalence (24.0%) and lowest cases of severe malaria (1.6%). The prevalence malaria by blood genotype, AbAA 99(39.6%) and severe malaria 20(8.0%), while HbAS 23(9.2%) and severe malaria 4(1.6%). Children with blood group O had more prevalence of malaria but less clinical episode than A and B. The HbAS genotype had less prevalence and less clinical episode than AbAA children.

INTRODUCTION

Malaria is a mosquito-borne infection of human and animals caused by genus *Plasmodium* (Fairhurst and Wallens, 2010). Malaria is presently endemic in a broad band around the equator, in areas of the Americans, many parts of Asia and much of Africa, in sub-Saharan Africa; were about 85 - 90% of malaria fatalities occur (Layne, 2006). Malaria is the most prevalent disease with high morbidity and mortality and high economic and social impact (WHO, 2001). Over 2 million fiberile episodes and one million deaths are caused by malaria in sub-Saharan Africa (Murray *et al.,* 2012). Five species of *Plasmodium* can infect and transmit malaria to humans and majority of death are caused by *P. falciparum, P. vivax, P. ovale, P. malaria* and *P. knowlesi* (Collins, 2012). Factors determining the mortality and survival of children are complex, but are related to both the host and parasite. Concerning the host factors, sickle cell trait (HbAS) have been shown to confer strong protection against *P. falciparum* infection (Luzatto, 1990). Sickle cell trait HbAS genotype confers high degree of resistance against severe and complicated malaria (Aidoo *et al.,* 2002).

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To some extent, the protection is related to physical or biochemical properties of HbAS red blood cells which prevent invasion, growth, and development of *P. falciparum* parasites (Shear *et al.,* 1993, Ayi *et al.,* 2004). The relationship of ABO blood groups and malaria parasitemia had been investigated by many workers. Severe malaria have been reported among blood group A individuals (Migot-Nabias *et al.,* 2000) and (Pathirana *et al.,* 2005) observed low parasitemia and uncomplicated malaria cases of *P. falciparum* among blood O group subjects. Significant advantage had been reported of *P. falciparum* with ABO-blood group O (Zerihum *et al*., 2011). Studies have suggested significant association between parasitemia, prevalence or antibody titer and ABO antigens (Rowe *et al*., 2007). Blood group O and HbAS may protects against severe malaria in children.

MATERIALS AND METHODS

Study Area: The study was carried out among pregnant women attending antenatal care at Agbonchia Health Centre in Eleme Local Government Area of Rivers State, Nigeria. Eleme Local Government covers an area of about 138km^2 and a population of 200,884. It is located between latitude $4^0$45S and $4^0$50N, longitudes $7^0$05E and 7.105W. Agbonchia, the largest community in Eleme is located in the fresh water zone with characteristic rainforest vegetation of the Niger Delta Area. The people are predominantly farmers.

Study Subjects: A total of 250 children attending Agbonchia Health Centre, Eleme from June – August, 2014 were selected randomly without prior knowledge of their clinical or family history. The children were of varying age ranging from 0 – 12 years.

Ethical concept: Ethical concepts are obtained from the authority of the Agbonchia Health Centre and parents whose children were used for study.

Collection of samples: Venous bloods were collected by capillary. Ethanol (70%) was used to clean site of collection, allowed to air dry and blood collected into hypodermic syringe and directly into EDTA bottle. This was mixed properly to avoid coagulation.

Preparation of blood smear: The preparation of thick and thin blood smears were done according to (Monica Cheesbrough, 2002).

Thin smear: The thin smear were allowed to air dry for 10 minutes and fixed with methanol by dipping the thin smear carefully in methanol for 5 seconds (to avoid methanol touching the thick smear). This was allowed to dry and Giemsa stain was applied for 8 – 10 minutes. This was rinsed with distilled water and allowed to dry.

Thick smear: The thick smear was air dried for about 30 minutes (not fixed in methanol) but dipped in water to de-haemoglobinize, allowed to dry and stained by the same procedure as the thin smear. The stained smears were allowed to air dry (Cheesbrough, 2002).

Microscopic examination: The back of each air dried blood film slides were carefully cleaned with cotton wool and examined using 10x and 40x firstly, morphology staining of cells and detection of malaria schizonts, trophozoites, and Gametocytes and100x objective (oil immersion) for *Plasmodium species*.

$$\text{Parasite per ml} = \frac{\text{Parasite count x 8000}}{\text{Set range of WBC (500)}}$$

Determination of Blood Genotype of Subjects: Haemolysate of each blood samples were prepared by washing the EDTA blood 3 times with normal saline. Distilled water was added to the test tube containing the blood; this was This was allowed for 5 minutes for complete lysis. The haemoglobin genotype separation was carried out using electrophoresis method as described by (Cheesbrough, 2002).

Determination of blood group: The blood groups of subjects were determined by rapid tile grouping method. Antisera A, B, AB, and D were reacted with the EDTA anticoagulated blood for the determination of ABO and Rhesus blood groups. For the Rhesus negative factor, the bloods were spun in a centrifuge and observed for agglutination both macroscopically and microscopically.

Statistical analysis: Statistical analysis was carried out using chi-square $P<0.05$ at 90% confidence limit.

RESULTS

Out of the 250 children whose blood samples were examined for malaria parasitemia, 122(48.8%) were positive for malaria parasites. The children were grouped into four age groups ranging from 0 – 13years. Age group 0 – 4 years were 200 children, 108(43.2%) were infected with malaria parasites. Age group 5 – 8 years, were 36, 11(4.4%) had malaria parasitemia, while children 9 – 12 years were 11, 2(0.8%0 were infected respectively. Age 13 years and above 3, 1(0.4%0 had malaria parasite.

Table 1. Prevalence of malaria among children by age groups

Age groups (yrs)	Number examined	Numbers positive
0-4	200	108(43.2)
5-8	36	11(4.4)
9-12	11	2(0.8)
13 above	3	1(0.4)
Total	250	122(48.8)

Numbers in parenthesis = percentages

Out of 250 children whose blood were examined for malaria parasites, 83(33.2%) were blood group A, 44(17.6%) were blood group B; 12(4.8%) were group AB and 111(44.4%) were blood group O respectively. The prevalence of malaria by ABO blood groups for A, B, AB and O were 4(16.4%), 19(7.6%), 2(0.8%) and 60(24.0%) respectively.

Table 2. Prevalence of malaria parasitemia and severe malaria by ABO blood groups among children

Blood group	Number examined	Number positive	Severe malaria in ABO group	Overall Severe malaria
A	83	41(16.40)	13(15.60)	13(5.20)
B	44	19(7.60)	7(15.91)	7(2.80)
AB	12	2(0.80)	0(0.00)	0(0.00)
O	111	60(24.00)	4(6.60)	4(1.60)
Total	250	122(48.80)	24(9.6)	24(9.6)

Numbers in parenthesis = percentages

Table 3. Prevalence of malaria parasitemia and severe malaria among children by blood genotype

Blood genotype	Numbers examined	Number positive	Severe malaria per genotype	Overall severe malaria
AA	197	99(39.6)	20(10.2)	20(8.0)
AS	53	23(9.2)	4(7.5)	4(1.6)
Total	250	122(48.8)	24(9.6)	24(9.6)

Number in parenthesis = percentages

The percentage prevalence of severe malaria among ABO blood groups A, B, AB and O were 12(4.8%), 5(2.0%), 0(0.00%) and 6(5.4%) respectively.

The prevalence of severe malaria among the ABO blood groups A, B, AB and O were 13(5.2%), 7(2.8%), 0(0.00%) and 4(1.6%) respectively. The percentage prevalence of severe malaria within each blood group for A, B, AB and O were 13(15.6%), 19(43.2%), 0(0.00%) and 4(3.6%) respectively. Of the 250 children examined, 197 (78.8%) had blood genotype AA, while 53 (21.2%) had blood genotype AS respectively. The prevalence of malaria by blood genotypes genotype are AA 99(39.6%) and AS 23(9.2%) respectively. The prevalence of severe malaria among the children by blood genotypes are AA 20(20.2%) of total infected in AA group and 20(8.0%) of overall, while AS 4(7.5%) were infected among AS children and 4(1.6%) of overall examined.

DISCUSSIONS

In this study, the percentage of blood group O observed among the children was (44.4%) phenotype, followed by A (33.2%), B (17.6%), and AB (4.8%). This was in line with the results of other studies on the frequency of O blood group as compared to other blood group. In Southern Ethiopia (Zerihun *et al.*, 2011) observed 45.7% of blood group O in Awash, Metehara and Ziway, Ethiopia; 55.8% was recorded in Amazon region, Brazil, and 54.4% in Zimbabwe (Tekeste and Petros, 2010) and (Otajevwo, 2013 observed) prevalence of 50% in Okada, Nigeria. The high percentage of O blood group obtained in this study was also in line with the findings of (Fischer and Boone, 1998). This shows high frequency of group O phenotype in tropical regions where malaria is prevalent. Various workers studying the prevalence of malaria parasitemia among children had observed different prevalence from various regions. Okafor and Oko-Ose (2012) examining children in Benin City, Edo State Nigeria, observed a prevalence of 36.4% of malaria among 2,788 children and 58.6% among age group ½ - 2years. In Uturu Abia State, Nigeria, (Etusin *et al*., 2013) examined 403 children, 338 (83.9%) were infected and age group 1 – 3 years had the highest prevalence of 172(89.5%). In another survey carried out in the Coast of Benin, West Africa, a prevalence of 68.9% was observed (Alain *et al.,* 2010). Nwaorgu and Orajaka, (2011), determining the prevalence rate of malaria parasitemia in Akwa North Local Government, Anambra, Nigeria, obtained a prevalence of 51.8% and age group 1-4 years was 71.2% respectively.

In this study, the percentage prevalence of malaria was 48.8% and for age group 0-4 years, the prevalence was 43.2%. The prevalence by age groups showed age 0-4 years had highest prevalence, which was in agreement with the results of (Hailu and Kebede, 2013, Okafor and Oko-Ose, 2012, Nwaorgu and Orajaka, 2011). The prevalence obtained in this study was lower than most prevalence obtained by others on children. This result might be influence by "the rollback malaria" program embarked by the Rivers State Government in which both insecticide treated net and malaria drug for treatments are given free of charge. The results of malaria infestation and severe malaria among ABO blood groups was investigated, the prevalence of malaria among blood group O was 60(24.0%), while cases of severe malaria among blood group O individuals was 4(1.60%) shown on table 3. Severe malaria was found to be less frequent in blood group O children. Blood group O is known to confer significant protection against severe malaria compared with non-blood group O (Zerihun *et al*., 2011, Row *et al*., 2007, Amala and Nwibani, 2015). The blood group O had more infestation rate but few clinical episodes. The reduced resetting observed in cases of malaria associated with blood group O, is known to confer protection against severe malaria by *P. falciparum*. In non O blood groups, the rosetting of infected blood cell with those of non malaria infected red cell, enhances severe malaria in blood groups A, B, and AB. Rosetting parasites and rosetting is strong risk factor to severe malaria in children with non group O blood. This occurs on the phenotype of host erythrocytes, whether rosetting receptors such as A and B triasaccharides are present, the resetting frequencies were found to be lower in O blood group (Rowe *et al.,* 2007). This shows that children with O blood group were prevented from severe malaria by reduced rosetting with *P. falciparum*.

Children with resetting frequency less than 5% could be subject to severe malaria attack episode by *P. falciparum* (Rowe *et al.,* 2007). Roseting parasites and rosetting are strong risk factors to severe malaria in children with non O blood group (Ayi *et al*., 2004). Statistical analysis $p< 0.05$ did not showed significant difference in rate of malaria infestation, but there was significant difference in the rate of severe malaria among O blood group, A and B groups. There was also significant difference in the rate of malaria infestation and severe malaria among blood group O children. From data obtained, the number of children examined were 250, 197(78.8%) were of blood genotype HbAA and 53(21.2%) were of blood genotype HbAS. Otajevwo, (2012) while investigating heterozygous haemoglobin associated with malaria parasitemia in Benin city, Nigeria, observed HbAA was 75(78.1%) and HbAS 11(24.4%), also in a similar study at University of Western Delta, Nigeria; HbAA was 265(66.7%), while HbAS was 99(27.5%) (Otajevwo and Enbulele, 2013).

The ratio of HbAA to HbAS is about 3:1 but the percentage varies from one population or ethnic group to another (Otajevwo, 2012, Anisa and Kwabene, 2013). The prevalence of malaria among HbAA and HbAS in this study are 99(39.6%) and 23(9.2%). In Yemen, the prevalence of malaria among HbAA and HbAS are 44(55.7%) and 18(37.5%) (Anisa and Kwaena, (2014). Otajevwo, (2012) observed a prevalence of 96(71.1%) among HbAA and 37(27.4%) HbAS. The prevalence of malaria in this study was 99(39.6%) for HbAA and 23(9.2%) HbAS and severe malaria among HbAA was 20(8.0%) and HbAS was 4(1.6%), which agrees with findings of others. There was significant difference at $P<0.05$ in both the rate of malaria infestation and severe malaria between HbAA and HbAS children. HbAS have been shown to protect against symptomatic *P. falciperum* malaria, mild clinical malaria and low parasite densities during such episode. These were significantly lowered in HbAS as equated to HbAA children. HbAS confers protection against serious clinical conditions such as cerebral malaria and severe anemia. One of the reasons is the reduced parasite ability to grow and multiply in HbAS (Friedman, 1978). Protection against hospital admission for *P. falciparum* and severe malaria episode were also noted in HbAS children (Possell *et al.,* 1978) which is attributed to early removal of erythrocytes from circulation by the immune system in HbAS children. Parasite infected HbAS cells have been shown to sickle 6 times more than un-parasitized HbAS cells (Luzzato *et al*; 1970, Rott *et al*; 1978).

This phenomenon may lead to intracellular parasite death and enhanced removal from circulation by the immune system. The suppression of immune system by bacterial invasion may enhance severe malaria in HbAS children (Berkley *et al;* 2005). Malaria parasite identified to cause malaria in this study was *P. falciparum* only as noted by (Amala and Nwibani, 2015).The protection offered by HbAS was found to *P. falciparum* specific (Williams *et al*., 2005). The pathogenesis of malaria related to anemia involves both bone marrow suppression and acute haemolysis (Menendez *et al*; 2000, Wheatherall and Abdulla, 1982). Children with HbAS benefit from two advantages such as, suffering few clinical attacks of *P. falciperum* malaria which means their baseline haemoglobin level may be higher and protection by lower parasite densities level achieved during incident infection (Aidoo et al; 2002). The above reasons accounts for the difference in the level of malaria parasitemia and severe malaria in HbAA and HbAS.

Conclusions

In children, although an immunocompromised group; the role of blood group O and haemoglobin genotype HbAS in protecting against *Plasmodium falciparum* malaria was not obscured or masked and the prevalence of malaria is high in children from age 0-4 years.

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