ASSESSMENT OF MALARIA PARASITEMIA AND GENOTYPE OF PATIENTS ATTENDING TWO HOSPITALS IN BENUE STATE, NIGERIA

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ABSTRACT

Malaria parasitemia and genotype of patients attending Federal Medical Center, Makurdi and General Hospital Otupko in Benue State was assessed between the months of November, 2017 and February, 2018. A total of 400 samples were collected from consented patients through venipuncture techniques. The blood samples were processed within 3 to 6 hours of collection. Infection status and genotype were ascertained using standard techniques. Their demographic characteristics and malaria control measures were determined using questionnaires. Chi-square test was used to determine the degree of association between malaria prevalence and other factors influencing its transmission. 162 patients (40.5 %) were positive. Females had higher prevalence than males and the difference was significant (x^2 = 11.993, df = 1, p = 0.00, p<0.05). The age group 0 – 9 had the highest prevalence of 46.8 %. The prevalence of malaria was not found to be dependent upon age group and the difference was not significant (x^2= 4.798, df = 6, p = 0.570, p<0.05). Individuals with genotype AA had the highest prevalence rate of 46.7 % followed by AS with 29.9 %, while no SS individual was infected. Prevalence between the three genotypes was significant (p = 0.001, p<0.05). The finding from the study showed that malaria prevalence depends on risk factors that promote transmission and that AS and SS individuals have a genetic advantage over AA individuals in relation to plasmodium parasitemia. Public enlightenment should be taken more seriously in both the urban and rural areas of Benue State.

Keywords: Malaria, Makurdi, Genotype, Hospital, Mosquito

INTRODUCTION

Malaria is a serious public health concern in sub-Saharan Africa, causing high morbidity and mortality (WHO, 2012). Some individuals in malaria academic zones are less susceptible to malaria infection despite the prevailing high morbidity and mortality rates compared to others who experience frequent malaria bouts. Some factors such as G-6-P-dehydrogences activity levels, sickle cell trait, host immune response and ABO blood groups have been linked to individuals with less or increased malaria susceptibility (Otajevwo, 2013). Malaria parasite does not thrive well in sickle cell trait because hemoglobin 'S' is known to interfere with the growth and reproduction of malaria parasite (Aidoo *et al.,* 2009).

The sheer scale of malaria in sub-Sahara Africa distribution and coexistence of several contributing factors such as ideal climatic conditions for its transmission, highly efficient *Anopheles gambiae* vectors, preponderance of the most virulent species *P. falciparum*, poverty and lack of health infrastructure (Snow *et al.,* 2001).

This study aimed at determining the frequency of malaria parasitemia among patients with various genotypes attending Federal Medical Centre (FMC), Makurdi and General Hospital, Otukpo, Benue State Nigeria. Benue State, like many other States in Nigeria, is burdened by predisposing factors such as the River Benue and its tributaries, seasonal ponds, waterlogged portions, overcrowding, illiteracy, poverty, bad drainages and improper sanitation which enhances vector breeding and transmission all year round, thus increasing the rate of malaria transmission. Thus, there's need to investigate the prevalence of malaria parasites in association with blood group and genotype in the study area so as to enhance efficacy for interventions against malaria.

MATERIAL AND METHODS

Study Area: The research was carried out in Federal Medical Centre (FMC) Makurdi and General Hospital (GH), Otukpo, Benue State, Nigeria. Makurdi lies within $8^0$30'E, $8^0$35 E and 7^0 30'N, 7^0 43'N. It has has estimated population of 428913 with growth rate of 3 % (NBS, 2011). The main monthly rainfall of Makurdi ranges from 150 mm to 180 mm and the main monthly temperatures ranges from 27^0 C to 38^0 C. Otukpo Local Government, lies within 7^0 00'N, $7^0$40'N and 7^0 30^1 E, 8^0 40'E with an annual rainfall of 1650 mm from April to October. It has estimated population of 390851 (NBS, 2011).

Study Population: The study population included patients who visited the two government hospitals and were referred to the laboratory for diagnosis between the months of November 2017 and February 2018. A total of 400 patients volunteered for the exercise. Samples were collected from between 8 am and 4 pm daily and analyzed in the Medical Laboratory of both hospitals. Sampling was done based on the availability of the individuals at a given period of time.

Ethical Consideration: Ethical clearance was obtained from the Hospital Management Board and from Benue State Ministry of Health. The study was conducted with strict adherence to the ethical standards and procedures for research with human beings participants (WHO, 2018). Recorded verbal consents were also gotten from the patients with a promise of non-disclosure of experimental outcome.

Study Design and Sampling: The study design was cross sectional epidemiological survey of two hospitals. The subjects included patients of all ages reporting to the hospital. They were directed to the hospital laboratory for blood screening. All individuals that volunteered to participate were recruited. Participant's consent was sought and they were duly informed of the significance of the study.

Questionnaire: Pre tested questionnaire was used to obtain demographic data (sex and age), socioeconomic status (civil servant, business, student, farmer, artisan and no occupation), educational status (primary, secondary, tertiary and no formal education)and other risk factors (used of insecticide treated nets (ITNs), untreated nets (UTNs), novan, insecticides, mosquito coils, traditional methods and none used of any of the above methods)associated with malaria infection from each volunteer. In case of infants, their mother or care giver that brought the infant was interviewed to obtain their data. The questionnaires were administered using a face to face interview approach and was collected back immediately to ensure 100 % return.

Parasitological Techniques

Sample Collection: Blood samples were collected from consented patients employing venipuncture technique (Cheesbrough, 2010) by the laboratory scientist. Soft tubing tourniquet was fastened to upper arm of the patient to enable the index finger feel a suitable vein. The

punctured site was then cleansed with methylated spirit (methanol) and venipuncture made with the aid of a 21 g needle attached to a 5 ml syringe. When blood had been collected, the tourniquet was released and the needle removed immediately while the blood was transferred into into labeled EDTA bottle.

Laboratory Analysis: The collected blood samples were analyzed within 3 – 6 hours of collection. Thin blood film was prepared according to the technique outlined by Cheesbrough (2010). The analyses used the following procedures:

Thin blood film preparation: A drop of each blood sample was placed in the center of a grease-free clean glass slide and spread with another clean glass slider (spreader) into a thin film, and the slide labeled accordingly. The thin films were fixed with methanol and all films were stained with Leishman stain (WHO, 2000). 7 - 8 drops of Leishman stain was added on the slide and it was left to stand for 2 minutes. Then, 15 drops of buffered water was added, it was mixed thoroughly and left to stand for 4 minutes. The slide was allowed to dry and was examined microscopically. The stained films were examined using x100 objective. The identification of *Plasmodium* species species was done using standard keys of Edington and Gills (1976). Many forms or crescent-shaped gametocytes indicated the presence of *P. falciparum.* Parasites surrounded by pale pink semi lunar containing pale red dots indicated the presence of *P. vivax*. Parasites more compact and smaller, some oval shaped parasitized cell with ragged ends indicated the presence of *P. ovale* and few thin compact rings or small round gametocytes with yellow-below pigment indicated the presence of *P. malariae*. In this way the blood films were examined and *Plasmodium* species abundance were noted. The genotypes were determined using allele-specific polymerase chain reaction (ASPCR) format together with low melting temperature agarose gel electrophoresis which allows rapid identification of the six major genotypes of the ABO blood group (Zhao and Chow, 1994).

Data Analysis: Data obtained were subjected to chi-square test to assess the prevalence of parasitemia amongst the study groups and analyzed at 95 % level of significance.

RESULTS

Out of the 400 persons examined, 162(40.5 %) were positive for malarial parasite. Females had higher prevalence (49.0 %) than male (32.0 %). However the difference in sex prevalence was significant (x^2 = 11.993, df = 1, p>0.05) (Table 1). The prevalence based on age groups indicated that the age group 0 – 9 years had the highest prevalence (48.7 %), followed age group 30 – 39 (45.2 %) and 60> years age group had the least prevalence (18.2 %). The prevalence of malaria was age group dependent but with no significant difference (x^2= 4.798, df = 6, p<0.05) (Table 2). Individuals with genotype AA had the highest prevalence (46.7 %), followed by AS (29.9 %), while no SS individual was infected. Prevalence between the three genotypes was significant (p<0.05) (Table 3). More individuals were positive for *P. falciparum* 79(48.8 %) at FMC Makurdi as compared to GH Otukpo 81(50.6 %). *P. malariae* recorded a prevalence of 2(1.2 %) at GH Otukpo, while no individual was infected at FMC Makurdi. The statistical analysis showed no significant difference (x^2= 1.927, d = 1, p>0.05) (Table 4). Artisans recorded the highest prevalence (80.0 %) followed by those with no occupation (52.0 %), farmers (50.8 %), business (49.4 %), students (32.6 %) and civil servant had the least prevalence (31.4 %). The difference in the rate of infection among the various occupation was statistically significant (x^2= 16.631, d = 5, p<0.05) (Table 5). The highest prevalence (100.0 %) was recorded among patients using no mosquito control measures and those using ITNs had the lowest prevalence (6.9 %). The difference was statistically significant (x^2 = 154.627, df = 6, p<0.05) (Table 6). Patients with non-formal education had the highest prevalence (51.9 %), followed by a patient with secondary education (50.6 %), primary education (35.1 %) and tertiary education (34.1 %). The difference was significant statistically (x^2 = 10.270, df = 3, p<0.05) (Table 7).

Table 1: Prevalence of malaria in relation to sex among health seekers at Federal Medical Centre, Makurdi and General Hospital, Otukpo, Benue State, Nigeria

Sex	FMC Makurdi		GH Otukpo		Total	
	Number Examined	Number Infected (%)	Number Examined	Number Infected (%)	Number Examined	Number Infected (%)
Male	90	33(36.7)	80	31(38.8)	170	64(32.0)
Female	110	46(41.8)	120	52(43.3)	230	98(49.0)
Total	200	79(39.5)	200	83(41.5)	400	162(40.5)

(x^2 = 11.993, df = 1, P-value = 0.0, p<0.05)

Table 2: Prevalence of malaria in relation to age of health seekers at Federal Medical Centre, Makurdi and General Hospital, Otukpo, Benue State, Nigeria

Age Group (Years)	FMC Makurdi		GH Otukpo		Total	
	Number Examined	Number Infected (%)	Number Examined	Number Infected (%)	Number Examined	Number Infected (%)
0 – 9	52	25(48.1)	59	29(45.8)	111	54(48.7)
10 – 19	36	11(30.6)	33	14(42.4)	69	25(36.2)
20 – 29	30	14(46.7)	35	13(30.1)	65	27.(41.5)
30 – 39	28	13.(46.4)	33	15(45.5)	62	28(45.2)
40 – 49	23	9(39.1)	25	8(32.0)	48	17(35.4)
50 – 59	15	4(26.7)	8	3(37.5)	23	7(30.4)
60 >	16	3(18.8)	6	1(16.7)	22	4(18.2)
Total	200	79(39.5)	200	83(41.5)	400	162(40.5)

(x^2 = 4.798, df = 6, p-value = 0.570, p>0.05)

Table 3: Prevalence of malaria in relation to genotypes among health seekers at Federal Medical Centre, Makurdi and General Hospital, Otukpo, Benue State, Nigeria

Genotype	FMC Makurdi		GH Otukpo		Total	
	Number Examined	Number Infected (%)	Number Examined	Number Infected (%)	Number Examined	Number Infected (%)
AA	140	64(45.7)	121	58(47.9)	261	122(46.7)
AS	58	16(27.6)	76	24(31.6)	134	40(29.9)
SS	2	-(0.0)	3	-(0.0)	5	0(0.0)
Total	200	80(40.0)	200	82(41.0)	400	162(40.5)

(x^2 = 13.93, df = 2, p-value = .0.001, p<0.05)

Table 4: Distribution of *Plasmodium* species among health seekers at Federal Medical Centre, Makurdi and General Hospital, Otukpo, Benue State, Nigeria

Plasmodium species	Number Examined	FMC Makurdi	GH Otukpo	Total
		Number Infected (%)	Number Infected (%)	Number Infected (%)
P. falciparum	162	79(48.8)	81(50.6)	160(98.8)
P. malariae	162	-	2(1.2)	2(1.2)
Total	162	79(48.8)	83(51.2)	162(100)

(x^2 = 1.927, df = 1, p-value = 0.165, p<0.05)

Table 5: Distribution of malaria parasite with respect occupation among seekers at Federal Medical Centre, Makurdi and General Hospital, Otukpo, Benue State, Nigeria

Occupation	FMC Makurdi		GH Otukpo		Total	
	Number Examined	Number Infected (%)	Number Examined	Number Infected (%)	Number Examined	Number Infected (%)
Civil servant	60	15(25.0)	26	12(46.2)	86	27(31.4)
Business	40	23(57.5)	41	17(41.5)	81	40(49.4)
Student	89	32(36.0)	49	13(26.5)	138	45(32.6)
Farmer	5	3(60.0)	60	30(50.0)	65	33(50.8)
Artisan	1	1(100)	4	3(75.0)	5	4(80.0)
No occupation	5	5(100)	20	8	25	13(52.0)
Total	200	79(39.5)	200	83(41.5)	400	162(40.5)

(x^2 = 16.631, d = 5, p-value = 0.000, p<0.05)

Table 6: Distribution of malaria parasite with relation to preventive methods among seekers at Federal Medical Centre, Makurdi and General Hospital, Otukpo, Benue State, Nigeria

Preventive methods	FMC Makurdi		GH Otukpo		Total	
	Number Examined	Number Infected (%)	Number Examined	Number Infected (%)	Number Examined	Number Infected (%)
ITNs	82	6(7.3)	48	3(6.3)	130	9(6.9)
UTNs	14	13(92.9)	47	31(66.0)	61	44(72.1)
Novan	29	11(37.9)	21	5(23.8)	50	16(32.0)
Insecticides	54	29(53.7)	36	3(8.3)	90	32(35.6)
Mosquito coil	21	20(95.2)	32	27(54.4)	53	47(88.7)
Traditional method	-	-	12	10(83.3)	12	10(83.3)
None	-	-	4	4(100)	4	4(100.0)
Total	200	79(39.5)	200	83(41.5)	400	162(40.5)

(x^2 = 154.627, df = 6, p-value = 0.000, p<0.05). Key: ITNs = Insecticide Treated Nets, UTNs =Untreated Nets

Table 7: Distribution of malaria parasite infection according to level of education among seekers at Federal Medical Centre, Makurdi and General Hospital, Otukpo, Benue State, Nigeria

Level of Education	FMC Makurdi		GH Otukpo		Total	
	Number Examined	Number Infected (%)	Number Examined	Number Infected (%)	Number Examined	Number Infected (%)
Primary	83	28(33.7)	88	30(34.1)	171	60(35.1)
Secondary	45	26(57.8)	42	19(45.2)	87	44(50.6)
Tertiary	61	20(32.8)	27	12(44.4)	88	30(34.1)
Non-formal education	11	5(45.5)	43	22(51.2)	54	28(51.9)
Total	200	79(39.5)	200	83(41.5)	400	162(40.5)

(x^2 = 10.270, d = 3, p-value = 0.016, p<0.05)

DISCUSSION

The overall prevalence of malaria recorded in this study was 40.5 %. The World Health Organization asserted that Nigeria is among the 31 countries where malaria is still endemic and that Nigeria accounted for almost 24 % of global malaria deaths in 2018 (WHO, 2019). The result agreed with this assertion. Studies by Jumbo *et al.* (2010) and Amuta *et al.* (2014) reported 42.4 % and 68.3 % prevalence respectively among children from the same hospital. The Lower prevalence in this study is an indication of positive response to roll back malaria in this community (RBM, 2015). However the infection rate among patients may be due to geographical location, seasonal variation, environmental conditions, socio-economic status of the study population such as housing, deplorable level of education, ignorance, poverty, neglect, socio-cultural lifestyle, poor utilization of ITNs and low ownership of ITNs which is a cardinal tool for the success of the roll back malaria (RBM) initiative (Deressa and Ali, 2009; Curtis *et al.*, 2003; Yewhalaw *et al.*, 2013). Male and female patients were equally at risk of getting infected. However, disease prevalence was slightly higher in female than male. This correlated with the work of Otajevwo (2013) and Jenkins *et al.* (2015) but contradicts the studies of Ebenezer and Amadi (2008) in Bayelsa State, Nigeria and Klinkenberg *et al.* (2006) in two cities in Ghana, who recorded males to be more infected than females. Malaria infection in both female and male recorded in this study may be due to the high rate of outdoor activities during the day and night which exposes both sex to mosquito bite. All age groups were exposed to the risk of malaria parasite. Patients aged 0 – 9 years of both sexes had the highest record of the infection at the two hospitals. This could be that they acquired the infection at school, residential houses and through other activities. In addition, the immunity in this age group is typically low

but as age increases, stronger immunity is built up due to proper nutritional balance, cumulative exposure to the parasite and adequate treatment for the disease. This was consistent with the findings of Pant *et al*. (1981) and Griffin *et al.* (2015), who reported age related pattern of biting and transmission of the disease and attributed it to host preference by the vector, level of immunological response of the host including differential activities of the age group.

Patients with genotype AA had the highest prevalence of malaria parasite followed by AS and lowest prevalence occurred in genotype SS. This finding was similar to those of Kolawole *et al.* (2006) in Ilorin, Nigeria, Akhigbe *et al*. (2011) in Ogbomoso, Nigeria, Otajevwo (2013) in Igbinedion University Okada, Nigeria, and Kolawole *et al*. (2017) who reported higher malaria parasitemia among people with AA genotype. This finding was in contrast with that of Ocheje and Mustapha (2016) in Duste, Jigawa State, Nigeria, where genotype AS had the highest infection with malaria.

The high prevalence rate of genotype AA could be attributed to the fact that during the peripheral blood stage of replication, malaria parasites have a high rate of oxygen consumption and ingest large amounts of haemoglobin A (normal haemoglobin gene). Unlike haemoglobin A (HbA), haemoglobin S (HbS) in endocytic vesicles of the parasite is deoxygenated, polymerizes and is poorly digested. In the digestive process toxic portion of the haemoglobin called haemin (ferri-protoporphyrin) is converted to the non-toxic substance called haemozoin by the parasite enzyme called malarial haem polymerase enabling the parasite to survive. However, HbS is poorly digested by the parasite and as a result haemin accumulate thereby inhibiting the replication and survival of the parasite in HbS containing red blood cell (Vaidaya and Mather, 2009).

In red cells containing abnormal haemoglobin (HbS) or which are glucose–6–phosphate dehydrogenase (G6PD) deficient, oxygen radicals are produce and malaria parasites induce additional oxidative stress and this result in changes in red cells membranes, including translocation of phosphatidylserine to their surface, followed by macrophage recognition and ingestion. This mechanism occurs earlier in abnormal (SS) than in normal red cells (AA), thereby restricting the multiplication of malaria parasite (Friedman, 1978). Translocation of sickle cell erythrocyte micro RNAs into *P. falciparum* contributes to malaria resistance to patients with genotype SS. Therefore, AS and SS individuals have a genetic advantage over AA individuals (LaMonte *et al*., 2012).

The low rate of other *Plasmodium* species may be because they tend to be selective in the type of blood cells they infect. *P. malariae* prefers leucocytes and young red blood cells than the old blood cells (Singh *et al.,* 2015). The non-usage of mosquito control measures and the failure to use ITNs was a significant risk factor for malaria in this study. This was consistent with previous reports of Loha and Lindtorn (2012) among residence of Chano Mille, South Ethiopia and Fana *et al*. (2015) among pregnant women in a semi-urban community of north-western Nigeria. The high malaria infection rate observed among patients that used mosquito coil, traditional methods, and insecticide spray could be due to mosquitos' resistance to the chemical or to the low quality of insecticides sold (Amuta *et al.,* 2014).

Occupationally, prevalence was highest among artisan and least among civil servants. This may be as a result of the differences in socio-economic status, illiteracy and negligence of the use of mosquito control measures by this group of people. Ricci (2012) stated that most households and individuals are prevented from using goods and services that otherwise would protect them against the risk of malaria because of poverty. Furthermore, malaria infection was significantly higher among patients with no-formal education. Kolawole *et al.* (2006) and RBM (2015) asserted that education holds the key to sustainable response to malaria awareness and prevention, while low level of education is associated with ignorance and increased vulnerability to malaria.

Conclusion: Finding revealed malaria prevalence of 40.5 %, thus indicating that malaria is still endemic in the study area. However, the prevalence might have probably reduced from over 80 % previously recorded in the national survey in the present level due to progress made in control efforts in the state. Females had higher prevalence was higher prevalence than males. Malaria prevalence was not dependent on socioeconomic status, but on the presence of risk factors that favour transmission of the diseases. Malaria due to *P. falciparum* was more common in this study. Malaria prophylactic and therapeutic strategies by government and non- governmental health agencies should be directed at individuals of both sex and all groups without any discrimination or preferential treatment. The monthly sanitation and public enlightenment should be taken more seriously in both the urban and rural areas of Benue State.

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