Prevalence of malaria parasite infection in pregnant women in three towns of the South West Region of Cameroon

ASOBA G. N., NDAMUKONG K. J.N. and ACHIDI E. A.
Faculty of Science, University of Buea, P.O. 63, Buea, Cameroon

ABSTRACT

The study was designed to investigate the prevalence and density of *Plasmodium* infection in 206 pregnant Cameroonian women and to assess their anaemic status. Blood samples were collected from the women during three consecutive antenatal clinical visits and examined for the detection and quantification of malaria parasites and determination of packed cell volume. The results revealed that the prevalence of malaria parasitaemia was 43.2% on the first antenatal visit, 25.2% on the second visit and 6.8% on the third visit. Significantly more primigravidae (54.5%) than multigravidae (32.7%), and more single women (55.3%) than married women (31.1%) were positive for malaria parasitaemia on the first visit ($P<0.001$). The infection rates varied significantly ($P<0.02$) with the location of the clinics, the highest prevalence being recorded in Muea. The prevalence decreased progressively during subsequent antenatal visits, irrespective of gravidity status, marital status or clinic of attendance. Significantly more younger women (= or < 20years) were positive for malaria parasitaemia than older women (> 20years) on the first and second visits ($P<0.001$). Geometric mean parasite density was higher in primigravidae and single women than in multigravidae and married women, but the difference was significant only between single and married women ($P<0.02$). The prevalence of anaemia (PVC<31%) was 53.4% on the first antenatal visit, 50.0% on the second and 28.2% on the third antenatal visit. The prevalence was higher among primigravidae, single women and women less than or equal to 20years of age than in multigravidae, married women and women above 20years of age. *Plasmodium falciparum* was the only species observed in this study

Key words: *Plasmodium,* parasitaemia, anaemia, packed cell volume, antenatal, primigravidae, multigravidae

RESUME

Le présent projet a pour objet d'étude la prévalence, la densité de l'infection au *Plasmodium* chez 206 femmes camerounaises en gestation, et l'évaluation de leur degré d'anémie. Des échantillons de sang ont été prélevés chez les sujets lors de trois visites prénatales consécutives et analysés en vue de la détection et de la quantification des parasites vecteurs du paludisme ainsi que l'évaluation de la valeur hématocrite. Les résultats obtenus ont révélé une prévalence de parasites vecteurs du paludisme de 43,2% lors de la première visite prénatale, de 25,2% lors de la deuxième et de 6,8% lors de la troisième. Les primipares (54, 5%) et les femmes célibataires (55,3%) se sont revelées beaucoup plus infectées lors de la première visite anténatale ($P<0,001$) que les multipares (32,7%) et les femmes mariées (31.1%). Le taux d'infection variait beaucoup ($P<0,02$) d'un Centre de Santé à l'autre; le taux de prévalence le plus important fut enregistré à Muea. Pendant les visites subséquentes à la première, le taux de prévalence diminuait progressivement, indépendamment de l'état de la grossesse, du statut ou de la clinique visitée. Les examens de laboratoire faits lors de la première et de la deuxième visites prénatales ont montré que les très jeunes femmes (20 ans ou moins) sont plus victimes de la parasitémie du paludisme que celles qui sont plus agées. La quantité de parasites était plus importante chez les primipares célibataires que chez les multipares mariées. Toutefois, la plus grande différence fut détectée lorsque l'on passait des femmes célibataires aux femmes mariées ($P<0,02$). La prévalence de l'anémie (PVC<31%) quant à elle, était de 53% lors de la première visite prénatale, de 50% lors de la deuxième visite, et de 28,2% lors de la troisième. Au sein de cette population exposée, il s'est avéré que les primipares, les célibataires et les agées de 20 ans ou moins preséntent les taux de prévalence les plus élevés comparativement à leurs congénères respectivement multipares, mariées ou plus agées. Le *Plasmodium falcipanum* fut la seule espèce découverte lors de cette investigation.

Mots clés: *Plasmodium*, parasitémie, anémie, valeur hématocrite, prénatal, primipare, multipare

INTRODUCTION

In sub-Saharan Africa, up to 24 million pregnant women may become infected with *Plasmodium* each year (Bundy *et al.*, 1995). Malaria in pregnancy is a major cause of maternal, foetal or offspring morbidity and mortality (Brabin, 1991; Mutabingwa, 1994). In malaria endemic areas, pregnant women, particularly primigravidae, are more susceptible to malaria, with higher frequency, density of parasitaemia, incidence of clinical malaria attack (McGregor, 1987) and a risk of chronic effects on the mother and the foetus such as anaemia and foetal growth retardation (Harrison, 1985). The high infection rate is often related to an increase in susceptibility of pregnant women to infections because pregnancy is a time of high hormone activity, which may exert immuno-suppressive effects (Beer & Billingham, 1978; McGregor *et al*., 1983) on the childbearing women.

Various factors interact to cause anaemia in pregnancy in tropical countries (Fleming, 1989). Considerable emphasis is generally given to deficiencies in nutritional factors (Brabin *et al.*, 2003) while the importance and contributing role of malaria in pregnancy has not yet been fully investigated. Randomized control trials have shown that effective antimalaria interventions can significantly reduce severe maternal anaemia (Shulman *et al.*, 1999). Studies in Fako Division of Cameroon (Achidi *et al.*, 2005) demonstrated that anaemia was prevalent in 69% of pregnant women, and 50% of these anaemic cases were attributed to malaria parasitaemia. A better understanding of the causes of anaemia will help reduce its incidence in pregnancy and improve on pregnancy outcome.

This study was designed to investigate the prevalence and density of *Plasmodium* infection in pregnant Cameroonian women in Fako division and assess their anaemic status.

MATERIALS AND METHODS

Study area

The study was carried out between August 2006 and August 2007 in Muea Integrated Health Centre, Buea Health Centre and Mutengene Integrated Health Centre, situated at the foot of Mount Cameroon in the South West of Cameroon. These health centres provide basic health care services, including immunization of children, antenatal surveillance, delivery services and family planning. Muea, Buea and Mutengene experience two seasons: the dry season (November to March) and rainy season (April to October). Mutengene is located at an altitude of 250m above sea level, Muea at 650m, while Buea has a patient catchment area spanning from 650m to 1096m above sea level.The poor environmental hygienic conditions existing in Muea and Mutengene, both semi-urban areas, expose the inhabitants to a high risk of malaria infection. Buea, on the other hand, is a regional capital with relatively improved hygienic conditions.

Study population

Women who came for antenatal clinic were recruited into the study on the day of their registration at the clinic. Informed consent to participate in the study was obtained from each participant. Ethical clearance was obtained from the Regional Delegation of Public Health for the South West. A total of 206 pregnant women participated in the study: 73 of the women attended clinic in Buea, 73 in Mutengene and 60 in Muea. Fifty-one of them were less than or equal to 20 years of age, while 155 were above 20years of age. Ninety-nine of them were primigravidae while 107 were multigravidae. The residents of Mutengene and Muea are predominantly peasant farmers and small scale traders while those of Buea are mainly civil servants and traders.

Control measures put in by the Government of Cameroon.

In compliance with Government health policy, all pregnant women who enrolled at these health centres were given iron, folate and fansidar on the day of antenatal enrolment and during subsequent antenatal visits. Fansidar was to be taken at the 16th week of gestation and repeated after 2months as chemoprophylaxis against malaria. The iron tablets and folate were to be taken daily during the whole period of pregnancy as safeguard against anaemia.

Study design

This was a longitudinal prospective study in which each of the subjects at the three health centres was followed from the day of registration in the health centre up to the third antenatal visit. The women reported at the health centres following their appointment dates given by the midwives. Prior to sample collection during each visit, a case report form was completed on each subject to obtain information on age, clinical symptoms of malaria, parity, gravidity status and axilla temperature. This was followed by collection of capillary blood for malaria parasite investigation and determination of packed cell volume.

Sample collection and processing

Some 3-5ml of blood was collected from each subject by venopuncture, using a sterile disposable syringe, and dispensed into an anticoagulant (EDTA) tube. A portion of the blood from the syringe was put into a heparinized capillary tube for the determination of packed cell volume (PCV) by microhaematocrit centrifugation as a measure of the degree of anaemia. The rest of the blood was used for white blood cell (WBC) count and preparation of Giemsa-stained thick and thin blood films for malaria parasite investigation. Parasite density was estimated by counting trophozoites concomitantly with white blood cells in each field. The number of trophozoites per 200 white blood cells was then multiplied by the average WBC count per microlitre of maternal blood (Larkin & Thuma, 1991).

Data analysis

The data was analyzed in relation to gravidity status, location of clinic, maternal age, marital status and axilla temperature. The Student's t test or analysis of variance was used as appropriate to compare the mean malaria parasitaemia while the chi-square test or Fisher's exact test was used to compare the prevalence of parasitaemia and anaemia in the different groups. A log transformation of parasitaemia was done to ensure a normal distribution.

RESULTS

Symptomatic status of the study population

Of the 206 pregnant women examined, 69.8% of them manifested malaria-related symptoms (headache, vomiting, fever and chill) at enrolment. Some 61.7%

Table 1: Effect of gravidity status, marital status and clinic attended on malaria parasite rates and densities in pregnant women.

Period	Description	Number examined	MPR (%)	Level of significance	MPD (GM per μl of blood)	Level of significance
1st Visit	**Gravidity**					
	Primigravidae	99	54.5	X^2 =9.99	253	t = 1.60
	Multigravidae	107	32.7	P = 0.002	187	P = 0.114
	Marital status					
	Married	103	31.1	X^2 = 12.36	171	t =2.35
	Single	103	55.3	P < 0.001	265	P = 0.021
	Antenatal Clinic					
	Buea	73	38.4	X^2 = 8.01	300	F = 3.77
	Muea	60	58.3	P = 0.018	238	P= 0.027
	Mutengene	73	35.6		156	
2nd Visit	**Gravidity**					
	Primigravidae	99	38.4	X^2 = 1.66	304	t =0.17
	Multigravidae	107	21.5	P = 0.198	318	P = 0.87
	Marital Status					
	Married	103	28.2	X^2 = 0.93	263	t = 1.42
	Single	103	22.3	P = 0.336	381	P = 0.163
	Antenatal Clinic					
	Buea	73	15.1	X^2 = 9.46	367	F = 4.44
	Muea	60	23.3	P = 0.009	518	P = 0.017
	Mutengene	73	37.0		221	
3rd Visit	**Gravidity**					
	Primigravidae	99	8.1	X^2 = 0.50	458	U = 18.50
	Multigravidae	107	5.6	P = 0.48	250	P = 0.47
	Marital Status					
	Married	103	7.8	X^2 = 0.31	231	U = 1200
	Single	103	5.8	P = 0.58	552	P = 0.12
	Antenatal Clinic					
	Buea	73	5.5	X^2 = 3.33	523	X^2 = 1.84
	Muea	60	11.0	P = 0.189	418	P = 0.40
	Mutengene	73	3.3		279	

NB: MPR = Malaria Parasite Rate GM = Geometric Mean
MPD = Malaria Parasite Density

of them were positive for fever/chill, 49.5% were positive for headache, and 51.0% were positive for vomiting.

Prevalence of malaria parasitaemia

The prevalence of patent parasitaemia was 43.2% (89/206) on the first antenatal visit, 25.2% (52/206) on the second visit and 6.8% (14/206) on the third visit (Table 1). *Plasmodium falciparum* was the only species observed in this study. .Significantly more primigravidae (54.5%) and single women (55.3%) than multigravidae (32.7%) and married women (31.1%) were found to be infected during the first antenatal visit (P<0.002 and P<0.001 respectively). The prevalence rate varied significantly (P<0.02) with the location of antenatal clinic, with the highest prevalence (58.3%) recorded in Muea.

On the second antenatal visit, infection rates with respect to gravidity status or marital status were not significantly different. Infection rates in the different locations of antenatal clinics were again significantly different (P<0.01), being highest in Mutengene (37%), followed by Muea (23.3%) and least in Buea (15.1%). On the third antenatal visit, there were no differences in infection rates between women of different gravidity status, marital status and location of antenatal clinic.

Malaria prevalence in pregnant women as affected by maternal age is shown in Table 2. The results revealed that the prevalence was higher in the younger pregnant women (=or< 20 years old) than in the older pregnant women (>20 years old), this being very significant during the first and second visits (P<0.001).

During the first antenatal visit, 60.9% of the women with axilla temperature $\geq 37.5^0$C (febrile) harboured malaria parasites while 38.1% of those who had axilla temperature $<37.5^0$C (afebrile) had patent parasitaemia (Table 2). The difference was very significant (P<0.001). There was a drop in the number of febrile cases with each subsequent antenatal visit, the third visit having the least number of febrile women with malaria parasites.

Overall, prevalence of malaria parasitaemia decreased progressively during the antenatal visits, irrespectively of gravidity status, maternal age, marital status or clinic of attendance.

Malaria parasite density.

The distribution of parasite density with respect to gravidity status, marital status and clinic attended is also shown in Table 1. Geometric mean parasite density was higher in primigravidae during the first visit but lower during the second visit compared to multigravidae, although the differences were not significant. The density was higher in single women than married women during the first two antenatal visits, the difference being significant only during the first visit (P<0.02). Women attending clinic in Muea had a significantly higher parasite density than those attending

Table 2: Malaria parasite rate in pregnant women in relation to maternal age and axilla temperature.

Description	1st Visit		2nd Visit		3rd Visit	
	No. examined	No.(%) infected	No. examined	No.(%) infected	No. examined	No.(%) infected
Age:						
<or=20yrs	51	50(98.0)	51	35(68.6)	51	9(17.6)
>20yrs	155	39(25.2)	155	17(11.0)	155	5(3.2)
Level of significance		X^2=9.11 P=0.001		X^2=10.12 P=0.001		X^2=0.97 P=0.32
Axilla temp:						
Febrile:($\geq 37.5^0$C)	46	28(60.9)	45	12(26.7)	42	8(19.0)
Afebrile: ($<37.5^0$C)	160	61(38.1)	161	40(24.8)	164	6(3.7)
Level of significance		X^2=7.53 P=0.006		X^2=0.03 P=0.862		P^{Fisher}=0.022

clinic in Buea and Mutengene during the first two antenatal visits (P<0.02). On the third antenatal visit, the differences in parasite density between women of different gravidity status, marital status and location of antenatal clinic were not significant.

Overall, malaria parasite density was found to be increasing at each antenatal visit, with the highest density registered on the third clinical visit in women of different gravidity status, marital status and places of antenatal clinical visits. However, the number of women carrying these infections decreased with each proceeding antenatal visit.

Anaemia

The prevalence of anaemia (PCV<31%) was 53.4% on the first antenatal visit, 50.0% on the second and 28.2% on the third antenatal visit. The prevalence was higher in younger women (= or< 20years of age) than in older women (> 20years of age) during the first visit (P<0.003). Significantly more primigravidae than multigravidae were anaemic on the first and second antenatal visits (P<0.003 and P<0.01 respectively) (Table 3). There were more anaemic single women than married women on the first and second antenatal visits, but the difference was not significant. There were significantly more anaemic cases among women attending clinic in Mutengene than in Muea and Buea (P<0.05).

Effects of malaria parasitaemia on anaemia

During the first antenatal visit, 58.4% (52/89) of the women who harboured malaria parasites were anaemic, with the highest geometric mean parasite density recorded in anaemic women (Table 4). The prevalence of anaemia decreased slightly on the second antenatal visit, but significantly on the third visit (P<0.005). The difference in the mean parasite density of anaemic and non-anaemic women during the second and third visits was not significant. However, significantly more anaemic women than non-anaemic women were still harbouring malaria parasites on the third visit (P<0.005). In both anaemic and non-anaemic women, the malaria parasite density increased with each antenatal visit while the prevalence of infection decreased.

DISCUSSION

A trend of decreasing malaria parasite rates in the course of pregnancy was observed in this study. The pregnant women enrolled in the study all received fansidar as chemoprophylaxis against malaria infection. Previous studies suggested that the second trimester is the time of maximum risk of malaria infection during gestation, with highest parasite prevalence and parasite density (Diagne *et al*., 2000; Saute *et al*., 2002). The results obtained in the present study indicate that although pregnant women are at high risk throughout their gestation period, the chemoprophylaxis given to them at the 16th week and repeated at 24th week of gestation

Table 3: Effect of gravidity status, marital status and clinic attended on anaemia in pregnant women.

Description	Number examined	Anaemia [n (%)]		
		1st visit	2nd visit	3rd visit
Gravidity				
Primigravidae	99	64 (64.6)	56 (62.9)	27 (40.3)
Multigravidae	107	46 (43.8)	47 (45.6)	31 (40.8)
Level of Significance		X^2=8.90 P= 0.003	X^2 = 5.74 P=0.01	X^2 = 0.004 P = 0.95
Marital status				
Married	103	48 (47.5)	53 (45.7)	34 (44.7)
Single	103	62 (60.2)	56 (54.3)	24 (35.8)
Level of significance		X^2 = 3.29 P = 0.07	X^2 = 0.04 P = 0.852	X^2 = 1.17 P = 0.279
Antenatal Clinic				
Buea	73	34 (47.2)	24 (34.3)	13 (20.6)
Mutengene	73	47 (65.3)	47 (67.1)	36 (66.7)
Muea	60	29 (48.3)	32 (61.5)	9 (34.6)
Level of significance		X^2 = 5.79 P = 0.001	X^2 16.98 P =0.001	X^2 = 26.02 P=0.001

Table 4: Effect of malaria parasitaemia on anaemia

Period	No. examined	Anaemic Women (PCV < 31)			Non-anaemic Women (PCV ≥ 31)		
		%	MPR (%)	MPD (per µl)	%	MPR (%)	MPD (per µl)
1st Visit	206	53.4	25.2	231	46.6	18.0	218
2nd Visit	206	50.0	19.4	309	50.0	05.8	328
3rd Visit	206	28.2	04.9	377	71.8	01.9	349

NB: **PCV**= Packed Cell Volume
MPR= Malaria Parasite Rate
MPD=Malaria Parasite Density

may have been responsible for the decrease in prevalence recorded on the subsequent visits.

Malaria parasite rates and densities tended to decrease with increasing parity in this study, but no significant association was observed between parity and parasite density. Previous studies (Steketee *et al.*, 1996) indicated that nulliparous women are at high risk of being parasitaemic. The lack of association between parity and parasite density confirms results of previous studies (Schleiemacher *et al.*, 2001; Saute *et al*., 2002).

Overall, malaria rate and density decreased with increasing maternal age (>20years), gravidity, parity and gravidity age. This does not only reinforce the suggestion that the ability to control malaria parasitaemia is parity and/or age dependent, but further indicates a possible role of gravidity age and gravidity status in the control of malaria in pregnancy. In a study carried out in the Centre region of Cameroon (Zhou *et al.*, 2002), age was identified as a major risk factor because women less than or equal to 20 years old were 1.8 and 3.4 times more likely to have malaria infection than women greater than 20 years old.

The prevalence of anaemia was 53.4% on the first antenatal visit, 50.0% on the second and 28.2% on the third visit. Among the anaemic women, 53.7%, 1.0% and 0.0% had mild, moderate and severe anaemia respectively. This indicates that the majority of anaemic cases in the community were mild to moderate. There was a significant difference in prevalence of anaemia between primigravidae and multigravidae, being higher in the former; this agrees with previous studies by Achidi *et al.* (2005) who showed that primigravidae had higher incidence of anaemia and lower mean haemoglobin levels than multigravidae. The progressive decrease in prevalence of malaria infection and level of anaemia with gestation age may be attributed to the iron tablets and folate which the pregnant women were taking to prevent anaemia.

Malaria parasite density increased with each antenatal visit; meanwhile the prevalence decreased in both anaemic and non-anaemic women. It is possible that the few women who still harboured infections were probably those using sub therapeutic doses of the drugs or who failed to complete a treatment regime.

From the results obtained in the study, it may be concluded that peripheral blood parasitaemia was detected in 43.2% of the samples studied. The ability to control malaria parasitaemia depends on parity, gravidity status, marital status and age of the pregnant woman. Primigravidae were more vulnerable to malaria than multigravidae. Chemoprophylaxis given to the women during antenatal visits appeared to play a significant role in the control of malaria.

ACKNOWLEDGMENTS

This study was funded by the African Renaissance Ambassador (ARA) Corp. We are indebted to the administration and staff of the health centres where the study was carried out for their assistance and collaboration.

REFERENCES

Achidi, E.A., Kuoh, A.J., Minang, J.T., Ngum, B., Achibom, B.M, Motane, S.C., Ahmadou, M.J. & Troye-Blomberg, M. (2005). Malaria infection in

pregnancy and its effects on haemoglobin levels in women from a malaria-endemic area of Fako Division, South West Province, Cameroon. Journal of Obstetrics and Gynecology, 25(3): 235-240.

Beer, A.E. and Billingham, R.E. (1978). Maternal immunological recognition during pregnancy. Symposium on maternal recognition of pregnancy. Foundation New series, pp 292-322.

Brabin, B.J., Fletcher, K.A. & Brown, N. (2003). Do disturbances within the folate pathway contribute to low birthweight in malaria? Trends in Parasitology, 19:39-43.

Brabin, B.J. (1991). The risks and severity of malaria in pregnant women. In: Applied Field Research in Malaria Reports No.1. UNDP/ World Bank/ WHO special Programme for Research and Training in Tropical Diseases (TDR). Geneva, Switzerland.

Bundy, D.A., Chan, M.S. & Savioli, L. (1995). Hookworm infection in pregnancy. Transactions of the Royal Society of Tropical Medicine and Hygiene, 89(2): 521-522.

Diagne, N., Rogier, C., Sokhna, C.S. et al. (2000). Increased susceptibility to malaria during the early postpartum period. New England Journal of Medicine, 343: 595.

Fleming, A.F. (1989). Tropical Obstetrics and gynaecology. I. Anaemia in pregnancy in tropical Africa. Transactions of the Royal Society of Tropical Medicine and Hygiene, 83:441-448.

Harrison, K.A. (1985). Child-bearing, health and social priorities: a survey of 22,774 consecutive hospital births in Zaria, Northern Nigeria. British Journal of Obstetrics and Gynaecology, 92 (suppl 5): 1-19.

Larkin, G.L. and Thuma, P.E. (1991). Congenital malaria in a hyperendemic area. American Journal of Tropical Medicine and Hygiene. 45:587-592.

McGregor, I.A. (1987). The significance of parasitic infections in terms of clinical disease: a personal view. Parasitology, 94: S159-S178.

McGregor, I.A., Wilson, M.E. and Billewiez, W. (1983). Malaria infection of the placenta in the Gambia,West Africa, its incidence and relationship to stillbirth, birth weight and placenta weights. Transactions of the Royal Society of Tropical Medicine and Hygiene, 77: 232-244.

Mutabingwa, T.K. (1994). Malaria and pregnancy: epidemiology, pathophysiology and control options. Acta Tropica, 57. 239-254.

Saute, F., Menender, C., Mayor, A., Aponte, J., Gomez Olive, X., Dgedge, M. & Alanso, P. (2002). Malaria in pregnancy in rural Mozambique: the role of parity in submicroscopic and multiple Plasmodium falciparum infections. Tropical Medicine and International Health, 7(1): 19-28.

Shulman, C.E., Dorman, E.K. & Cutts, F. (1999). Intermittent Sulfadoxine-pyrimethamine to prevent sever. Lancet, 353: 632-636.

Schleiermacher, D., Roger, C. & Spiegel, A. et al. (2001). Increased multiplicity of year and infections and skewed distribution of individual MSP1 and MSP2 alleles during pregnancy in Ndiop, a Senegalese village with seasonal mesoendemic malaria. American Journal of Tropical Medicine and Hygiene, 64: 303-309.

Steketee, R.W., Wirima, J.J., Slutsker, L., Noteman, J.G. & Heymann, D.L. (1996). Comparability of treatment groups and risk factors for parasitaemia at the first antenatal clinic visit in a study of malaria treatment and prevention in pregnancy in rural Malawi. American Journal of Tropical Medicine and Hygiene, 55: S17.

Zhou, A., Megnekou, R., Leke, R., Fogako, J., Metenou, S., Trock, B., Taylor, D.W. & Leke, R.F. (2002). Prevalence of *Plasmodium falciparum* infection in pregnant Cameroonian women. American Journal of Tropical Medicine and Hygiene, 67 (6): 566.

Recieved: 15/08/2008

Accpeted: 03/12/08