Tanzania Journal of Health Research
Volume 20, Number 2, April 2018
Doi: http://dx.doi.org/10.4314/thrb.v20i2.4

Impact of asymptomatic *Plasmodium falciparum* on haematological parameters of pregnant women at first antenatal visit in South-western Nigeria

OLUWASOLA O. OBEBE[1*], OLUFARATI O. FALOHUN[1], OLAITAN O. OLAJUYIGBE[2], MIKE A. LAWANI[3] and OLUBUNMI A. AJAYI [4]
[1]*Department of Veterinary Parasitology, University of Ibadan, Nigeria*
[2]*Nigeria Institute for Oceanography and Marine Research, Victoria Island, Lagos, Nigeria*
[3]*Centers for Control and Prevention of Zoonoses, University of Ibadan, Nigeria*
[4]*Department of Haematology, College of Medicine, University of Ibadan, Nigeria*

Abstract
Background: Pregnant women in malaria-endemic areas are at high risk of *Plasmodium falciparum* infection and its complications. This study investigated the impact of asymptomatic *P. falciparum* on haematological parameters of pregnant women at first antenatal visit in south-western Nigeria.
Methods: Hospital-based cross-sectional evaluation of 130 asymptomatic pregnant women was conducted. *Plasmodium* infection was diagnosed using Giemsa-stained blood smear microscopy and rapid diagnostic test, while haemoglobin levels (Hb), Packed Cell Volume (PCV), white blood cells (WBC) count, red blood cell (RBC), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) were determined using semi-automated haematology analyser.
Results: Multigravid pregnant women presented more during their second trimester. Overall malaria prevalence in the sampled population was 14.6%. Prevalence of malaria parasitaemia was highest (68.4%) in the second trimester. *P. falciparum* infected pregnant women had significantly lower mean values of packed cell volume (PCV), haematocrit (Hb), and total white blood cell (WBC) compared to non-infected individuals ($t = -4.07$, $p = 0.001$; $t = 3.30$, $p = <0.001$; $t = -2.77$, $p <0.001$).
Conclusion: There is asymptomatic *P. falciparum* infection in the study area and this may form a reservoir for transmission**.** Pregnant women infected with malaria parasites exhibited important changes in haematocrit level, haemoglobin concentration, and total white blood cells. Use of anti-malaria therapy and insecticide-treated bed net would result in greater haematological benefits.

Keywords: asymptomatic, malaria, *Plasmodium falciparum,* haematological parameters, pregnant women, Nigeria

Introduction

Malaria is one of the most severe global public health problems worldwide, particularly in Africa, where Nigeria suffers the highest malaria burden, with approximately 30% of the total malaria burden in Africa (WHO, 2014). Malaria is transmitted throughout Nigeria, (approximately 97% of the population at risk) with children and pregnant women having the highest morbidity (NFMOH, 2009; WHO, 2014). In Nigeria, malaria accounts for 60 % of outpatient visits to hospitals and lead to approximately 11% maternal mortality and 30 % child mortality, especially among children less than 5 years (WHO, 2014).

Malaria in pregnancy is a known cause of maternal anaemia (Guyatt & Snow, 2001). Massive sequestration of *Plasmodium falciparum* parasites in the placenta, with or without detectable parasites in the peripheral circulation, is a distinct feature of pregnancy-associated malaria (Fried & Duffy, 1996), and is assumed to be responsible for an increased risk of adverse pregnancy outcomes such as low birth weight, miscarriage, foetal distress, congenital malaria and stillbirths (Sullivan *et al*., 1999; Verhoeff *et al*., 1999; Rogerson *et al*., 2007). In addition, asymptomatic carriers

* Correspondence Email: olashol@yahoo.com

 Doi: http://dx.doi.org/10.4314/thrb.v20i2.4

can serve as silent reservoirs of gametocyte for the continuous spread of malaria parasites (Alves *et al*., 2005; Cucunuba *et al*., 2013).

Haematological changes are important features in *P. falciparum* infection in pregnant women and they play important role in malaria pathology. These abnormalities involve the major cell lines such as red blood cells, leucocytes and thrombocytes (Maina *et al*., 2010). This results in anaemia, thrombocytopenia, and leucocytosis or leucopenia in patients with malaria (Maina *et al*., 2010; Ladhani *et al*., 2002). Haematological changes in malaria, such as anaemia, thrombocytopenia and leucocytosis or leukopenia are well recognized. However, there is need to determine the impact of *P. falciparum* infection on various blood indices in pregnancy in our setting. Determining the haematological changes in our setting may provide support for pregnant women to be screened for asymptomatic *Plasmodium* infection and be treated promptly via the antenatal care services. The objective of the present study was to determine the impact of asymptomatic *P. falciparum* on haematological changes of pregnant women at first antenatal visit in south-western Nigeria.

Materials and Methods

Study area and design

The study was carried out at Oluyoro Catholic Hospital, Oluyoro, Oke-Ofa located in Ibadan, Oyo State, Nigeria. Oluyoro Catholic hospital is a private secondary healthcare facility offering antenatal care, preventive and curative services at affordable costs for the middle and low- income population. The health facility is highly accessible, facilitating the utilisation of antenatal services. This cross-sectional hospital based study was conducted among asymptomatic pregnant women. All asymptomatic pregnant women with axillary temperature ≤ 37.5^{o} C, who reported for first antenatal clinic visit on scheduled clinic days (Wednesdays and Fridays) and who gave their consent to participate in the study were enrolled. Those who refused consent, with symptomatic infection (fever, chills, rigour, nausea, vomiting, and headache and axillary temperature above > 37.5) and those with any prior evidence of antimalarial treatment were excluded from the study.

Using a prevalence rate of 8.4% of malaria parasitaemia at booking from a study in Ibadan (Falade *et al*., 2008), the precision of 5% and standard normal deviate of 1.96 at 95% confidence intervals was used. In consideration of non-response rate of 10%, a total of 130 pregnant women took part in the study at their first antenatal booking.

Data collection

Each study participant had a complete medical examination including the axillary temperature, height and weight measurements as well as blood pressure by trained midwives who work with the hospital. Venous blood samples were also collected by trained Medical Laboratory Scientist according to standard procedures (Ibhanesebhor *et al*., 1996; Okocha *et al*., 2005; Epidi *et al*., 2008). Giemsa-stained blood smear microscopy and rapid diagnostic tests were employed for the diagnosis of asymptomatic malaria parasitaemia. Drops of whole blood were applied to grease free slides for the preparation of thin and thick smears for detection of *P. falciparum* as described by Cheesbrough (1998). Rapid diagnostic tests (RDTs) were performed according to manufacturer instruction. RDTs used in this study were SD BIOLINE, standard diagnostic, Inc., Korea and CareStart, Access Bio, Inc., USA. This test is one step, rapid, and qualitative test for the detection of Histidine-rich protein II (HRP-II) specific to *P. falciparum* in human blood.

A complete blood count was ran using BC-2800 Haematology Analyser (Mindray, China) following the manufacturer's instructions, that automatically generated values for Packed Cell Volume (PCV), Haemoglobin (Hb) levels, white blood cell (WBCs), red blood cell (RBC), mean corpuscular volume (MCV), mean corpuscular haemoglobin concentration (MCHC), as well as platelets count.

Doi: http://dx.doi.org/10.4314/thrb.v20i2.4

Doi: http://dx.doi.org/10.4314/thrb.v20i2.4

Data analysis
Data entry and analysis were carried out using SPSS version 21.0. Frequency tables and percentages were used to display categorical data. Means ± SD was compared using independent sample t-test. Statistical significance was accepted at $p<0.05$.

Ethical considerations
Ethical clearance (No: OCH/EC/16/05) was obtained from the ethical review committee of Oluyoro Catholic Hospital. Written informed consent was also obtained before enrolment into the study.

Results

Socio-demographic characteristic of the pregnant women
A total of 130 pregnant women participated in the study. The mean age ± standard deviation of the pregnant women was 30.40±4.7 years (range = 19-42 years). The pattern of first registration of the pregnant women showed that majority of them belonging to all gravidies presented at the second trimester. However, multigravid pregnant women (36.9%) presented more during their second trimester, 16.9% during the first trimester and 10.0% during the third trimester.

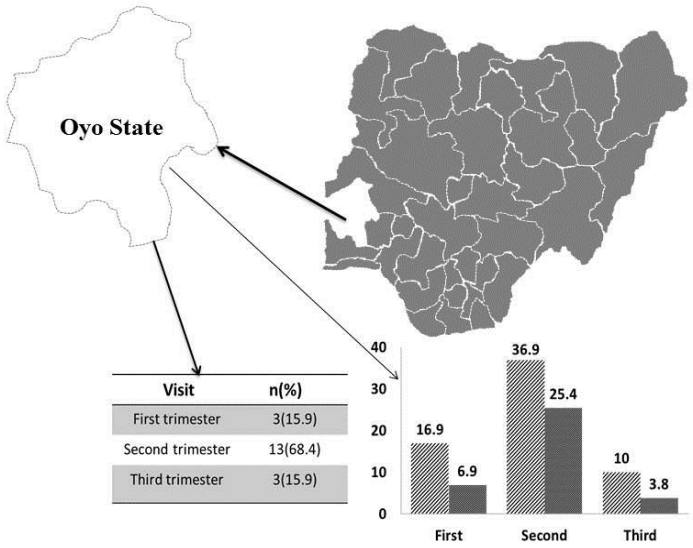

Fig 1: Prevalence of asymptomatic *P. falciparum* and pattern of first registration among pregnant women

Prevalence of malaria
Overall malaria prevalence in the sampled population was 14.6%. Twelve women (9.2%) had *P. falciparum* infection by direct microscopy while 17 (13.1%) were *P. falciparum* positive with the qualitative rapid diagnostic test. Prevalence of malaria parasitaemia was highest (68.4%) in the second trimester (Figure 1).

Anthropometric characteristic of pregnant women
The mean height, weight and body mass index (BMI) were found to be 1.61 ±.060m, 72.16 ±16.3kg, 27.92 ±5.79m^2, respectively (Table 1). The mean values of all the haematological parameters were found to be within the reference values expected in pregnancy (Table 2).

Doi: http://dx.doi.org/10.4314/thrb.v20i2.4

Table 1: Ranges and means of anthropometric and clinical values of pregnant women

Variables	Range	Mean ±SD
Age (yrs.)	19-42	30.4 (±4.7)
Temperature (°C)	35-37.6	36.1 (±.62)
Weight (kg)	42-132	72.2 (±16.3)
Height (m)	1.40-1.87	1.6 (±.060)
BMI (kg/m^2)	17.7-45.4	27.9 (±5.79)
Systolic (mm Hg)	80-150	106.4 (±12.17)
Diastolic (mm Hg)	50-90	64.8 (±8.30)

Table 2 Ranges and means of haematological parameters of pregnant women

Variables	Range	Mean ±SD
Haemoglobin (g/dl)	7.5-12.7	10.2 (±.93)
Packed Cell Volume (%)	22-42	33.6 (±3.22)
White blood cell (10^3/µL)	2.5-12.9	7.5 (±1.66)
Neutrophil (%)	29-83	63.9 (±8.56)
Lymphocyte (%)	12-62	28.2 (±7.861)
Monocyte (%)	3-11	7.3 (±1.58)
Eosinophil (%)	0-6	0.01 (±1.13)
Basophil (%)	0-2	0.01 (±.26)
Platelet (10^{3}µ/L)	49-387	218.4 (±)
Mean corpuscular volume (fl)	69-105	88.5 (±7.22)
Mean corpuscular haemoglobin (pg)	20-33	27.0 (±2.56)
Mean corpuscular haemoglobin concentration (g/dl)	27.5-34.5	30.5 (±1.44)
Red blood cell (10^3/µL)	2.5-4.9	3.8 (±.43)

Haematological parameters of asymptomatic malaria positive and malaria negative pregnant women

The mean of Hb (9.46 g/dL) in asymptomatic malaria pregnant women was significantly lower than that of their non-malaria infected (10.35 g/dL) individuals. Similarly, there was a significant difference in the PCV (31.42%) of asymptomatic malaria pregnant women compared to their non-malaria (33.95%) counterpart (P = 0.001)**.** There was a significant difference in total WBCs count between the two groups of patients (P value < 0.001). The mean Platelets, RBCs, MCV, MCH, were also found to be lower in asymptomatic *Plasmodium falciparum*-infected pregnant women (200.26 × 10^3/µL, 3.65, 86.84fl, and 26.21pg) compared to their negative individuals (221.58 × 10^3/µL, 3.87, 88.86fl and 27.14pg) respectively. However, there was no significant difference between them (P value > 0.05) (Table 3).

Table 3: Mean values of haematological parameters of the pregnant women with asymptomatic malaria (AM) and negative results of malaria tests (NEG)

Variables	Malaria status	N	Mean (SD)	T-test P value	95% CI of difference
Haemoglobin (g/dl)	AM	19	9.46 (0.81)	-4.07	-1.31- -0.45
	NEG	111	10.35 (0.88)	<0.001[b]	
PCV (%)	AM	19	31.42 (2.85)	-3.30	-4.05--1.01
	NEG	111	33.95 (3.13)	<0.001[b]	
White blood cell (10^3/µL)	AM	19	6.57 (1.35)	-2.77	-1.91--0.32
	NEG	111	7.69 (1.65)	<0.001[b]	
Neutrophil (%)	AM	19	64.58(10.45)	0.35	-3.46-4.94
	NEG	111	63.84 (8.20)	>0.05[a]	
Lymphocyte (%)	AM	19	27.42 (9.31)	-0.44	-4.71-3.01
	NEG	111	28.27 (7.59)	>0.05[a]	
Monocyte (%)	AM	19	7.05 (1.39)	-0.81	-1.09-0.46
	NEG	111	7.37(1.60)	>0.05[a]	

Doi: http://dx.doi.org/10.4314/thrb.v20i2.4

Eosinophil (%)	AM	19	0.94 (1.41)	1.68	-0.08-1.02
	NEG	111	0.42(1.07)	>0.05[a]	
Basophil (%)	AM	19	0.05 (0.23)	0.02	-0.13-0.13
	NEG	111	0.05(0.26)	>0.05[a]	
Platelet (10^3/µL)	AM	19	200.26(69.17)	-1.41	-51.20-8.57
	NEG	111	221.58(59.35)	>0.05a	
Mean corpuscular volume (fl)	AM	19	86.84 (8.06)	-1.13	-5.55-1.52
	NEG	111	88.86 (7.04)	>0.05a	
Mean corpuscular haemoglobin (pg)	AM	19	26.21 (2.64)	-1.47	-2.17-0.32
	NEG	111	27.14 (2.52)	>0.05[a]	
Mean corpuscular haemoglobin concentration (g/dl)	AM	19	30.158 (0.88)	-1.06	-1.08-0.33
	NEG	111	30.532 (1.50)	>0.05[a]	
RBC ×10^3/µL	AM	19	3.653 (0.38)	-1.85	-0.41-0.01
	NEG	111	3.850 (0.44)	>0.05[a]	

[a] Significant at P <0.05 level, [b] significant at P <0.001 level

Discussion

Malaria is transmitted throughout Nigeria, with majority of the population at risk. Though the pregnant women in the present study were apparently healthy, *P. falciparum* infection prevalence of 14.6% was observed. The overall prevalence of 14.6% obtained in this study is lower than the 22.4% in Cameroon (Anchang-Kimbi *et al*., 2015) and 29% reported in elsewhere in Nigeria (Nwonwu *et al*., 2009). Differences in the reported prevalence of *Plasmodium* infection may be due to different ecological characteristics of the areas and by the group of pregnant women studied (only healthy pregnant women who had no complaints were included in our study). Moreover, it could be further explained by the fact that, fewer participants were screened in our study than in the other two studies. The prevalence of *P. falciparum* infection was highest in the second trimester because most of the women book at this stage of pregnancy which is in keeping with a previous report by Egwunyenya *et al* (2001).

This present study showed changes in the mean PCV and Hb among asymptomatic *P. falciparum*-infected patients. This is consistent with reports of other investigators in the Democratic Republic of the Congo (Matangila *et al*., 2014), Sudan (Adam *et al*., 2005) and Ethiopia (Nega *et al*., 2015). Possible explanation could be found in the immune mechanisms associated with anaemia in *P. falciparum* infection elsewhere (Price *et al*., 2001; Oh *et al*., 2001; Erhabor *et al*., 2010). In addition, the current study detected a statistically significant decrease in the mean value of WBC count among the infected pregnant women. This is contrary to the reports of another study from Nigeria (Mbanefo *et al*., 2009). The reason for decrease in mean WBC count reported in our study is not known. However, possible explanation for the decrease in mean WBC count in our study could be due to apoptosis as reported elsewhere (Dodoo *et al*., 2002; Abdalla & Pasvol, 2004).

Some of the limitations of the study included the fact that with cross sectional hospital-based nature of the study, the information on malaria infection prevalence and haematological changes may not be generalizable for the whole area. Further, large, community based studies in this setting are highly necessary. In conclusion, infection with *P. falciparum* produces significant changes in haematological parameters in pregnant women attending antenatal visit for the first time. The most commonly affected parameters are haemoglobin, packed cell volume and total WBC

Conflict of Interest

None declared

Authors' contributions

 Doi: http://dx.doi.org/10.4314/thrb.v20i2.4

OOO, OOF, OO and MAL participated in the design of the work. OOO and OAA did data collection and laboratory work. OOO and MAL performed statistical analysis and interpretation of the data. OOO wrote the first draft of the manuscript. OO and OOF provided critical revision of the manuscript. All authors read and approved the final version of the manuscript.

Acknowledgments

The authors appreciate the assistance and collaboration of staff of the laboratory unit, antenatal unit of O.L.A hospital as well as the pregnant women who gave their consent during the course of the research work

References

Abdalla, S.H. & Pasvol, G. (2004) *Malaria: A haematological perspective*. London: Imperial

Adam, I., Khamis, A.H. & Elbashir, M.I. (2005) prevalence and risk factors for anaemia in pregnant women of eastern Sudan. *Transaction of the Royal Society of Tropical Medicine and Hygiene* 99:739–743.

Alves, F.P., Gill, H., Marrelli, M.T., Ribolla, P.E., Camargo, E.P. & DaSilva, L.H. (2005) asymptomatic carriers of *Plasmodium* species as infection source for malaria vector mosquitoes in the Brazilian Amazon. *Journal of Medical Entomology* 42:777–779.

Anchang-Kimbi, J.K., Nkweti, V.N., Ntonifor, H.N., Apinjoh, T.O., Tata, R.B., Fru Chi, H. & Achidi, E.A. (2015) *Plasmodium falciparum* parasitaemia and malaria among pregnant women at first clinic visit in the mount Cameroon Area. *BMC Infectious Diseases* 15:439.

Cheesbrough M. (1998) *District Laboratory Practice in Tropical Countries*. Cambridge University press: part 1& 2.

Cucunuba, Z.M., Guerrab, A.N.P., Riverab, J.A. & Nichollsa, R.N.S. (2013) Comparison of Asymptomatic *Plasmodium* species Infection in Two Malaria-Endemic Colombian Locations. *Transaction of the Royal Society of Tropical Medicine and Hygiene* 107: 129–136.

Dodoo, D., Omer, F.M. & Todd, J. (2002) Absolute levels and ratios of pro-inflammatory and anti-inflammatory cytokine production in vitro predict clinical immunity to *Plasmodium falciparum* malaria. *Journal of Infectious Disease* 185: 971–979.

Egwunyenya, A.O., Ajayi, J.A., Mnorsi, O.P. & Duhhinksla–popoola, D.D. (2001) *Plasmodum*/intestinal helminth co infection among pregnant Nigeria women. *Memórias do Instituto Oswaldo Cruz* 96:1055-1059

Epidi, T.T., Nwani, C.D. & Ugorji, N.P. (2008) Prevalence of malaria in blood donors in Abakaliki Metropolis, Nigeria. *Scientific Research and Essay* 3(4): 162-164.

Erhabor, O., Adias, T.C. & Hart, M.L. (2010) Effects of falciparum malaria on the indices of anaemia among pregnant women in the Niger Delta of Nigeria. *Journal of Clinical Medicine and Research* 2 (3):35-41

Falade, C.O., Olayemi, O., Dada-Adegbola, H.O., Aimakhu, C.O., Ademowo, O.G. & Salako, L.A. (2008) Prevalence of malaria at booking among antenatal clients in a secondary health care facility in Ibadan, Nigeria. *African Journal of Reproductive Health* 12:141-152.

Fried, M. & Duffy, P.E. (1996) Adherence of Plasmodium falciparum to chondroitin sulfate A in the human placenta. *Science* 272: 1502–1504.

Guyatt, H.L. & Snow, R.W. (2001) The epidemiology and burden of Plasmodium falciparum-related anaemia among pregnant women in sub-Saharan Africa. *American Journal of Tropical Medicine and Hygiene* 64: 36–44.

Ibhanesebhor, S.E., Otobo, C.S. & Ladipo, O.A. (1996) Prevalence of malaria parasitaemia in transfused blood donor in Benin-City, Nigeria. *Annals of Tropical Paediatrics* 16: 93-95.

Doi: http://dx.doi.org/10.4314/thrb.v20i2.4

Ladhani, S., Lowe, B., Cole, A.O., Kowuondo, K.& Newton, C.R. (2002) Changes in white blood cells and platelets in children with falciparum malaria: Relationship to disease outcome. *British Journal of Haematology* 119:839-847.

Maina, R.N., Walsh, D., Gaddy, C., Hongo, G., Waitumbi, J., Otieno, L., Jones, D. & Ogutu, B.R. (2010) Impact of *Plasmodium falciparum* infection on haematological parameters in children living in Western Kenya. *Malaria Journal* 9(3): S4.

Matangila, J.R., Lufuluabo, J., Ibalanky, A.L., Luz, R.A., Lutumba, P. & Geertruyden, J.P.V. (2014) Asymptomatic *Plasmodium falciparum* infection is associated with anaemia in pregnancy and can be more cost-effectively detected by rapid diagnostic test than by microscopy in Kinshasa Democratic Republic of the Congo. *Malaria Journal* 13:132.

Mbanefo, I.E.C., Umeh, I.J.M., Oguoma, V.M. & Eneanya, I.C.I. (2009) Antenatal malaria parasitaemia and haemoglobin profile of pregnant mothers in Awka, Anambra State, south-east Nigeria. *American-Eurasian Journal of Scientific Research* 4: 235-239.

Nega, D., Dana, D., Tefera, T. & Eshetu T. (2015) Anaemia associated with asymptomatic malaria among pregnant women in the rural surroundings of Arba Minch Town, South Ethiopia *BMC Research Notes* 8:110.

Nigeria Federal Ministry of Health National Malaria Control Programme. (2009) Strategic plan 2009–2013: "a road map for malaria control in Nigeria", abridged version. Abuja: Yaliam Press Ltd, Federal Ministry of Health, Nigeria.

Nwonwu, E.U., Ibekwe, P.C., Ugwu, J.I., Obarezi, H.C., & Nwagbara, O.C. (2009) Prevalence of malaria parasitemia and malaria related anaemia amongst pregnant women in Abakaliki, Southeast Nigeria. *Nigeria Journal of Clinical Practice* 12:182-186.

Oh, M.D., Shin, H., Shin, D., Kim, U., Kin, N., Chol, M.H., Chai, J.Y. & Choe, K. (2001) Clinical features of vivax malaria. *American Journal of Tropical Medicine and Hygiene* 65:143–146

Okocha, E.C., Ibeh, C.C., Ele, P.U. & Ibeh, N.C. (2005) The prevalence of malaria parasitaemia in blood donors in a Nigerian teaching hospital. *Journal of Vector Borne Disease* 42: 21-24

Price, R.N., Simpson, J.A., Nosten, F., Luxemburger, C., Hkirjaroen, ter Kuile, F., Chongsuphajaisiddhi, T. & White, N.J. (2001) Factors contributing to anaemia after uncomplicated Falciparum malaria. *American Journal of Tropical Medicine and Hygiene* 65:614-622.

Rogerson, S.J., Hviid, L., Duffy, P., Leke, R. & Taylor, D. (2007) Malaria in pregnancy: pathogenesis and immunity. *Lancet infectious diseases* 7: 105-117.

Sullivan, A.D., Nyirenda, T., Cullinan, T., Taylor, T., Harlow, S.D., James, S.A. & Meshnick, S.R. (1999) Malaria infection during pregnancy: intrauterine growth retardation and preterm delivery in Malawi. *Journal of Infectious Diseases* 179:1580–1583.

Verhoeff, F.H., Brabin, B.J., Chimsuku, L., Kazembe, P. & Broadhead, R.L. (1999) Malaria in pregnancy and its consequences for the infant in rural Malawi. *Annals of Tropical Medicine and Parasitology* 93 (Suppl1): S25–S33.

WHO (2014) WHO policy brief for the implementation of intermittent preventive treatment of malaria in pregnancy using sulfadoxine-pyrimethamine (IPTp-SP), April 2013 (revised January 2014). World Health Organization, Geneva. Available at: http://www.who.int/malaria/publications/atoz/policy_brief_iptp_sp_policy_recommendation/en/.