

Does radical cure of asymptomatic *Plasmodium falciparum* place adults in endemic areas at increased risk of recurrent symptomatic malaria?

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Summary

A cohort of 197 adults in Kassena-Nankana District (northern Ghana) was radically cured of malaria parasites to study subsequent incidence of malaria infection. During the following 20 weeks of the malaria transmission season, 49% experienced clinical attacks associated with *Plasmodium falciparum* parasitaemia. In a group of 202 adults identically followed-up 1 year later without being treated, only 38% experienced such episodes (log-rank test for equality of survivor functions, $P = 0.035$). Clinical attacks in radically cured individuals presented with lower parasite densities but more symptoms. Randomized studies are needed to test the hypothesis that radical cure of *P. falciparum* enhances the risk and severity of subsequent clinical malaria attacks.

keywords malaria, adults, radical therapy, treated and untreated groups, recurrent symptomatic malaria

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Introduction

In trials of insecticide-treated mosquito nets (Msuya & Curtis 1991; Stich *et al.* 1994), anti-malarial drugs (Taylor *et al.* 1999) and malaria vaccines (Alonso *et al.* 1994) participants are often pre-treated with antimalarial drugs to determine time to infection. The impact of such therapy on incidence and severity of subsequent malaria episodes is unclear. Does the elimination of parasitaemia render the host more susceptible to disease caused by new inoculations? Can the 'cocktail' of drugs lead to important side-effects or immune suppression? These questions are important in terms of both consequences for the volunteers and relevance of trial results to situations where parasites are not cleared.

In May 1996 in Kassena-Nankana District in northern Ghana, radical antimalarial therapy was administered to 197 adults between the ages of 18 and 55 years, to

determine the incidence of infection and clinical malaria during the peak malaria transmission period (until October) (Owusu-Agyei *et al.* 2001). Exactly 1 year later, malaria-related morbidity was recorded in a further 202 adults who had not been pre-treated. We report here a comparison of patterns of clinical malaria in these two cohorts.

Subjects and method

Study site and population

Kassena-Nankana (Upper East Region of Ghana) lies within the Guinea Savannah woodlands between latitudes 10°30'–11°00' North and longitudes 1°00'–1°30' West. Malariological surveys have consistently found the area to be holoendemic for *Plasmodium falciparum* malaria (Colbourne & Wright 1955; Binka *et al.* 1994).

Subject selection, consent and enrolment

Using the Navrongo Demographic Surveillance System (NDSS) (Binka *et al.* 1999), 16 index compounds were randomly selected from four geographical zones of the district with the number of compounds per zone proportional to the 18–55-year-old population. Verbal informed consent was sought from chiefs, opinion leaders and the community members (communal consent).

Potential volunteers were selected by visiting compounds radially in concentric circles around the index compounds until a pre-determined number of subjects were recruited. Each gave individual consent by signing or thumb-printing a written consent form. Volunteers were screened for vital signs, physically examined by a physician, had a clinical history taken and thick and thin blood smears prepared.

In the 1996 study, blood samples were collected and tested for G-6-PD status, and urine was collected from all women for pregnancy tests. Those enrolled received therapy designed to clear all malaria infections. The 18-day regimen administered by fieldworkers at the volunteers' homes consisted of oral quinine sulphate (650 mg, three times daily for 4 days) followed by doxycycline (100 mg, twice daily for 10 days). Subjects testing normal for G-6-PD (NADP + spot test, Sigma Chemical Co., St Louis, MO, USA) received in addition primaquine (0.5 mg base per kg body weight once daily for 14 days), given concurrently with doxycycline. Success of the treatment was confirmed by microscopic examination of blood films collected on day 15. All but five subjects completed therapy, and were found to have cleared all parasites by microscopy.

The second cohort of adults was enrolled 1 year later using the same procedures, with the exceptions that G-6-PD status and pregnancy tests were omitted as there was no pre-treatment of this cohort.

Follow-up

Fieldworkers visited volunteers three times a week at home, inquired about their general health and measured axillary temperature. Blood films were collected every 2 weeks for the 20-week duration of the study, and also if any illness or axillary temperature ≥ 37.5 °C was reported. In that case a detailed symptoms questionnaire was administered, and the patient was referred to a project physician at the district hospital.

Giemsa-stained blood films were examined by 1000 \times light microscopy using oil immersion. Parasitaemias were scored per microlitre of blood by counting asexual parasites against 200 leukocytes and multiplying the parasite count by 40. A slide was considered negative if no parasites

were observed in 100 high-power fields. A 10% random sample of all slides was re-examined for quality control.

Asymptomatic subjects with malaria parasitaemia at routine 2-weekly visits were not treated. Symptomatic (one or more of: reported/documentated fever, headache, chill, nausea, dizziness, myalgias) subjects with microscopy-confirmed parasitaemia were diagnosed as clinical malaria cases and promptly treated with chloroquine diphosphate (25 mg/kg body weight over 48 h). The first dose of 600 mg was taken in the presence of the doctor's assistant and the remainder taken home by the patient.

Patients treated with chloroquine were evaluated clinically and parasitologically on days 2 and 7 after initiation of therapy. Treatment failures were given three tablets of Fansidar[®] (sulphadoxine 500 mg + pyrimethamine 25 mg per tablet, Hoffman–La Roche), and followed up until smear-negative.

Data analysis

The incidence of clinical attacks in the two groups was compared using Kaplan–Meier (KM) survival curves. The first 10 days after curative therapy among the treated group were excluded from analyses as a risk-free period. Surveillance for clinical malaria among the untreated cohort started on the day of recruitment. Statistical significance of the differences was tested using a log-rank test. Comparisons of proportions used the usual Pearson χ^2 statistics or Fisher's exact test when the expected value for any cell in a table was less than 5. Two-sided *t*-tests were used to compare log-transformed parasite densities and a Wilcoxon test was used to compare age distributions (which are clearly non-normal).

Results

The two cohorts had similar age distributions (Table 1). Forty-four percent (44%) of those who completed radical therapy were females compared with 52% of the untreated cohort (Table 1). Environmental conditions relevant for malaria transmission were similar during the 2 years, with 910 mm of rainfall in 1996 and 861 mm in 1997. In both years, the rainy season started in April and ended in October with similar monthly rainfall.

The main difference at enrolment was that 56% of the volunteers who underwent radical therapy were parasitaemic compared with only 28% among the other cohort, presumably because of different levels of transmission during the preceding dry season. Parasite densities at enrolment were similar in the two cohorts.

Forty-nine percent (97 of 197) of volunteers in the treatment group were diagnosed with clinical malaria in

Table 1 Characteristics of adults in the two cohorts

	Radical therapy group	No radical therapy group	Significance test of difference
Number of cohort members	197	202	
Mean age (range)	38.6 (18–55 years)	36.8 (18–54 years)	Wilcoxon test, $P = 0.3$
Percentage male	56%	48%	$\chi^2_1 = 1.6$, $P = 0.2$
Percentage parasitaemic at enrolment	56%	28%	$\chi^2_1 = 32.5$, $P < 0.001$
Geometric mean malaria parasites at enrolment (parasites/ μ l blood, 95% CI)	116 (90, 150)	161 (114, 226)	$t_{397} = 1.5$, $P = 0.14$
Geometric mean malaria parasite density at first malaria clinical episode	201 (125, 325)	669 (516, 869)	$t_{174} = 4.2$, $P < 0.001$

the 20 weeks, compared with 38% (77 of 202) in the non-pre-treated group [log-rank test of equality of survivor functions, $\chi^2 = 4.42$, $P = 0.035$; RR (95% CI) = 1.29 (1.03–1.61) (Figure 1)]. Among the latter, 33.9% (19 of 56) of those with initial parasitaemia and 39.7% (58 of 146) of those aparasitaemic at enrolment were diagnosed with clinical malaria within the 20-week period [log-rank test $\chi^2 = 0.71$, $P = 0.4$; RR (95% CI) = 1.17 (0.77–1.78)].

Though geometric mean parasite densities at enrolment were similar, at the first symptomatic episode the geometric mean density was three times as high in the untreated group as in the treated group (Table 1). However, substantially more symptoms were associated with each episode among the treated group than the untreated (Table 2).

All volunteers who received treatment prior to follow-up and were diagnosed with clinical malaria during follow-up cleared parasitaemia by day 7 when they were treated with a full dose of chloroquine. This compared with a 9% failure rate in those who did not receive treatment at enrolment.

Discussion

While young children are highly susceptible to both malaria infection and disease, adults who have lived in endemic areas have considerable clinical immunity (Day & Marsh 1991; Trape *et al.* 1994). We were therefore surprised when 20% of the pre-treated volunteers had clinical attacks at first re-infection (Owusu-Agyei *et al.* 2001). Within the 20-week follow-up, clinical malaria was diagnosed in 49%. The high incidence of clinical attacks among this treated cohort raised the question of whether clearance of malaria parasitaemia can increase the risk of clinical malaria in adults when they subsequently become reinfected.

The study of the initial cohort was not designed to investigate the effects of radical therapy, and the second cohort was recruited only because of the surprising responses in the first year. Selection of volunteers, follow-up in the two cohorts, observation techniques, data collection

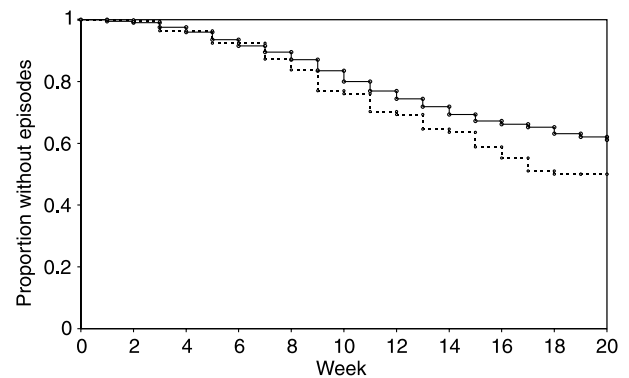


Figure 1 Kaplan–Meier survival curves of clinical malaria incidence in the two cohorts (solid line, untreated cohort; dotted line, treated cohort).

and clinical monitoring in the two cohorts were identical, with the same field workers, microscopist and physicians.

However, there was a substantial difference between the two cohorts in parasite prevalence at enrolment (56 and 28%). The most obvious sources of bias would decrease the relative risk of episodes in the treated cohort. Many clinical episodes may have been caused by other pathogens but were diagnosed as clinical malaria because of incidental parasitaemia (Smith 1996). We would expect more overdiagnosis of clinical malaria in the untreated than in the treated group, as the former were parasitaemic for much more of the follow-up. Similarly, it is remarkable that the episodes in the treated cohort occurred after less time at risk as parasites were present in the controls from the start of follow-up.

A second test of the effect of pre-existing parasitaemia on subsequent clinical malaria was the analysis of the effect of baseline parasitaemia in the untreated cohort on subsequent risk of clinical malaria. The observed tendency was in the direction of a protective effect of baseline parasitaemia, but this was not statistically significant. This analysis may have been affected by misclassification of

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Symptom histories	Treated (%)	Not treated (%)	Pearson χ^2	P-value
Documented fever	10/97 (10)	1/77 (1)	–	0.024*
Reported fever within 48 h	82/97 (85)	42/77 (55)	18.9	< 0.001
Chill	76/97 (78)	30/77 (39)	27.8	< 0.001
Headache	83/97 (86)	55/77 (71)	5.2	0.02
Nausea	36/97 (37)	13/77 (17)	21.5	< 0.001
Myalgias	39/97 (40)	1/77 (1)	36.5	< 0.001

* Fisher's exact test.

Table 2 Comparison of symptom reports between the two cohorts

individuals with very low parasitaemia as parasite densities at baseline were very low.

The fact that the parasite density at first clinical malaria among the untreated group was much higher than the enrolment density indicates that these were mainly malaria episodes. Conversely, the similarities between densities at first clinical malaria and at enrolment in the treated cohort could indicate that these are not really malaria episodes. However, as these episodes occurred disproportionately concurrently with the first reinfections, an alternative explanation for the low densities is that the treatment group became sick at lower parasite densities as a result of a loss of parasite tolerance.

The responses to chloroquine treatment are also of interest. Though completion of the dose schedule was not directly observed, there was no reason to suspect a difference in compliance between the cohorts. The 9% parasitological failure rate is comparable with the findings of other studies in the sub-region (Afari *et al.* 1992). Untreated individuals in holoendemic areas often harbour many clones of parasites (Ntoumi *et al.* 1995; Beck *et al.* 1997), and so the chance of a chloroquine-resistant strain being present is higher than the resistance rate for single infections. The absence of resistance among the treated group is probably because of these patients being reinfected with only one or a few strains of parasites.

Clinical malaria occurring after radical therapy may thus differ from the 'natural' disease, and the implications of treating asymptomatic individuals may be very different in adults than for young children, for whom intermittent treatment is beneficial (Schellenberg *et al.* 2001). However, the fact that the cohorts were not studied concurrently and the lack of comparability in baseline parasitaemia mean that this should be considered for the moment only as a hypothesis. A properly randomized study with concurrent controls is urgently needed.

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