Sulphadoxine-Pyrimethamine and the prevalence of malaria (in blood and placenta) among booked women who have completed intermittent preventive treatment in Zaria, northern Nigeria

Ranyang Akafa[1], Adekunle O. Oguntayo[1], Modupe O.A.Samaila[2]

[1]Department of Obstetric and Gynecology, Ahmadu Bello University Teaching Hospital, Shika-Zaria. Kaduna State. Nigeria.
[2]Department of Histopathology, Ahmadu Bello University Teaching Hospital, Shika-Zaria. Kaduna State. Nigeria.

ABSTRACT

INTRODUCTION. Malaria is currently regarded as the most common and potentially the most serious infection occurring in pregnancy in sub Sahara African countries.

OBJECTIVE. The objective of this study was to look at Sulphadoxine pyrimethamine and the prevalence of malaria (in blood and placenta) among booked women who had completed intermittent preventive treatment in Zaria, Nigeria. To determine the prevalence of malaria at booking, peripheral blood & placenta parasitaemia of those who had completed Intermittent Preventive Therapy and compare the sensitivity of placental tissue biopsy and peripheral blood smear method for the diagnosis of malaria infection at delivery.

METHOD. The study took place in ABUTH Zaria. It was a longitudinal study of 108 consecutive mother-baby pair who had antenatal care and delivered during the study period of seven months. Participants were enrolled from the booking clinic of ABUTH Zaria. A structured questionnaire was administered to obtain information about the socio-demographic factors and other relevant information about the participants. Base line packed cell volume as well as blood film for malaria parasite was done at booking. They received 2 adult doses of SP orally as direct observation therapy (DOT) during clinic visits. They also received routine haematinics and continued with their routine antenatal visits. At delivery, blood smear for malaria parasites was prepared from maternal peripheral blood and Placental biopsy was examined for malaria pigments and parasites. Gestational ages at delivery as well as the weight of the infants were recorded. The packed cell volume of the parturient was determined at delivery.

RESULTS. The prevalence of malaria parasitaemia at booking was 36.1%. The prevalence of malaria parasitaemia after completion of IPT with SP was 25.9% and 16.6% in peripheral blood and placenta tissue biopsy respectively. By simple proportion, peripheral blood smears method was more sensitive than placental tissue biopsy in the diagnosis of malaria infection at delivery.

There was no significant difference in peripheral parasitaemia before and after administration of IPT with SP. The prevalence of maternal anaemia (pcv<30%) was 8.3%, while that of preterm delivery (GA<37weeks) was 3.7%. No infant was born with a birth weight of less than 2500g.

Correspondence to: Adekunle O. Oguntayo
fayokunmi@yahoo.co.uk

RIASSUNTO

INTRODUZIONE. La malaria è attualmente considerata come l'infezione più comune e potenzialmente più grave che si verifica in gravidanza nei paesi africani subsahariani.

OBIETTIVO. L'obiettivo di questo studio era di esaminare la Sulphadoxine pirimetamina e la prevalenza della malaria (nel sangue e nella placenta) tra le donne reclutate che avevano completato il trattamento preventivo intermittente a Zaria, Nigeria. Per determinare la prevalenza della malaria al momento del reclutamento, sangue periferico e parassitemia placentare di coloro che avevano completato la Terapia Preventiva Intermittente e confrontare la sensibilità della biopsia del tessuto placentare e del metodo di striscio di sangue periferico per la diagnosi di infezione da malaria al momento del parto.

METODO. Lo studio ha avuto luogo in ABUTH Zaria. E' stato uno studio longitudinale di 108 coppie consecutive madre-bambino sottoposte a cure prenatali e che hanno partorito durante il periodo di studio durato sette mesi. I partecipanti sono stati arruolati dall'ospedale di ABUTH Zaria. Un questionario è stato somministrato per ottenere informazioni sui fattori socio-demografici e altre informazioni pertinenti sui partecipanti. L'ematocrito di base così come per la ricerca del parassita della malaria sullo striscio di sangue è stato fatto al momento del reclutamento. Hanno ricevuto 2 dosi per adulti di SP per via orale come terapia osservazionale diretta (DOT) durante le visite cliniche. Hanno inoltre ricevuto ematinici di routine e hanno continuato con le loro visite prenatali di routine. Al parto, la ricerca del parassita della malaria sullo striscio di sangue è stato effettuato da sangue periferico materno e la biopsia placentare è stata esaminata per i pigmenti e i parassiti della malaria. Età gestazionale al parto e peso dei neonati sono stati registrati. L'ematocrito della partoriente è stato determinato al momento del parto.

RISULTATI. La prevalenza della parassitemia malarica al reclutamento era del 36,1%. La prevalenza della parassitemia malarica dopo il completamento della IPT con SP è stata del 25,9% e del 16,6% rispettivamente nel sangue periferico e nella biopsia dei tessuti della placenta. Con una semplice proporzione, la ricerca del parassita sullo striscio di sangue periferico è risultata essere una metodica più sensibile della biopsia del tessuto placentare nel diagnosticare l'infezione malarica al momento del parto.

Non vi era alcuna differenza significativa nella parassitemia periferica prima e dopo la somministrazione di IPT con SP. La prevalenza di anemia materna (PCV <30%) è stata del 8,3%, mentre quella da parto pretermine (GA <37 settimane) è stato del 3,7%. Nessun bambino è nato con un peso alla nascita inferiore a 2500g.

CONCLUSIONE

IPT con SP riduce la prevalenza di parassitemia periferica e

CONCLUSION. IPT with SP reduces the prevalence of peripheral and placental parasitaemia and improves pregnancy outcome. However, this is based on simple proportions, it was statistically insignificant.

Key-words: sulphadoxine-pyrimethamine, malaria, placenta, blood, parsitaemia, prevalence.

placentare e migliora l'esito della gravidanza. Tuttavia ciò è basato su semplici proporzioni ed è stato statisticamente insignificante.

Parole chiave: sulphadoxine-pirimetamina, malaria, placenta, sangue, parassitemia e prevalenza.

INTRODUCTION

Falciparun malaria in pregnancy is an important cause of maternal and perinatal morbidity and mortality in areas where malaria is endemic. Pregnant women in malaria endemic areas may experience a variety of adverse consequences from malaria including anaemia and accumulation of parasites in the placenta. The outcomes of placental invasion by parasites, inflammatory cells and cytokines include: abortion, premature labour, small-for-date babies, congenital malaria and foetal/maternal death in some instances. This unfavourable pregnancy outcomes in the mother and in her baby[1] is the reason why this condition need to be treated and prevented as a matter of routine in all women at risk of infection[2]. Because of the consequences of Plasmodium Falciparum infection during pregnancy, the world health organization (WHO) recommends that women living in malarious areas receive chemoprophylaxis during pregnancy[3]. Since, Schultz and colleagues[4] demonstrated the efficacy of SP in decreasing placental malaria, SP is still the drug of choice for prophylaxis in pregnancy. IPT for preventing malaria reduces the incidence of maternal anaemia, spontaneous abortion, preterm birth, still birth and low birth weight. The aim of this study was to look at SP and the prevalence of malaria parasitaemia (in blood and placenta) of booked women who had completed intermittent preventive therapy in Zaria, Nigeria.

This study was carried out at Ahmadu Bello University Teaching Hospital Zaria.

Zaria is one of the major cities in Kaduna state of Nigeria. The ABUTH Zaria serves as a tertiary/ referral health facility for Zaria and its environs.

SUBJECTS AND METHODS

Participants were enrolled from the booking clinic of ABUTH Zaria. Booked women in their index pregnancy who were between 16 and 24 weeks gestation were enrolled, those excluded from the study included those with sickle cell anaemia, HIV positive women, previous adverse reaction (hypersensitivity) to Sulphonamides, multiple gestations and those who did not give their consent.

Eligible participants were required to give informed verbal consent after explanation of study procedures. Enrolled participants completed the routine ANC measurements, examinations and investigations. Gestational age was ascertained by last menstrual period and abdominal palpation. A questionnaire was administered to collect information about the socio-demographic factors and other relevant information about the participants. A finger stick blood sample was drawn for packed cell volume and malaria thick blood smear. They received 2 adult doses of SP orally by (DOT) during clinic visits administered by the supervision of midwives and recorded in subjects ANC case notes and signed. Antenatal records of subject were stamped for easy identification during delivery and the use of SP was confirmed from it. The first dose of SP was given in the second trimester and the second dose was given in the third trimester at least four weeks apart up to 34 weeks gestation. All participants of this group received Sulphadoxine Pyrimethamine obtained from the hospital pharmacy. The same batch was used. Each dose of SP consists of 3 tablets containing 500mg of sulphadoxine and 25mg of pyrimethamine per tablet. They also received prescription for folic acid and iron supplements and continued with their routine antenatal visits. Participants who developed malaria during the study period were treated with Artemisinin/ Lumefantrine combinations and noted. Case definition of malaria in this study was defined as the participants who develop fever, headaches, joint pains, nausea/vomiting in the absence of other causes of the above symptoms with or without peripheral malarial parasitaemia. At delivery, thick and thin film for malaria parasites was prepared from maternal peripheral blood and placental impression smear (within 1 hour of delivery). Placental biopsy was examined for malaria pigments and parasites. Gestational age at delivery as well as the weight of the baby was recorded. The packed cell volume of the parturient was determined again. Babies were weighed to the nearest gram using a weighing scale (Way Master, made in England.) and for the purpose of this

study; LBW was defined as neonatal birth weight less than 2,500 g. Babies born before 37 weeks of gestation were considered pre-term while those born after 37 weeks of gestation and above were considered term, while a packed cell volume reading of <30% in the parturient was considered as anaemia.

LABORATORY PROCEDURE

Maternal peripheral venous blood (2 ml) was collected by venopuncture within 24 hours of parturition and used to prepare thick blood films. Immediately following delivery, the placenta was obtained and a small piece of placental tissue (2 by 2 by 1 cm) was excised from the centre of the placenta and fixed in 10% neutral buffered formalin for histopathological studies. Fixed placental biopsies was processed, embedded in paraffin wax and sectioned onto slides by standard techniques. Sections were stained later with haematoxylin-eosin stain for analysis. Microscopic examination of blood smears was done under oil immersion for parasite detection. When no parasite was found, 200 high power fields were examined before the smear was considered negative. Parasites were counted against 200 leucocytes assuming an average leukocyte count of 8,000 per microlitre of blood[5]. Placental histological sections were examined by a Histopathologist without knowledge of the blood film microscopy results. One thousand intervillous cells (IVS) were counted to determine the level of parasitaemia in placenta tissue sections. Past infection was defined as the presence of malaria pigment in fibrin or monocyte/macrophage without malaria parasites[6]. Sections were observed under light microscopy to assess the presence of malaria pigment[7].

The packed cell volume (PCV) was determined using blood collected into heparinized capillary tubes and spun with a Hawksley micro-hematocrit centrifuge for 5 minutes and read using the heamatocrit reader. Haemoglobin concentration was calculated from PCV values as described by Topley[8].

STATISTICAL ANALYSIS

Data obtained were analyzed using SPSS version 15.0. Frequencies and percentages were presented in tables and charts, statistical significance of differences between qualitative variables was tested using Chi Square and level of significance was set at $p < 0.05$. P. falciparum infection was defined as the presence of malaria parasites as detected in thick peripheral blood or placental tissue sections. Malaria infection on placental tissue section was defined as positive, only when active infection was found (presence of infected erythrocyte in the intervillous space).

RESULTS

During the period of the study, a total of 150 participants who fulfilled the inclusion criteria were recruited. Out of these, 22 (14.6%) did not come for delivery, placenta biopsy was not taken in 12 (8%) clients and the other 8 (5.3%) has no blood sample taken for malaria parasitaemia. Only 108 results were analysed. All the infant birth weight were 2500g and above. One client had intra-uterine foetal death at term. She had a positive malaria parasite at booking but negative at delivery both in the peripheral blood and placenta. The birth weight was 2500g. One hundred and five (97.3%) delivered after 37 weeks gestation while 3 (2.7%) delivered below 37 weeks gestation.

Table I shows the socio-demographic profile of the client. They were between ages 19 and 41 years with a mean age of 27.75 years. primigravidae were 35.2%, multipara constituted 40.7% and grandmultips 11.1% percent.

Figure 1.
PCV at Booking and at Delivery.

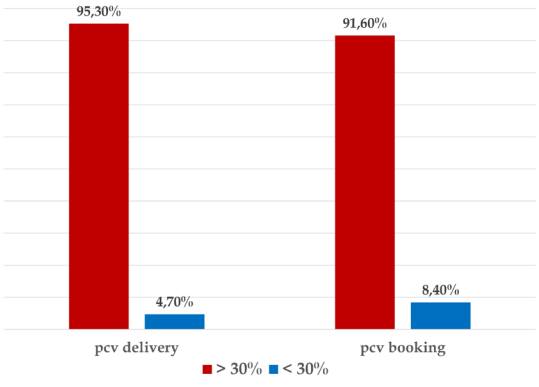

Table II shows symptomatic malaria among clients at booking. During the first visit, 2 client (1.9%) compliant of headache/fever, 1 (0.9%) compliant of dizziness/weakness and another 1 (0.9%) had joint pains and 2 (1.9%) were pale. These patients were treated with Artemisinin/ Lumefantrine combination and they had their 2 doses of SP at the scheduled intervals. Only one

Table I.
Socio demographic profile of clients.

Characteristic	Frequency	Percentage
Age		
<20	4	3.7
20-29	62	61.4
30-39	39	36.1
>40	3	2.8
Parity		
0	38	35.2
1	14	13.0
2-4	44	40.7
≥ 5	12	11.1
Marital Status		
Single	6	5.6
Married	102	94.4
Educational Status		
Quranic	3	2.8
Primary	8	7.4
Secondary	37	34.3
Tertiary	60	55.6
Occupation		
House Wife	56	51.9
Business	13	12.0
Civil Servant	27	25.0
Religion		
Islam	75	69.4
Christianity	32	29.6
Other	1	0.9
GA at booking		
<20 weeks	15	14.2
20-24 weeks	93	86.1

Table II.
Symptomatic malaria among clients at booking.
Relevant history

	Frequency	Percentage
Symptoms		
Headache/fever	2	1.9%
Dizziness/weakness	1	0.9%
Joint pains	1	0.9%
None	104	96.3%
Signs		
Pallor	2	1.9%
None	106	98.1%

client (0.9%) took quinine after completion of SP.

Figure 1 show PCV at booking and at delivery. Ninety-nine of the client (91.6%) had a PCV of greater 30% at booking, while 103 (95.3%) had a PCV of greater than 30% at delivery.

Figure 2 shows result of Malaria Parasitamia. At booking 39 (36.1%) of the participants had positive malaria parasitaemia in their blood, at delivery this figure was reduced to 28 (25.9%). For placental parasitaemia, 18 (16.6%) was positive at delivery.

Table III shows results of sensitivity (by simple proportion) of placental tissue biopsy and peripheral Blood smear methods for the diagnosis of malaria at delivery. At delivery the peripheral blood detected 25.9% of malaria parasitaenia while the placental tissue biopsy detected 16.6%. By this simple proportion, peripheral blood smears method was more sensitivity than placental tissue biopsy in the diagnosis of malaria infection at delivery.

Table IV shows relationship between sensitivity of placental tissue biopsy and peripheral blood smear at delivery. The sensitivity of placental tissue biopsy was 16.6% and that of Malaria Parasitamia at delivery was 25.9%. The P value was 0.0000 and this was statistically significant

Figure 2.
Result of Malaria Parasitamia.

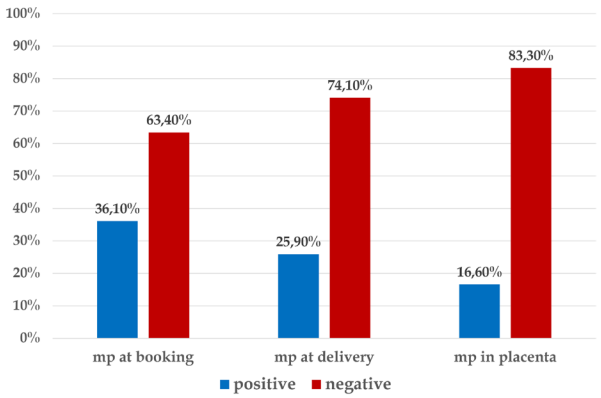

Table V shows the relationship between malaria parasitaemia in the peripheral blood before and after administration of SP. Before administration of SP (at booking), 39 of the participants had positive malaria parasitaemia in their blood, after administration of SP (at delivery), 28 of the participant had positive malaria parasitaemia in their blood. The P value was 0.138 and this was not statistically significant.

DISCUSSION

The prevalence of malaria at booking was 36.1%. This figure was lower than 58% reported by Nwagha et al.[9] and 59.9% reported by Ogbodo et al[10]. Nnaji and colleagues[11] has reported a

Table III.
Results of sensitivity (by simple proportion) of placental tissue biopsy and peripheral Blood smear methods for the diagnosis of malaria at delivery

	Positive	Negative	Total
Placental tissue biopsy	18 (16.6%)	90 (83.3%)	108
Peripheral Blood Smear	28 (25.9%)	80 (72.1%)	108
Total	46	170	

Table IV.
Relationship between sensitivity of placental tissue biopsy and peripheral Blood smear at delivery

		Placental parasitaemia		Total
		Positive	Negative	
Peripheral parasitaemia at delivery	positive	12	16	28
	negative	16	74	80
	Total	18	90	108

Pearson's chisquare ($\chi 2$) = 18.669, df=1, P-value=0.0000
This was statistically significant

Table V.
Relationship between malaria parasitaemia in the peripheral blood before and after administration of SP

		Malaria parasitaemia after SP		Total
		Positive	Negative	
Malaria parasitaemia before SP	positive	13	26	39
	negative	15	54	69
	Total	28	80	108

Pearson's chisquare ($\chi 2$) = 1.744, df=1, P-value=0.138
This was not statistically significant

prevalence rate as high as 79.3%. The prevalence rate less than 36.1% gotten in this study have been reported in various studies. Taye and colleagues[12] reported a prevalence rate of 34.2% while Nwonwu and colleagues[13] reported a prevalence rate of 29%. Prevalence rate as low as 2.9%, 4.8% and 8.4% have been reported by Laminkara[14], Isah et al[15] and Falade et al[16] respectively. It is worthy to note that there is a wide variation in prevalence rate of malaria at booking and this is not related to the sample size. Isah et al[15] have speculated that the group of pregnant women selected (symptomatic or asymptomatic) and the sampling method used might have contributed to this. Perhaps, the pattern of malaria (stable or unstable) in that region and the period of the year in which the study was conducted might also influence the prevalence.

The prevalence of malaria after completion of IPT with SP was 25.9%. There was a reduction in peripheral malaria parasitaemia from 36.1 to 25.9%. Bako et al[17] reported peripheral malaria parasitaemia of 28.8% after sp with reduction in peripheral parasitaemia from 60.3% to 28.8%. Challis et al[18] reported an incidence of 6.3% of peripheral parasitaemia after SP with a reduction from 30.6% to 6.3%.

The prevalence of placental parasitamia was 16.6%. This value was more than 13.8% and 10.4% reported by Van Ejik et al[19] and Falade et al[20] respectively. Other workers have reported higher incidence of placental parasitaemia on histology. Shulman et al[21], Okoko et al[22] and Judith et al[23] reported incidences of 64%, 51% and 60.4% respectively. A higher incidence of placental parasitaemia was reported by these authors because they recorded both active and past placenta infection as positive, while this study as well as those by Van Ejik et al[19] and Falade et al[20] only reported active placental infection. Also, they were not strict on whether their subjects took SP or not. By summing up active and past placental infections, the percentage of positivity will be higher for placental parasitaemia relative to peripheral blood parasitaemia and this is what was done and reported by those three authors[21,22,23]. In this study, the past placental infection constituted 18.5% (20/108), but it was not added to active placental infection (but rather counted as negative) since the study was looking at the prevalence of malaria parasitaemia in blood and placental after the completion of IPT with SP. Counting past infection as positive does not reflect drug use. If past placental infection were added to active infection, it will give a prevalence rate of 34% which is higher than that of peripheral blood. Since there is no means of diagnosing past infection in peripheral blood yet, it will fair to use the same yard stick of measurement.

The prevalence of malaria parasitaemia in the peripheral blood (25.9%) was higher than that of placental parasitaemia (16.6%) and this result was statistically significant attesting to the fact that the peripheral blood smear method was more sensitive that the placental tissue biopsy. The peripheral blood smear method has the advantage of being cheaper, easier or quicker (within an hour) to perform and requires less training compared to placental histology which is more expensive, takes days to perform and requires technicalities with high expertise which are not widely available.

The result of peripheral parasitaemia before and after the administration of SP showed a

reduction from 36.1% at booking to 25.9% at delivery; this result however, was not statistically significant, but the resultant effect of SP was a reduction in malaria parasitaemia.

CONCLUSION AND RECOMMENDATION

In conclusion, the prevalence of malaria parasitaemia in the peripheral blood and placental tissue reduced with IPT using SP though this effect was not statistically significant. SP use also improved pregnancy outcome. By simple proportion, peripheral blood smear method was more sensitive than placental tissue biopsy in the diagnosis of malaria infection at delivery.

While the use of SP for IPT may suffice for now, there is a need for further research in other to revalidate or invalidate the efficacy of SP or to consider combination therapy or a newer drug.

ACKNOWLEDGEMENT

I want to declare that no any special interest in this study or any clash of interest what so ever (either financially or otherwise).

REFERENCES

1) **WHO. A strategic framework for malaria prevention and control during pregnancy in the African region. Brazzaville 2004**, WHO Regional office for Africa (01).
2) Shulman CE. Malaria in pregnancy: Its relevance to safe-motherhood programmes. Ann.Trop.Med.Parasitol. 1999 ; 93 (1): 559-66.
3) **World Health Organization, 1986. WHO Expert Committee on Malaria, 18th Report.** World Health Organ Tech Rep Ser 735.
4) Schultz LJ, Steketee RW, Macheso A, Kazembe P, Chitsulo L, Wirima JJ. **The efficacy of antimalarial regimens containing sulfadoxine-pyrimethamine and/or chloroquine in preventing peripheral and placental P. falciparum infection among pregnant women in Malawi.** Am J Trop Med Hyg, 1994; 51: 515–522
5) Rooth I, Perlmann H, Bjorkman A: P. falciparum reinfection in children from holoendemic area in r**elation to seroreactivities against oligopeptides from different malaria antigens.** Am J Trop Med Hyg, 1991; 45:309-18
6) Ismail MR, Ordi J, Menendez C, Ventura PJ, Aponte JJ, Kahigwa E, Hirt R, Cardesa A, Alonso PL: **Placental pathology in malaria: a histological, immunohistochemical, and quantitative study**. Human Pathol ,2000; 31:85-93.
7) Romagosa C, Menendez C, Ismail MR, Quintó L, Ferrer B, Alonso PL, Ordi J: **Polarisation microscopy increases the sensitivity of hemozoin and P. detection in the histological assessment of placental malaria.** Acta Trop, 2004; 90:277-284
8) Topley E. **Anaemia in rural Africa: community support for control activities where malaria is common. Available through FSG.** Medimedia Vine house. Fair Green Reach. Cambridge CB5 OJD. UK 1998 , 2.
9) Nwagha UI, Ugwo VO, Nwagha TU, Anyachie BU. **Asymptomatic plasmodium parasitaemia in pregnancy in pregnant Nigerian women: Almost a decade after roll back malaria.** Trans R Soc Trop and Med Hyg, 2009; 103: 16-20
10) Ogbodo SO, Nwagha Ul, Okaka AN, Ogenyi SC, Okoko RO, Nwagha TU. **Malaria parasitaemia amongst pregnant women in a rural community of eastern Nigeria; need for combine measure.** Niger J. Physiol Sci, 2009; 24:95-100
11) Nnaji GA, Ikechebelu JI, Okafor CI. **A comparison of the prevalence of malaria parasitaemia in pregnant and non pregnant women.** Niger J. Med, 2009; 18(1): 47-51.
12) Taye AO, Akinola OI, Shittu LAJ, Ottun TA, Bankole MA et al. **Prevalence of malaria parasitaemia in the booking antenatal patients at the Lagos University Teaching Hospital.** African journal of biotechnology , 2009;8(15): 3628-3631.
13) Nwonwu Eu, Ibekwu PC, Ugwu JI, Obarezi HC, Nwagbara OC. **Prevalence of malaria parasitaemia and malaria related anaemia amongst pregnant women in Abakaliki, Southwest Nigeria.** Niger J. Clin Pract, 2009; 12:182-6.
14) Laminkaro OT. **A study of malaria parasithemia in pregnant women, placentae, cord blood and new born babies in Lagos, Nigeria.** West Africa J med 1993; 12:213-7
15) Isah AY, Amanabo MA, Ekele BA. P**revalence of Malaria parasitaemia amongst asymptomatic pregnant women attending a Nigerian Teaching Hospital.** Annal of African Medicine, 2011; 10(2):171-174
16. Falade CO, Olayeni O, Dada—adegbola HO, Aimakhu CO, Ademowo OG and Solako LA. **Prevalence of Malaria at booking among antenatal clients in a secondary health care facility in Ibadan, Nigeria.** Afr J Reprod Health, 2008; 12(2): 141-152.
17) Bako BG, Geidam AD, Mariga AG, Malah AB, Ngadda HA, Musa AB et al. **The effect of intermittent preventive treatment for malaria on pregnancy outcome at university of Maiduguri Teaching Hospital**. KJMS ,2009; 3 :1.
18) Challis K, Osman NB, Cotiro M, Nordahl G, Dgedge M, Bergstrom S. **Impact of double dose sulphadoxine-pyrimethamine to reduce the prevalence of pregnancy malaria in southern Mozambique.** Trop Med Int. Health, 2004; 9(10): 1066-73.
19) Van Ejik Am, Ayisi Ja, Kuile FO, Otieno Ao, Odonjijo, Misore AO, etal. **Effectiveness of intermittent preventive treatment with S-P for control of malaria in pregnancy in western Kenya; a hospital-based study.** Trop Med.Int.Health, 2004; 9(3): 351-60.
20) Falade C. O, Yusuf B. O, Francis F. F, Olugbenga A. M, Davidson H. H, Lateef A. S. **Intermittent preventive treatment with sulphadoxine-pyrimethamine is effective in preventing maternal and placental malaria in Ibadan, south-western Nigeria.** Malar J, 2007; 6: 88.
21. Shulman CE, Marshall T, Dorman EK, JN Bulmer, Cutts F, Peshu N, Marsha K. Malaria in Pregnancy: **adverse effect in haemoglobin level and birth weight in primigravida and multigravida.** Tropical medicine and international health, 2001;6(10): 770-778.
22. Okoko BJ, Ota MO, Yamuah LK, Idiong D, Mkpanam SN, Avieka A, Banya WA, Osinusi K. **Influence of placental Malaria infection on foetal outcome in the Gambia: twenty years after Ian Megregor.** J Health Popul Nutr, 2002; 20 (1): 4-11.
23) Judith KAK, Eric AA, Blaise N, Eva SME, Marita TB. **Diagnostic Comparison of Malaria Infection in Peripheral Blood, Placental blood and placental biopsies in Cameroonian Parturient Women.** Malaria Journal, 2009; 8:126.