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Congenital Malaria Among Newborns Admitted for Suspected Neonatal Sepsis In Abuja.

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Summary

Background: Signs and symptoms of congenital malaria do not differ much from those of neonatal sepsis: both can co-exist, and most times very difficult to differentiate clinically.

Objective: To document the prevalence, risk factors for congenital malaria among neonates admitted for suspected neonatal sepsis, and determine sensitivity, specificity and predictive values of some common signs and symptoms.

Method: Blood for culture, thin and thick blood film for malaria parasite were taken for all cases of suspected neonatal sepsis at the special care baby unit of the hospital, from August 2007 to December 2009.

Results: A total of 266 newborns (150 males and 116 females) with suspected neonatal sepsis were recruited. Their mean admission weight was 2.5±0.87 kg, gestational age was 36.1±3.5 weeks, and age of 5.1±2.3 days. While 76 (28.6%) had malaria parasites in their blood film, 97 (36.5%) were blood culture positive, and 12 (4.5%) had both positive blood culture and malaria parasite. Among the recruited babies, 82.4% were noticed to have low parasite density, 13.2% had moderate density, while 2.6% had high density. Peripartum pyrexia, prematurity and intrauterine HIV exposure, were found to have significant association with congenital malaria, (OR = 7.9, 7.2, 4.4) for peripatum pyrexia, prematurity and HIV exposure, p values <0.05. None of the clinical feature had good sensitivity, specificity or predictive value for congenital malaria, and only 1.6% death was recorded in a baby with high parasite density.

Conclusion: Congenital malaria is common in newborns with suspected neonatal sepsis. History of peripartum pyrexia, prematurity and intrauterine HIV exposure was associated with increased risk of the disease.

Introduction

Neonatal sepsis (NS) has remained one of the commonest reason for admission into most special care baby unit (SCBU) in Nigeria.[1] It is responsible for over 25% of all neonatal mortality,[2,3] and a major problem in many developing countries partly because of non-availability of standard neonatal care. Malaria has also been recognized as a leading cause of infant morbidity and mortality in African [4] but neonatal malaria (NM) was taught to be rare.[5,6] This was believed to be partly due to the protective effect of fetal haemoglobin F present in the blood of the neonates,[7] as well as the transplacental transferred maternal antibodies.[6] Congenital malaria (CM) is defined as the presence of malaria parasite (MP) in the peripheral blood smear of a new born baby within the first seven days of life,[8-10] Recently, there are many

reported cases of CM in most endemic malaria areas, ranging from 1.5% in Maputo Mozambique,[11] to 28.2% in Jos, Nigeria[12] 35% in Calabar [13] and as high as 46.7% in Ile-Ife.[14] The reported increase has been attributed to increased resistance of *plasmodium falciparium* (PF) to anti-malaria drugs resulting in increase in maternal parasitaemia,[15] mothers on regular malaria chemoprophylaxis having low malaria antibody titers and hence transfer little protective antibody to their newborns, [16] and most importantly increased reporting of cases.[17] CM is thought to have resulted from MP crossing the placenta from maternal blood to the fetal circulation. The exact mechanism of this crossing is not know, but damage to the syncytiotrophoblast occurring during active placental infection has been suggested.[14,18] This type of malaria has been shown to occur in babies of clinically healthy mothers who are delivered in malaria endemic areas.[19] In babies of these women with high level of immunity, CM is generally asymptomatic, majority of parasites being rapidly cleared from the infant's circulation as a result of passive protection of the baby by maternal antibody crossing the placenta, active immunity developing from exposure to soluble malaria antigens in utero, and high proportion of fetal haemoglobin present in the babies which retards the growth of the parasite.[7,18]

Clinical signs of CM may be indistinguishable from that of NS.[13,20] This has lead to many researchers suggested screening for MP to be included as part of routine investigation in newborn infants with fever.[20] The increasing reported cases of CM in hyperendemic areas, and the difficulty in differentiating NS from this disease condition necessitated the need to determine the prevalence of CM in babies admitted for NS, assess the risk factors associated with it and determine sensitivity, specificity, and positive predictive values of some common clinical signs and symptoms. It is envisaged that the result(s) of this findings will assist health care providers in identifying newborns at risk of this disease for early investigation and management.

Subjects and Methods

An 18month prospective study was carried out at University of Abuja Teaching Hospital (UATH), Gwagwalada between the months of August 2007 to December 2009. Neonates with features of NS and admitted into the special care baby unit (SCBU) of the hospital were recruited into the study. For the purpose this study, analysis was on newborns with congenital malaria (ie those babies with positive MP in their blood stream in their first seven days of life). Majority of the babies admitted into the unit were referrals from the neighbouring general, private, and mission hospitals. Referrals were also received from maternity homes, nearby primary health centers in addition to self referrals from home. SCBU is a 32-bed capacity key service ward in our health institution. It is an area where newborns are promptly managed on 24 hour basis, and represents a high volume, high stress service area of the hospital. UATH is a 350-bed capacity tertiary health institution located in the Federal Capital Territory (FCT), Abuja in the north central zone of the country. It is sub-serving many neighbouring states including Nassarawa, Niger, Kogi, Benue, parts of Kaduna state and FCT, Abuja

Blood for culture, total white blood cell count/ differentials, and malaria parasite (MP) were collected from the study patients. Blood for culture was collected in 2 bottles, one containing glucose broth and the other thio-glycolate medium, and both were incubated at 37°C for 48 hours. Growth in either of the media was sub-cultured on Mc-Conkey, blood or chocolate agar and further tested for antibiotic sensitivity. However, if no growth was observed, further incubation was done for another 48 hours. Other investigations like urine, stool or cerebrospinal fluid culture were determined by clinical presentations of the neonate. Thin and thick blood films were prepared immediately upon collection of blood on separate slide. For thick films, 12 µl of blood was spread over a diameter of about 15 mm, while 2 µl of blood was used for thin films. The thick film smear was allowed to air dry before being dehaemoglobinized with buffered distilled water at PH of 7.2. The thin film was fixed in absolute methanol for 1-2 minute before air drying. Both thick and thin blood films were stained after 24-48 hr with 3% Giemsa stain solution at pH 7.2. For the thick film the dilution was in the ratio of 1 volume of Giemsa to 9 volume of buffered distilled water, while that of thin film the dilution was at 1 : 29 of Giemsa and distilled water. The stained slides were read by a Laboratory Technologist. The method adopted by Greenwood and Armstrong[21] and described by Cheesbrough[22] was used to determine MP density. The average number of parasites counted per high power field (100 x objectives) was multiplied by 500.[21,22] Between 10 and 15 fields were counted for each slide, and results given per µl of blood. A definitive diagnosis of malaria was made when a reddish chromatin dot with a purple or blue cytoplasm of the malaria parasites are seen together. A slide was pronounced negative when 100 high power fields have been examined using ×100 oil immersion objective lens. Malaria parasite density was considered significant when MP count was greater than 1,000 parasites per µl of blood. Low parasite count was when count of 500 parasite/µ/ of blood and below was obtained, moderate count was a count of >500-<1,500 parasite/µl of blood, while high parasite count was a count of >1,500->3,000 parasite/µl.

Other information collected from the patients includes: age at onset of symptoms (days), presenting problem and duration of such problem, gender, admission weight (AW), mother's parity, history of maternal peri-partum pyrexia (PPP), history of rupture of membrane (ROM), whether the mother attended/ received antenatal care, gestational age (GA), treatment given/ outcome of the illness, HIV status of the mother, and socio-economic status (SES) of the parents using Olusanya's two factor index: husband's occupation and mother's level of education.[23] All newborns admitted into SCBU for CM and found to have moderate and high MP density in their blood film were treated for malaria with oral quinine.

Ethical approval was obtained from the Ethics committee of the hospital. Signed or thumb-printed informed consent was also obtained from the mothers of the subjects after adequate explanation of the purpose of the research in the language they understood best. The research data was coded, privacy protected and confidentiality maintained. Data analysis was conducted using SPSS version 13.5. Tests for associations and differences were done by chi-square analysis. Statistical significance was set at p value < 0.05.

Results

The characteristics of recruited newborns are shown in table 1. A total of 266 newborns with suspected NS and accounting for 25.8% of the total admissions were recruited for the study. There were 150 males and 116 females with male to female ratio of 1.3:1. The mean admission weight(AW) was 2.5±0.87 kg, with mean GA of 36.1±3.5 weeks, and age of 5.1±2.3 days.

Babies with Malaria Parasitaemia

A total of 76 neonates (35 males and 41 females: 0.9 to 1) were positive for malaria parasitaemia (MP) while 109 had culture-proven sepsis accounting for 26.8% and 41.0% of the screened population respectively. Of these, 64 had MP alone, 97 had bacterial sepsis alone and 12 had MP in association with culture proven sepsis. The mean AW for babies with CM was 2.4±0.24 kg, GA of 35±4.3 weeks and Age of 3±0.7 days. Further analysis of babies with CM showed that 52 (81.3%) of them had low PD (parasite count of 500 parasite/µ/ of blood), 10 (15.6%) had moderate PD (parasite count of >500-<1,500 parasite/µl of blood), while 2 (3.1%) had high PD (parasite count of > 1,500-> 3,000 parasite/µl) fig 1. There was no significant difference in malaria PD between male and female newborns, p >0.05 (table: 2).

Maternal/ Neonatal Factors and 64 Babies with Congenital Malaria Only

Table: 3 shows maternal and neonatal factors and malaria parasite density among 64 newborns with CM. Congenital malaria was identified in 71.9% of neonates from low socio-economic class (LSEC), in 67.2% of mothers on anti malaria prophylaxis (daraprim) during pregnancy, in 65.6% of preterm deliveries, in 59.4% of para1 and 2 mothers, and in 39.1% of mothers with peri-partum pyrexia (PPP). Low birth weight (LBW), maternal PPP, and intra uterine HIV exposure, were the maternal and neonatal factors found to have significant association with CM (OR of 7.2 with CI of 1.20 18.47, p value 0.002 for LBW, OR of 7.6 with CI of 1.32 18.37, p value 0.002 for PPP, OR of 4.4, 95% CI of 0.98 -9.82 and p value 0.35 for HIV exposure. No association was seen in mothers with rupture of membrane (OR of 0.66, 95% CI of 0.19 - 2.14, p value 0.55), and those on anti malaria prophylaxis (OR of 0.33, 95% CI 0.12 - 2.01, p value 0.65). Moderate and high parasitaemia were seen more in newborns of mothers with PPP, preterm deliveries, HIV exposed babies of mothers from low socio-economic class (LSEC).

Sensitivity, Specificity and Positive Predictive Values of Common Presenting Signs and Symptoms

Sensitivity, specificity and positive predictive values (PPV) of common clinical signs and symptoms of CM in newborns was shown in table 4. While hepatomegaly, fever, jaundice, fast breathing, poor feeding, pallor, and irritability were the common symptoms and signs seen in newborns with CM, jaundice, pallor, fever, and hepatomegaly were the only three clinical features that had fairly good sensitivity, specificity with PPV, 81.3%, 34.2% and 28.1% for hepatomegaly, 50.0%, 43.5%, and 21.9% for fever, 39.0%, 67.8% and 38.5% for jaundice, and 23.4%, 83.7% and 31.3% for pallor.

Table 1

Profile of Recruited Newborns with Presumed Sepsis.

	Male	Female	Total
Total no recruited	150	116	266
Age (days)	*4.7±3.1	*5.5±4.3	*5.1±3.7
Weight (kg)	*2.6±0.84	*2.4±0.89	*2.5±0.87
Gestational age (weeks)	*36.9±2.8	*35.3±4.2	*36.1±3.5
Mother's parity	*2.0±1.9	*3.0±1.1	*2.5±1.5
SVD	82	77	159
C/S	33	28	61

*Values are mean ±SD

SVD = Spontaneous vertex delivery.

C/S = Caesarian section

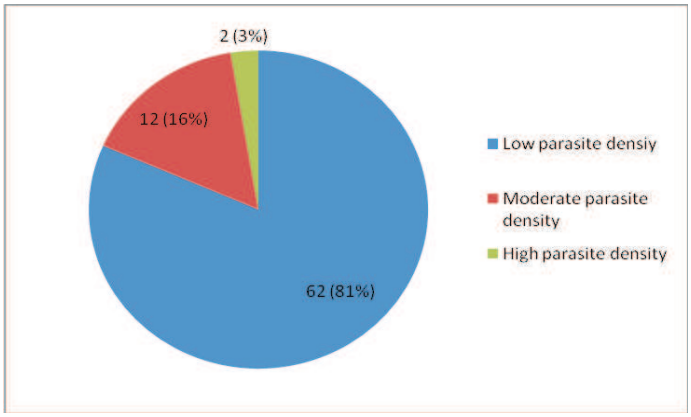

Figure 1: Malaria parasite densiy distribution among 76 neonates with congenital malaria

Table 2
Malaria Parasite Density in Babies Admitted For Neonatal Sepsis according to sex.

	Male (%)	Female (%)	Total %)
No of recruited patients	150(56.4)	116(43.6)	266(100)
Total no with CM	36(47.4)	40(52.6)	76(28.6)
No with only CM	30(46.9)	34(53.1)	64(24.1)
Only CM with low PD	23(44.2)	29(55.8)	52(81.3)
Only CM with moderate PD	3(30.0)	7(70.0)	10(15.6)
Only CM with high PD	2(100.0)	0(0.0)	2(3.1)
No of BCP patients	61(62.9)	36(37.1)	97(36.5)
No of BCP with CM	8(66.6)	4(33.3)	12(12.4)
CM with Low PD and BCP	7(77.7)	2(22.2)	9(75.0)
CM with moderate PD and BCP	1(33.3)	2(66.6)	3(25.0)
CM with high PD and BCP	0(0.0)	0(0.0)	0(0.0)

MP= Malaria Parasite, CM= Congenital Malaria, PD= Parasite Density, BCP= Blood culture positive.
Low= 500 parasite/µ/ of blood.
Moderate=>500- <1,500 parasite/µl of blood.
High=> 1,500-> 3,000 parasite/µl.

Table: 3 *Maternal/Neonatal factors and Malaria Parasite Density in 64 Newborns with Congenital Malaria only.*

Maternal/Neonatal factors	Low (%)	Moderate (%)	High (%)	Total (%)
Peripartum pyrexia	14(56.0)	10(40.0)	1(4.0)	25(30.1)
Rupture of membrane	6(66.7)	33(33.3)	0(0.0)	9(14.1)
Para1	17(77.3)	5(22.7)	0(0.0)	22(34.4)
Para 2	13(1.3)	3(18.8)	0(0.0)	16(25.0)
Para 3	12(80.0)	1(6.7)	2(13.3)	15(23.4)
Para >3	10(0.9)	1(9.1)	0(0.0)	11(17.2)
Birth weight <2,500gms	31(73.8)	9(21.4)	2(4.8)	42(65.6)
Birth weight > 2,500gms	21(95.5)	1(4.5)	0(0.0)	22(34.4)
HIV exposed babies	6(50.0)	4(33.3)	2(16.7)	12(18.8)
Mothers on AM prophylaxis during pregnancy	41(95.3)	2(4.7)	0)0.0)	43(67.2)
SES 1	3(75.0)	1(25.0)	0(0.0)	4(6.3)
SES 11	5(100.0)	0(0.0)	0(0.0)	5(7.8)
SES 111	8(88.9)	1(11.1)	0(0.0)	9(14.1)
SES 1V	16(76.2)	4(19.1)	1(4.8)	21(32.8)
SES V	20(80.0)	4(16.0)	1(4.0)	25(39.1)

Low= 500 parasite/µ/ of blood
Moderate=>500- <1,500 parasite/µl of blood
High=> 1,500-> 3,000 parasite/µl

Table: 4
Frequency of selected maternal and neonatal factors among 64 neonates who had congenital malaria only.

Maternal/Neonatal factors	Low	Moderate	High	Total (%)
Peripartum pyrexia	14	10	1	25(39.1)
Rupture of membrane	6	33	0	9(14.1)
Para1	17	5	0	22(34.4)
Para 2	13	3	0	16(25.0)
Para 3	12	1	2	15(23.4)
Para >3	10	1	0	11(17.2)
Birth weight <2,500gms	31	9	2	42(65.6)
Birth weight > 2,500gms	21	1	0	22(34.4)
HIV exposed babies	6	4	2	12(18.8)
Mothers on AM prophylaxis during pregnancy	41	2	0	43(67.2)
SES 1	3	1	0	4(6.3)
SES 11	5	0	0	5(7.8)
SES 111	8	1	0	9(14.1)
SES 1V	16	4	1	21(32.8)
SES V	20	4	1	25(39.1)

Low= 500 parasite/µ/ of blood
Moderate=>500- <1,500 parasite/µl of blood
High=> 1,500-> 3,000 parasite/µl
SES- Socio-economic class.

Table 5:*Clinical Signs and Symptoms and Malaria Parasite Density in 64 Neonates with Only Congenital Malaria.*

Symptoms	Malaria Parasite Density						
And signs	Low (%)	Moderate (%)	High (%)	Total (%)	Sensitivity %	Specificity %	PPV %
Fever	20(62.5)	10(31.3)	2(6.3)	32(50.0)	50.0	43.5	21.9
Diarrhoea	3(75.0)	1(25.0)	0(0.0)	4(6.2)	6.3	89.6	16.0
Jaundice	19(76.0)	4(16.0)	2(8.0)	25(39.0)	39.0	67.8	38.5
Irritability	2(15.4)	9(69.2)	2(15.4)	13(20.3)	20.3	57.4	13.3
Pallor	6(40.0)	7(46.7)	2(13.3)	15(24.3)	23.4	83.7	31.3
Fast breathing	10(50.0)	8(40.0)	2(10.0)	20(31.3)	39.0	32.2	15.4
Poor feeding	12(70.6)	5(29.4)	2(11.8)	17(26.6)	26.6	24.8	10.1
Hepatomegaly	20(62.5)	10(31.3)	2(6.3)	32(50.0)	81.3	34.2	28.1

Low=500 parasite/µ/ of blood and below
Moderate= >500- <1,500 parasite/µl of blood
High= >1,500- >3,000 parasite/µl

Discussion

The data generated from the study shows that CM is common in newborns with suspected NS (28.6%). This was similar to 35.0% from a similar study in Calabar[13] and 28.2% from another study in hyperendemic area of Jos [12] in the same geographical zone. The finding was however higher than 5.1% reported in a multi-center study in the country,[24] 8.25% from Northern part of the country,[20] and 16.7% from Tanzania,[25] but much lower than 46.7% reported from Ile-Ife.[14] The differences in the reported prevalence rates of CM in different parts of the country and even in the same geographical zones may be attributed to the skill and experience of the laboratory personnel involved in blood film preparation, staining, and reading of the slides, as results of training programmes on malaria microscopy have shown low levels of sensitivity and specificity of those involved in malaria diagnosis routinely and for research,[24] or it could be as a result of increased resistance of *plasmodium falciparium* (PF) to anti-malaria drugs in some parts of the country resulting in increase in maternal parasitaemia.[15] However, all these reports support the growing number of cases of CM in hyperendemic areas.

Early workers found primary attack of CM to be mild, and in most instances confined to a transient asymptomatic parasitaemia.[25,26] In the majority of asymptomatic cases, the parasites were rapidly cleared from the infant's blood from the transplacentally transferred maternal antibodies.[25,26] In support of above statement was the findings that 62.1% of smear positive newborns had remained asymptomatic after three days of delivery in a multi-centered study in the country.[27] In the present study, 81.3% of newborns with CM had low parasite count of 500 parasite/μ/l of blood or less, thus this may represent the transient asymptomatic parasitaemia phase that may be cleared off with time. Only 18.7% presented with moderate and high malaria PD of >500 parasite/μ/l of blood which could be the symptomatic form of CM.

Many risk factors have been identified to increase the risk of CM in newborns, notably among them includes primigravidae, LBW and PPP.[27] Similar risk factors including intrauterine HIV exposure were also found to have increased association with CM (OR = 4.4) in the present study. Peripartum pyrexia which results from maternal infective conditions notably malaria may damage the syncytiotrophoblast during active placental infection. This damage as previously documented will facilitate the entry of MP from the placenta into the fetal circulation, thus increasing the chances of CM in her offspring.[28,29] Primigravidas have also been reported to be at a [f] Greatest risk of malaria in pregnancy because they lack the specific immunity to placental malaria which is acquired from exposure to malaria parasites during pregnancy.[30,31] This immunity accumulates with successive pregnancies, provided there is exposure to malaria infection.[32]

Literature exist between maternal/placental malaria parasitaemia during pregnancy and LBW.[28,29] It is therefore not surprising to find a strong association between malaria parasite and low birth weight, as placental parasitaemia has been reported as a major cause of LBW in malaria endemic areas. Maternal HIV infection has also been found to increases the prevalence and intensity of malaria infections in pregnancy.[33] Both HIV infection and malaria in the mother are well known independent risk factors for both the mother and her baby, and when both co-exist, the risk of damage to the placental syncytiotrophoblast will be increased so also will be there be more chances of entry of MP into the feotal circulation, and hence CM.

Clinical signs of CM may be indistinguishable from those of NS whose main presentation is fever, this has led to the suggestion that screening for MP should be included as part of routine investigation in newborn infants with fever, as Nyirjesy et al [34] has reported increased risk of perinatal deaths (RR=7.2), and low birth weight from CM. In the present study, most blood culture positive neonates and those with moderate and high density parasitaemia in their blood smear presented with fever, thus justifying the recommendation that all newborns with fever should be screened for malaria. There is wide documented similarity in the clinical presentation of these two disease entities and because of the difficulties encountered clinically in differentiating the two especially in hyperendemic malaria areas, the sensitivity, specificity as well as the PPV of common clinical signs and symptoms tested. Though none of the clinical signs and symptoms showed good sensitivity/ specificity and PPV for CM, however, fever, hepatomegaly, jaundice and pallor had fairly varying sensitivity, specificity and PPV for CM. The present study therefore justifies the recommendation that screening for MP be part of septic work up in newborns who presented with fever, especially when there is an associated history of maternal PPP, prematurity or intrauterine HIV exposure.

References

1 Akindele JA. Predisposing factors to neonatal septicaemia; a 4 year review in a special care unit. *Nig J Paediatr* 1988;15:35-9..

2. Airede AI. Neonatal Septicemia in an African city of high altitude. *J Trop Pediatr* 92; 38:189-91. YEAR?

3. Okechukwu AA, Achonwa A. Morbidity and mortality patterns of admissions into the special care baby unity of the University of Abuja Teaching Hospital, Gwagwalada, Nigeria. *Nig J Clin Pract* 2009;12(4):389-3.

4 Bruce-Chwatt LJ. Malaria in African infants and children in southern Nigeria. Ann Trop *Med Parasitol* 1952; 46: 173-75.

5. Covell G. Congenital malaria. *Trop Dis Bulletin* 1950;47:1147-65.

6. McGregor FA. Congenitally acquired malaria. *Postgrad Doctor Afr* 1986;8:52-54.

7. Jellife EF. Low birth weight and malaria infection of the placenta. *Bull World Health Org,* 1968; 38:69-8.

8. McGregor FA, Wilson MME, Billewicz WZ. Malaria infection of of the placenta in the Gambia,West Africa. Its incidence and relationship to still birth, birth weight and placental weight. *Trans R Soc Trop Med Hyg*, 1983; 77:232-44.
9. Ibhanesebor SE. Clinical characteristics of neonatal malaria. *Trop Paediatr, 1995; (16): 330-333.*
10. Sodeinde O, Dawodu AH. Neaonatal transfusional malaria: A growing clinical problem. *Nig J Paediatr, 1985; 12:57-60.*
11. Bergstrom S, Fernandes A, Schwaibach J, Perez O, Miyar, R. Materno-fetal transmission of pregnancy malaria: an immune parasitologically study on 202 patients in *Maputo Gynecol Obstet Invest, 1993;* 35:103-7.
12. Egwunyanga O A, Ajayi J A, Oluyinka A and Popoola D D. Transplacental passage of *Plasmodium faciparum* and Sero evaluation of Newborns in Northern Nigeria. *J Commun Dis 1995;* 27:77-3.
13. Ekanem A D, Anah M U, Udo JJ. The prevalence of congenital malaria among neonates with suspected sepsis in Calabar, Nigeria. *Trop Doct*, 2008; 38:73-6.
14. Obiajunwa P O, Owa J A, Adeodu O. Prevalence of congenital malaria in Ile-Ife, Nigeria. *J Trop Paediatr,* 2005; 51 (4):219-22.
15. Nahlen B I, Akintunde A, Alakija T I. Lack of efficiency of pyrimethamine prophlaxis in pregnant Nigerian women. *Lancet,* 1998; 2: 830-34.
16. Ogala WN. Malaria. In Azubuike JC, Nkangineme KEO. Paediatrics and child health in the tropical region 1st edition. Nigeria. African Educational Services. 1991; 426-437.

1. Please pay close attention to the convention for citing books 2. Is the information not available in the second edition?

17. Mukhtar M. The growing incidence of neonatal malaria- A situational review in developing countries. *Nig J Med*, 2007; 16 (1): 25-30.
18. Shulman C, and Dorman E. Clinical features of malaria in pregnancy. In Warrell D A and Gilles H M (Editors), Essential Malariology, 4th ed. London, BookPower formerly ELST, 2002; pg 223.
19. Larkin G.L, Thuma P.E. Congenital malaria in a hyperendemic area. *Am J Trop Med Hyg* 1991; 45 (5): 587-92.
20. Orogade A A. Noenatal malaria in a mesoendemic malaria area of Northern Nigeria. *Annals of Afr. Med, 2004; 3(4):170-73.*
21. Greenwood, B. M. and Armstrong J. R.M. Comparison of two single methods for determining malaria parasite density. Transactions Royal Soc *Trop Med and Hyg 1991; 85:186-*88.
22. Cheesbrough, M. District Laboratory Practice in Tropical Countries. 2nd Edition. Cambridge University Press,United Kingdom, 2005; pp.244-51.
23. Olusanya O, Okpere E, Ezimokhai M (1985). The importance of social class in voluntary fertility control in developing countries. *West Afr J Med; 4: 20512.*
24. Agomo C O, Oyibo W A, Anorlu R I, Agomo PU. Prevalence of Malaria in Pregnant Women in Lagos, South-West *Nigeria. Korean J Parasitol. 2009; 47(2): 179183.*
25. Thapa B R., Narang A, Bhakoo O N. Neonatal malaria: a clinical study of neonatal and transfusional malaria. *J Trop Paediatr 1987; 33: 266-69.*
26. Fischer P R. Congenital Malaria: an African survey. *Clin Pediatr Phila, 1997; 36(7): 411-3.*
27. Falade C, Mokuolu O, Okafor H, Orogade A, Falade A, Adedoyin O et al. Epidemiology of congenital malaria in Nigeria: a multi-centre study. *Trop Med & Int Hlth, 2007; 12 (11): 1279 87.*
28. Marsh K. Immunogy of human malaria. In : Gilles HM and Warrel DA (editors) 4th edition. Essentials malariology. London: Edward Arnold (Publishers) Ltd, 1993; 60-77.
29. Akum A E, A Kuoh J A, Minang J T, Achimbom B M , Ahmadou J M, Troye-Blomberg M. The effect of maternal, umbilical cord and placental malaria parasitaemia on the birthweight of newborns from South-western Cameroon. *Acta Pædiatrica,2005; 94 (7): 917-23.*
30. Staalsoe T, Shulman CE, Buhner JN, Kawuondo K, Marsh K, Hviid L. Variant surface antigen-specific IgG and protection against clinical consequences of pregnancy-associated Plasmodium falciparum malaria. *Lancet. 2004;363:283-289.*

31. Elliott SR, Brennan AK, Beeson JG, Tadesse E, Molyneux ME, Brown GV, Rogerson SJ. Placental malaria induces variant-specific antibodies of the cytophilic subtypes immunoglobulin G1 (IgG1) and IgG3 that correlate with adhesion inhibitory activity. *Infect Immun. 2005;73:5903-5907.*

32. Beeson JG, Duffy PE. The immunology and pathogenesis of malaria during pregnancy. *Curr Top Microbiol Immunol. 2005;297:187-227.*

33. Steketee R.W., Wirima J.J., Bloland P.B. Impairment of a pregnant woman's acquired ability to limit plasmodium falciparium by infection with human immunodeficiency virus type-1. *Am J Trop Med Hyg 1996a ;55 (1):42-9.*

34. Nyirjesy P, Kasasiya T, Axelord P. Malaria during pregnancy; Neonatal morbidity and mortality and the efficacy of chloroquine chemoprophylaxis. *Clin Infec Dis 1993; 16 (1):127-32.*