

## **EFFECT OF INTERMITTENT PREVENTIVE TREATMENT OF MALARIA ON THE OUTCOME OF PREGNANCY AMONG WOMEN ATTENDING ANTENATAL CLINIC OF A NIGERIAN TEACHING HOSPITAL**

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### **ABSTRACT.**

**Background:** Malaria is a public health problem globally especially in the Sub-Saharan Africa and among the under five children and pregnant women. Malaria in pregnancy is well known to be associated with a lot of maternal and fetal complications. Intermittent Preventive Treatment with Sulphadoxine-Pyrimethamine is the currently recommended regimen for prevention of malaria in pregnancy in the endemic areas.

**Objective:** The study was on the effect of intermittent preventive treatment of malaria in pregnancy on the prevalence of malaria in pregnancy and the outcome of pregnancy.

**Method:** It was a prospective descriptive cross-sectional study and a semi-structured questionnaire was administered to women in the lying in ward University Teaching Hospital, Ado-Ekiti, who booked in the hospital, attended at least two antenatal clinic visits and delivered in the hospital.

**Results:** The study revealed that about 75% of the pregnant women studied had access to intermittent preventive treatment of malaria and also that among the women attending the antenatal clinic that received the IPT, about 78% of them took the recommended dose of the IPT. The prevalence of malaria was statistically higher in women who did not receive intermittent preventive treatment with sulphadoxine-pyrimethamine during pregnancy (44.7% vs 31.3%,  $p=0.0001$ ) and among women who had one dose of the drug instead of two doses (40.0% vs 28.7%,  $p=0.0001$ ). There was no statistical significant difference in the mean age in years ( $31.53\pm 5.238$  vs  $31.07\pm 4.751$ ,  $p=0.09$ ) and the gestational age at delivery ( $38.76\pm 1.784$  vs  $38.85\pm 1.459$ ,  $p=0.122$ ) between the women who did not receive IPT and those who had it.

There was a statistical significant difference in the outcome of pregnancy among women who had IPT and those who did not viz a viz in the duration of labor ( $8.6\pm 1.491$  vs  $8.7\pm 1.634$ ,  $p=0.011$ ) and the birth weight of the babies ( $3.138\pm 0.402$  vs  $3.263\pm 0.398$ ,  $p=0.0001$ )

**Conclusion:** Intermittent preventive of malaria with Sulphadoxine-pyrimethamine in pregnancy is effective as a prophylaxis against malaria and improves the outcome of pregnancy in malaria's endemic areas.

**Keywords:** Pregnancy, Malaria, Intermittent Preventive Treatment, Sulphadoxine-Pyrimethamine.

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## **INTRODUCTION**

Malaria is an important public health problem globally and especially in Sub-Saharan Africa due to climatic factors, poor environmental sanitation and cultural habits which provide conducive atmosphere that allows transmission of the parasite throughout the year<sup>1</sup>. It has been estimated that about 90% of the annual 500 million cases of malaria occur in the Sub-Saharan Africa with 80% of the 1.5-3.0 million annual deaths due to malaria<sup>1</sup>. Most studies from Sub-Saharan Africa showed that about 25 million pregnant women are at risk of malaria infection every year<sup>2</sup> while it is estimated that 40% of the world's pregnant women are exposed to malaria infection during pregnancy<sup>3</sup>.

In Nigeria, at least 50% of the population has malaria infection annually with under-five children and pregnant women at greater risk of the debilitating effects of the infection<sup>3</sup>. Malaria accounts for 30% of childhood mortality and 11% of maternal deaths in Nigeria. In many African countries, malaria is holo-endemic and non-pregnant female adults have a significant level of immunity against malaria. However, during pregnancy, these women experience a considerable decline in their levels of immunity to malaria<sup>4</sup> and several studies have reported that 1<sup>st</sup> and 2<sup>nd</sup> pregnancies are associated with a higher prevalence of malaria in the first half of pregnancy in women living in endemic malarious areas<sup>5-7</sup>.

Malaria is an important cause of maternal anemia, intrauterine growth retardation, intrauterine death, still birth, premature delivery, low birth weight(LBW), perinatal and neonatal morbidity and mortality and post-partum morbidity<sup>4,8,9</sup>. In Sub-Saharan Africa, poor nutrition, micronutrient

imbalances (especially vitamin A, Zinc, Iron and Folate), HIV co-infection, poverty and limited access to effective primary health care and emergency obstetric services exacerbate the impact of malaria in pregnancy<sup>8</sup>. Women are particular at risk of cerebral malaria, hypoglycemia, pulmonary edema and severe hemolytic anemia. Fetal and perinatal loss has been documented to be as high as 60-70% in non-immune women with malaria<sup>3</sup>. These complications are commoner in primigravidae than multigravidae<sup>3,4</sup>.

The renewed interest in protecting and promoting both maternal and child health has led to the three pronged approach of tackling malaria in pregnancy, namely: intermittent preventive treatment of malaria using an effective antimalarial drug to address the heavy burden of asymptomatic infections among pregnant women living in areas of moderate to high transmission of *Plasmodium falciparum*; the use of insecticide treated nets by all pregnant women and effective case management of malaria illness and anaemia.<sup>10</sup> In 1986, World Health Organization recommended that pregnant women living in malaria endemic areas receive chemoprophylaxis with a safe and effective antimalarial drug as part of routine antenatal care<sup>11</sup>. Although this recommendation was widely adopted as policy across Sub-Saharan Africa, program implementation was often poor or non-existent especially in East-Africa. In 2000, the WHO Expert committee on malaria recommended that malaria control during pregnancy should emphasize a preventive package that included either intermittent preventive treatment or chemoprophylaxis<sup>12</sup>.

Intermittent Preventive Treatment (IPT) involves the administration of therapeutic doses

of an antimalarial drug to a population at risk whether or not they are known to be infected, at specific point intervals usually with the aim of reducing morbidity and mortality<sup>13</sup>. In 2002, WHO developed a strategic framework for the control of malaria during pregnancy in Africa. The document recommends that pregnant women receive at least two doses of Sulphadoxine-Pyrimethamine (SP) as intermittent preventive treatment during the second and third trimesters during the routine antenatal visit while chemoprophylaxis is no longer recommended for a no of reasons including the difficulty in the delivery of this strategy, poor adherence with weekly drug dosing and rising rate of resistance to most of the chemoprophylaxis regimens including chloroquine<sup>14</sup>. Recently, intermittent preventive treatment (IPT) has been shown to be better than malarial chemoprophylaxis and has replaced it<sup>3,4,13</sup>.

The objective of this study was to determine the influence of the use of intermittent preventive treatment of malaria with sulphadoxine-pyrimethamine during pregnancy on the prevalence of malaria in pregnancy and the outcome of pregnancy. The outcome of this would help to strengthen the use of IPT by the pregnant women during routine antenatal care in University Teaching Hospital, Ado-Ekiti.

## **MATERIALS AND METHODS**

The study was a prospective descriptive cross-sectional study carried out at the maternity department of the University Teaching Hospital, Ado-Ekiti, Nigeria over a period of 13 months. Ado-Ekiti is the capital city of Ekiti-State located in the south-western part of Nigeria with a population of about

3,500,000. The people of Ado-Ekiti are mainly Yoruba ethnic group and the city is the commercial nerve centre for farm produce from the neighboring towns and villages of the state, an agrarian state. The University Teaching Hospital, Ado-Ekiti is an emerging teaching hospital that was established about two years ago and it serves as a referral centre for all the specialist and general hospitals in the state. About five thousand women booked annually for antenatal care in the hospital with an average of about 80 attendees at each of the two consultants' clinic day in a week.

The tool for data collection from the respondents was a semi-structured questionnaire which was administered to women in the lying in ward of the maternity department who were booked in the hospital, attended at least two antenatal clinic visits and delivered in the hospital. The questionnaires were administered by house-officers and registrars in the department who had been trained on how to do so. The research instrument had three sections: the first section elicited information about the socio-demographic characteristics of the respondents; the second section inquired about the current antenatal history and the third section about the labour history and the neonatal outcome. About 4200 women who met the inclusion criteria were recruited. The data collected were entered and analyzed with the use of Statistical Package for Social Sciences (SPSS) software, version 15. The results were summarized using relevant descriptive statistics (such as means) and presented using frequency tables and percentages. The association between discrete variables was tested using chi-square test. Statistical significance was accepted at  $p$  value  $< 0.05$ .

## **RESULTS**

About 4200 women recruited for the study participated by filling the questionnaires giving a response rate of 100%.

Table 1 showed that 3136 (74.7%) pregnant women received intermittent preventive treatment while 1064 (25.3%) did not receive this. 2800 women (66.7%) were Christians while 1400 women (33.3%) were Muslims. 2716 (64.7%) of the women were Yoruba, 812 (19.3%) were Igbo and 672 (16%) were Hausas. 504 (12%) pregnant women had no formal education or primary school education while 1092 (26%) and 2604 (62%) women had secondary and tertiary education respectively. 868 (20.7%) women were nulliparous and 3332 (79.3%) were multiparous women. 2744 (65.3%) women belonged to the upper social class, 756 (18%) women were in the middle social class and 700 (16.7%) women belonged to the lower social class.

Of the women that received the IPT drug, 727 (23.1%) of them were nulliparous while 2409 (76.9%) were multiparous. 700 (22.3%) of them received one dose of the drug while 2436 (77.7%) received 2 doses of the IPT drug.

The age range of the respondents was between 19-41 years with a mean age of  $31.19 \pm 4.882$  years, the parity of the women was para 0-4 with a mean parity of  $1.61 \pm 1.095$ , their gestational age at booking ranged from 9-34 weeks with a mean gestational age of  $21.13 \pm 4.562$  weeks and the gestational at presentation in labor was between 32-43 weeks with the age at  $38.83 \pm 1.548$ . The duration of labor was between 5-12 hours with a mean duration of  $8.63 \pm 1.53$  and the birth weight of their babies ranged from 2.2-4.4 with a mean birth weight of  $3.277 \pm 0.4019$ .

Table 2 showed the comparison of the characteristics between the women who received intermittent preventive treatment and the women who did not receive the treatment.

There was no significant difference in the mean age, mean gestational age at booking and delivery between the women that used IPT drug and those who didn't use but there was significant difference in the mean parity, birth weight and duration of labor of the women.

More women who are multiparous with higher educational status and social class and of Yoruba race used IPT during pregnancy in this study and this was found to be statistically significant. However, even though more women who are Christians used the IPT during pregnancy, it was not statistically significant.

Table 3 showed the incidence of malaria in pregnancy among the women who had IPT and those who did not have IPT.

There was a higher incidence of malaria in pregnancy among women who did not receive intermittent preventive treatment of malaria compared to women who used it and this was statistically significant.

Table 4 showed the incidence of malaria in pregnancy among the women who had a single dose of IPT and those women who had double dose of IPT.

Among the women who used IPT and had malaria in pregnancy, the incidence of malaria was higher in those who had a single dose of the drug compared to those had two doses and this was statistically significant.

Table 5 showed the outcome of pregnancy among the women who had IPT and those who did not have during pregnancy.

The mean gestational age at presentation in labor were comparable between the women who received IPT and those women who did not receive even though the women who received IPT presented at a higher gestational age in labor, however this was not statistically significant.

The duration of labor was higher in women who did not use IPT and the birth weight of babies was also lower in them, this was statistically significant.

TABLE 1 showing the sociodemographic characteristics of the pregnant women involved in the study.

Characteristics	Frequency ( n= 4200 )	Percentage (%)
<b>Religion</b>		
Christians	2800	66.7
Muslims	1400	33.3
<b>Tribe</b>		
Yoruba	2716	64.7
Igbo	812	19.3
Hausa	672	16.0
<b>Educational status</b>		
No formal/ Primary	504	12.0
Secondary	1092	26.0
Tertiary	2604	62.0
<b>Parity</b>		
Nulliparous	868	20.7
Multiparous	3332	79.3
<b>Social class</b>		
Upper (I & II)	2744	65.3
Middle	756	18.0
Lower (IV & V)	700	16.7

TABLE 2 showing the comparison of demographic characteristics and obstetric outcome between pregnant women who used IPT and those who did not use IPT.

PARAMETER	YES IPT ( n= 3136)	NO IPT (n=1064)	P value	
Mean Age (years)	31.07±4.751	31.53±5.238	0.09	NS
Mean Parity	1.52±1.094	1.87±1.056	0.0001	S
Mean GA at booking	21.09±4.498	21.26±4.747	0.283	NS
Mean GA at delivery	38.85±1.459	38.76±1.784	0.122	NS
Birth Weight (Kg)	3.263±0.3976	3.138±0.4019	0.0001	S
Duration of labor (hrs)	8.6±1.491	8.7±1.634	0.011	S

GA= Gestational age (weeks); NS= Not Significant; S= Significant

Characteristics	Yes IPT n= 3136		No IPT n= 1064		X <sup>2</sup>	df	P value
	%		%				
<b>Religion</b>							
Christian	2072	74.0	728	26.0	1.974		1
Muslim	1064	76.0	336	24.0	0.610		
(NS)							
<b>Tribe</b>							
Yoruba	2072	76.3	644	23.7	17.77	2	
Igbo	560	69.0	252	31.0	0.0001		
Hausa	504	75.0	168	25.0			(S)
<b>Educational Status of women</b>							
None	243	48.2	261	51.8			
Primary					12.942	2	
Secondary	659	60.3	433	39.7	0.023		
Tertiary	2234	85.8	370	14.2			(S)
<b>Social Class</b>							
I	812	79.3	212	20.7			
II	1216	70.6	504	29.4	27.129	3	
III	530	70.1	226	29.9	0.0001		
IV & V	478	68.3	222	21.7			(S)
<b>Parity of the women</b>							
Nulliparous	727	23.1	142	13.3	46.844	1	
Multiparous	2409	76.9	922	76.7	0.0001		(S)



TABLE 3 showed the prevalence of malaria in pregnancy among women who had IPT and those who did not have IPT.

Characteristics	Yes IPT Use		No IPT Use		X <sup>2</sup>	df	P value
	n= 3136	%	n= 1064	%			
<b>Treated for Malaria ?</b>							
Yes	980	31.3	476	44.7	63.803	1	
No	2156	68.7	588	55.3	0.0001		

(S)

TABLE 4 showed the prevalence of malaria in pregnancy among women who had single dose of IPT and those who had double dose of IPT.

Characteristics	Single dose IPT		Double dose IPT		X <sup>2</sup>	df	P value
	n= 700	%	n= 2436	%			
<b>Treated for Malaria?</b>							
Yes	280	40.0	700	28.7	32.114	1	
No	420	60.0	1736	71.3	0.0001		

(S)

TABLE 5 showed the outcome of pregnancy among women who had IPT and those who did not have IPT.

Mean GA at delivery	38.85±1.459	38.76±1.784	0.122	NS
Duration of labor(hrs)	8.6±1.491	8.7±1.634	0.011	S
Birth Weight(Kg)	3.263±0.3976	3.138±0.4019	0.0001	S

## DISCUSSION

The study revealed that more than two-thirds of the pregnant women studied had access to intermittent preventive treatment of malaria and also that among the women attending the antenatal clinic that received the IPT, more than two-third of them took the recommended dose of the IPT. The high coverage recorded in this study is similar to that reported by Takem et al<sup>14</sup> and Falade et al<sup>15</sup> but higher than that in previous hospital based studies by Nganda et al<sup>16</sup> and Van Ejik et al<sup>17</sup>. This higher coverage was due to the fact that the study was done in the antenatal clinic where the IPT drug was being distributed. It also showed

that the program implementation is improving gradually over time.

There was a low prevalence of malaria among women who used IPT compared to those who did not use it and this was statistically significant. Even among the women that took IPT, those that took two doses of IPT experience a lower prevalence of malaria compared to those who took one of the drug. This demonstrates the efficacy of the IPT drug in improving the outcome of pregnancy. This is comparable to reports by Takem et al<sup>14</sup> and Mbonye et al<sup>18</sup> who reported a reduction in the prevalence of peripheral parasitaemia and parasite density among women of all parities.

The increased use of intermittent preventive treatment with increasing level of education found in this study is not surprising since those with higher educational status are more health conscious and can easily apply health education programs to their daily living. This finding is consistent with report in previous studies by Takem et al<sup>14</sup> and Marchant et al<sup>19</sup>. Women in the higher social class and parity were also associated with greater tendency to use the IPT drug. This is because women in that class tend to know and appreciate the benefits of the preventive measures against malaria in pregnancy like the use of insecticide treated nets and IPT amongst others. The multiparous women comply more with antenatal instructions including the use of prescribed drugs compared to the primigravid women since they would have discovered the benefits associated with these drugs in their previous pregnancies (experienced they say is the best teacher). This is similar to finding earlier reported by Marchant et al<sup>19</sup> but not consistent with that of Takem et al<sup>14</sup> who reported that the effect of socioeconomic status on IPT use was close to null in their studies.

Women who received IPT during pregnancy had better outcome of their pregnancy. Low birth weight and prematurity are the greatest risk factors for neonatal mortality and a major contribution to infant mortality<sup>15</sup>. In this study, babies born to mothers who received IPT-SP on the average weighed more than babies born to women who did not receive the drug. They also had shorter duration of labor which reduced the length of time the babies had to be exposed to the stress of labor. The birth weight of babies in this study may not only be attributed to the low prevalence of malaria in pregnancy in them since other factors such as socio-economic level may also play a role but this was statistically significant. This is similar to reports from Falade et al<sup>15</sup>, Mbonye et al<sup>18</sup> and Shulman et al<sup>20</sup>.

High educational status and gravidity are associated with the use of intermittent preventive treatment among pregnant women attending antenatal clinic in this study and that use of Sulphadoxine-Pyrimethamine as intermittent preventive treatment of malaria during pregnancy has been accepted by women as a malaria control strategy in pregnancy. However, much work still need to be done to improve the uptake of the drug especially among the pregnant women who are of low educational status and low parity, who incidentally had a higher prevalence of malaria in this study and to ensure that all pregnant women received the recommended two doses of the IPT during pregnancy.

The results from the study also showed that intermittent preventive treatment of malaria during pregnancy with Sulphadoxine-Pyrimethamine is beneficial in improving pregnancy outcome and is widely used by the pregnant women attending antenatal clinic

though not optimal. Therefore, health education programs for pregnant women in this area should be intensified in women of low educational status and low parity to improve the uptake among them. Direct observation therapy system (DOTS) can also be employed in which the pregnant women would be asked to take the drug under the observation of the nurses in the antenatal clinic. This would help in improving the compliance rate since some women may get the drug and not use it.

Government should be encouraged to make the drug available in the various antenatal clinics to be distributed to pregnant women free or at subsidized rate as part of efforts at improving maternal and child health in order to achieve the millennium development goals (MDGS) 4 and 5.

## REFERENCES

1. Asa, O.O., Onayande, A.A., Fatusi, A.O., Ijadunola, K.T., Abiona, T.C. Efficacy of Intermittent Preventive Treatment of Malaria with sulphadoxine-Pyrimethamine in preventing Anaemia in Pregnancy among Nigeria women. *Matern Child Health J.* 2008; 12: 692-698.
2. Desai, M., Terkuili, F.O., Nosten, F., McGready R., Asamoah, K., Brabin, B., Newman, R.D. Epidemiology and burden of Malaria in Pregnancy. *Lancet Infect Dis* 2007; 7(7): 93-104.
3. Shulum, C.E., Dorman, E.K. Importance and prevention of Malaria in Pregnancy. *Trans R. Soc Trop Med Hyg* 2003; 97(1): 39-35.
4. Nwonwu, E. U., Ibekwe, P.C., Ugwu, J. I., Obarezi, H. C, Nwangbara, O.C. Prevalence of Malaria Parasitaemia and Malaria related Anaemia among Pregnant Women in Abakaliki, South-East Nigeria. *Nig Journ of*

- Clinical Practice 2009; 12(2): 182-186.
5. Okonofua, F.E. Malaria in Pregnancy in Africa. A review. *Nig J. Med.* 1991(suppl): 59-62.
  6. Schulman, C.E., Dorman, E.K. Intermittent Sulphadoxine-Pyrimethamine to prevent Severe Anaemia secondary to Malaria in Pregnancy: a randomized placebo-controlled trial. *Lancet* 1999; 353: 632-636.
  7. Okwu, O.O. The status of Malaria among Pregnant Women: a study in Lagos, Nigeria. *Afr J Reprod Health*, 2003; 7(3): 77-83.
  8. Valley, A., Valley, L., Changalucha, J., Greenwood, B., Chandramohan, D. Intermittent Preventive Treatment for Malaria in Pregnancy in Africa: What's new, what's needed? *Malaria Journal* 2007; 6:16.
  9. Guyatt, T.K., Snow, R.W. The epidemiology and burden of Plasmodium Falciparum related Anemia among Pregnant Women in Sub-Saharan Africa. *Am. J. of Tropical Med and Hygiene* 2001; 64: 36-44.
  10. Oyibo, W.A., Agomo, C. A. Scaling up of Intermittent Preventive Treatment of malaria in Pregnancy using Sulphadoxine-Pyrimethamine: Prospects and Challenges. *Matern Child Health J* 2010;
  11. WHO. WHO Expert Committee on Malaria. 18<sup>th</sup> Report. Who Health Organization 1986. Geneva, Switzerland.
  12. WHO. WHO Expert Committee on Malaria. 20<sup>th</sup> Report. Technical Report Series 892. World Health Organization, Geneva, Switzerland.
  13. Newman, R. D., Moran, A.C., Kayenta, K., Benga-De, E., Yameogo, M., Gaye, O., Faye, O., Lo Y., MarcMoreira, P., Doumbo, O., Parise, M.E., Steketee, R.W. Prevention of Malaria during Pregnancy in West-Africa. Policy change and the power of sub-regional action. *Euro Journ of Trop Med and Int Health* 2006; 11(4): 462-469.
  14. Takem, E.N., Achidi, E.A., Ndumbe, P.M. Use of Intermittent Preventive Treatment for Malaria by Pregnant Women in Beua, Cameroun. *Acta Tropica* 2009; 112: 54-58.
  15. Falade, C. O., Yusuf, B.O., Fadero, I.F., Mokoulu, O.A., Hamer, D.A., Salako, L.A. Intermittent Preventive Treatment with Sulphadoxine-Pyrimethamine is effective in preventing maternal and placental malaria in Ibadan, South-West Nigeria. *Malar. J.* 2007; 6:88.
  16. Nganda, R.Y., Drakeley, C., Reyburn., Marchant, T. Knowledge of Malaria influences the use of Insecticide Treated Nets but not Intermittent Preventive Treatment of Malaria by Pregnant women in Tanzania. *Malar J.* 2004; 3: 42.
  17. Van Ejik, A.M., Ayisi, J.G., Ter kuile, F.O., Slutsker, L., Otieno, J.O., Misore, A.O., Odondi, J.O., Rosem, D.A., Kager, P.A., Steketee, R.W., Nahlen., B.L. 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 : 351-360.
  18. Mbonye, A.K., Bygbjerg, I.B., Magnussen, P. Intermittent Preventive Treatment of Malaria in Pregnancy: a community based delivery-system and its effects on parasitaemia, anaemia and low birth weight in Uganda. *Int. J of infectious Dis.* 2008; 2: 22-29.
  19. Marchant, T., Nathan, R., Jones, C., Mponda, H., Bruce, J., Sedekia, Y., Schellenberg, J., Mshinda, H., Hanson, K. Individual, facility and policy level influences on national



coverage estimates for Intermittent Preventive Treatment of Malaria in Pregnancy in Tanzania. *Malar J.* 2008; 7: 260.

20. Shulman, C. E., Marshall, T., Dorman, E.K., Bulmer, J.N., Cutts, E., Peshu, N. Malaria in Pregnancy: adverse effects on Haemoglobin levels and birth weight in Primigravidae and Multigravidae. *Trop. Med. Int. Health* 2001; 6: 770-778.