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Chapter 4  In-vivo parasitological response to sulfadoxine-pyrimethamine in pregnant women in southern Malawi.

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IN-VIVO PARASITOLOGICAL RESPONSE TO SULFADOXINE-PYRIMETHAMINE IN PREGNANT WOMEN IN SOUTHERN MALAWI.

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ABSTRACT

Malaria in pregnancy is a significant cause of maternal and infant morbidity and mortality. Malawi adopted intermittent preventive treatment with sulfadoxine-pyrimethamine (SP) for the control of malaria in pregnancy in 1993. We conducted a cross-sectional and prospective cohort study of women attending an antenatal care clinic at Montfort Hospital in the Lower Shire Valley, Malawi in order to determine the prevalence of malaria and anaemia at the first antenatal visit, and the rate of parasitological failure to SP in pregnancy.

Of 961 pregnant women screened, 9% were parasitaemic, 77% had mild anaemia (Hb <11.0g/dl), 24% moderate anaemia and 6% had severe anaemia (<7.0g/dl). Malaria parasitaemia was significantly more frequent in primigravidae, the second trimester and in the post-rainy season (all p <0.05). Moderate anaemia (Hb ≤ 9.0g/dl) was significantly more common in adolescents and primigravidae (both p <0.05). In the in vivo follow up study, 6 of 77 women (7.8%) had parasitological failure at day 7, 8 of 73 (10.9%) women who completed the day 14 follow up had parasitological failure, and at day 28, 12 of 61 (19.7%). Parasitological failures were all R1 category except for one participant with R2 failure.

Anaemia prevalence was high at first antenatal visit. Parasitological failure to SP in pregnant women increased in this area from 5% in 1996 to 19.7% in 2004.
INTRODUCTION

In Malawi, anti-malarial chemoprophylaxis during pregnancy has been the primary strategy for malaria control in pregnancy for many years with chloroquine initially introduced in 1987. In 1993 sulfadoxine- pyrimethamine (SP) replaced chloroquine with a change from weekly chloroquine prophylaxis to intermittent preventive treatment (IPTp-SP) (Verhoeff et al. 1997, Schultz et al. 1994).

Since that time surveillance data in children with uncomplicated clinical malaria indicated changing prevalence of malaria resistance to SP, from less than 5% in 1993 (Bloland et al. 1993, Nwanyanwu et al. 1996) to 14-20% in 2000 (Nwanyanwu et al. 2000, Takechi et al. 2001, MacArthur et al. 2001, Sulo et al. 2002, Plowe et al. 2004). It is uncertain whether treatment failure estimates in children indicate comparable risk of parasitological failure in pregnant women and information on the rate of SP treatment failure in pregnant women with peripheral parasitaemia is currently not available, although SP continues to be recommended for IPTp in 2010. The primary aim of the present study was to determine the rate of parasitological failure after SP treatment in pregnant women.

MATERIALS AND METHODS

Study location

The study was conducted at Montfort Hospital in Chikwawa District in the lower Shire Valley-southern Malawi. This is a hot, dry, rural area whose population is mainly engaged in subsistence farming of maize, sorghum, sugar cane and cotton. This area lies between 100 and 300m above sea level and the rainy season extends from December to March. Anopheles gambiae, Anopheles funestus and Anopheles arabiensis are the main malaria vectors (Spiers et al. 2002).

Enrolment

Women who attended the antenatal clinic at Montfort Hospital from June, 2004 to February, 2005 were enrolled at their first antenatal visit after written informed consent was obtained. After receiving routine antenatal care, consenting women were interviewed for information on age, gravida, parity, history of fever, headache and use of antimalarials in the preceding two weeks. Gestation was estimated by abdominal palpation. A sub-sample of women with P. falciparum infection were included in the in vivo follow-up study if more than 14 weeks and less than 37 weeks gestation, had a haemoglobin concentration over 8.0 g/dl, single infection with P. falciparum and no history of taking antimalarials in the previous 2 weeks. All women received SP by directly observed therapy (sulfadoxine 500 mg, pyrimethamine 25 mg) and haematinics supplements (200 mg ferrous sulphate, 250 µg folic acid) according to Malawi Government malaria and anaemia control policies.

Follow up

The WHO 2001 guidelines for in vivo studies of malaria drug resistance were followed with follow-up at days 3, 7, 14, 21 and 28. At each visit, information was collected on history of fever, headache or use of other antimalarial drugs. A finger prick blood sample was collected for a malaria thick smear and haemoglobin estimation (HemoCue, Angelholm, Sweden). Quinine was prescribed to women with parasitological failure.
Laboratory tests
Thick films were stained with Field's stain. Parasite density was estimated by parasite counts per 200 white blood cells (WBC) (Trape, 1985) and geometric mean parasite densities (GMPD) assuming a white cell count of 8000/µL blood.

Definitions
Anaemia was defined as a haemoglobin concentration <11.0 g/dl, moderate anaemia as 7.0-8.9 g/dl, and severe anaemia as < 7.0 g/dl (Stoltzfus, 1997). Parasitological response was classified as a success with parasite clearance by day 7 with a negative thick smear on all subsequent follow-up days. Parasitological failure was defined as parasitaemia on or after day 7. Parasitological failure was sub-classified as R1, R2 or R3 (WHO, 2001):
- **R1:** Clearance then re-appearance of parasites as indicated by negative then positive thick smears on or after day 7;
- **R2:** No clearance of parasites from day 0 to day 28 with reduction in parasite density on day 3 to < 25% of day 0;
- **R3:** No clearance of parasites from day 0 to day 28 with no reduction, or reduction on day 3 to ≥25% of the day 0 parasite density.

Analysis
Data were analysed using SPSS for Windows release 11.0.0 (SPSS, Chicago, IL) and Epi-info 2004 (Centres for Disease Control and Prevention, Atlanta). Chi-square or Fisher's exact tests were used to evaluate differences in proportions and the student's t-test or Mann-Whitney for differences in means. 95% confidence intervals for percentage values were estimated by $P \pm 1.96 \sqrt{P(1-P)/n}$ where $P$ is the percentage and $n$ is the sample size.

Ethical Approval
Ethical approval was granted by the College of Medicine Research and Ethics Committee (COMREC) and the Liverpool School of Tropical Medicine.

RESULTS

Maternal characteristics
A total of 961 women were screened for malaria and anaemia at first ANC visit. Mean age, and gestation were 24.1 years (SD 5.6), and 24.9 weeks (SD 4.4) respectively. One in five women were adolescent (<20 years) and 27.7% were primigravidae. One third commenced antenatal care in the third trimester and only 1.0% in the first trimester.

Malaria parasitaemia and anaemia at first ANC visit
Prevalence of peripheral parasitaemia was 9.3 % (95% CI 7.5-11.1%) at first antenatal visit. Anaemia was present in 76.5% of women, 23.8% had moderate anaemia and 5.7% had severe anaemia. All women with parasitaemia were asymptomatic. Parasitaemia was more frequent in first than later pregnancies (14.7% versus 7.4%, $p<0.05$). Prevalence was highest in the second trimester (70/634, 11.0%) compared to either the first (0/10, 0%) or last trimester (19/316, 6.0%), and in the post-rainy season (26/208, 12.5%), compared to the dry season (35/375, 9.3%) or rainy season (28/378, 7.4%). Geometric mean parasite density at enrolment was 401 parasites per µl. Moderate anaemia was more common in adolescents than adults (35.2% versus 27.3%, $p<0.05$), in primigravidae than multigravidae (35.3% versus 24.6%, $p<0.05$) and in the post-rainy season (34.1% versus 28.0% in other periods, $p<0.05$).
In vivo parasitological response

All women with malaria parasitaemia at the first antenatal visit (day 0) (n = 89) were eligible for the 28-day follow up study. Four women (4.5%) refused to participate because of the distance to travel to the hospital. Of 85 consenting women, 8 did not complete the day 7 follow up (9.4%), 12 did not complete the 14 day follow up (14.1%) and 24 did not complete the 28 day follow-up (28.2%). Women who failed to complete the follow-up did not differ at the first antenatal visit from women with known outcomes with respect to geometric mean parasite density, haemoglobin concentration, maternal age or gravidity.

At day 7, 6 of 77 women who completed the follow up had parasitological failure (7.8%), at day 14, 8 of the 73 women who completed the follow up had parasitological failure (10.9%), and at day 28, 12 of 61 (19.7%) who attended at all follow-up times (table 1). If all women with loss to follow up were considered as treatment failures, then the day 7, 14, and 28 failure rates would be 16.5% (95%CI 8.7-24.3%), 23.5% (95%CI 14.5-32.5%) and 42.3% (95%CI 31.8-52.8%) respectively.

Table 1: Loss to follow-up, parasitological failure and category at day 7, 14 and 28 in 85 women enrolled for in vivo testing

<table>
<thead>
<tr>
<th></th>
<th>Day 7</th>
<th>Day 14</th>
<th>Day 28</th>
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<tbody>
<tr>
<td>Cumulative loss</td>
<td>8/85 (9.4%)</td>
<td>12/85 (14.1%)</td>
<td>24/85 (28.2%)</td>
</tr>
<tr>
<td>to follow up</td>
<td></td>
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<tr>
<td>Cumulative total</td>
<td>6/77 (7.8%) [95%CI 1.8-13.8%]</td>
<td>8/73 (10.9%) [95%CI 3.8-18.0%]</td>
<td>12/61 (19.7%) [95%CI 9.7-29.7%]</td>
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<tr>
<td>parasitological</td>
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<td>failure</td>
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<tr>
<td>Resistance</td>
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<tr>
<td>category R1</td>
<td>5</td>
<td>7</td>
<td>11</td>
</tr>
<tr>
<td>R2</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>R3</td>
<td>0</td>
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Key: R= resistance, CI= confidence interval

DISCUSSION

Study Sample

This sample represents primarily second trimester attendances as few women attended early in pregnancy. Late attendance for antenatal care is an important reason for failing to complete the uptake of two SP doses during pregnancy (Ashwood-Smith et al. 2002, van Eijk et al. 2004). Most women were anaemic with a characteristic pattern of higher prevalence in primigravidae which frequently occurs in malarious areas (Savage et al. 2006). Malaria parasite prevalence at first antenatal visit was lower than in a previous prevalence study in this area during the mid-1990s (Verhoeff et al. 1999) which may relate to changing malaria transmission patterns and increased use of impregnated bed nets. Parasite densities at enrolment were low.

In-vivo parasitological response

Loss to follow-up for a proportion of women may have resulted in a selected study sample for assessment of parasitological response. Resolution of clinical symptoms was
unlikely to explain early loss to follow-up as women enrolled were asymptomatic and there were no differences at enrolment between women who complied with follow-up compared with those who missed visits. Pregnant women as a specific group may show reluctance to attend the several additional attendances required for these studies in addition to their routine monthly visits for antenatal care.

At the time of this study there was little published data for in vivo follow-up studies in pregnant women, and as a consequence in vivo follow-up studies in children with uncomplicated malaria were generally used as a proxy assessment in order to assess antimalarial drug efficacy in pregnancy. A comparison of in vivo results in children from five African countries (Kenya, Nigeria, Tanzania, Zambia and Malawi) has reported a good statistical correlation with in vivo results in pregnant women living in the same study area (Kalanda et al. 2006), although one study from Ghana has reported much higher failure rates in children than in pregnant women (Tagbor et al. 2007). The present study observed a 7.8% parasitological failure at day 7 indicating recrudescent infections. At day 14 and 28 falciparum parasitaemia was present in 10.9% and 19.7% respectively, with one $R_2$ failure in a primigravid woman. Without data on PCR correction an unknown proportion of these late infections could be re-infections rather than recrudescence. PCR confirmation would be required to distinguish these from true failures.

On assumption that all infections were recrudescent, then these SP parasitological failure rates in pregnant women were similar to those observed in children with clinical malaria from published studies between 1998-2002 undertaken in Malawi (Nwanyanwu et al. 2000, Takechi et al. 2001, MacArthur et al. 2001, Sulo et al. 2002, Plowe et al. 2004). In this study drug resistance was almost wholly type (R1), whereas reports in children have shown substantial numbers with moderate or severe ($R_2/R_3$) resistance. The parasite density was low in most women with geometric mean densities below 1000 parasites per µl, which is much lower than values usually seen in children with uncomplicated malaria. The low density of infection is consistent with the type of resistance pattern observed. A previous in vivo study of SP sensitivity amongst pregnant women attending this same hospital and completed in 1996 and using the same methodology (without PCR correction) showed a 5% parasitological failure rate at day 28 (Howarth, 1996). Eight years later this had increased to between 8-20% and paralleled changes in SP drug resistance in children. This supports the conclusion that changes in resistance patterns in pregnant women follow those observed in children, although prevalence of resistance appears to be lower. A meta-analysis of African studies comparing chloroquine or SP resistance in pregnant women and children, for studies conducted in the same location and at the same approximate time, supports this conclusion (Kalanda et al. 2006).

All of these studies were undertaken without PCR correction for identification of recrudescent infections. This is a limitation of the present and previous studies. Currently, PCR correction of drug efficacy estimation is a standard requirement for in vivo studies with longer follow up times to day 56 required in order to identify late recrudescent infections. If an intention to treat analysis is used including all women with loss to follow up as failures, then estimates of parasitological failure were doubled for all follow up intervals (days 7, 14 and 21). This assumption would give a maximum mean estimate for parasitological failure at day 28 of 42.3% (95%CI 31.8-52.8%).

Although malaria parasitaemia prevalence at first antenatal visit was lower than in previous studies conducted in this area, nevertheless anaemia at first antenatal visit was still a common problem with one in twenty women experiencing severe anaemia in early pregnancy. This emphasises the need to improve anaemia control activities in women of child-bearing age, especially prior to commencement of child-bearing. The relatively low (<10%) malaria parasitaemia prevalence at first antenatal visit compared to previous studies
could indirectly reflect an acceptable level of SP drug sensitivity within this population contributing to lower transmission, as well as the increasing use of insecticide treated bed nets by pregnant women. At present (2010) SP still remains the drug of choice for IPTp in Malawi. In view of rising levels of parasite resistance to SP, safe alternative combination of antimalarials will be required for IPTp in order to improve malaria control in pregnancy.

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