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Original Research Article

Asymptomatic malaria among students of Federal University of Technology, Owerri(FUTO), Imo State, Nigeria

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The study aimed at determining the prevalence of asymptomatic malaria among students of Federal University of Technology Owerri, was carried out between July and September 2015. Blood samples of 160 students who lived both in school hostels and off campus residents were examined using Giemsa stained thick and thin films. A total of 40 students (25%) were infected with *Plasmodium falciparium*, with an overall asymptomatic case of 11.25%. Students of age 16-22 years had the highest prevalence of 28.28%, with an asymptomatic case of 46.43%, age group 23-29 years had a prevalence of 19.67%, with an asymptomatic case of 41.67%. It was observed that male students had the higher prevalence of 26.15%, with an asymptomatic case of 47.06%, while that of the females was 24.21%, with an asymptomatic case of 43.48%. On the relationship of malaria parasites and genotype, students with genotype 'AA' had an infection rate of 27.69%, with an asymptomatic case of 47.22% and students with genotype 'AS' had a prevalence of 13.33% with an asymptomatic case of 25%. The result shows that students with blood group O had highest prevalence of 60%, with an asymptomatic case of 61.1%. Blood group A has a prevalence of 27.5% with an asymptomatic case of 27.8%, and Blood group B has a prevalence of 12.5%, with an asymptomatic case of 11.1%, while blood group AB which was 5.62% of the study population had no case of prevalence. Asymptomatic malaria is prevalent in this community as reflected in this study; this could impact negatively on the health of the population. More efforts are needed to encourage public enlightenment on malaria diagnosis and treatment.

Key words: Parasitaaemia, prevalence, asymptomatic, malaria, students, plasmodium, anopheles.

INTRODUCTION

Malaria is a life-threatening disease of man caused by a parasite of the genus *Plasmodium,* which is transmitted through the bite of infected female *Anopheles* mosquitoes. It is a killer and debilitating disease and remains a formidable health and socio-economic problem in the world (Nebe et al., 2007). Over 90% of all deaths caused by malaria occur in sub – Saharan Africa and about 85% of deaths globally are in children under 5 years of age (WHO, 2010).The five important species of the parasite that cause this disease are; *Plasmodium falciparum, P. vivax, malariae,P. ovale and P. knowlesi* (this plasmodium causes malaria in long-tailed macaques but it may also infect humans, either naturally or artificially). Various species of the malaria parasites such as *P. falciparum* and *P. malariae* are reported in Nigeria (Eneanya, 2008; Matur et al., 2011). *Anopheles gambiae*, *An. funestus* and *An. arabiensis* have been implicated for malaria transmission in Nigeria with major impacts (Umaru et al., 2007). Scientific investigations revealed many

pathological effects of malaria on man which include varying degrees of anaemia, splenic enlargement and various syndromes resulting from physiological and pathological involvements of certain organs like the brain, liver and the kidneys (Adams and Macgraith, 2005). *Plasmodium falciparum* malaria is the most prevalent and virulent in Nigeria (Chukwura et al., 2008), capable of causing mental apathy, weakness and generally slowing down economic development; accounting for up to 98% of severe cases with significant mortality and morbidity (WHO, 2010). Malaria accounts for several deaths daily in Nigeria, especially in children under five years of age in the rural, peri-urban and urban settlements; with high index of child mortality from the disease (Cooker et al., 2011; Salako, 2006).

Asymptomatic parasitemia, the presence of malaria parasites in the blood in the absence of symptoms, is prevalent in highly endemic areas of Africa, reaching over 90% in children, with only a small percentage of individuals ever exhibiting clinical symptoms. The clinical consequences of asymptomatic malaria may vary across different epidemiological settings and are not fully understood. It is generally assumed that in endemic areas asymptomatic parasitemia is involved in the development of partial immunity (Staalsoe and Hviid, 1998) and may protect against clinical disease from new infections (Farnert et al., 2009). On the other hand, asymptomatic parasitemia provides a reservoir for transmission and may be a precursor in the progression to symptomatic disease (Bousema et al., 2004).

Asymptomatic parasitaemia causes diagnostic challenges. In a clinical setting, a patient presenting with fever or other symptoms of malaria should always be treated for malaria when having a positive test result for malaria. However, a patient with asymptomatic parasitaemia may present with a febrile illness with a different cause and with symptoms clinically indistinguishably from malaria, but requiring a different treatment despite a positive malaria result (Smith et al., 2004, Chandramoham et al., 2012). The prevalence of asymptomatic parasitaemia in areas with high malaria endemicity varies greatly among study locations. A study done in western Kenya identified a very high prevalence of asymptomatic asexual parasitaemia (73.8%) and gametocytaemia (33.8%) in children under five as assessed by thick blood film microscopy (Bousema et al., 2004). In Gabon, a study done in 2011 showed 1.7 - 8.7% asymptomatic parasitaemia in children when thick and thin blood films were examined (Nkoghe et al., 2011). They argued that the large variation might to some degree be attributable to the diagnostic methods used, such as the thick blood films, which are commonly used in many studies on asymptomatic parasitaemia (Nkoghe et al., 2011, Bousema et al., 2004, Missinou et al., 2013), are known to be difficult to interpret (Wongsrichanalai et al., 2007). Patients with asymptomatic gametocytaemia, with or without concurrent asexual parasitaemia, may serve as reservoirs for the perpetuation of the malaria life cycle (Alves et al., 2005). Treating asymptomatic gametocytaemia has been shown to have some value in reducing malaria transmission when combined with other interventions (Kern et al., 2011). Basically, individual susceptibility to malaria infection and disease is regulated by hereditary and acquired factors such as human blood groups (ABO) and genotypes (AA, AS, and SS)(Adu et al., 2014). Blood group antigens are hereditary determined and plays a vital role in transfusion safety, understanding genetics, inheritance pattern and disease susceptibility (Deshpande and Wadde, 2013; Adu et al., 2014).The study is aimed at determining the prevalence of asymptomatic malaria and other haemotological indices associated with the disease among students of Federal University of Technology, Owerri (FUTO), Imo State.

MATERIALS AND METHODS

The study area

The study area was Federal University Technology Owerri, located at Ihiagwa, Owerri west Local Government Area in Imo State, Nigeria. It is bounded in the North by Obinze, in the South-West by Eziobodo and in the South-South by Nekede. Federal University of Technology Owerri is a large institution with an average of not less than 25,000 students, who live at different locations within the perimeters of the school.

Sample Population and size

The exercise was carried out among students of the Federal University of Technology, Owerri, Imo State, Nigeria, which included those living in school hostels and off campus residents (Umuchima, Eziobodo, Ihiagwa and other places as they may have indicated in questionnaire). A total of 160 students were randomly sampled for the study. Running of the sample tests was done at Pillars Medical Laboratory located at number 8 Umezeronini Street, Ikenegbu layout, Owerri, Imo State.

Study group; were those who do not have symptoms of malaria.

Control group: were those that complained of having some signs and symptoms of malaria (fever, weakness, headache etc.).

Ethical Considerations

Permission from the hostel administrators were sought for after explaining to them the motive of the study. Oral consent of both the students living in the school hostels and off campus were sought and obtained before commencement of the study.

Recruitment and Data Collection

Inclusion criteria: Students who gave their consent for this study irrespective of having symptoms or no symptoms

of malaria.

Exclusion criteria: Students who are on anti-malarial drugs or have taken any within two weeks prior to sample collection and also those who declined consent.

Procedure

Before the collection of the blood, questions were put across to the students whether any anti-malarial drugs or chemoprophylaxis had been taken recently (in the past two weeks).Those who answered in the positive were dropped while blood samples were collected from those who answered in the negative. And if they were presently having fever or any symptoms of malaria (in order to help determine those who may be asymptomatic).

Laboratory Test

About 2ml of blood was collected from a peripheral vein into an ethamine diamine tetra acetic acid (EDTA) bottle for preparation of thick and thin blood film as well as packed cell volume. Thick and thin blood films were prepared according to the technique described by Hanscheid (1999) and Cheesbrough.(2006). Two glass slides were labeled for each participant. A drop of blood was then placed on the clean, grease free glass slide and allowed to dry. Precaution was taken to maintain a constant volume as much as possible. Thick and thin blood smear were made together on each slide with 6µl and 2µl of blood respectively. The thin smear was made to spread on the glass slide so that newsprint could be read through it. This was immediately fixed in absolute methanol for 5 seconds and allowed to air dry completely before staining. The dried slides were then placed on a rack in preparation for staining.

Packed Cell Volume estimation

Using two heparinized capillary tube, 4-5cm column of blood was obtained from blood already collected. This was to ensure that the average of the two values obtained is used for calculation. One end of the capillary tube was sealed with plasticin, several samples were assembled in the Centrifuge (haematocrit machine) and spinned at 5000 revolution per minute for 5 minutes. PCV was read using Hawksley's micro haemotocrit reader. Anaemia was diagnosed when packed cell volume was below 33%, according to World health Organization recommendation (WHO, 2002).

Staining Technique for Thick and Thin Blood Films

The thin films were fixed with absolute methanol for 2seconds and the entire smear(both thick and thin films) were air-dried before staining with 10% and 3% Giemsa working solution for 10mins and 45- 60mins respectively(WHO, 2010). The stained slides were removed and rinsed in buffer water (pH 7.2), kept vertically on the rack to air- dry before examination. The slides stained with 10% Giemsa stain was used for preliminary slide reading while the second slide stained with 3% Giemsa stain was read by two skilled and independent malaria microscopists and archived for relevant records.

Reading of Slides and Counting of Parasites

When the slides were completely dried, a drop of oil immersion was placed on each slide and examined first with low magnification(10X ,40Xobjetive lens) to ascertain a definite field with even distribution of WBC(10 - 20WBC) before finally examining with X100 oil immersion objective lens(WHO, 2010). Hundred high power fields were examined before a slide was reported negative and blood sample were reported positive only when the same result was produced by two independent skilled microscopists. Thin blood films were examined to confirm the species identification on the thick films. Parasite densities were documented as a ratio of parasites to WBC in thick films. Malaria parasites(*Plasmodium falciparum*) were counted against 200WBC in thick films. However, 500WBC were counted where less than nine parasites were counted after counting against 200WBC (WHO, 2010). Parasite densities were calculated as follows:

$$\frac{\text{Parasite count} \times 8000}{\text{WBC counted}} = \text{Parasite/µl of whole blood}$$

Qualitative Data Collection

Structured questionnaires were used. The section A of the questionnaire was for Bio-data such as: age, sex etc. Questions such as Blood group and Genotype were asked in section B. Questions on diagnosis and regularity of antimalarial intake in section C while section D was for knowledge, attitude and control measures.

Data Analysis

The data were analyzed using: tabulations, percentages, and test of statistical significant differences using chi-square (X^2). The statistical software used was the Statistical Program for Social Sciences (SPSS). The significance was taken at $p = 0.001$, $p < 0.05$.

RESULTS

Out of the 160 students whose peripheral blood samples were examined in this study, the overall prevalence of malaria parasitaemia was 25% (40/160), with symptomatic cases having a higher prevalence of 55% (22/40) while the asymptomatic case had only 45% (18/40) prevalence. The overall prevalence of asymptomatic malaria is 11.25% (18/160) of the study population (Table 1). Only asexual stages of *Plasmodium falciparium* were found in this study. Thus, there is a low prevalence of asymptomatic malaria among FUTO students.

Table 1. Prevalence of malaria parasites

Study Population	No. Examined (%)	No. Infected (%)
Subjects with symptoms (Symptomatic)	34 (21.25)	22 (55)
Subjects without symptoms (Asymptomatic)	126 (78.75)	18 (45)
Total	**160**	**40**

**The tables below were drafted in accordance with the study (i.e. those who were infected but are asymptomatic) **

Table 2. Prevalence of malaria parasites on the study population according to age

Age in years	No. Examined (%)	No. Infected (%)	No. Asymptomatic (%)
16 – 22	99 (61.88)	28 (28.28)	13 (46.43)
23 – 29	61 (38.12)	12 (19.67)	5 (41.67)
Total	**160**	**40**	**18**

Df=1, p=0.008, p<0.05

Table 3. Relationship between blood group and malaria parasites

Blood Group	No. Examined (%)	No. Infected (%)	No. Asymptomatic (%)
A	40 (25)	11 (27.5)	5 (27.8)
B	16 (10)	5 (12.5)	2 (11.1)
AB	9 (5.62)	0 (0.0)	0 (0.0)
O	95 (59.38)	24 (60.0)	11 (61.1)
Total	**160**	**40**	**18**

Table 4. Association between genotype and parasitaemia

Genotype	No. Examined (%)	No. Infected (%)	No. Asymptomatic (%)
AA	130 (81.25)	36 (27.69)	17 (47.22)
AS	30 (18.75)	4 (13.33)	1 (25)
Total	**160**	**40**	**18**

The result also showed that students between 16 – 22 years old had the highest infection rate with 28.28% (28/99), with an asymptomatic case of 46.43% (13/28). While students between the ages of 23-29 years had an infection rate of 19.67% (12/61), with an asymptomatic case of 41.67% (5/12) as shown in Table 2. There was no record of students aged 30& above. The prevalence of malaria with regards to age groups were found to be statistically significant (p<0.05).

Based on malaria parasitaemia relationship with blood group, the result shows that students with blood group O had highest prevalence of 60% (24/40), with an asymptomatic case of 61.1% (11/18). Blood group A has a prevalence of 27.5% (11/40) with an asymptomatic case of 27.8% (5/18), and Blood group B has a prevalence of 12.5% (5/40), with an asymptomatic case of 11.1% (2/18), while blood group AB which was 5.62% (9/160) of the study population had no case of prevalence as shown in Table 3.

On the relationship of malaria parasitaemia and Genotype, it was discovered that students who have genotype AA had the highest infection rate of 27.69% (36/130), with an asymptomatic case of 47.22% (17/36) and students with genotype AS had a prevalence of 13.33% (4/30) with an asymptomatic case of 25% (1/4) as shown in Table 4. There was no record of any student with genotype SS.

Considering the parasite density count, 22.73% (5/22) of the symptomatic student had a low parasite count below 100parasite/µl, while those that were asymptomatic had 38.89% (7/18). Symptomatic students having a parasite count of 101 – 200parasite/µl, is 45.45% (10/22) while the asymptomatic student had 61.11% (11/18). 18.18% (4/22) of symptomatic had 201 – 300, while there was no such record for the asymptomatic. 4.55% (1/22) of the symptomatic students had 301 – 400, asymptomatic had no record. Lastly, 9.09% (2/22) of the symptomatic students had a parasite count of 400 – 500, while the asymptomatic had no record, as shown in Table 5.

Table 6 shows the association between age, anemia and parasitaemia. 22.22% (22/99) of those between the ages of 16 – 22 were anemic, while 21.31% of those between the ages of 23 – 29 (13/61) were anaemic. The overall prevalence of anemia in the study population is 21.88%.

Table 5. Parasite Density

Parasite/µl	Symptomatic (%)	Asymptomatic (%)	Total
10 – 100	5 (22.73)	7 (38.89)	**12**
101 – 200	10 (45.45)	11 (61.11)	**21**
201 – 300	4(18.18)	0 (0.00)	**4**
301 – 400	1 (4.55)	0 (0.00)	**1**
401 – 500	2 (9.09)	0 (0.00)	**2**
>500	0 (0.00)	0 (0.00)	**0**

Table 6: Association between the study population and packed cell volume

Age in years	No. Examined	No. Anaemic
16 – 22	99	22 (22.22)
23 – 29	61	13 (21.31)
Total	**160**	**35 (21.88)**

Table 7. Association of parasites density and severity of anemia

Parasite density (µl)	PCV			
	20–33 (%)	**34-43 (%)**	**>40 (%)**	**Total**
10 – 100	4 (14.81)	7 (46.67)	3 (100)	14
101 – 200	8 (24.63)	8 (53.33)	0 (0.00)	16
201 – 300	5 (18.52)	0 (0.00)	0 (0.00)	5
301 – 400	6 (22.22)	0 (0.00)	0 (0.00)	6
401 – 500	4 (14.81)	0 (0.00)	0 (0.00)	4
Total	**27**	**15**	**3**	**45**

Severity of anaemia can be traced to its influence by parasite density. Table 7 shows that higher parasite density had more effect on the packed cell volume

DISCUSSION

Malaria is indeed by far the most important tropical parasitic disease causing great suffering and loss of lives. Asymptomatic or subclinical malaria parasitaemia is the presence of malaria parasites in blood in the absence of symptoms (Bottius et al.,1996).

From this study, the overall prevalence rate of asymptomatic malaria is 11.25% which compares with 17.4% previously reported among University of Maiduguri students (Adesina, 2013) and the 17% reported in Uganda children (Denise et al., 2004). The prevalence rate obtained from this study is lower when compared with similar work done in Senegal by Fernando et al. (2003) with prevalence rate of 77% and 83.3% in a similar work conducted by (Omolade et al., 2010) in southern Nigeria. The fact that this study was conducted in a university environment, among individuals of higher learning and understanding, may have contributed to the low level of asymptomatic prevalence obtained in this study. High standard of education usually affect health awareness and therefore has a positive impact on health since they are probably better informed about vector control such as the use of insecticide treated nets (ITN) and other control measures.

Interestingly, only the asexual stage of *Plasmodium falciparium* was found in this study, which represents major problem in Nigeria. This corroborates other studies carried out in Awka South East Nigeria (Mbanugo and Ejims, 2000), and in Lagos state South West Nigeria (Asianya et al., 2006) and in Azia, Anambra State (Aribodor et al., 2004) where only infections of *P. falciparium* were reported. On the other hand Ukpai and Ajoku (2001) and Matur et al. (2011) reported cases of *P. falciparium* and *P.malariae* or mixed infections of *P.falciparium* and *P. malariae*.

The transmission of this parasite in this study could be influenced by socio-economic and cultural factors when considering hostel location and its structures which possibly play a vital role in influencing susceptibility to infection. From this study, as also supported by Carter et al. (2000) that malaria transmission is not homogenous through an endemic area but spotty and depends on two primary factors; location of the breeding sites and clustering of human habitations where people are serving as reservoirs of malaria parasites. From the study sites, there is poor or inadequate drainage system. Also in the school hostels, there is overpopulation with ratio of 8 to 12 students per room thus making use of mosquito net as preventive measure cumbersome.

In comparison of age and prevalence of asymptomatic parasitaemia, Table 2 reveals that the results of students between ages 16 – 22 (46.43%) is almost comparable to that of the students between the ages of 23 – 29 (41.67%) and also comparable to other studies which had 43.3% for students between the age 17 – 25 by (Adesina, 2013). These results are comparable to the findings of Ntoumi et al.(1995) which reported an association of age with parasite load, stating further that prevalence of subclinical malaria was age dependent and that age increase was associated with decrease parasite load and complexity of infections(Bruce et al., 2000). From other studies, asymptomatic malaria has been reported in areas with high malaria transmission. It is believed to serve as a reservoir for continued transmission, and furthermore complicates diagnostics, as not all individuals with a positive malaria test are necessarily ill due to malaria, although they may present with malaria-like symptoms.

Anaemia was significantly associated with malaria parasitaemia in this study which is also similar to other studies (Ogbu et al*.,* 2015; Isah et al*.,* 2011). The prevalence of anaemia among students with parasitaemia was 21.88% (Table 6). Despite the presence of anaemia in these students, at least 18 of them were asymptomatic for malaria. Parasite density in this study may have influence on the severity of anaemia. Higher parasite density had more effect on the packed cell volume with a higher degree of anaemia. Ogbu et al. (2015) state that asymptomatic malaria parasitaemia is one of the major causes of anaemia in malaria hyper endemic environment. A higher proportion of those with malarial parasitaemia were anaemic compared with those without malarial parasitaemia. Ogbu et al*.* (2015) also reported that higher density of malaria parasitaemia lead to increased red blood cell haemolysis ultimately leading to anaemia, which is usually normochromic and normocytic and accompanied by reticulocytosis.

From the result of this study as shown in Table 4, students with genotype 'AA', had the highest infection rate of 27.69% with an asymptomatic case of 47.22%, while the students with genotype 'AS', had a lower infection rate of 13.33% with asymptomatic case of 25%. This may suggest that, patients with genotype 'AA' are more susceptible to asymptomatism, but other findings seem not agree with this opinion (Nwanne et al., 2015). On blood group relationship with parasitaemia (Table 3), students who have blood group 'O' had highest subclinical case followed by 'A', while those who are 'B' had no case of asymptomatism, the higher prevalence of infection in blood group O shows that they are most susceptible to infection. This corresponds with the result gotten by Rowe et al. (1997) in Mali, where blood group O had a higher prevalence of uncomplicated malaria.

CONCLUSION

There is a prevalence of asymptomatic malaria in this FUTO community as reflected by the high malaria parasitaemia among the students. Malarial infection affects more teenagers than adults and older age group. Anaemia is also a serious problem with asymptomatic parasitaemia.

Therefore this study has indicated that malaria is a contributor to anaemia among young people. This could impact negatively on the health of this population. Improving hygienic conditions and periodic insecticides spray in and around the hostels can go a long way in reducing the prevalence of asymptomatic malaria and indirectly symptomatic malaria.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of the paper

REFERENCES

Adams, ARD and Macgraith, BG (2005). *Clinical Tropical Disease. Malaria Parasite.* 8th Edition. Prentice Hall International Inc. Georgia: 424

Adesina, OO (2013). Subclinical malaria infection among University of Maiduguri students: prevalence and parasite density. Bor. Mal. J, 10(1):6- 11.

Adu EM, Ezie E, Onyekpe JO, Oseodion J (2014). Distribution of ABO and rhesus blood groups among HIV seropositives patients in Agbor, Delta state, Nigeria. Int. J. Infect Trop Dis., 1:13-17.

Adu EM, Isibor CN, Ezie E (2014). Prevalence of haemoglobin variants among the Ika ethnic nationality of Delta state. Int J Med Biomed Res, 3:63-67.

Alves FP, Gil LHS, Marrelli MT, Ribolla PEM, Camargo EP, Pereira Da Silva LH (2005). Asmptomatic carriers of *Plasmodium spp* as infection source for malaria vector mosquitoes in Brazilians Amazon, J. Med Ent, 42: 777-779.

Aribodor DN, Njoku OO, Eneanya CI, Onyali IO (2004). Studies on The Prevalence of Malaria and Management Practices of the Azia community in Ihiala L.G.A., Anambra State, South-East Nigeria. Nig. J. Parasitol, 24: 33-38.

Asianya VN, Agomo PU, Mafe CA, Akindele AG, Agomo SK, Bottius E, Druilhe P (2006). Malaria: even more chronic in nature than previously thought: evidence for sub-patent parasitaemia detectable by Polymerase chain reaction. Ame. J. Tropical Med. and Hygiene, 90: 15-19.

Bottius E, Guanzirolli A, Trape JF, Rogier C, Konate L, Druilhe P (1996). Malaria: even more chronic in nature than previously thought : evidence for sub-patent parasitaemia detectable by Polymerase chain reaction. Mal. J., 90: 15-19.

Bousema JT, Gouagna LC, Drakeley CJ, Meutstege AM, Okech BA, Akim INJ, Beier JC, Githure JI, Sauerwein RW (2004). *Plasmodium falciparum* gametocyte in asmptomatic children in western Kenya, Mal. J. 3: 18

Bruce MC, Donnelly CA, Packer M, Lagog M, Gibson N, Narara A, Walliker D, Alpers MP, Day KP (2000). Age- and species-specific duration of infection in asymptomatic malaria infections in Papua New Guinea, Parasitol., 121(3): 247-256.

Carter R, Mendis KN, Roberts D(2000). Spatial targeting of interventions against malaria. WHO Bull, 78:1401-1411.

Chandramoham D, Jaffar S, Greenwood B (2012). Use of clinical algorithms for diagnosing malaria. Trop. Med. Int. Health, 7:45-52.

Cheesebrough M (2006). District Laboratory Practice in Tropical Countries, part 1. University Press, Cambridge, pp.239-258.

Chukwura BM, Fidock DA, Kyle DE, Kappe SH, Alonso P, Collins FH, Duffy PE (2008). Malaria: progress, perils and prospects for eradication. J. Clin. Invest, 118:1266-1276.

Cooker HAB, Chukwuani CM, Ifudu HD, Aina BA (2011). The Malaria Scourge. Concept in Disease Management. Nigeria J. Pharma. 32:19-48.

Denise NM, Moses RK, Grant D (2004). Asymptomatic parasitaemia as a risk factor for symptomatic malaria in a cohort of Uganda children. J. Trop. Med. Int. Health, 9(8): 862-864.

Deshpande RH, Wadde SK (2013). Distribution of Blood Groups in Blood Donors in Blood Banks of Latur. Sch J. Appl. Med. Sci, 1:276-279.

Eneanya CI (2008). Seasonal Variation in Malaria Episodes among Residents in a semi-urban community in southeast Nigeria. The Nigeria J. Parasitol. 19:39-43.

Farnert A, Arez AP, Correia AT, Bjorkman A, Snounou G, Do Rosario V (2009). Sampling and storage of blood and the detection of malaria parasites by PCR. Transactions of the royal Society of Tropical Medicine and Hygiene: 93:50-53.

Fernando SD, Gunawardena DM, Bandara MR, De Silva D, Carter R, Mendis KN, Wickremasinghe AR (2003). The impact of repeated malaria attacks on the school performance of children. Ame. J. Tropical Med. Hygiene, 69(6):582-588.

Hanscheid T (1999). Diagnosis of Malaria: A review of alternatives to conventional microscopy. Clin. Lab Haematol, 21: 235-245.

Isah AY, Amanabo MA, Ekele BA (2011). Prevalence of malaria parasitemia amongst asymptomatic pregnant women attending a Nigerian teaching hospital. Annual J. Afr. Med.10:171-174.

Kern SE, Tiono AB, Makanga M, Gbadoe AD, Premji Z, Gaye O, Ogutu B (2011). Community screening and treatment of asymptomatic carriers of *Plasmodium falciparum* with arthmether-lumefantrine to reduce malaria disease burden: a modelling and simulation analysis. Malar J., 10:210.

Matur BM, Azare BA, Ugbong I (2011). The Prevalence of Asymptomatic Malaria Parasitaemia Amongst Undergraduate Students of University of Abuja. The Nigeria J. Parasitol., 22:44-52.

Mbanugo JI, Ejims DO. (2000). *Plasmodium* Infections in Children Aged 0- 5 years in Awka Metropolis, Anambra State. Nigeria. The Nigeria J. Parasitol. 21:55-59.

Missinou K, Vegter AR, Van Genderen PJ, Visser LG (2013). Treatment outcome of intravenous artesunate in patients with severe malaria in the Netherlands and Belgium. Malaria J., 11: e102

Nebe OJ, Adeoye GO, Agomo PU, Mosanya ME (2007).Malaria in Coastal Area of Lagos State, Nigeria: A Survey of Perception and Practice Among the Mothers Caregivers of Children under five years old. Nigeria J. Parasitol.23:69-80.

Nkoghe D, Akue JP, Gonzalez JP, Leroy EM (2011). Prevalencce of *Plasmodium Falciparum* infection in asymptomatic rural Gabonese populations. Malaria J. 10:33.

Ntoumi F, Contamin H, Rogier C, Bonnefoy S, Trape J, Odile MP (1995). Age dependent carriage of multiple plasmodium falciparium merozoite surface antigen-2 alleles in asymptomatic malaria infections. Ame. J. Tropical Med., 52(1):81-88.

Nwanne A, Njoku IO, Charles CC, Egbo NU, (2015): Prevalence of gestational and placental malaria among pregnant mothers attending health facilities in Owerri. African J., 65(42): 548

Ogbu GI, Aimaku CO, Anzaku SA, Ngwan S, Ogbu DA (2015): Prevalence of malria parasitaemia among among asymptomatic women at booking in tertiary hospital, North-central Nigeria. J. Reproductive Biol. and Health, 3(1):1-7.

Omolade O, Okwa D, Adejoke CI (2010). The malaria situation, perception of cause and treatment in a Nigeria University; Borno Malaria J., 9(18): 124.

Rowe JA, Moulds JM, Newbold CI, Miller LHP (1997)Plasmodium falciparum resetting mediated by parasite-variant erythrocyte membrane protein and complement-receptor 1. Nat, 388:292–295.

Salako SA (2006). Malaria in Nigeria: Proceedings of the conference on Malaria in Africa. American Institute of Biological Sciences, Eleventh Street, N.W.Washington, D.C. 131-141.

Smith T, Schellenberg JA, Hayes R (1994). Attributable fraction estimate case definitions for malaria in endemic areas. Statistical Medicine, 13:2345-2358

Staalsoe T, Hviid L (1998). The role of variant-immunity in asymptomatic Malaria infections: maintaining a fine balance. Parasitol. Today, 14:177-178.

Ukpa OM, Ajoku EI (2001). The Prevalence of Malaria in Okigwe andOwerri Areas of Imo State. The Nigeria J. Parasitol., 22:43-48.

Umaru AT, Mafe CA, Akindele AG, Agomo SK, Bottius E, Druilhe P (2007). Malaria: even more chronic in nature than previously thought: evidence for sub-patent parasitaemia detectable by Polymerase chain reaction. Tropical Med. Hygiene, 90: 15-19.

WHO (2002). A strategic framework for malaria diagnosis in African region. WHO Document. AFR/MAL/02/01: 6-9.

WHO (2010). World Malaria Report 2010.www.cdc/malariaepidemiology.com. Assessed 6th July 2015.

Wongsrichanalai C, Barcus, MJ, Muth S, Sutamihardja A, Wensdorfer WH (2007). A review of malaria diagnostic tools: microscopy and rapid diagnostic test (RDT). Ame. J.