# Impact of Some Pretreatment Clinical Factors on Artemether - Lumefantrine Treatment Failure in Malaria Patients attending some Health Care Centres in Kano State, Nigeria

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**Abstract:** The efficacy of antimalarial drugs may be influenced by other factors independent of the parasite susceptibility to the drugs. This study aimed at evaluating the effect of some clinical factors on Arthemeter – Lumefantrine (AL) treatment failure in patients with uncomplicated malaria receiving care in selected community pharmaceutical shops and hospitals from Kano Municipal, Gwale, Tarauni and Kura Local Government Area. Four hundred (400) consenting patients with AL prescription were randomly selected for the study. *Plasmodium falciparum* positive subjects were confirmed by microscopic examination using Giemsa stained blood films techniques. Clinical and parasitological responses of the enrolled patients were evaluated using 28 days follow up according to WHO protocols for therapeutic efficacy. Structured questionnaire was used to record clinical details of each of the patients. Hematological parameters were assessed using automated hematology analyzer and blood group using antisera agglutination test kit. Among the 400 subjects enrolled, 220 (55%) completed the 28 days follow-up, out of which 170 (77.3%) had adequate clinical and parasitological response (ACPR) and 50 (22.7%) had treatment failure. The mean duration of symptoms before treatment, pre-treatment parasite density, parked cell volume(PCV),erythrocyte sedimentation rate(ESR) and white blood cell count (WBC) of patients with ACPR (4.3±2.1 days, 8,300±3,250/µl, 36±8.2%, 10.9±2.9mm/H and 5.8±2.5.1x$10^9$/L) were found to be significantly different (P<0.05) from that of patients with treatment failure (6.7±4.2days, 12,210±2,160/µL, 24±6.1%, 14.1±5.3mm/H and 8.4±4.1x$10^9$/L) respectively. Treatment failure was found to be more common among patients suffering from malaria and other diseases such as typhoid and hypertension (36%) when compared to those with malaria only (20.8%).It was also found to be less common among patients with O blood group (11.8%) when compared with those of AB, A and B blood groups with treatment failure rate of 27%, 31% and 29% respectively (P<0.05). Treatment failure was found to be not significantly associated with pretreatment body temperature (P>0.05).This study revealed that some pretreatment clinical factors such as high parasitaemia level, long duration of symptoms and abnormal hematological parameters could predispose individual to failed treatment and consequently increases the risk of developing antimalarial drug resistance in a population.

**Key words**: Treatment failure, Malaria, Artemether-Lumefantrine, clinical factors

## INTRODUCTION

Malaria is a major public health problem with estimated cases of 228 million worldwide and estimated death of 405, 000 in 2018 (World Malaria Report, 2019). According to World Malaria Report (2017) Nigeria contributed to 27% of the global malaria burden and accounted for 23% of the global estimated malaria death. In Nigeria the national malaria control program (2011) has estimated that malaria is still responsible for 60% of outpatients visit to health facilities, 30% of childhood death and 11% of maternal death (FMOH, 2011). It is also estimated that 50% of Nigerians adult population will have at least one episode of malaria each year and children under five(5) years will have 2 – 4 attacks annually ((FMOH, 2011; WHO, 2017).

Artemisinin-based combination therapies (ACTs) are widely recommended as first line treatment for uncomplicated malaria. However, evidence of clinical and parasitological failure after treatment with some ACTs have already emerged in some countries such as Burkina Faso (Zango *et al.* 2007), Tanzania (Humphrey *et al.* 2007) and in Nigeria (Aminu *et al.* 2017). Decreased sensitivity to ACTs could be a serious threat to controlling malaria in this era. Since there is no alternative classes of antimalarial that are ready to replace them thus intensive research is required to monitor the causes of ACTs treatment failure.

The outcome of antimalarial treatment could be effective or ineffective classified as either adequate clinical and parasitological response (ACPR) or treatment failure according to World Health Organization (1996) criteria.

Treatment failure can be further categorized as either early treatment failure, late clinical failure or late parasitological failure (WHO, 1996; WHO, 2008).

The main cause of treatment failure is resistance to the active drug or in the case of combination therapy resistance to one or more of the active components. However, the efficacy of antimalarial drugs may be influenced by other factors independent of the parasite susceptibility to the drugs (O'flaherty *et al.,* 2017). Response to antimalarial therapy might be influenced by host factors such as immunity, adherence to chemotherapy and presence of concomitant diseases (O'flaherty *et al.,* 2017). Base line clinical parameters of malaria patients varied greatly and may have impact on treatment outcomes for example, a higher parasite burden at the time of treatment may be associated with less favorable outcomes (Dorsey *et al.*, 2004)

Several studies revealed treatment failure after administration of different ACTs against *falciparum* malaria without evidence of drug resistance (Aminu *et al.,* 2017; Sutherland *et al.,* 2017). This necessitated the need to search for other causes of ACT treatment failure apart from drug resistance.

The wide spread failure in malaria chemotherapy has led to an increase in mortality and morbidity associated to malarial infection. Many factors such as incorrect dosage of drugs, parasite burden and host immunity to malaria have been reported to contribute significantly to malaria treatment failure. These factors may probably contribute to the development and spread of drug resistance (WHO, 2006; O'flaherty *et al.,* 2017). Knowledge on the risk factors associated with antimalarial treatment failure is essential when developing guidelines for effective control of malaria in endemic areas like Nigeria. This study therefore attempt to provide information on the possible impact of base line clinical parameters on treatment failure in subject with uncomplicated malaria treated with Artemether – Lumefantrine (AL).

## MATERIALS AND METHODS

Ethical permission for the research was sought and granted by Kano State Hospital Management Board, Ministry of Health (MOH/OFF/797/T.I/284). Consent for the study was obtained from each participant or parents/guardians in the case of children.

The study was conducted between October, 2017 and October, 2019 among out patients attending Murtala Muhammad Specialist Hospital, Hasiya Bayero Paediatric Hospital and Volunteers who attended some community pharmaceutical shops situated in Gwale, Tarauni, Wudil and Kura L.G.A.

Subject's enrollment, blood sample collection, treatment, follow- up and other laboratory procedure were carried out according to the procedure of WHO (2006) Cheesbrough (2008), Yeka *et al.* (2008), and Aminu *et al.* (2017).

Inclusion criteria were consented subjects of different ages with symptoms of uncomplicated malaria including fever or history of fever within 48 hours, body pain and headache and monoinfected with *P. falciparum.*. Subjects who were on antimalarial drugs within two weeks before presentation were excluded. Enrolled subjects were also patients to whom Arthemether - Lumefantrine have been prescribed by any of the health care practitioners from the hospitals or community pharmaceutical shops.

A total of 1000 subjects were screened, out of which four hundred met the study criteria and were eventually enrolled.

Structured questionnaire was used to record details of symptoms and their duration, vital signs, axillary temperature, information of the antimalarial drug used and presence or absence of other concomitant diseases or infections such as typhoid, HIV, diabetes and hypertension.

Blood samples were collected aseptically (finger pricking and venipuncture). *Falciparum* malaria was diagnosed using rapid malaria test kit (care start HRP2) according to manufacturer's protocols.

Thick and thin blood films were made from finger prick blood; stained with 10% Giemsa stain and observed microscopically for confirmation of the presence of *P. falciparum,* parasite density and stage. Venous blood collected were sent to hematology laboratory for full blood count(PCV, ESR,WBC count) and blood grouping using automated hematology analyser (Abbott cell – $Dy_n$ CD - 1800) and agglutination method using antisera (Span, India) respectively.

Enrolled subjects with prescription of arthemether – lumefantrine were educated on the dosage based on their prescription forms and manufacturer's protocols and consequences of not completing their antimalarial dosage. Subjects were followed up for 28 days to assess the therapeutic efficacy of the ACT used. They were asked to return to the health centers for clinical and parasitological response evaluation on day 3, 4, 7, 14, 21 and 28 post treatments respectively. Patients using another antimalarial drug, withdrawal of informed consent or lost to follow up were excluded during follow up. Blood samples were collected on each follow – up day via finger pricking to identify parasite clearance through microscopic examination of thick and thin Giemsa stained blood films. Treatment responses were recorded as classified by WHO (1996).Early treatment failure ETF (present of parasitemia > 25% of day 0 level on day 3 with auxiliary temperature > 37.5$^{o}$C), adequate clinical and parasitological response ACPR (absence of parasitemia and all other clinical symptoms from day 14) and late treatment failure (Present of parasitaemia after day 4).

**Statistical Analyses**

Data obtained were analyzed using SAS software general linear model version 9.3 and open Epi version 2.3. The data were summarized and interpreted using means, frequency, range, standard deviation and percentage. The result was compared using T – test, Chi-square and the level of significance was fixed at 0.05.

**RESULTS**

Base line clinical characteristics of the subjects enrolled were presented in Table 1. The table showed mean duration of symptoms, body temperature and parasite density of the subjects enrolled as 4.4 days, 38.5$^{o}$C and 9,300 parasite /µL respectively. Some hematological parameters of the subject enrolled including mean PCV (36%), mean WBC count (9.1 x $10^{9}$/L) and ESR (15.3mm/µ) were also presented in Table 2. Highest number of subjects enrolled have O blood group (38%) followed by A blood group (35%) and B blood group (18%) and the least number of subjects have AB blood group (10%). Treatment outcome of the subject enrolled is presented in table 2.Of 400 subjects enrolled, 220(55%) completed 28 days follow up and 180(45%) were lost to follow up. One hundred and seventy(55%) from subjects who completed 28 days follow up had adequate clinical and parasitological response and 50(22.7%) had treatment failure, 20(9.1%) early treatment failure and 30(13.6%) late treatment failure. Therapeutic indices of the subjects who completed 28- days' follow-up were compared between subjects with adequate clinical and parasitological response (ACPR) and subjects with treatment failure in Table 3. Mean parasite density/µL of blood (8,300) and mean duration of symptoms (4.3$\pm$2 days) were significantly different (P<0.05) from that of subjects with treatment failure (12, 210/µL and 6.7$\pm$4.2 days respectively). Mean body temperature before treatment was not statistically different between the 2 – groups (38.3$^{o}$C and 38.8$^{o}$C) (P > 0.05).

Result of the occurrence of treatment failure with respect to blood group of subject who completed 28 – day's follow-up is presented in Table 4. Subject with O blood group had significantly lower risk of treatment failure (11.8%) P< 0.05 compared to AB, A and B blood groups with treatment failure rate of 27%, 31% and 29% respectively.

The result of the incidence of treatment failure with respect to patient with malaria only and patients with additional health condition is presented in Table 5. Treatment failure was found to be more common

among subjects with malaria and another disease (36%) than subject with malaria infection only (20.8%) P<0.05.

Table 5 shows the results of comparison of some hematological parameters among subjects with ACPR and subject with treatment failure. PCV (36%), total WBC ($95.8x10^9$L) and ESR (10.9mm/μ) of subjects with ACPR were significantly different from value of subject with treatment failure 24%, $8.4x10^9$/L and 14.1mm/H respectively (P< 0.05).

**Table 1: Baseline Clinical Characteristics of the Patients Enrolled (n = 400)**

| Characteristics | Number/ Mean/Range |
|---|---|
| Mean duration of symptom (range) in days | 4.4 ( 1 – 14) |
| Mean body temperature (range) in $^oC$ | 38.5 (37.5 – 40.1) |
| Mean parasite density (range) per μl of blood | 9,300 (1,240 – 14, 400) |
| Mean PCV (range) in % | 360 (19 – 42) |
| Mean WBC Count (range) x $10^9$/L | 9.1 (4.0 – 12.0) |
| ESR (range) (mm/H) | 15.3 (4 – 22) |
| **Blood group** | |
| O | 150 (38%) |
| A | 140 (35%) |
| B | 70 (18%) |
| AB | 40 (10%) |

**Table 2: ACT Treatment outcome among Malaria Patients Enrolled (N = 400)**

| Treatment outcome | Number (%) |
|---|---|
| Subjects who completed 28 days follow up | **220 (55)** |
| • ACPR | 170 (77.3) |
| • Treatment failure | 50 (22.7) |
| ETF | 20 (9.1) |
| LTF | 30 (13.6) |
| Subject excluded/Lost to follow – up | 180 (45) |

**Key – ACPR = Adequate Clinical Parasitological Response, ETF=Early treatment failure**
**LTF= Late treatment failure**

**Table 3: Comparison of Pretreatment Clinical Characteristics among Patients with ACPR and those with Treatment Failure**

| Therapeutic indices | ACPR(n=170) | Subject with TF ( n = 50) | P – value) |
|---|---|---|---|
| Mean body temperature $^oC$ | 38.3 ± 1.2 | 38.7±1.1 | P > 0.05 |
| Mean parasite density/μL of blood | 8,300 ± 3,250 | 12,210 ±2,160 | P < 0.05 |
| Mean duration of symptoms (days) | 4.3±2.1 | 6.7 ± 4.2 | P < 0.05 |

**Key: ACPR = Adequate Clinical and Parasitological Response, TF = Treatment Failure**

**Table 4: Occurrence of Treatment Failure Based on Blood Group**

| Blood group | Number of subject | Subject with ACPR (%) | Subject with TF (%) |
|---|---|---|---|
| AB | 30 | 22 (73) | 08 (27) |
| A | 74 | 51 (69) | 23 (31) |
| B | 31 | 22 (71) | 09 (29) |
| O | 85 | 75 (88) | 10 (11.8) |

**Key: ACPR = Adequate Clinical and Parasitological Response, TF = Treatment Failure p < 0.05**

**Table 5: Occurrence of Treatment Failure with Respect to Malaria Patients with additional Diseases/infection**

| Characteristics | Number of subjects (n = 220) | Subjects with ACPR (%) | Subjects with TF (%) | P = value |
|---|---|---|---|---|
| Malaria only | 192(87.3%) | 152 (79.2) | 40 (20.8) | |
| Malaria and other diseases | 28(12.7%) | 18 (64) | 10 (36) | P < 0.05 |

**Key: ACPR = Adequate Clinical and Parasitological Response, TF = Treatment Failure**

**Table 6: Comparison of some Hematological Parameters among Patients with ACPR and those with TF (Mean ± SD)**

| Parameters | ACPR (n = 17) | TF (N = 50) | P = Value |
|---|---|---|---|
| PCV (%) | 36 ± 8.2 | 24 ± 6.1 | P < 0.05 |
| Total WBC ($X10^9$/L) | 5.8 ± 2.5 | 8.4 ± 4.1 | P < 0.05 |
| ESR (mm/H) | 10.9 ± 2.9 | 14.1 ± 5.3 | P < 0.05 |

**Key: ACPR = Adequate Clinical and Parasitological Response, TF = Treatment Failure, WBC= White Blood Cells Count, ESR= Erythrocyte Sedimentation Rate, PCV= Parked Cell Volume**

## DISCUSSION

Recognizing factors that determine antimalarial efficacy is essential for monitoring the occurrence of treatment failure and emerging resistance. Antimalarial treatment failure amongst other factors hinders control efforts and increases the risk of morbidity and mortality from malaria (Dorsey *et al.*, 2004).

The rate of AL treatment failure observed in this study is relatively high (22.7%) compared to 5% reported in north western Nigeria (Aminu *et al.,* 2017), 7% in western Nigeria (Happi *et al.* 2008) and 5.2% in Tanzania (Sisowath *et al.,* 2005) .

The world health organization has set two standards for antimalarial drugs that a total failure rate (adjusted for new infection) of > 10% should trigger a change of first line drug policy and that a new drug being adopted as a policy should have a total failure rate (adjusted for new infection) of <5% (Sinclair, 2009). This suggested the need for adjustment in malaria treatment policy in the study area. The high AL treatment failure rate observed could be due to limitation of this study to conduct PCR genotyping to differentiate true treatment failure from new infections/re- infection. This could be justified by the fact that most of the treatment failure observed in this study is late treatment failure (13.1%), which might be as a result of new infection not genuine recrudescence of the parasites. According to the WHO guidelines in evaluating the efficacy of antimalarial treatment, PCR genotyping of polymorphic loci is required to distinguish true treatment failure from new infection/re infection especially in endemic areas like Nigeria where transmission occur all year round (WHO, 2008).

Thus, failure to consider parasite genotyping might have resulted in underestimation of the efficacy of AL in this study.

Comparison of some parameters (age, parasite density and duration of symptoms) between patients with ACPR and those responding with treatment failure showed significant risk factors for therapeutic failure.

Parasite density and duration of symptoms before treatment of subjects with treatment failure (12,210/µL, 6.7days) are significantly higher than that of subjects with ACPR (8,300/µL, 4.3 days) respectively ($P< 0.05$). Long duration of malaria symptoms before treatment may cause an increase in parasite density and the likely hood of developing complication that could lead to poor treatment outcomes (Adesola *et al.,* 2014). Early treatment of diagnosed cases of malaria has remained the ultimate control strategy against *falciparum* malaria infection (WHO, 2008). The finding of this research is in line with the work of Zwang *et al.* (2014), who reported high pretreatment parasitemia as a risk factor for failing to clear parasites after treatment with ACTs. In this study, higher parasitemia at presentation was significantly associated with an increased risk of treatment failure. According to White (1997) treatment with antimalarial drugs will optimally reduce the number of parasites between 100- and 10,000-fold per asexual cycle.

Parasite reduction appears to be a first-order process, such that a fixed fraction of the parasite population is removed with each successive cycle provided that the minimum parasiticidal concentration of the drug is exceeded. A higher parasite density at the time of treatment initiation could therefore increase the risk of treatment failure (White, 1997). Similar report by Dorsey *et al.* (2004 ) revealed that higher parasitemia at presentation has been associated with an increased risk of early and late treatment failure in patients treated with different regimens of antimalarials .Pre treatment body temperature was found to be not significantly associated with treatment failure in this study. This is in line with the work of Dorsey *et al.* (2004) who observed that increasing baseline temperature was an independent predictor of treatment failure across many antimalarial treatment regimens. They also stated that higher temperature at presentation may represent a surrogate marker of a less effective host immune response to infection.

The mean values of hematological parameters (PCV, WBC count and ESR) were compared between subjects with ACPR and subjects with treatment failure. The significant increase in the WBC count of subjects with treatment failure ($8.4\pm4.1x10^9$/L) when compared to subjects with ACPR ($5.8\pm2.4x10^9$/L) could be an indication of the risk of developing severe malaria which is associated with massive increase in WBC count. Reduction of WBC is common during acute malaria. White blood cell count (WBC) plays a crucial role in the body's ability to fight infection. Alteration of WBC count has been associated with severity of infection, concurrent of infection and response to treatments (Teddy *et al.,* 2013). Elevated ESR of subjects with treatment failure ($14.1\pm5.3$mm/µ) compared to subjects with ACPR ($10.9\pm2.9$mm/µ) could be an indication of subsequent development of malaria complication which may result in treatment failure (Vemuna *et al.,* 2016). Significantly lower PCV level of subjects with treatment failure (24%) compared to subjects with ACPR (36%) may reflect anaemia which is mainly related to mechanical destruction of parasitized RBC and spleenic clearance of parasitized and defected erythrocytes( White *et al*., 2018). Although anaemia is a common feature of all types of malaria infections, the lower PCV level of subjects with treatment failure correlate with high parasitemia level and severity of the infections. This may lead to complication and may result in poor treatment outcomes. Teddy *et al.* (2013) were able to correlate some hematological parameters with the parasite density, malaria severity and artesunate treatment response. Zwang *et al.* (2014) also reported anaemia as a risk factor for failure to clear parasite after

treatment with ACTs. Subjects with O blood group in this study have significantly lower risk of treatment failure (P< 0.05) compared to others. Meta analysis's study confirmed an increase in severity of *P. falciparum* infection among individuals with blood group A,B and AB in comparison with those of blood group, O (Degarege *et al.,* 2019). Absence of A and B antigens on the surface of blood group O erythrocyte reduce cytoadherence and resetting of parasitized erythrocytes in individuals with blood group O. This can lead to less malaria complication and reduce the chances of treatment failure (Richmond *et al.,* 2016).

Patients presented with other concomitant diseases or infections such as diabetes, hypertension, typhoid fever, HIV and pneumonia have significantly higher risk of treatment failure (36%) than subjects with malaria infection only (20.8%). This could be as a result of mounting evidence that co-infection adversely affects either disease and causes excess morbidity and mortality particularly among extreme ages. Drugs may also behave differently in the context of co-infection or when concomitant treatment is given. Combination of disease and drug interaction makes treatment less effective (Gasasira *et al.,* 2008). Gasasira *et al.* (2008) also reported that ACT treatment was highly effective for patient with malaria who did not have HIV infection than patient with HIV malaria co-infection. Ukaga *et al.* (2006) showed that the presence of concomitant bacteria in malaria positive cases results in persistence of malaria symptoms after treatment with antimalarial. Concomitant infection should therefore be considered in the management of malaria.

## CONCLUSION AND RECOMMENDATION

This study reveals that some factors can predispose individuals to failed treatment and consequently increase the risk of developing antimalarial drug resistance in population. Artethemeter- lumefantrine treatment failure was found to be significantly associated with high pretreatment parasitemia, long duration of symptoms before treatment, non O blood group, presence of other concomitant diseases and abnormal haematological parameters (PCV, ESR and WBC count). It is therefore recommended that health care administrators should pay special attention to this high risk group during the course of malaria treatment in the study area.

## REFERENCES

Adesola, O., Sangowawa, O.K., Sabulade, A.O., Folarkemi, A.A., Peter, E.O. and Olayemi, O.O. (2014). Factors Associated with a Poor Treatment Outcome Among Children Treated for Malaria in Ibadan *Epidemiology Research International* 10(1): Pp115.

Aminu, B.M., Mukhtar, M.D. and Deeni, Y.Y. (2017): Assessment of Therapeutic Efficacy of ACT against *Plasmodium falciparum* Malaria in Kano and Katsina State, Nigeria. *UMYU Journal of Microbiology Research:* 2 (2): Pp 56 – 64.

Cheesbrough, M. (2008): District Laboratory Practice Manual in Tropical Countries Cambridge University Press Pp 235 – 245.

Degarege, A., Gebrezgi, M.Y. Ibanez, G.C and Wahlgren, M. (2019): Effect of the ABO Blood group on Susceptibility of Severe Malaria. *A Systematic Review and Meta analysis: Blood Review* 33:Pp101.

Dorsey, G; Gasasira, A. F; Machekano, R; Kamya, M. R; Staedke, S. G; Hubbard, A; (2004) The impact of age, temperature, and parasite density on treatment outcomes from Antimalarial clinical trials in Kampala, Uganda. *American journal of tropical medicine And hygiene* 71(5).Pp531-536

Federal Ministry of Health (FMOH). (2011): National policy on malaria diagnosis and treatmentAbuja.

Gasasira, A.F., Kamya, M.R. Archan, J. and Mebratitu, Y. (2008): High Risk Neutropenia in HIV Infected Children Following Treatment with Artesunate Plus Amodiaquine for Uncomplicated Malaria in Uganda: *Clinical Infectious Disease*. 46: Pp 985 – 91.

Happi, C.., Gbotosho, G., Folann, O.A; Sawunmi, A. Hudson, T., Neil, M.O. Milhouse, W., Wirth, D.F., and Oduola, A.M.J. (2008). Selection of *Plasmodium falciparum* Mdr1 in Asexual Stages and Gametocytes by Arthemether – Lumefantrine in Nigerian children with Uncomplicated *Falciparum Malaria. Journal of Antimicrobial Agents and Chemotheraphy*, 42: Pp 682 – 686.

Humphreys, G.S., Marinopoulos, I. Ahmed, J., Whittyu, L.J. Mutabingwa, T.K., Sutherland, C.J. and Hallet, R.L. (2007). Amodiaquine and Arthemether Lumefantrine Select Distinct Alleles of the *Plasmodium falciparum* Mdr1 gene in Tanzanian Children Treated for Uncomplicated Malaria. *Antimicrobial Agent and Chemotheraphy* 51: Pp 991 – 997.

O'flaherty, K.O., Maguire, J. Simpson, A.J. and Fowkes F.J.I. (2017): Immunity as a Predictor of Antimalarial Treatment Failure: *A Systematic Review Malaria Journal* 16: Pp 158.

Richmond, A., Edmond, A., James, P., Ellene, K.N. and Johnson, N.B. (2016): Relative Susceptibility of ABO Blood groups to *Plasmodium falciparum* in Ghana. *Advance in Haematology* Volume 2016. Article ID 5368793, 4 pages. Hindawi.

Sinclair, D. Zani, B., Donegan, S., Olliaro, P. (2009). Artemisinin – based Combination Theraphy for Treating Uncomplicated Malaria. *Review cochrane Database System Review* 8(1):Pp 220.

Sisowath, C.J., Stromberg, A., Martensson, M., Msellem, C., Ohondo, A. and Gill, J.P. (2005). *In vivo* Selection of *Plasmodium falciparum pFmdr1* 86N Coding Alleles by Artemether – Lumefantrine (Co-artem) *Journal of Infectious Disease* 191: Pp 1014 – 1017.

Sutherland, C.J. Landell, P., Sanders, M. Muwanguzi, J. and Van Schalkwyk, D.A. (2017). *Pfk13* Independent Treatment Failure in four Imported Cases of *P. falciparum* treated with Artemether – Lumefantrine in the United Kingdom. *Antimicrobial Agents and Chemotherapeutcs.* 61(3).

Teddy, A.C., Moses, B., Diweni, P.D., Precious., O., Chioma, E.O. and Joseph, O. (2013): Effects of *Plasmodium falciparum* on Haematological Parameters on Patients Treated with Artesunate in the Niger Delta of Nigeria. *International Journal of Medical Sciences and Healthcare* 1(12).

Ukaga, C.N., Orgi, C.N. Orogwi, S; Nwoke, B., Anosike, J.C., Udujih, O.S., Onyeka, P.K.K. and Awujo, N.C. (2006). Concomitant Bacteria in the Blood of Malaria Patients in Owerri, South Eastern Nigeria. *Tanzania Health Research Bulletin* 8(3).

Vemula, S., Katarau, B.U. Adappa, S., and Chakrapani, (216). Pretreatment Elevated ESR and C-reaction Protein as a Predictor of Malaria Complication *J. Infect. Dev. Ctries* 10(12): Pp 1332 – 1337.

White, N. J (2018): Anamia and Malaria. *Malaria Journal* 17(371) https:/doi.org/10.1186/s12936-018-2509-9

White, N .J (1997). Assessment of the pharmacodynamic properties of antimalarial drugs *in vivo*. *Antimicrobial agent and chemotherapy* 41:Pp1413-1432

WHO (1996): Assessment of Therapeutic Efficacy of Antimalarial Drugs for Uncomplicated *falciparum* malaria in areas with Intense Transmission. World Health Organization https://apps.who.int/iris/handle/10665/63295WHO/MAL/96.1077:32P.

WHO (2006): Guidelines for the Treatment of Malaria WHO press Geneva, Switzerland Pp/20 WHO/HTM/MAL/2006.1108.

WHO (2008): Methods and Techniques for Clinical Trials on Antimalarial Drug Efficacy. Genotyping to Identify Parasite Pop. https://www.ww.int/in/handle/10665/43824.

WHO (2017): World Malaria Report, Global Malaria Program. Geneva WHO 2017 http://www.who.int/malam

WHO (2019): Regional and Global Trends in Burden of Malaria Cases and death. World Malaria Report 2019. https://www.who.int.

Yeka, A., Benek, K. Bakhaita, N. Staedke, S.G., Kamya, M.R. Talisuna, A., Kironde, F., Nsobiya, S.L., Kilian, A., Slater, M., Reingold, A., Rosenthal, P.J., Wabire-mangin, F. and Dorsey, G. (2005): Artemisinin Versus non Artemisinin Combination Therapy for Uncomplicated Malaria Randomized Clinical Trials for Sites in Ugandan Public Library of Science Medicine, 2(7): Pp190.

Zongo, I., Dorsey, G., Rouamba, N. Dokomajilar, C., and Sere, Y., (2007): Randomized Comparison of Amodiaquine Plus Sulfadoxine – Pyrimethamine, Artemether – Lumefantrine and Dihydroaartemisinin – Piperaquine for the Treatment of Uncomplicated *Plasmodium falciparum* Malaria in Burkina Faso. *Clinical Infectious Disease* 45: Pp1453 – 1461.

Zwang, J. Dorsey, G., Martensson, A., Alexandro, U., Ndiaye, J., Karema, C. Djimde, A., Phillippe, B., Sodiomon, B. and Olliaro, P. (2014) : *Plasmodium falciparum* Clearance in Clinical Studies of Artesunate amodiaquine and Comparator Treatments in Sub – Saharan Africa. *Malaria Journal* 13:Pp 114.