



Research Article

Prevalence of Immunoglobulin G and M among children
infected with *Plasmodium falciparum* in some Hospitals in
Maiduguri, Borno State

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ABSTRACT

The threat of Malaria to the public health especially in Sub-Saharan Africa is enormous and Nigeria in particular is considered a bit hotspot. The diseases are transmitted by *Plasmodium* species with severe disease outcome children under 5 years. In this study, we undertook a cross-sectional study using representative cohort of children in Maiduguri aged 5 – 59 months to determine the seroprevalence and risk factors associated with infection with *P. falciparum* in this vulnerable aged group. A cross-sectional study was conducted on participants from four hospitals in Maiduguri, Borno State. In total, plasma was collected from 182 and 91 participants for the detection of IgG and IgM antibodies, respectively using Enzyme-Linked Immunosorbent Assay for the measurement of IgG and IgM antibody production against *P. falciparum*. The result indicates that the overall seroprevalence was (75.3%; 137/182) and (86.8%; 79/91) corresponding to IgG and IgM respectively. Based on gender, male 24(13.2%) and 8(8.8%) were positive with respect to IgG and IgM while 21(11.5%) and 4(4.4%) of the females were positive to IgG and IgM antibodies respectively. Additionally, both University of Maiduguri Teaching Hospital and State Specialist Hospital Maiduguri, recorded the highest prevalence of 15 (8.2%) for IgG while only UMTH recorded the highest prevalence of 5(5.5%) to IgM antibodies. In conclusion, the overall seroprevalence was found to be low for both IgG and IgM. However, our study observed differences in the seroprevalence of *P. falciparum* in children according to age and study locations for IgM and IgG respectively. Also, males have high seroprevalence for both IgG and IgM.

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INTRODUCTION

The threat of Malaria to the public health especially in Sub-Saharan Africa is enormous and Nigeria in particular is considered a bit hotspot. The diseases are transmitted by *Plasmodium* species with severe disease outcome children under 5 years. There are five different human *Plasmodium* species such as *P. falciparum*, *P. vivax*, *P. malariae*, *P. knowlesi* and *P. ovale* (Elhussein *et al.*, 2015). Focus has been on control and elimination of the disease; however, the efforts could not tame the threat to the public health. According to global record of 2018, it was reported that 228 million cases of malaria occurred globally, with a slight decrease of cases from the previous year, 2017 in terms of mortality and morbidity. The burden of malaria cases reported as 93% and deaths 94% occurred in the Africa Region with *Plasmodium falciparum* accounting for 99.7% of the cases (WHO, 2019). Annually, in Sub-Saharan Africa, about 25 million pregnant women are at risk of *P. falciparum* malaria infection (Steketee *et al.*, 2001). According to World Health Organization recorded Malaria as the most highly prevalent tropical disease with high morbidity and mortality, accompanied with high economic and social impact (WHO, 2010). The mortality rate of malaria in Sub-Saharan Africa is tagged at 90% of all deaths caused by malaria, in addition to this, about 85% of deaths globally is reported to be among children under 5 years of age (WHO, 2010).

In Malaria endemic regions, children at age of 4 to 6 months are the most vulnerable group to malaria and exchange of maternal antibodies is considered as the contributing factor (Dobis, *et al.*, 2016). Thereafter, the transfer of maternal antibodies could lead to the development of antibodies (Doolan, *et al.*, 2009). Antibodies are effector immune concept that mediates in acquired form to

malaria. On the surface of infected erythrocytes, two important antigens; Blood-stage merozoite antigens and variant surface antigens (VSA) are expressed. The antibodies involved in antigens inhibit invasion of red blood cells (RBCs), as well as preventing inter-erythrocytic growth (Richards, & Beeson, 2009). Furthermore, the antibodies involved in organization for phagocytic clearance and complement fixation (Osier, *et al.*, 2014; Boyle, *et al.*, 2015). Interestingly, the reaction of antibodies to merozoite antigens is sufficient to contribute to the community (Richards, 1998). The burden of exposure of Young children made them to act as reservoir of malaria (Stanisic *et al.*, 2015), with the potential to inform surveillance and control activities (Elliott, *et al.*, 2014). Among the tested antigens, merozoite surface protein 1 (MSP 1) is the most copious protein found on the surface of the merozoite (Gilson, *et al.*, 2016).

Nigeria is a breeding ground of malaria and resulted to partial immunity. Thus, people living at malaria endemic areas develop naturally acquired immunity to *Plasmodium* spp. Based on age, the infections occurs slowly and after repeated exposure (Marsh and Kinyanjui, 2006; Doolan *et al.*, 2009), then capability of controlling parasitemia in the blood is then developed at a later stage.

Studies have indicated that antibodies involved in protection against clinical malaria due to *P. falciparum*. The main response of antibodies against cell surface proteins of either the merozoite form of the parasite or of infected red blood cells is responsible acquired protective immunity against malaria (Richards and Beeson, 2009). The development of partial immunity to clinical malaria is reported to be antigen-specific, and it is mediated by exposure to various genetically distinct parasite subpopulations (clones). In regions, where malaria is endemic, individuals are at

risk of being to a diversity of clones, which results in a rapid development of anti-malaria immunity and asymptomatic infections (Magesa *et al.*, 2002). In areas of low transmission, however, there is a lower rate of multiclonal infections and anti-disease immunity develops more slowly (Hamad *et al.*, 2002).

This study aims at determining the seroprevalence of immunoglobulin (IgG) and immunoglobulin (IgM) antibody responses against *P. falciparum* merozoite antigens. Understanding of the basis of acquired immunity would contribute to the global elimination and control strategy of malaria. This study seeks to provide baseline data on the seroprevalence and acquired immunity due to *P. falciparum* infection in a Maiduguri, Borno state targeting four secondary health facilities in the State.

MATERIALS AND METHODS

Study Area and population

The study was carried out at the pediatric units of the general out-patient department (GOPD) of the four hospitals in Maiduguri, Borno State. The State lies approximately between latitude 10° 2'N and 13° 4'N and longitude 9° 8' E and 14° 4'E, while Maiduguri lies on latitude 11° 40'N and 13° 5'E longitude. Maiduguri is the capital of Borno State in northeast Nigeria. It has a mean annual rainfall and temperature of about 650 mm and 32°C respectively. The month of March and April are the hottest periods of the year with temperatures ranging between 30°C and 40°C. It is usually cold and dry during the harmattan, November to January being the coldest months (Borno State Ministry of Information, 2015). All participants recruited into the study were children within the age ranges from 6 to 59 months from the four health centers selected for the study.

Study design and sampling

The study design is a case-control study conducted between December 2021 to April 2022. Consent to participate in the study was obtained through parents or guardians. Before enrolment, information about study objectives and procedures was provided and explained to the guardians in the local language. Study staff read and explained the consent form in the local language for subjects and parents or guardians who didn't acquire formal education. After this, the subjects were submitted to laboratory scientists for blood sample collection. Initially, four hundred and twenty-one (421) children within the age range of 6-59 months were enrolled in this study. After microscopy, 182(43.23%) of the samples were confirmed positive for the presence of *P. falciparum* parasite. Hence, plasma from these subjects were used for serological detection of antibodies (IgG and IgM) to *P. falciparum*.

Collection of blood sample

Venous blood was collected using hypodermic needle and 5 mL syringe into a well-labelled sterile tube which contained EDTA bottle. The tubes were centrifuged at 3,000 rpm for 10 min using a bench centrifuge (Centromix, UK). Plasma was collected into plain tubes that were properly labeled and stored in the freezer at - 20 °C until required for use.

ELISA for the detection of anti-*P. Falciparum* antibodies

The plasma collected from the children enrolled in the study were tested using ELISA Kits (Sunlong Biotech CO., Ltd, China) to determine the presence of *P. falciparum* specific IgG and IgM antibodies, resulting from infection with *P. falciparum*. A 96-well plate was prepared for both test and control specimens containing the test and control specimens at same time, before transferring them into the ELISA microplate using a multichannel pipette. Optical densities

(OD) were read at 450 nm. The test was validated if the mean value of the positive control O.D. (ODPC) is greater than or equal to 1.00 (≥ 1.00) and the mean O.D. values of the negative controls is less than or equal to 0.10 (≤ 0.10). According to the manufacturer's recommendation, plasma with OD value $<$ CUT OFF be considered as negative while plasma with OD value \geq CUT OFF, be considered as positive.

The critical value is computed using the formula:

CV= the average value of negative control + 0.15.

Ethical Clearance

Ethical clearance was obtained from the study locations and approval was duly given before the commencement of the study.

Statistical analysis

Chi-square was used to determine whether there are associations between malaria status and sero-prevalence of Immunoglobulin G and M. among the children. Graphs were plotted using GraphPad Prism ver. 8. The difference was considered significant when $P \leq 0.05$.

RESULTS

The overall prevalence of immunoglobulin (IgG) among positive children in some hospitals in Maiduguri, Borno State was 24.7% while those of negative children were 75.3% (Figure 1a & b). On the other hand, the overall prevalence of immunoglobulin (IgM) among positive children in some hospitals in Maiduguri, Borno State was 13.2% while that of negative children was 86.8% (Figure 2).

A significant association was observed between *P. falciparum* malaria status and immunoglobulin G (IgG) with respect to study locations (hospital) ($\chi^2= 8.062$; $p=0.045$) as shown in table 1. On the other hand, a non-significant association was observed between the malaria status and gender of the subjects ($\chi^2= 0.235$; $p=0.628$) and age of the subjects ($\chi^2= 1.814$; $p=0.628$) (Table 1). A highly significant association was observed between *P. falciparum* malaria status and immunoglobulin M (IgM) with respect to age of the subjects in the study area ($\chi^2= 39.08$, $p=0.000$), with subjects within the age group 6 – 10 months having the highest prevalence of 11.0%, Whereas the non-significant association was observed between the malaria status and gender of the subjects ($\chi^2= 0.096$; $p=0.757$) as well as study locations (hospital) ($\chi^2= 4.632$; $p=0.201$) (Table 2).

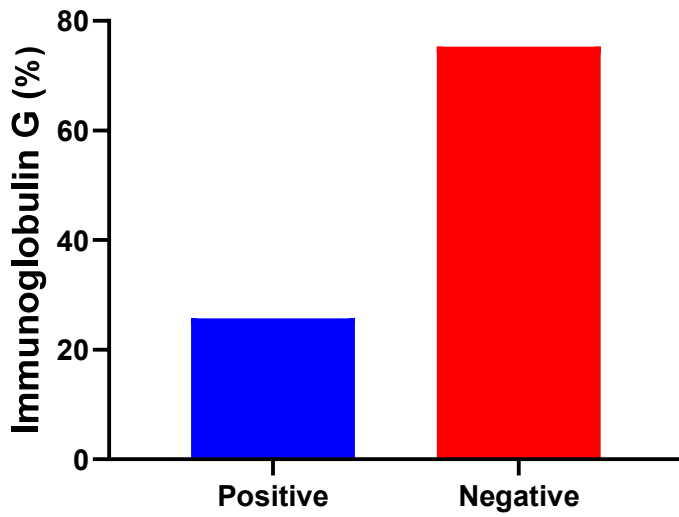


Figure 1a: The overall prevalence of immunoglobulin (IgG) among children in some hospitals in Maiduguri, Borno State

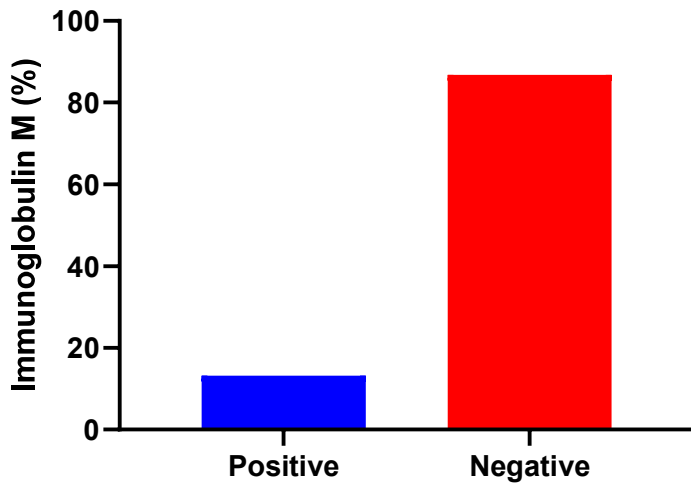


Figure 1b: The overall prevalence of immunoglobulin (IgG) among children in some hospitals in Maiduguri, Borno State

Table 1: Association between malaria status and Immunoglobulin G in children (6-59 months) in Maiduguri, Borno State, Nigeria

Variables	No. examined	No. of positive (%)	No. of negative (%)	χ^2	p-value
Age (months)					
6 – 10	26	2 (1.1)	24 (13.2)	1.814	0.936
11 – 20	44	10 (5.5)	34 (18.7)		
21 – 30	43	9 (4.9)	34 (18.7)		
31 – 40	22	6 (3.3)	16 (8.8)		
41 – 50	16	6 (3.3)	10 (5.5)		
51 – 60	31	12 (6.6)	19 (10.4)		
Total	182	12 (24.7)	137 (75.3)		
Gender					
Male	104	24 (13.2)	80 (44.0)	0.235	0.628
Female	78	21 (11.5)	57 (31.3)		
Total	182	45 (24.7)	137 (75.3)		
Hospital					
UMTH	36	15 (8.2)	21 (11.5)	8.062	0.045
SSHM	66	15 (8.2)	51 (28.0)		
USUMH	38	7 (3.8)	31 (17.0)		
MSMH	42	8 (4.4)	34 (18.7)		
Total	182	45 (24.7)	137 (75.3)		

Table 2: Association between malaria status and Immunoglobulin M in children (6-59 months) in Maiduguri, Borno State, Nigeria

Variables	No. examined	No. of positive (%)	No. of negative (%)	χ^2	p-value
Age (months)					
6 – 10	17	10 (11.0)	7 (7.7)	39.08	0.000
11 – 20	23	2 (2.2)	21 (23.1)		
21 – 30	22	0 (0.0)	22 (24.2)		
31 – 40	81	0 (0.0)	8 (8.8)		
41 – 50	9	0 (0.0)	9 (9.9)		
51 – 60	12	0 (0.0)	12 (13.2)		
Total	91	12 (13.2)	79 (86.8)		
Gender					
Male	57	8 (8.8)	49 (53.9)	0.0969	0.757
Female	34	4 (4.4)	30 (33.0)		
Total	91	12 (13.2)	79 (86.8)		
Hospital					
UMTH	19	5 (5.5)	14 (15.4)	4.632	0.201
SSHM	26	3 (3.3)	23 (25.3)		
USUMH	22	3 (3.3)	19 (20.9)		
MSMH	24	1 (1.1)	23 (25.3)		
Total	91	12 (13.2)	79 (86.8)		

DISCUSSION

Malaria remains the biggest public health concern in Nigeria. Malaria is account for up to 30% of childhood death, 25% of death in children under one year, and 11% of maternal death. In Nigeria, data available indicates that a child could experience malarial infection between 2 and 4 times annually and 70% of pregnant women suffer from malaria; contributing to maternal anaemia, low birth weight, stillbirth, abortion and other pregnancy-related complications (Federal Ministry of Health, National Malaria Control Program, 2005).

The overall sero-prevalence (IgG and IgM) was low with 24.7% and 13.2% respectively. This pattern of low prevalence has been documented in the Gambia and Sierra Leone. The low antibodies may be in part due to the short life of anti- p fMpl9 antigens which are mainly found after clinical episodes (Cavanagh *et al.*, 1998) and low immunogenicity or lack of adequate T cell help for antibody protection in these infected children (Egan *et al.*; 1997; Hui *et al.*, 1996).

A significant association was observed between *P. falciparum* malaria status and immunoglobulin G (IgG) in some hospital in the area. This finding corroborates with Nmorsi, *et al.*, (2019) who reported a positive association between the level of malaria parasitaemia and IgG profile. Furthermore, he reported children within 1-5 years recorded the highest parasite locally and this implicates high IgG to be associated with the severity of malaria. These observations appear to derive from the association that IgG appears to play a role in protection against malaria diseases (Cohen *et al.*, 1962; Miller *et al.*, 1994).

Significant associations between malaria status and immunoglobulin M (IgM) were observed with respect to age of the subjects in the study location. This finding is consistent with studies done in Nigeria (Morakiyo *et al.*,

2018) and Uganda (Robbers and Mathews, 2016), where children had a higher proportion of malaria as well as increased the risk of infection. These findings could be due to two factors; maternal antibodies transferred to children in their first 12 months of life and inadequate protection of older children. A study reported that the immunoglobulin M, which has a half-life of six (6) months, is actively transferred during pregnancy and nadir occurs at months of life explains the reason why malaria is low in children in their first 12 months compared to older children (Charnaud *et al.* (2016). Furthermore, children who are still being breastfed are more likely to be protected with lactoferrin and secretory immunoglobulin A (IgG) which have all been shown to impede the growth of malaria parasite *in vitro* (Kassim *et al.*, 2000).

However, a non-significant association was observed between malaria status and gender of the subjects with respect to IgG and IgM in this study. Furthermore, reports have it that the prevalence of malaria is often higher in males than females (WHO, 2005; WHO 2006), and not influenced by gender statistically (Giles and Warell, 1996). The higher prevalence rate among males could just be by chance. However, Krogsad (1996) attributed sex differences to malaria, most of the studies that reported sex differences whereof statistically significant.

CONCLUSION

Generally, the overall seroprevalence was found to be low for both IgG (24.7%) and IgM (13.2%). However, our study observed significant association between the seroprevalence of *P. falciparum* in children according to age and study locations for IgM and IgG respectively. Also, males (13.2 % and 8.8%) have high seroprevalence compared to their females counterpart (11.5% and 4.4%), for both IgG and IgM respectively.

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REFERENCES

- Boyle, M.J., Reiling L., Feng, G., Langer, C., Osier, F.H., Aspelng-Jones, H., *et al.* (2015). Human Antibodies fix Complement to Inhibit *Plasmodium falciparum* Invasion of Erythrocytes and are Associated with Protection Against Malaria. *Immunity*.**42**:580–90.
- Cavanagh, D.R., Elhassan, I. M., Cally Roper, V. Robinson, J., Giha H., Anthony, A., Holder, A. *et al* 1998 . A Longitudinal Study of Type-Specific Antibody Responses to *Plasmodium falciparum* Merozoite Surface Protein-1 in an Area of Unstable Malaria in Sudan. *The Journal of Immunology*, **161**: 347–359.
- Charnaut, S.C., MCGreedy, R., Herten-Crab A., Powel R., Guy, A., Langer C., *et al.* (2016). Maternal fetal transfer of *Plasmodium falciparum* and *Plasmodium vivax* antibodies in a low transmission setting. *Scientific report*, **6**:20859.
- Cohen, S., MC Gregor, I., Carrington, S. (1962). Gamma globulin and acquired immunity to human malaria nature. *192*:733-737.
- Dobis, K.R., Dent, A.E. (2016). *Plasmodium* Malaria and Antimalarial Antibodies in the First Year of Life. *Parasitology*.**143**:129–38.
- Doolan., D.L., Dobano, C., and Baird, J.K. (2009). Acquired Immunity to Malaria *Clinical Journal of Parasitology* **22**:13–36.
- Egan, A.F., Moris, J., Barnish, G., Allen, S. Green wood, B.M., Kaslow, D.C., Holder, A.A, Riley, E.M. (1996). Clinical immunity to *Plasmodium falciparum* malaria is associated with Serum antibodies to 19-KDA C terminal fragment of the merozoite surface antigen PFMSP-1. *Journal Infections Diseases*. *173*:765-769.
- Egan, A.M., Waterfall, M., Pinder, M., Holder A., Riley, E. (1997). Characterization of human T and B cell epitopes in the C-terminus of *Plasmodium falciparum* merozoites surface protein I: evidence for poor T-cell recognition of polypeptides with numerous disulfide bonds. *Infection and immunity*. *65*:3024-3031.
- Elhussein, A. B., Huneif, M. A., Naeem, A., Fadlseed, O. E., Babiker, W. G., Rahma, N. E. A. A., ... & Shalayel, M. H. F. (2015). Correlation of interleukin-4 levels with *Plasmodium falciparum* malaria parasitaemia in Sudanese children. *Acta Clinica Belgica*, *70*(6), 414-418.
- Elliott, S.R., Fowkes, F.J.I., Richards, J.S., Reiling, L, Drew, D.R., Beeson, J.G. (2014). Research Priorities for the Development and Implementation of Serological Tools for Malaria Surveillance. *F1000Prime Rep*. **6**:100.
- Gilles, H.M., Warrell, D.A. (1993). In Bruce-Chwatt's Essential Malariaology, 3rd Edition. Edward Arnold pp. 19-24.
- Gilson P.R., Nebl, T., Vukcevic, D., Moritz, R.L., Sargeant, T., Speed, T.P., *et al.* (2016). Identification and Stoichiometry of Glycosyl Phosphatidylinositol-Anchored Membrane Proteins of the Human Malaria Parasite *Plasmodium falciparum*. *Mol Cell*
- Hui, G.S, Nikardo, S.C., Hashiro, C., Kaslow, D.C., Collins, W.E. (1996). Dominance of G conserve B-cell episodes of *Plasmodium falciparum* merozoite surface protein MSPI, in blood stage infections of naïve Aofus Monkeys infection and immunity. *64*:1502-1509.
- Kassim, O., Ako-Anai, K. Torimiro, S., Hollowell, G., Okoye, V., Martin, S. (2000). Inhibitory factors in breastmilk, maternal and infant sera against in vitro growth of *Plasmodium falliparum* malaria parasite. *Journal of Tropical Pediatrics*. *46*:92-6.

- Krongstad, D.J. (1996). Malaria as a re-emerging disease epidemiology. Review, **18**:77-89.
- Magesa, S.M., Mdira, K.Y., Famert, A, Simonsen, P.E., Bygbjerg, I.C., & Jakobsen, P.T.I. (2001). Distinguishing of *Plasmodium falciparum* Treatment Failures from Reinfections by Using Polymerase Chain Reaction Genotyping in a Holoendemic Area in Northeastern Tanzania. *American Journal Tropical Medicine and Hygiene* **65**, 477-483.
- Miller, L., Good, M., Milon, G. (1994). Malaria Pathogenesis. *Science* 264:1878-1883.
- Morakinyo, O. M., Balogun, F. M., and Fagbamigbe, A. F. (2018). Housing Type and Risk of Malaria Among Under-five Children in Nigeria: Evidence from the Malaria Indicator Survey. *Malaria Journal*, **17** (1), 311
- Nmorsi, O.P.G., Nkwandu, N.C.D., Isaac, C., Koma C., and Asibor V. (2008). Immunoglobulin profile of Nigerian children with *Plasmodium falciparum* infection. *African Journal of Biotechnology*, **7**(2): PP. 077-080.
- Osier, F.H., Feng, G., Boyle, M.J., Langer, C., Zhou, J., Richards, J.S., *et al.* (2014). Opsonic phagocytosis of *Plasmodium falciparum* Merozoites: Mechanism in Human Immunity and Correlate of Protection Against Malaria. *BMC Medicine* **12**:108.
- Ramasamy, R. (1998) Molecular Basis for Evasion of Host Immunity and Pathogenesis in Malaria. *Biochimica Biophysica Acta General Subject Journal* **1406**, 10-27.
- Richards, J.S., Beeson, J.G. (2009). The Future of Blood-stage Vaccines Against Malaria. *Immunology Cell Biology* **87**:377-90.
- Richards, J.S., Stanisic, D.I., Fowkes, F.J., Tavul, L., Dabod, E., Thompson, J.K., *et al.* (2010) Association Between Naturally Acquired Antibodies to Erythrocyte-Binding Antigens of *Plasmodium falciparum* and Protection from Malaria and High-Density Parasitemia. *Clinical Infection Disease* **51**: e50-60.
- Roberts, D., & Matthews, G. (2016). Risk Factors of Malaria in Children Under the Age of Five Years Old in Uganda. *Malaria Journal*, **15**, 246
- Staalsoe, T., Hamad, A.A., Hviid, L., Elhassan, I.M., Arnot, D.E., & Theander, T.G. (2002). In Vivo Switching Between Variant Surface Antigens in Human of *Plasmodium falciparum* Infection. *Journal Infections Disease*. **186**, 719-722.
- Stanisic, D.I., Fowkes, F.J., Koinari, M., Javati, S., Lin, E., Kiniboro, B., *et al.* (2015). Acquisition of Antibodies Against *Plasmodium falciparum* Merozoites and Malaria Immunity in Young Children and the Influence of Age, Force of Infection, and Magnitude of Response. *Infections Immunity* **83**:646-60.
- Steketee, R.W., Nahlen, B.L., Parise, M. E., Menendez, C. (2001). The Burden of Malaria in Pregnancy in Malaria Endemic Areas. *American Journal Tropical Medicine Hygiene* **64**:28-35.
- WHO (2006) World health statistics, NHs, Nigeria fact sheet, No .3, p.7.
- WHO 2005. Susceptibility of *Plasmodium falciparum* to antimalarial drugs: report on global monitoring, WHO/HTM/MAL/1103.
- World Health Organization. World Malaria Report, 2019; World Health Organization: Geneva, Switzerland.