Evaluation of the adhesion of pregnant women to intermittent presumptive treatment with Sulfadoxine-Pyrimethamine in a rural area of Gabon.

Fleuramie Mirembou-Boukoumba
Ecole Doctoral Régionale D'Afrique Centrale En Infectiologie Tropicale

Sydney Maghendji-Nzondo
Département d'Epidémiologie Biostatistique et Informatique médicale, Université des Sciences de la Santé

Pierre Blaise Matsiegui
Centre de Recherches Médicale de la Ngounie

Irene Pegha-Moukandja
Centre de Recherches Médicale de la Ngounie

Davy Tanguy Mendene-Abessolo
Centre de Recherches Médicale de la Ngounie

Jean Bernard Lekana-Douki (lekana_jb@yahoo.fr)
Unit of Evolution, Epidemiology and Parasitic Resistances (UNEEREP), Centre International de Recherches Médicales de Franceville (CIRMF) B.P. 769 Franceville Gabon. Phone : 00241 06 25 95 90.

Research Article

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Abstract

Background: Gestational malaria remains one of the most complex forms of malaria. To fight it, several African countries adopted intermittent presumptive treatment with Sulfadoxine-Pyrimethamine (IPT-SP) and the use of preventive measures such as insecticide-treated bed nets (ITNs), indoor residual sprays (IRS) and popular education on good practices to fight against malaria. In Gabon a country of central Africa, no study has investigated the use of IPT-SP in rural areas since its implementation. The aim of this study was to investigate the adhesion level of pregnant women to IPT-SP, coverage of ITNs and IRS, and knowledge on the good practices about malaria in a rural area of Gabon.

Using a questionnaire, we led a retrospective study including pregnant women from January 5th 2016 to January 31st 2018 and a cross-sectional survey including women seen for antenatal care and all febrile patients in consultation from February 2nd to May 31st 2018. Malaria was diagnosed using rapid diagnostic tests. Statistical analyses were done.

Results: We included 607 pregnant women before their delivery. Women between 20 and 25 years old were the most prevalent (37.26%, n=229). Among them, 74.53% were unemployed and 47.21% living in the villages surrounding the rural town of Fougamou. The rate of adhesion to IPT-SP was 94.37% (n=573). Among them, 47.8% (n=274) had received 3 doses of IPT. Among the pregnant women included during the cross-sectional survey, only 8.7% (n=14) were infected with *Plasmodium*. Bed nets were used by 80.12% (n=129) of women.

Conclusion: Data showed a near complete adhesion of IPT-SP in the rural area of Fougamou. Clinical trials are needed to investigate the efficacy of IPT-SP and antimalarial drug markers.

Introduction

In 2016, the global count of malaria reached 212 million cases and 429 000 deaths among which more than half were recorded in sub-Saharan Africa [1]. Pregnant women are among the most vulnerable individuals to malaria, with children under five years old and non-immunized travelers [2].

During pregnancy, women must tolerate the fetus, which carries 50% of the father's genetic material [3], they are therefore prone to immune suppression. This situation increases the risk of susceptibility to infectious pathogens, such as *Plasmodium falciparum* [4–6]. Gestational malaria is often externalized by hyperthermia, which is related to parasitemia. Susceptibility to malaria decreases with the number of pregnancies [7], suggesting the acquisition of a specific immunity directed against this pathology [8–10]. Anemia is common during pregnancy [11, 12], it affects primiparae more than multiparae. Cerebral malaria is not frequent among pregnant women in areas of high transmission, both in multiparae and primiparae.

One of the pathophysiological factors of gestational malaria is placental sequestration of parasitized red blood cells [13]. Numerous studies have shown that this preferably occurs through the cytoadherence of

parasitized red blood cells on placental syncytiotrophoblates via Chondroitin Sulfate A [14]. Thus, the placenta behaves like a niche concentrating parasitized red blood cells, called CSA cytoadherence phenotypes [15, 16]. The consequences of gestational malaria are therefore increased morbidity and mortality in pregnant women [17]. For fetuses, placental infection leads to spontaneous abortions, *in utero* growth delays, and children born with low weight [18–21].

To prevent placental malaria, WHO recommends the use of intermittent presumptive treatment with Sulfadoxine-Pyrimethamine (IPT-SP), in addition to the use of insecticide-treated bed nets (ITNs). However, in 2014, among the 28 million pregnant women registered, 15 million pregnant women had not received IPT-SP [22]. Moreover, among the pregnant women who had received IPT-SP, 50% had received one dose, 35% two doses and only 15% had received at least three doses.

In Africa, the Sulfadoxine-Pyrimethamine (SP) therapeutic combination was adopted in most countries. However, these two molecules are subject to *P. falciparum* resistance. Pyrimethamine resistance is the result of mutations in codons 16, 51, 59, 108, 164 of the *P. falciparum* Dihydrofolate Reductase (*PfDHFR*) gene [23–25], while mutations in codons 436, 437, 540, 580 and 613 of the *P. falciparum* Dihydropteroate synthase (*PfDHPS*) gene generate resistance to sulfadoxine [26].

In Gabon, IPT based on SP in pregnant women was adopted in 2003. As a result, the prevalence of *P.falciparum* infection declined among pregnant women both in urban and rural areas [27].

The aim of this study was to determine the level of adhesion of pregnant women to IPT-SP in the rural area of Fougamou, Gabon.

Methods

Area and study population

The study was conducted in a Gabonese semi-urban town named Fougamou located in the Ngounié province, in the western central part of Gabon. Fugamou is the chief town of the Tsamba-Magotsi department, as such, its hospital receives patients from villages throughout the department.

The study population was composed of pregnant women who received antenatal care at the maternity of the Fougamou medical center.
The women met the following criteria: they had been seen in consultation at the maternity ward and had been entered in the consultation registers. Women who did not fill the criteria were excluded from the study.

Study Design And Sampling Collection

We conducted a prospective study from February to May 2018 at the maternity of the Fougamou medical center. Retrospective data from 2018 to January 2018 from maternity registries were also included in this study. Blood samples from the pregnant women were collected in ethylene diamine tetra acetic acid (EDTA) tubes during the prenatal consultation after the pregnant women gave their informed consent.

Questionnaire Interview And Ethical Consideration

An interview based on a questionnaire was given to all the participants.
The questionnaire included questions on socio-demographic data (age, education and family income/month), medical history and clinical data (the number of IPT-SP doses taken). Data on their use of IPT-SP, their use of ITNs, indoor residual sprays (IRS) and their knowledge, attitudes and practices regarding malaria were collected.

This study was approved by the Gabonese national committee of ethics and registered under PROT 0020/2015/SG/CNE; it was performed in accordance with the principles of Committee.
To ensure their voluntary participation, an informed consent form was signed by all the participants.

Malaria Diagnosis

The Optimal-IT® rapid diagnostic test was used. The sensibility and specificity of the tests were 94 and 97 % [28]. Previous work in Gabon showed that this test is a good tool to diagnose malaria [29]. Parasite load was determined on blood smears using the Lambarene method [30]. All blood smears were read by two independent technicians and quality control was done for 10 % of slides by a third reader. Fever and *P. falciparum* infection (1000 parasites per µl of blood) were considered as malaria.

Statistical analysis

Statistical analysis was performed with the use of Stat view 5.0 1992–1998 (SAS Institute, Cary, USA). A chi-square test was used to compare quantitative variables among groups.

The average values of the qualitative variables were compared by the Student's t-test. Quantitative variables were compared by the chi-square test and when the numbers were lower than 5, Fisher's exact test was used. The threshold of significance was established for $\alpha = 5\%$.

Results

Study population

During the study period, 607 pregnant women were received in prenatal consultation in the medical center of Fougamou. The general characteristics of the women are described in Table 1. Unemployed pregnant women were the most numerous (60.9%), then came high school students and employed pregnant women ($p < 0.001$). The proportion of women aged between 20–25 years (37.7%) was significantly higher than the proportion of women of the other age groups ($p < 0.001$). The proportion of women aged between 20–25 years was similar to the proportion of women aged between 26–30 years (19.9%) and 31–35 years (16.0%) ($p > 0.05$). Hematological values were normal. The interval between the prenatal consultations was of 1 month.

Table 1: Socio demographical and biological characteristics of included pregnant women.

	Pregnant women included
General parameters	
Mean age ± SD (month)	*309.5 ± 80.4*
Hemoglobin (g/dl)	*9.7± 2.1*
*WBC (x 10^3/µl)	*6.2 ± 2.6*
**RBC(x 10^6/µl)	*4.1 ± 0.8*
Platelet (x 10^3/µl)	*159.7 ± 60.1*
Age groups (years) ; %(n)	
14-19	*16.1% (98)*
20-25	*37.7% (229)*
26-30	*19.9% (121)*
31-35	*16.0% (97)*
36-40	*6.6% (40)*
>40	*3.6% (22)*
Profession (N= 604)	
Student high school	*25.2% (152)*
Employed	*13.9% (84)*
unemployed	*60.9% (368)*
Parity (N=603) ; % (n)	
large multipara***	*6.8 (41)*
Multipara	*48.6% (293)*
Nullipara (Primigravidea)	*24.9% (150)*
Primipara	*19.7% (119)*

*White blood cell, ** Red blood cell, *** More than 4 deliveries.

Level Of Adherence To IPT-SP Treatment

The level of adherence was analyzed from 427 women who gave birth in the Fougamou medical center. Ninety-four percent (94.4%; n = 403) of women included in the study had taken at least one dose of IPT-SP and 3.3% (n = 14) had not taken the treatment. This information was unavailable for 10 women. There was a significant difference between women who took the treatment and women who did not take IPT-SP ($p < 0.05$). The prevalence of women who took 1 dose (16.1%; n = 65) of IPTp-SP was significantly lower than the prevalence of women who took 2 doses (29.5%; n = 119) and 3 doses (47.9%; n = 193) of IPT-SP

(P < 0.001) (Fig. 2). The prevalence of women who took 2 doses of IPT-SP was significantly lower than the prevalence of women who took 3 doses (p < 0.001). Two women (2.1%; n = 2/96) had received IPT-SP between the first and 15th week of amenorrhea (WA). In addition, the majority of women who had not received a dose of IPT-SP were those with one to 25 WA.

Plasmodium characterization

The overall prevalence of *Plasmodium* infection was 11.7% upon blood smear examination (n = 67/508). The mean values of parasitemia were calculated based on the 67 pregnant women with positive thick films. Mean parasitemia was low at 1052 (33–19,880) p/lL of blood. We found that 100% of infections were due to *P. falciparum*. Table 2 shows that *Plasmodium*-infected women were less old than uninfected women (p < 0.05). Figure 1 shows that the proportion of *Plasmodium*-infected women aged between 14–19 years (22.8%) was significantly higher than the proportion of *Plasmodium*-infected women aged between 20–25 years (11.0%), 26–30 years (8.8%), 31–35 years (7.6%) and 36–40 years (7.9%) (p < 0.05). The Kruskal–Wallis test showed a significant difference in mean parasitemia according to age groups (p = 0.02). The mean values of red blood cell were significantly lower in infected women than uninfected women (p < 0.01) whereas white blood cells were higher in infected women than uninfected women (p = 0.0007),

Influence of IPT-SP treatment on plasmodial infection.

In this study, 29.9 % (n = 20/67) of women having received at least one dose of IPTp-SP were infected by *P. falciparum* with a mean parasitemia of 229 (33-19880) p/µL. Among the *Plasmodium*-infected women (Fig. 2), the prevalence of women who took 3 doses of IPTp-SP (5.0%; n = 1/20) was not significantly different than the prevalence of *Plasmodium*-infected women who took 1 dose (50.0%; n = 10/91) and 2 doses (45.0%; n = 9/20) of IPT-SP (p > 0.05).

Influence Of Parity On Plasmodial Infection

In this study, multipara women (48.6%) were more represented than other women (grand multiparae, nulliparae and primiparae) (P < 0.001) (Table 1). *Plasmodium*-infected nulliparae (primigravidea) (38.8%; n = 26/67) were more represented than *Plasmodium*-infected multiparae (34.2%; n = 23/67) and the other *Plasmodium*-infected women [primiparae (22.4%; n = 15/67) and grand multiparae (4.5%; n = 3/67) (P < 0.05)].

Plasmodium-infected multiparae (35.0%; n = 7/20) and *Plasmodium*-infected nulliparae (primigravidea) (30.0%; n = 6/20) having received at least one dose of IPT-SP were more represented than *Plasmodium*-infected primiparae (25.0% ; n = 5/20) and *Plasmodium*-infected grand multiparae (10.0% ; n = 2/20), but without statistical significance (p > 0.05).

Preventive Measures

We analyzed the patients' use of preventive measures (Table 2). The overall coverage of bed nets was 68.2% (n = 413/606). The proportion of insecticide house spray use was 44.7% (n = 268/600) and the proportion of pregnant women showing essential information on malaria was 74.3% (n = 437/588). Univariate and multivariate analysis showed no association between the use of preventive measures and malaria infection (p > 0.05). The use of preventive measures had no preventive effect in pregnant women without and with malaria.

Table 2: Socio demographical and biological characteristics of uninfected versus infected pregnant women.

	Uninfected versus infected pregnant women		P
	uninfected	*Infected*	
Mean age ± SD (month)	312.7± 79.6	284.1± 80.5	***0.006***
Hematological parameters	***n= 64***	***n= 475***	
Hemoglobin (g/dl)	9.8±2.0	9.1±2.5	***0.004***
*WBC (x 10^3/µl)	6.2±2.4	6.6±3.5	***0.0007***
**RBC(x 10^6/µl)	4.1±0.8	3.8±0.9	***0.01***
Platelet (x 10^3/µl)	161.3±60.7	147.4±54.3	0.08
Prevention measures			
Bed net	67.5 (342)	68.7 (46)	0.84
Insecticides	42.9 (216)	44.8 (30)	0.78
Received an IEK on malaria***	77.1 (380)	73.4 (47)	0.52

*White blood cell, ** Red blood cell; ***IEK: Information, education and knowlegment on malaria.

Discussion

Gestational malaria remains a major public health problem since it leads to severe risks for the mother, the fetus or the newborn child. This study investigated the epidemiology of malaria in Gabon and estimated the level of adherence to IPT-SP in pregnant women. We noted an average of about 200 deliveries per year, which represents between four and five deliveries per week and is representative of the low demography of Fougamou.

The mean age of the pregnant women in our study was about 25.8 ± 6.7 years. This result is consistent with previous data from rural areas in Burkina Faso, in Benin and in the capital of Gabon, Libreville, and its surroundings, in 2011 [31–33]. In Gabon, this data highlights the fact that there does not seem to be a significant difference in the distribution of age of the pregnancy between urban and rural areas.

In this work, we noticed that nearly half of the women included in the study lived in villages. The rural context of Fougamou is confirmed by the socio-demographical factors (a majority of women were unemployed).

However, the analysis of the hematological parameters revealed a good general health state, suggesting an efficient medical follow-up of these women.

The excellent level of adhesion to IPT-SP consolidates this hypothesis. Previous data from Gabon showed that the level of adherence to IPT-SP was very high, resulting in a minimization of the consequences of malaria associated with pregnancy [34]. In other African countries such as Kenya or Burkina Faso, similar results were reported [35–37]. The level of adherence to IPT-SP in our study was 94.4%. This proportion is higher than the value previously reported in Libreville and in Burkina Faso [31, 37]. This result in Fougamou fits the goal set by OMS, which aimed to have 80% of women receive at least 2-doses of IPTp-SP during pregnancy.

Although the national directive indicates that pregnant women have to receive at least 3 doses of IPT-SP, in this study, we found that women received a maximum of 3 doses, which is an erroneous reading of the national directive. In this study, the proportion of women having received IPT-SP during the first quarter of pregnancy is similar to that observed in Libreville where 5.9 % of the women had received IPT-SP in the first quarter. After delivery, half the women had received 3 doses of IPT-SP.

Data showed that the majority of women without IPT-SP were between one and 25 weeks of amenorrhea, which could suggest a late start in their antenatal care.
This observation is revealing of the rural context of Fougamou. The time between consultations confirms the high attendance of women to prenatal visits in Fougamou, which ranged from one to nine visits.

The low prevalence of plasmodial infection observed in our study could be a consequence of the adherence of women to IPT-SP. Moreover, nulliparae (primigravidea), without IPT or under IPT, remain the most exposed to malaria. Similar results were observed in Burkina Faso, in Benin, in Malawi and in Gabon [31, 33, 38–41]. This could be explained by the fact that multigravidae develop, over subsequent pregnancies, a protective immunity against placental malaria infection. These women develop antibodies which are able to specifically inhibit the cytoadhesion on Chondroitin Sulfate A [42–44]. The more women age and have parity, the more they are protected from malaria.

The overall malaria rate in women having benefited from IPT-SP obtained in our study is similar to the rates of malaria infections reported in Burkina Faso, in Mali, in Kenya and in Malawi [39, 41, 45–47]. However, lower rates than ours were reported in Burkina Faso in 2013 (4.7 %) and in Benin (4,1 %) [33, 37].

The low prevalence of plasmodial infection observed in this study could also be a consequence of adhesion to other recommended malaria prevention measures. Indeed, an overall coverage rate in bed nets of about 70 % was observed. This very high coverage rate could have reduced the development of plasmodial infection. Several studies reported the association of IPT-SP and the use of bed nets to protect women against plasmodial infection during their pregnancy [48, 49]. However, this rate contrasts the women's knowledge on malaria because during the questionnaire only 74.3 % of the women answered the three questions which define the level of knowledge on malaria correctly (What is malaria? How can you avoid it? How does it manifest itself?). These data suggest that women follow IPT without a clear understanding of its role and might indicate that the impact of public health messages is erroneous.

The positive impact of IPT-SP in pregnant woman is confirmed in this study by the fact that all the pregnant women infected by *P. falciparum* were asymptomatic and that the average parasitemia was low. This is consistent with previous data indicating that in areas of stable transmission, plasmodial infection in pregnant women is often asymptomatic [50].

Conclusion

This study shows that almost all women giving birth in the Fougamou medical center receive at least one dose of IPT-SP during their pregnancy. About 80% of them were 14 to 30 years old with a peak between 20 and 25 years. We also confirm the efficiency of IPT-SP in association with preventive measures against malaria. It would be necessary to pursue these malaria control strategies in pregnant woman by looking at the impact of IPT-SP on the genetics of the parasite.

Abbreviations

FGM: Fougamou; **IPT:** Intermittent preventive treatment; **SP:** Sulfadoxine-pyrimethamine; **WHO:** World Health Organization; **WA:** Week of amenorrhea.

Declarations

Ethics approval and consent to participate: This study was approved by the Gabonese national Committee of ethics and registered under PROT 0020/2015/SG/CNE; it was performed in accordance with the principles of Committee. To ensure their voluntary participation, an informed consent form was signed by all the participants.

Consent for publication: All authors read the last file of this paper and agree the submission for publication

Availability of data and materials: The authors declare that the data will be made available after the article's acceptance and publication

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Authors' contributions: FMB: conducted the study and participated in writing the paper; **SMN** and **IPM**: participated in the study and in writing the paper; **PBM**: coordinated the study and the writing of the paper; **DTMA**: participated in the study and in the data collection; **JBLD**: conceived and conducted the study and wrote the paper. All authors read and approved the final manuscript.

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Authors' information (optional): MMF: PhD student, **IPM**: PhD; **MNS**: PhD, **PBM**: PhD physician; **DTMA**: Master; **JBLD**: PhD, associate professor.

References

1. WHO: **World Malaria Report** *World Malaria Report* 2017.
2. WHO: **World Malaria Report**. *Vaccins et vaccination: la situation dans le monde* 2009.
3. Gaunt G, Ramin K: **Immunological tolerance of the human fetus**. *American journal of perinatology* 2001, **18**(6):299-312.
4. Fievet N, Moussa M, Tami G, Maubert B, Cot M, Deloron P, Chaouat G: ***Plasmodium falciparum* induces a Th1/Th2 disequilibrium, favoring the Th1-type pathway, in the human placenta**. *The Journal of infectious diseases* 2001, **183**(10):1530-1534.
5. Luft BJ, Remington JS: **Effect of pregnancy on resistance to Listeria monocytogenes and Toxoplasma gondii infections in mice**. *Infection and immunity* 1982, **38**(3):1164-1171.
6. Luft BJ, Remington JS: **The adverse effect of pregnancy on macrophage activation**. *Cellular immunology* 1984, **85**(1):94-99.
7. Fried M, Nosten F, Brockman A, Brabin BJ, Duffy PE: **Maternal antibodies block malaria**. *Nature* 1998, **395**(6705):851-852.
8. Ricke CH, Staalsoe T, Koram K, Akanmori BD, Riley EM, Theander TG, Hviid L: **Plasma antibodies from malaria-exposed pregnant women recognize variant surface antigens on *Plasmodium falciparum*-infected erythrocytes in a parity-dependent manner and block parasite adhesion to chondroitin sulfate A**. *Journal of immunology* 2000, **165**(6):3309-3316.
9. Ibitokou S, Oesterholt M, Brutus L, Borgella S, Agbowai C, Ezinmegnon S, Lusingu J, Schmiegelow C, Massougbodji A, Deloron P *et al*: **Peripheral blood cell signatures of *Plasmodium falciparum***

infection during pregnancy. *PloS one* 2012, **7**(12):e49621.

10. Rovira-Vallbona E, Monteiro I, Bardaji A, Serra-Casas E, Neafsey DE, Quelhas D, Valim C, Alonso P, Dobano C, Ordi J *et al*: **VAR2CSA signatures of high *Plasmodium falciparum* parasitemia in the placenta**. *PloS one* 2013, **8**(7):e69753.
11. Guidotti RJ: **Anaemia in pregnancy in developing countries**. *BJOG : an international journal of obstetrics and gynaecology* 2000, **107**(4):437-438.
12. Nazir G, Naz S, Ali S, Aziz S, Malik SA, Qari IH, Irum S: **Anaemia: the neglected female health problem in developing countries**. *Journal of Ayub Medical College, Abbottabad : JAMC* 2011, **23**(2):8-11.
13. Brabin BJ, Romagosa C, Abdelgalil S, Menendez C, Verhoeff FH, McGready R, Fletcher KA, Owens S, D'Alessandro U, Nosten F *et al*: **The sick placenta-the role of malaria**. *Placenta* 2004, **25**(5):359-378.
14. Jakobsen PH, Rasheed FN, Bulmer JN, Theisen M, Ridley RG, Greenwood BM: **Inflammatory reactions in placental blood of *Plasmodium falciparum*-infected women and high concentrations of soluble E-selectin and a circulating *P. falciparum* protein in the cord sera**. *Immunology* 1998, **93**(2):264-269.
15. Khunrae P, Dahlback M, Nielsen MA, Andersen G, Ditlev SB, Resende M, Pinto VV, Theander TG, Higgins MK, Salanti A: **Full-length recombinant *Plasmodium falciparum* VAR2CSA binds specifically to CSPG and induces potent parasite adhesion-blocking antibodies**. *Journal of molecular biology* 2010, **397**(3):826-834.
16. Srivastava A, Gangnard S, Round A, Dechavanne S, Juillerat A, Raynal B, Faure G, Baron B, Ramboarina S, Singh SK *et al*: **Full-length extracellular region of the var2CSA variant of PfEMP1 is required for specific, high-affinity binding to CSA**. *Proceedings of the National Academy of Sciences of the United States of America* 2010, **107**(11):4884-4889.
17. Kassebaum NJ, Bertozzi-Villa A, Coggeshall MS, Shackelford KA, Steiner C, Heuton KR, Gonzalez-Medina D, Barber R, Huynh C, Dicker D *et al*: **Global, regional, and national levels and causes of maternal mortality during 1990-2013: a systematic analysis for the Global Burden of Disease Study 2013**. *Lancet* 2014, **384**(9947):980-1004.
18. Black RE, Cousens S, Johnson HL, Lawn JE, Rudan I, Bassani DG, Jha P, Campbell H, Walker CF, Cibulskis R *et al*: **Global, regional, and national causes of child mortality in 2008: a systematic analysis**. *Lancet* 2010, **375**(9730):1969-1987.
19. Singh J, Soni D, Mishra D, Singh HP, Bijesh S: **Placental and neonatal outcome in maternal malaria**. *Indian pediatrics* 2014, **51**(4):285-288.
20. Nakimuli A, Mbalinda SN, Nabirye RC, Kakaire O, Nakubulwa S, Osinde MO, Kakande N, Kaye DK: **Still births, neonatal deaths and neonatal near miss cases attributable to severe obstetric complications: a prospective cohort study in two referral hospitals in Uganda**. *BMC pediatrics* 2015, **15**:44.
21. Schwarz NG, Adegnika AA, Breitling LP, Gabor J, Agnandji ST, Newman RD, Lell B, Issifou S, Yazdanbakhsh M, Luty AJ *et al*: **Placental malaria increases malaria risk in the first 30 months of life**. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America* 2008, **47**(8):1017-1025.
22. WHO: **World Malaria report**. 2015.

23. Reed MB, Saliba KJ, Caruana SR, Kirk K, Cowman AF: **Pgh1 modulates sensitivity and resistance to multiple antimalarials in *Plasmodium falciparum***. *Nature* 2000, **403**(6772):906-909.
24. Cowman AF, Morry MJ, Biggs BA, Cross GA, Foote SJ: **Amino acid changes linked to pyrimethamine resistance in the dihydrofolate reductase-thymidylate synthase gene of *Plasmodium falciparum***. *Proceedings of the National Academy of Sciences of the United States of America* 1988, **85**(23):9109-9113.
25. Wu Y, Kirkman LA, Wellems TE: **Transformation of *Plasmodium falciparum* malaria parasites by homologous integration of plasmids that confer resistance to pyrimethamine**. *Proceedings of the National Academy of Sciences of the United States of America* 1996, **93**(3):1130-1134.
26. Triglia T, Wang P, Sims PF, Hyde JE, Cowman AF: **Allelic exchange at the endogenous genomic locus in *Plasmodium falciparum* proves the role of dihydropteroate synthase in sulfadoxine-resistant malaria**. *The EMBO journal* 1998, **17**(14):3807-3815.
27. Ramharter M, Schuster K, Bouyou-Akotet MK, Adegnika AA, Schmits K, Mombo-Ngoma G, Agnandji ST, Nemeth J, Afene SN, Issifou S *et al*: **Malaria in pregnancy before and after the implementation of a national IPTp program in Gabon**. *The American journal of tropical medicine and hygiene* 2007, **77**(3):418-422.
28. Moody AH, Chiodini PL: **Non-microscopic method for malaria diagnosis using OptiMAL IT, a second-generation dipstick for malaria pLDH antigen detection**. *British journal of biomedical science* 2002, **59**(4):228-231.
29. Mawili-Mboumba DP, Bouyou Akotet MK, Ngoungou EB, Kombila M: **Evaluation of rapid diagnostic tests for malaria case management in Gabon**. *Diagnostic microbiology and infectious disease* 2010, **66**(2):162-168.
30. Planche T, Krishna S, Kombila M, Engel K, Faucher JF, Ngou-Milama E, Kremsner PG: **Comparison of methods for the rapid laboratory assessment of children with malaria**. *The American journal of tropical medicine and hygiene* 2001, **65**(5):599-602.
31. Bouyou-Akotet MK, Mawili-Mboumba DP, Kombila M: **Antenatal care visit attendance, intermittent preventive treatment and bed net use during pregnancy in Gabon**. *BMC pregnancy and childbirth* 2013, **13**(1):52.
32. Coulibaly SO, Gies S, D'Alessandro U: **Malaria burden among pregnant women living in the rural district of Boromo, Burkina Faso**. *The American journal of tropical medicine and hygiene* 2007, **77**(6_Suppl):56-60.
33. Huynh B-T, Fievet N, Briand V, Borgella S, Massougbodji A, Deloron P, Cot M: **Consequences of gestational malaria on birth weight: finding the best timeframe for intermittent preventive treatment administration**. *PloS one* 2012, **7**(4):e35342.
34. Ramharter M, Schuster K, Bouyou-Akotet MK, Adegnika AA, Schmits K, Mombo-Ngoma G, Agnandji ST, Nemeth J, Afène SN, Issifou S: **Malaria in pregnancy before and after the implementation of a national IPTp program in Gabon**. *The American journal of tropical medicine and hygiene* 2007, **77**(3):418-422.

35. Coulibaly SO, Kayentao K, Taylor S, Guirou EA, Khairallah C, Guindo N, Djimde M, Bationo R, Soulama A, Dabira E: **Parasite clearance following treatment with sulphadoxine-pyrimethamine for intermittent preventive treatment in Burkina-Faso and Mali: 42-day in vivo follow-up study**. *Malaria journal* 2014, **13**(1):41.
36. Parise ME, Ayisi JG, Nahlen BL, Schultz LJ, Roberts JM, Misore A, Muga R, Oloo AJ, Steketee RW: **Efficacy of sulfadoxine-pyrimethamine for prevention of placental malaria in an area of Kenya with a high prevalence of malaria and human immunodeficiency virus infection**. *The American journal of tropical medicine and hygiene* 1998, **59**(5):813-822.
37. Bamba S, Séré A, Nikiéma R, Halidou T, Thiéba B, Dao B, Guiguemdé RT: **Traitement préventif intermittent à la sulfadoxine–pyriméthamine du paludisme chez les femmes enceintes: efficacité et observance dans deux hôpitaux urbains du Burkina Faso**. *Pan African Medical Journal* 2013, **14**(1).
38. Jäckle MJ, Blumentrath CG, Zoleko RM, Akerey-Diop D, Mackanga J-R, Adegnika AA, Lell B, Matsiegui P-B, Kremsner PG, Mombo-Ngoma G: **Malaria in pregnancy in rural Gabon: a cross-sectional survey on the impact of seasonality in high-risk groups**. *Malaria journal* 2013, **12**(1):412.
39. Gies S, Coulibaly SO, Ouattara FT, D'Alessandro U: **Individual efficacy of intermittent preventive treatment with sulfadoxine–pyrimethamine in primi-and secundigravidae in rural Burkina Faso: impact on parasitaemia, anaemia and birth weight**. *Tropical medicine & international health* 2009, **14**(2):174-182.
40. 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 *Plasmodium falciparum* infection among pregnant women in Malawi**. *The American journal of tropical medicine and hygiene* 1994, **51**(5):515-522.
41. Rogerson SJ, Chaluluka E, Kanjala M, Mkundika P, Mhango C, Molyneux ME: **Intermittent sulfadoxine-pyrimethamine in pregnancy: effectiveness against malaria morbidity in Blantyre, Malawi, in 1997–1999**. *Transactions of the Royal Society of Tropical Medicine and Hygiene* 2000, **94**(5):549-553.
42. Fievet N, Cot M, Ringwald P, Bickii J, Dubois B, Le Hesran J, Migot F, Deloron P: **Immune response to *Plasmodium falciparum* antigens in Cameroonian primigravidae: evolution after delivery and during second pregnancy**. *Clinical & Experimental Immunology* 1997, **107**(3):462-467.
43. Fried M, Duffy PE: **Adherence of *Plasmodium falciparum* to chondroitin sulfate A in the human placenta**. *Science* 1996, **272**(5267):1502.
44. Lagerberg RE: **Malaria in pregnancy: a literature review**. *Journal of Midwifery & Women's Health* 2008, **53**(3):209-215.
45. Kayentao K, Kodio M, Newman RD, Maiga H, Doumtabe D, Ongoiba A, Coulibaly D, Keita AS, Maiga B, Mungai M *et al*: **Comparison of intermittent preventive treatment with chemoprophylaxis for the prevention of malaria during pregnancy in Mali**. *The Journal of infectious diseases* 2005, **191**(1):109-116.

46. van Eijk AM, Ayisi JG, ter Kuile FO, Otieno JA, Misore AO, Odondi JO, Rosen DH, Kager PA, Steketee RW, Nahlen BL: **Effectiveness of intermittent preventive treatment with sulphadoxine-pyrimethamine for control of malaria in pregnancy in western Kenya: a hospital-based study**. *Trop Med Int Health* 2004, **9**(3):351-360.
47. Diakite OS, Kayentao K, Traore BT, Djimde A, Traore B, Diallo M, Ongoiba A, Doumtabe D, Doumbo S, Traore MS *et al*: **Superiority of 3 over 2 doses of intermittent preventive treatment with sulfadoxine-pyrimethamine for the prevention of malaria during pregnancy in mali: a randomized controlled trial**. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America* 2011, **53**(3):215-223.
48. Aitken EH, Mbewe B, Luntamo M, Kulmala T, Beeson JG, Ashorn P, Rogerson SJ: **Antibody to *P. falciparum* in pregnancy varies with intermittent preventive treatment regime and bed net use**. *PloS one* 2012, **7**(1):e29874.
49. Feng G, Simpson JA, Chaluluka E, Molyneux ME, Rogerson SJ: **Decreasing burden of malaria in pregnancy in Malawian women and its relationship to use of intermittent preventive therapy or bed nets**. *PloS one* 2010, **5**(8):e12012.
50. Takem EN, D'Alessandro U: **Malaria in pregnancy**. *Mediterranean journal of hematology and infectious diseases* 2013, **5**(1).

Figures

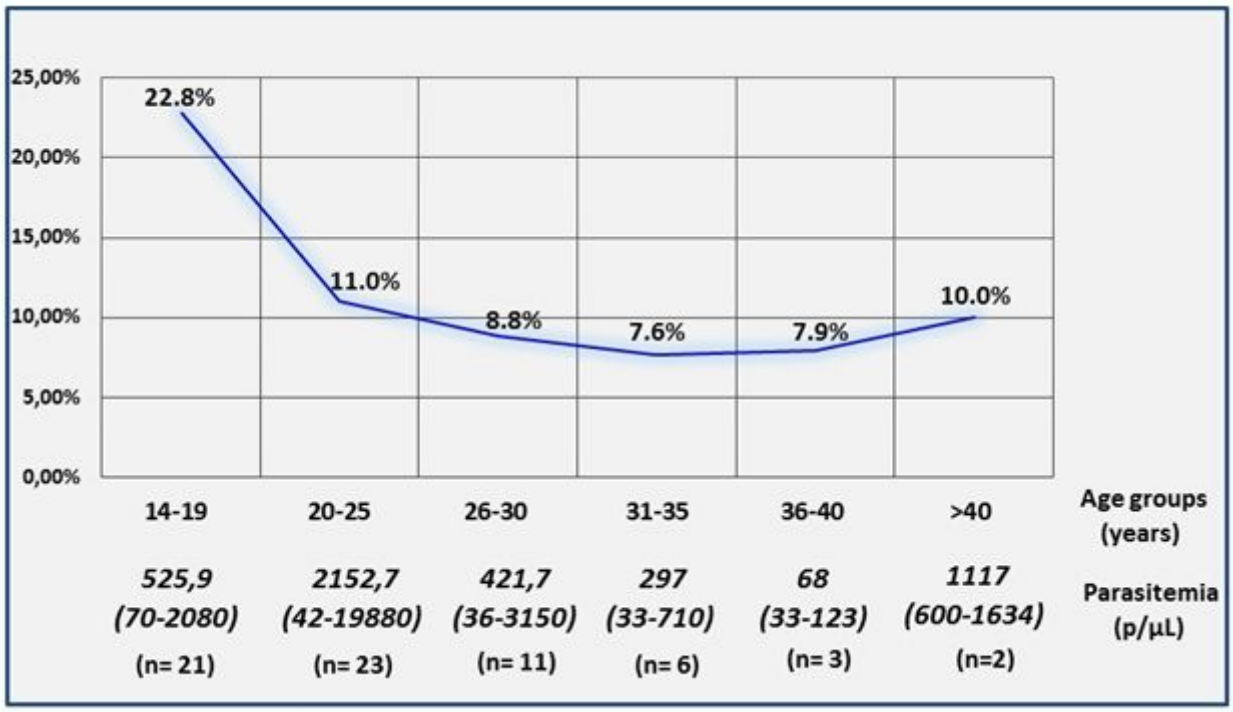

Figure 1

Prevalence of malaria infection and means parasitemia according to age groups in pregnant women (N= 575) (women diagnosed by RDT and blood smear).

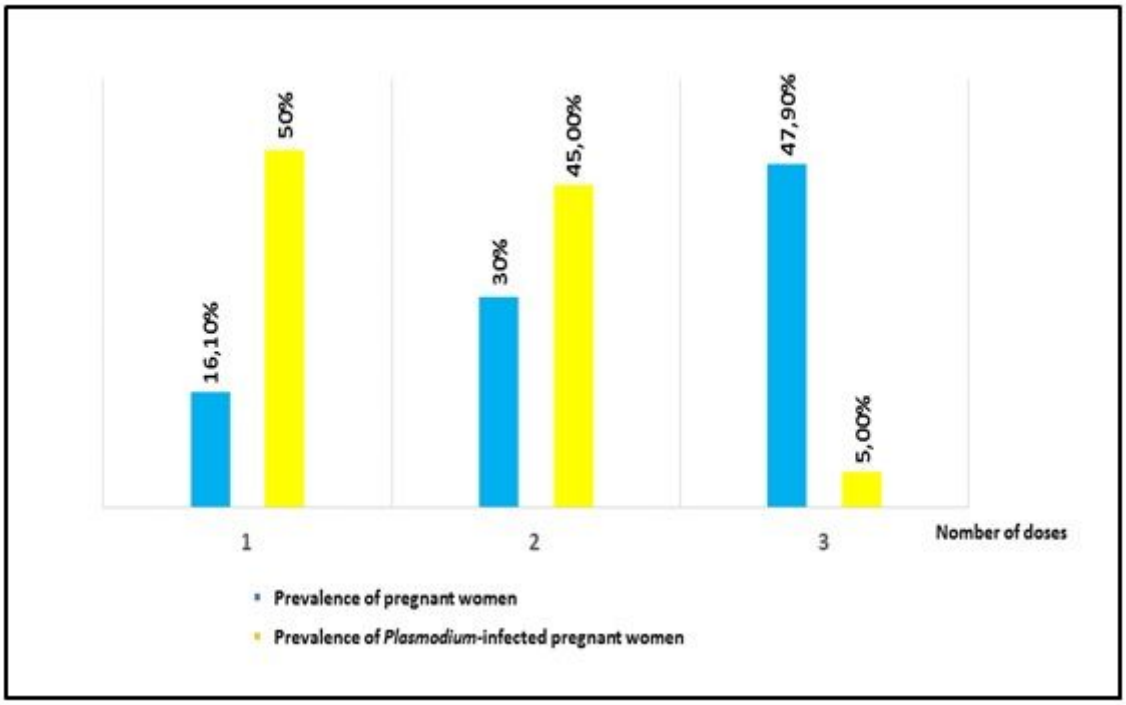

Figure 2

Adherence of women to treatment and the influence on plasmodial infection