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In vivo efficacy of sulphadoxine-pyrimethamine for the treatment of asymptomatic parasitemia in pregnant women presenting for first antenatal care visit in Malawi.

By

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Abstract

In vivo efficacy of sulphadoxine-pyrimethamine for the treatment of asymptomatic parasitemia in pregnant women presenting for first antenatal care visit in Malawi.

By Joseph Abdallah

Background: *In vivo* assessments of anti-malarial treatments for pregnant women are important to ensure continued efficacy, especially in areas of increasing drug resistance.

Methods: Pregnant women with asymptomatic parasitemia who presented at the Machinga District Hospital, Malawi were treated with Sulphadoxine-pyrimethamine (SP) and followed for 42 days to determine the drug efficacy. Eligible participants were between 16 and 26 weeks gestation, eligible to take SP in the antenatal clinic on day of enrollment, had asymptomatic malaria defined as a parasitemia of 2,000-200,000/µl asexual forms and an axillary temperature below 37.5°C. Study endpoint was parasite-free status after the follow-up period. Survival analyses were performed to assess the effect of gravidity on the efficacy of SP treatment. The results of analyses were expressed as hazard ratios (HRs) accompanied by the corresponding 95% confidence intervals (CIs)

Results: Two hundred and forty five pregnant women were included in the analysis [245 = intention to treat (ITT); 150 = per-protocol (PP)]. The overall cure rate (adequate clinical and parasitologic response, ACPR) in the PP analysis was 42% (34.0-50.3) and the corresponding estimate in the ITT analysis was 25.7% (20.4-31.7). Multivariable analysis revealed statistically significant interaction between age and gravidity. Among women younger than 21 years, primigravid women were more likely to have parasitemia at study end when compared to multigravid women (Hazard Ratio (HR) =5.06; 95% CI, 1.76-14.57). By contrast, the corresponding analysis for older women demonstrated an no statistically significant association with a Hazard Ratio (HR) of 0.24 and a 95% CI between 0.04 and 1.34. Similarly, bed net use was associated with a statistically significant decrease in parasitemia among women over 20 years of age (HR=0.35; 95% CI, 0.14-0.86), but not in younger women (HR=1.05; 95% CI, 0.51-2.16).

Conclusion: SP efficacy may be waning in Malawi. The lower failure rate in multigravid women suggests that further SP efficacy studies should focus on the protection for primigravid women. Age-specific effect modification should be explored further.

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BACKGROUND

Malaria is an acute febrile illness that causes 225 million clinical infections and approximately 800 thousand deaths per year, mostly in sub-Saharan Africa [1]. The disease is caused by the *Plasmodium* parasite transmitted by an infective bite of the female *Anopheles* mosquito. When an infected mosquito bites a human, it injects the infective form of the parasite, the sporozoite, from the mosquito salivary gland into the blood stream. The sporozoites then travel to and penetrate the liver cells. Once in the liver cells, the sporozoites mature into liver-stage schizonts and rupture the liver cells, releasing merozoites into bloodstream. Merozoites are responsible for the clinical manifestations of malaria. Clinical manifestations of uncomplicated malaria include, but are not limited to, fever, headache, malaise, chills, sweats, aches, and vomiting [2].

Approximately 25 million women live in malarious areas and are at risk for infection during pregnancy [3]. It has been estimated that the prevalence of malaria infection during pregnancy in Africa is 27.6% [4]. In both low and high transmission areas, primigravid and secundigravid women are at an increased risk for infection and elevated parasitemia compared to pregnant women of higher gravidities or non-pregnant women [3, 5]. Malaria in pregnancy has been associated with severe maternal anemia, placental malaria, low birth weight, maternal mortality and infant morbidity and mortality [4].

To reduce the risks associated with malaria among pregnant women living in high transmission settings, the World Health Organization (WHO) recommends a package of interventions, which includes the use of insecticide-treated nets (ITNs), intermittent

preventive treatment with sulphadoxine-pyrimethamine (IPTp-SP) and medical management of malaria and anemia [6]. IPTp-SP has been shown to reduce third-trimester maternal anemia, placental parasitemia, and the prevalence of low birth weight infants [7-9]. IPTp has been shown to be of greater benefit to primigravid compared to multigravid women, with no statistically significant benefit among the multigravidae in several studies [14,15].

IPTp-SP requires delivery of at least two doses of SP not less than a month apart administered to HIV negative women in the second and third trimesters of pregnancy.

SP delivery schedules vary by country. In Malawi the policy is to give SP at the first visit after quickening (about 20 weeks) and again during the third trimester, with a total of two doses during pregnancy regardless of prior pregnancies [10].

Due to increasing resistance to SP, it is no longer used as a treatment for symptomatic malaria, however, there is evidence that IPTp-SP remains effective even in areas with SP resistance, and thus, it is still recommended as a preventative measure [8, 9, 11]. However, newer data from Tanzania and Malawi indicate that the advantageous effects of IPTp-SP are negated by increased resistance to SP [12-14].

As resistance to SP increases, it is important to continue to monitor the efficacy of IPTp-SP to determine when this intervention is no longer providing any benefit. As pregnancy specific anti-parasitic immunity increases with each pregnancy, it is important to account for this by assessing the efficacy of IPTp-SP separately among primigravid and multigravid women. The purpose of this study is to test the efficacy of IPTp-SP in an area of Malawi with a high level of resistance to SP, and to determine how gravidity affects IPTp-SP efficacy.

METHODS

Study design and study site

This is a retrospective analysis of clinical, epidemiological, and genetic data, which were collected during the prospective *in vivo* arm of the "Assessment of the efficacy and effectiveness of sulphadoxine-pyrimethamine for intermittent preventive treatment of malaria in pregnancy in Malawi" study (IPTp study). The study was conducted by the US Centers for Disease Control and Prevention (CDC) in 2010.

Study population

Pregnant women were enrolled in the study if they: 1) presented at the Machinga District Hospital, Malawi between 16 and 26 weeks gestation (based on last menstrual period (LMP) or quickening); 2) were eligible to take SP in the antenatal clinic on the day of enrollment; 3) had asymptomatic malaria defined as a parasitemia of 2,000-200,000/µl asexual forms and an axillary temperature below 37.5°C; 4) had a viable fetus documented by detection of a fetal heartbeat or fetal movement; and 5) provided informed consent for participation. Women were excluded if they had a history of a hypersensitivity reaction to SP or components of SP, had received antimalarials or antibiotics with antimalarial activity in the past month, or had known HIV infection. At the time of enrollment, basic demographic and clinical information was recorded on a standardized data collection form. Women were given three tablets of SP (1 tablet = 25 mg of pyrimethamine and 500 mg of sulfadoxine) under direct observation; those who vomited within 30 minutes were redosed according to WHO guidelines [15]. All women without a history of hypersensitivity reaction to SP or components of SP or axillary

temperature ≥37.5°C, regardless of their decision to participate in this study, were offered the IPTp regimen as per WHO recommendation: one follow up dose of SP after gestational week 16, a second follow up dose in the third trimester.

Follow-up

Women were seen at seven-day intervals from enrollment until day 42 as well as on any other day if they presented with fever or other complaints. At each visit, study participants were asked about the presence of any symptoms of malaria, an axillary temperature was measured, and patients were tested for hemoglobin (Hb) levels (by Hemocue) and parasitemia (by microscopy of thick blood smear). All blood smears were read by two microscopists who were unaware of the other's reading; if there was a greater than 10% discordance in recorded parasite density between the two readings, a third microscopist read the slide to determine the final result. Parasite density was determined by counting the number of parasites against 200 white blood cells; 100 high power fields were reviewed before calling a slide negative.

Outcomes

Therapeutic efficacy was assessed by clinical and parasitological outcomes during the 42-day follow-up using modified WHO definitions. Clinical failure was defined as presence of parasitemia on any day between day 4 and day 42 with axillary temperature ≥ 37.5 °C in patients who did not previously meet any of the criteria for parasitologic failure. Parasitologic failure was defined as the presence of parasitemia on any day between day 7 and day 42 with axillary temperature <37.5 °C in patients who did not

previously meet any of the criteria of clinical failure. Participants who were aparasitemic throughout the 42-day follow-up, irrespective of axillary temperature, and did not reach other study outcomes were classified as having adequate clinical and parasitological response (ACPR). Patients who took an antimalarial during the course of the study were classified as protocol violations and were censored on the day of the last study visit prior to taking the drug.

Statistical analysis

The primary study outcome was the proportion of women with asymptomatic malaria parasitemia at the time of their first antenatal care (ANC) visit who remained free of parasites for the duration of the study, 42-day follow-up, following treatment with SP. The effect of gravidity on the efficacy of treatment of asymptomatic malaria in pregnancy with SP was also assessed. Data cleaning and analysis were performed using SAS software version 9.2 (SAS, Inc., Research Triangle Institute, Research Triangle Park, N.C.). De-identified epidemiological and clinical data from 245 patients who were *P. falciparum* smear positive on the day of enrollment were used for this analysis. Baseline characteristics of participants were examined and compared by gravidity status. Differences in the distributions of categorical variables between primigravidae and multigravidae were tested using the chi-square or Fisher's exact tests (for tables with at least one cell containing an expected value less than five). Differences between continuous variables were tested using the Wilcoxon-Mann-Whitney test.

Unadjusted analyses of the association between treatment failure and various patient characteristics were conducted using simple Cox proportional hazard models and

by constructing Kaplan-Meier survival curves. The results of Cox model-based analyses were expressed as crude hazard ratios (HR) with 95% confidence intervals (CI). The statistical significance of Kaplan Meier survival curves were tested with log-rank tests. Multivariable Cox proportional hazards models were used to calculate adjusted HRs and 95% CIs reflecting the association between treatment failure and gravidity after controlling for covariates. All models were tested for the proportional hazards assumption for each variable. In addition all models were examined for collinearity and interactions.

All enrolled women meeting the inclusion criteria who presented for at least 1 follow-up visit were included in the intention-to-treat analysis, while only women meeting a defined study end-point (failure or ACPR at 42 days) were included in the per-protocol analysis. The outcome (dependent variable) in these analyses was time to treatment failure while the main exposure variable was gravidity (primigravid compared to multigravidae). The covariates (i.e. possible confounders and effect modifiers) included age, log parasite density, bednet use, number of schooling years, and wealth status perception.

Age was dichotomized at the median value as >20 yrs or to ≤ 20 yrs to provide sufficient numbers for analysis across gravidity groups. The parasite density variable was skewed and for this reason it was normalized by log transformation (log[parasites/mL]). Bed net use variable was dichotomized as “used last night” compared to the combined category of “sometimes or no use”. The number of years of schooling was treated as a continuous variable. Lastly, wealth status perception was dichotomized as “average and above” compared to “below average.”

RESULTS

Study population

Two hundred and forty five women were enrolled and determined to be eligible for inclusion in the intention to treat (ITT) analysis. Of those, 114 (46.5%) were primigravid and 131 (53.4%) were multigravid (Figure 1, Table 1). Forty-six participants were lost to follow-up and 49 received SP before completing the study (protocol violation). Thus, 150 women completed the study per protocol and were included in the per protocol (PP) analysis. Baseline characteristics of the study participants are shown in Table 1 and compared by gravidity. Most primigravid participants (56.1%) were 18 years old or younger compared to only 4.6% of multigravid participants. Relative to primigravidae, multigravidae were statistically significantly older, more educated, more likely to have used a bednet the previous night, had higher gestational age, lower parasite density, higher hemoglobin level, and were less likely to be anemic. Almost all of the participants lived in rural areas (244; 99.6%). There were no significant differences between primigravidae and multigravidae with respect to key assets that are used as indicators of socio-economic status. Among multigravid women, 92.4% did not have electricity, 85.5% had earth or sand floors in their houses, and 80.2% had that or grass roofs. Among primigravid women, 95.6% had no electricity, 89.5% had earth or sand floors in their houses, and 80.7% had that or grass roofs.

Efficacy of IPTp-SP

In ITT analysis, the cure rate (ACPR) was 25.7% (20.4-31.7). Clinical failures were seen in 0.8% (0.01-2.9) and parasitologic failures in 34.7% (28.8-41.0). In PP analysis, the cure rate (ACPR) was 42% (34.0-50.3). Clinical failures were seen in 1.3% (0.2-4.7) and parasitologic failures in 56.7% (48.3-64.7) (Table 2).

Gravidity and IPTp-SP

Unadjusted estimates (Table 3, Figure 2) reveal that primigravidae were twice as likely to develop parasitemia than multigravidae (HR= 2.02; 95% CI, 1.32-3.10). Additionally, pregnant women who used a bed net the previous night were less likely to have parasitemia at study end when compared to those who had not used a bednet on the previous night (HR=0.54, 95% CI, 0.33-0.89). The association between woman's age and parasitemia at study end was not statistically significant (HR=0.71; 95% CI, 0.46-1.09).

In the multivariable analyses there was statistically significant interaction between age and gravidity (Table 4). For this reason all adjusted analyses are presented stratified on age (Table 5). Among women 20 years old or younger, primigravidae were more likely to have parasitemia at study end when compared to multigravidae (HR=5.06; 95% CI, 1.76-14.57). In contrast, in women older than 20 years, there was no significant difference in the hazard ratio for parasitemia between older and younger women (HR= 0.24; 95% CI, 0.04-1.34). Bed net use was associated with a statistically significant decrease in parasitemia among women over 20 years of age (HR=0.35; 95% CI, 0.14-0.86), but not in younger women (HR=1.05; 95% CI, 0.51-2.16).

DISCUSSION

Our study findings indicate that different risk factors affect anti-malaria treatment failure differentially depending on age. We found the effect of gravidity on SP efficacy, defined as the absence of parasites at the end of the 42-day follow-up, is clearly present but only among women who are under the age of 21 years. Conversely, when women were above 20 years of age, the association between gravidity and treatment failure was more pronounced in primigravid women compared to multigravid women, although not statistically significant. Other studies have found that multigravidae are relatively protected from malaria compared to primigravidae. This is expected as multigravidae have pregnancy specific anti-parasitic immunity which helps to clear the parasites. Bed net use, in conjunction with IPTp-SP, has been shown to protect against parasitemia in a previous study in Malawi [12]. Data from our study show that the previously described protective effect of bed net use is maintained, but only among women who are beyond 20 years of age. The lack of protection in younger women is unexpected and requires further exploration.

The main strength of our study is that it provides needed data on SP efficacy in an area of increasing resistance to SP. The prospective collection of this data is also a strength of this study. Additionally, use of multivariable models and, in particular, systematic evaluation of effect modification, which is not commonly performed in this area of research. One previous study utilized multivariate analysis and assessed effect modification in the context of delivery outcomes or resistance to SP in cohort of pregnant women in Muheza, Tanzania [13]. The effect of IPTp on birth weight was stronger

among infants born to women with pregnancy malaria (PM) compared with infants born to PM-negative women; however, there was no effect modification by parity. The study also showed that IPTp-SP did not improve overall pregnancy outcomes [13].

This study has several weaknesses. The study included only a single arm, as it would not be ethical to withhold treatment from a parasitemic pregnant woman. Without a randomized control group that did not receive SP, estimates of treatment failure could only be compared between groups characterized by gravidity where everyone in each group received SP. Another weakness is the very high rate of loss to follow-up and protocol violations resulted in a sample size (150 women in the per protocol group) that fell short of the targeted enrollment of 179.

The proportions of patients who violated the protocol or withdrew from the study present potential sources of selection bias whereby comparison groups may not be relatively equal across most, if not all, measureable and unmeasurable characteristics, granted this was not a randomized control trial with a true placebo group. For example, women who felt ill would have been more likely to self-treat; this group would have been more likely to have malaria but would have been preferentially excluded from analysis.

This study suggests that although SP may initially clear parasites, it is not effective at suppressing parasitemia for the long term. Although additional investigation still needs to be done to determine if the recurrent parasitemias were due to reinfection with a new strain of parasite, or recrudescence of the initial strain (suggesting drug failure), given the extremely high failure rate, it is likely that even after adjusting for new infections, SP is falling well below the threshold set by WHO for an effective antimalarial drug. For treatment drugs, WHO recommends that countries consider adopting a new drug once the

drug failure rate is 10%. In both primi and multi-gravidae in this study, failures were well above this threshold. This study also demonstrates that primigravidae remain much more susceptible to malaria, and suggests that SP no longer provides sufficient protection for these vulnerable women. New methods of preventing malaria in pregnancy must be identified in order to prevent the adverse outcomes associate with malaria in pregnancy.

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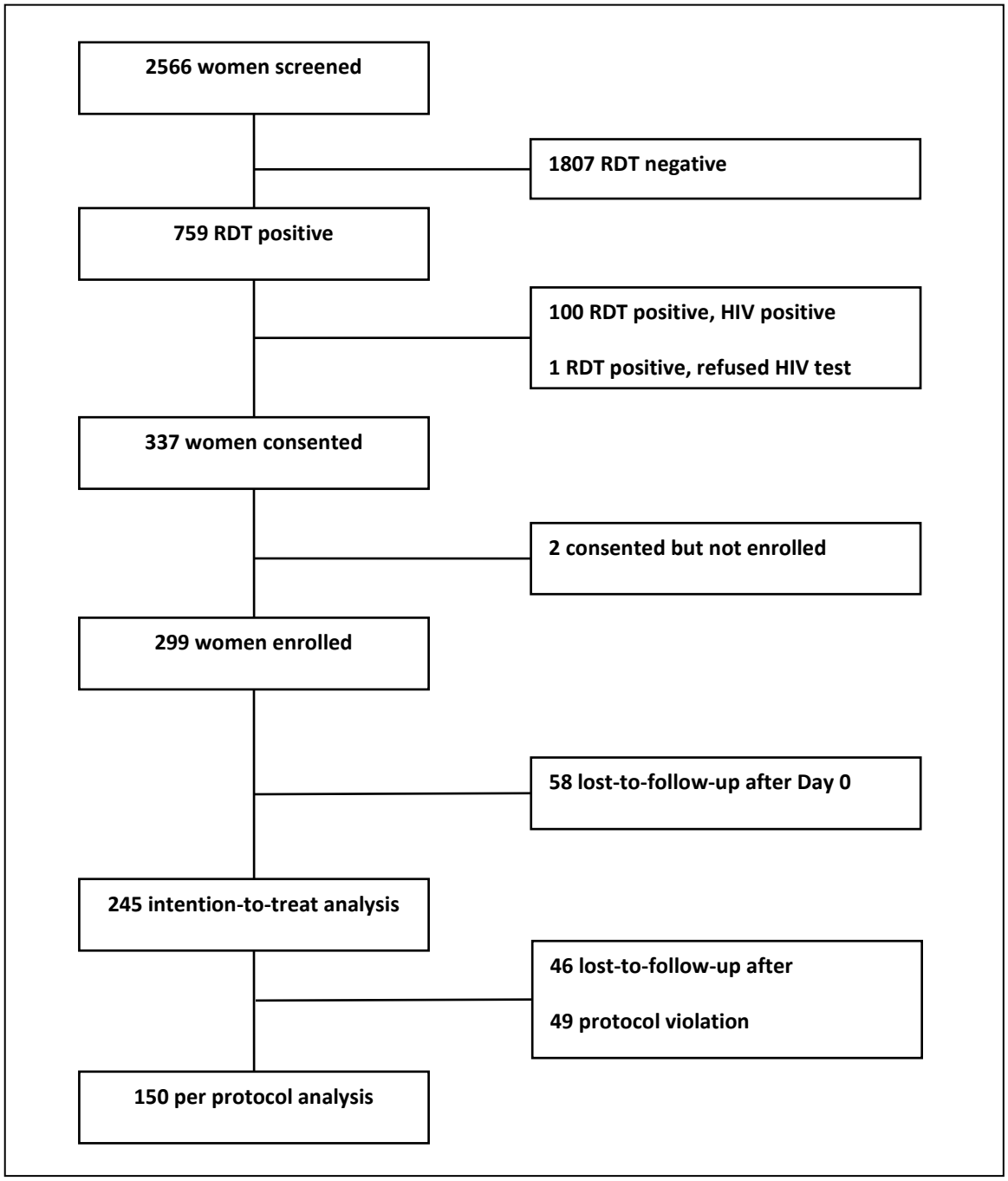
TABLES AND FIGURES

Figure 1: Enrollment flow diagram for Sulfadoxine-Pyrimethamine (SP) in vivo efficacy study*



*Study included pregnant women with asymptomatic malaria parasitemia attending antenatal care for their first dose of intermittent preventive treatment in pregnancy with SP (IPTp-SP), Machinga District Hospital, Malawi 2009-2010.

Table 1. Baseline characteristics of enrolled patients, stratified by gravidity

	Total (N=245)	Primigravid (N=114)	Multigravid (N=131)	p-value
Age (years)				
≤ 20	54.3%	87.7%	25.2%	< .0001
> 20	45.7%	12.3%	74.8%	
Education (years), mean (range)*	6.2 (0 - 12)	6.9 (0 - 12)	5.6 (0 - 12)	0.0005
Wealth status perception*				
Below Average	78.0%	81.6%	74.6%	0.1911
Average and Above	2.1%	18.4%	25.4%	
Bednet Use*				
Never or Sometimes Use	67.8%	78.1%	58.8%	0.0013
Used Last Night	32.2%	21.9%	41.2%	
Maternal weight (kg), median (IQR) *	55 (50 - 60)	54 (50 - 60)	55 (50 - 60)	0.3341
Gestational age at enrollment (weeks), median (IQR)	20 (18 - 24)	20 (17 - 23)	21 (18 - 24)	0.0027
Hb at enrollment, median (IQR)	9.9 (9.0 - 10.7)	9.2 (8.6 - 10.0)	10.5 (9.6 - 11.3)	<.0001
Percent of women with anemia (Hb <11) at enrollment	81.2%	55.3%	44.7%	<.0001
Percent of women with gametocytemia	0.42%	0.0%	0.008%	-
Parasite Density (parasites/ml), geometric mean (range)	208 (32 - 12800)	304 (48 - 12800)	176 (32 - 8960)	<.0001

IQR – interquartile range

*Indicates variables with <2% missing values.

Table 2. Main Outcomes

			Confidence Interval				Confidence Interval	
	Per-Protocol (N=150)	**(%)**	**Lower Limit**	**Upper Limit**	**Intention-To-Treat (N=245)**	**(%)**	**Lower Limit**	**Upper Limit**
Clinical Failure (par + fever)	2	1.33	0.16	4.73	2	0.82	0.01	2.92
Parasitologic Failure (w/o fever)	85	56.67	48.34	64.73	85	34.69	28.75	41.02
Appropriate Clinical and Parasitic Response	63	42	34.00	50.32	63	25.71	20.36	31.66
Loss to follow-up (parasite negative)	-	-	-	-	95	38.78	32.64	45.19

Figure 2: Kaplan-Meier curves comparing treatment failure in primigravid and multigravid study participants

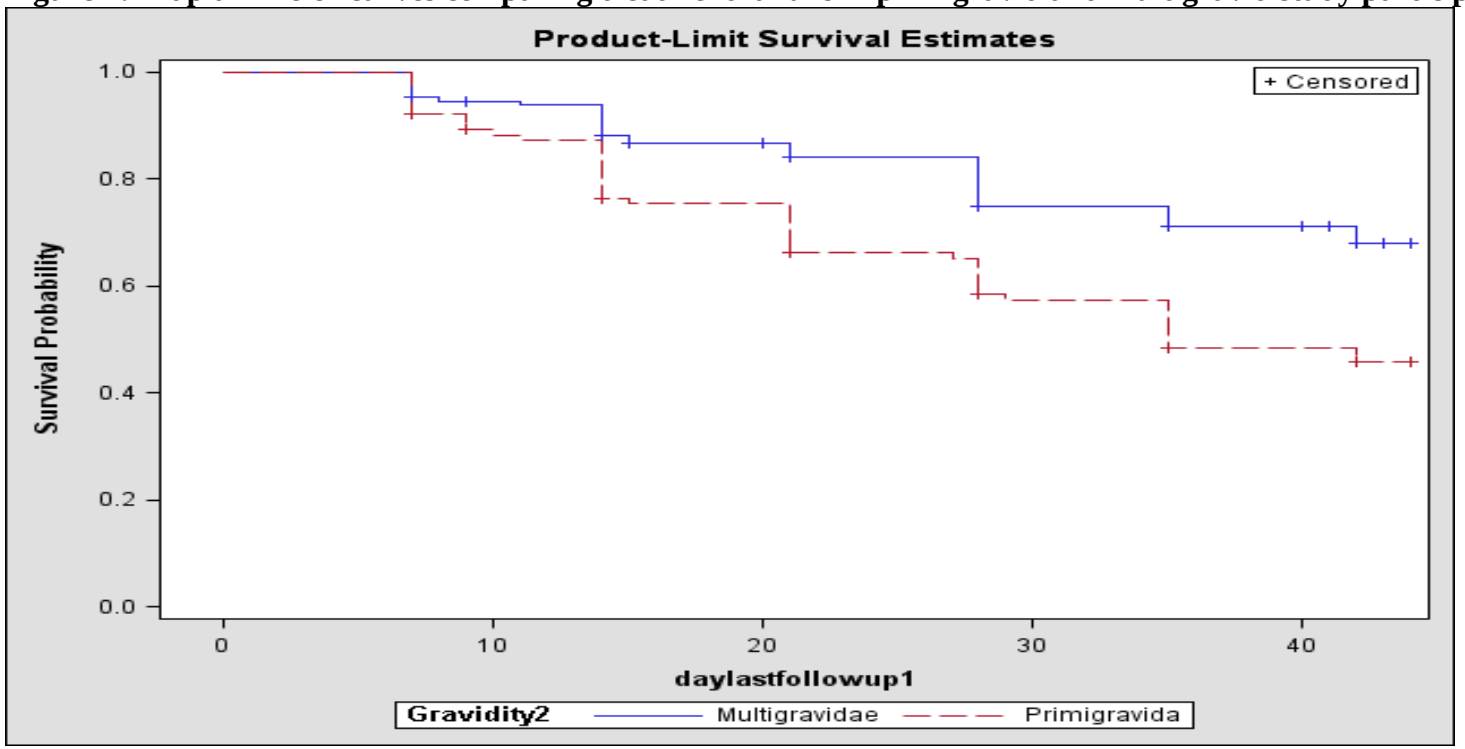


Table 3. ITT unadjusted analysis of the association between treatment failure and various subject characteristics

Subject Characteristics *	**HR**	**Confidence Interval**		**p-value**
Gravidity: Comparing Primigravid to Multigravidae	2.02	1.32	3.10	0.0013
Age: Comparing >20 years to ≤ 20 years	0.71	0.46	1.09	0.1200
Log Parasite Density (Continuous)	0.95	0.78	1.16	0.6135
Net use: Comparing used last night to sometimes or never use	0.54	0.33	0.89	0.0151
Schooling years (Continuous)	1.02	0.95	1.09	0.6830
Wealth Status: Comparing average and above to below average	1.14	0.70	1.86	0.5998

Table 4. ITT Multivariable analysis of the association between treatment failure and various subject characteristics: Full model

Subject Characteristics	HR	Confidence Interval		p-value
Gravidity: Comparing Primigravidae to Multigravidae	5.27	1.83	15.22	0.0021
Age: Comparing >20 years to ≤ 20 years	2.61	0.88	7.73	0.0825
Log Parasite Density (Continuous)	0.85	0.68	1.06	0.1424
Net use: Comparing used last night to sometimes or never use	0.72	0.41	1.25	0.2412
Interaction term: Age*Gravidity	0.09	0.01	0.60	0.0133

Table 5. ITT multivariable analysis of the association between treatment failure and various subject characteristics: Stratified on age

	20 years old or younger				Older than 20 years			
	HR	**Confidence Interval**		**p-value**	**HR**	**Confidence Interval**		**p-value**
Gravidity: Comparing Primigravidae to Multigravidae	5.06	1.76	14.57	0.0027	0.24	0.04	1.34	0.1047
Log Parasite Density (Continuous)	0.87	0.67	1.13	0.3062	0.66	0.41	1.06	0.0869
Net use: Comparing used last night to sometimes or never use	1.05	0.51	2.16	0.9006	0.35	0.14	0.86	0.0224