

Safety, efficacy and population pharmacokinetics of fixed-dose combination of artesunate-mefloquine in the treatment of acute uncomplicated *Plasmodium falciparum* malaria in India

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ABSTRACT

Background & objectives: India has switched over to artemisinin-based combination therapy (ACT) for the treatment of acute uncomplicated *Plasmodium falciparum* malaria and the ACT used in the national programme is artesunate + sulphadoxine-pyrimethamine. Since the efficacy of ACT is dependent also on the partner drug, there is a need to evaluate and deploy multiple ACTs.

Methods: This multicentre, single-arm, open-label clinical trial was carried out to assess the efficacy, safety and population pharmacokinetics of a fixed dose combination (FDC) artesunate mefloquine (ASMQ) in *P. falciparum* infected, Indian adults at Panjim, Goa, and Mangalore, Karnataka between December 2007 and November 2008.

Results: A total of 77 patients (males 74) were screened and enrolled: 42 at Goa and 35 at Mangalore with a median age of 25 yr (range 18–55 yr). One patient failed in treatment on D53, a PCR proven new infection, seven developed recurrent vivax parasitaemia and 11 did not have a parasitological endpoint. By per protocol analysis, the D63 cure rate was 58/59 (98.3; 95% C.I. 90.9–99.9%), and 58/58, with PCR correction. ASMQ was well-tolerated and no serious adverse events were reported.

Interpretation & conclusion: The study showed that the ASMQ FDC was efficacious and well-tolerated for the treatment of acute, uncomplicated *P. falciparum* malaria in highly endemic, chloroquine resistant areas of Goa and Mangalore. It is a viable option for India.

Key words Artesunate; India; malaria; mefloquine; *P. falciparum*; pharmacokinetics

INTRODUCTION

Out of 86 malaria endemic countries, 77 have switched to artemisinin-based combination therapy (ACT) for the treatment of acute, uncomplicated *Plasmodium falciparum* malaria¹. The fixed dose combinations (FDCs) are strongly recommended over the blister packs to reduce the potential use of monotherapy². FDCs are preferred to loose tablets because fewer tablets are involved and patient adherence can be improved³. Accordingly, all ACTs, except artesunate + sulphadoxine-pyrimethamine have been developed as FDCs. Since the efficacy of the ACT is partly dependent on the efficacy of its partner drug, there is a need to develop multiple ACTs. In India, artemether-lumefantrine⁴, artesunate-amodiaquine⁵, pyronaridine-artesunate⁶, dihydroartemisininpiperazine⁷

and loose ASMQ^{6–7} have all achieved cure rates of 95% and above.

Mefloquine has been consistently shown to be effective for the treatment of *P. falciparum* malaria⁸ even in pregnant women⁹ and children¹⁰. The present study was conducted in two malaria endemic districts of Goa and Karnataka to evaluate the efficacy of ASMQ fixed-dose combination for the treatment of uncomplicated *P. falciparum* malaria.

MATERIAL & METHODS

This was a single-arm, two site, open-label clinical trial to assess the efficacy, safety and population pharmacokinetics of ASMQ. It was carried out at Panjim, Goa and Mangalore, Karnataka between December 2007 and

November 2008. Goa is situated on the western coast of the country and has almost round-the-year transmission of malaria. *P. falciparum* contributes to about 25% of the malaria cases of the state¹¹. Mangalore is also situated along the western coast and active transmission of malaria is recorded during the post-monsoon months¹². Chloroquine failure rates in falciparum malaria in Goa and Karnataka, as shown by the therapeutic efficacy studies carried out earlier, were about 20 and 39% respectively^{11, 13}.

Patients

The study was carried out in patients of >18 yr of age having *P. falciparum* mono-infection (asexual parasitaemia of 1000–100,000) and fever $\geq 37.5^{\circ}\text{C}$ (99.5°F). Patients with signs of severe malaria, febrile conditions due to diseases other than malaria, history of hypersensitivity to study drug(s), positive pregnancy test or lactating women and history of antimalarial treatment in past 15 days were excluded. Patients were also excluded from the study if they had anaemia (haemoglobin < 7 g/dl), hepatic (ALT/AST ≥ 2.5 ULN) or renal impairment (serum creatinine ≥ 1.2 ULN). Patients having a history of clinically significant disorders such as cardiovascular, respiratory, gastrointestinal, immunological, neurological, endocrine, malignancy, psychiatric disorders, and history of convulsions were also excluded. Potential sexually active individuals were included only if they agreed to use a medically acceptable form of contraception during the study and for at least 15 days after Day 63.

Treatment

The subjects were orally administered two tablets of ASMQ, containing 100 mg of AS and 200 mg of MQ base (220 mg of MQ hydrochloride), once daily for three consecutive days. The treatment was administered under supervision. If vomiting occurred within 30 min, a full dose (two tablets) was re-administered; if it occurred between 31 and 60 min, one tablet was re-administered. Patients remained hospitalized for at least three days. Other drugs were allowed to be given as clinically indicated, e.g. paracetamol for fever and an antiemetic for nausea and vomiting.

Patients were followed on Days 7, 14, 21, 28, 35, 42, 49, 56 and 63. In case of adverse events (AEs) reported and unresolved on D63, patients were followed up for another 30 days or until resolution/stabilization of the event, whichever was earlier. Serious adverse events (SAEs) were followed up to resolution/stabilization.

Safety and efficacy assessments

The classification of treatment outcomes was based on the assessment of parasitological and clinical outcomes of antimalarial treatment according to the guidelines of WHO¹⁴. Accordingly, patients were classified as having one of the following: an early treatment failure (ETF), a late treatment failure (LTF), a late clinical failure (LCF), a late parasitological failure (LPF), or an adequate clinical and parasitological response (ACPR), i.e. those without falciparum parasites on Day 63 who were not treatment failures.

The primary endpoints of the study were the PCR uncorrected cure rate (proportion of patients with ACPR) on Day 63 and the PCR corrected cure rate on Day 63 (proportion of patients without recrudescent or new falciparum infections/inconclusive/no result as classified by PCR genotyping).

The secondary endpoints included the 28 day cure rate based on ACPR with and without PCR correction, parasite reduction ratio (PRR) at 48 h, parasite clearance time (PCT), fever clearance time (FCT), percentage of patients without gametocytes on Day 28, proportion of patients with ETF, LTF and LPF, proportion of patients with mixed infections in the follow up assessments and proportion of patients with the development of severe malaria.

Procedures

Blood samples were collected by fingerprick for parasitological assessment at enrolment during admission and follow up visits. Smears were prepared, Giemsa stained and examined for parasite density. This was done by counting the number of asexual parasites per 200 leucocytes on the thick blood film. Parasite density per microlitre was calculated as (Number of parasites counted $\times 8000$)/(Number of leukocytes counted). Two qualified microscopists independently examined all the slides and parasite densities were calculated by averaging the two counts. The parasitologic assessment was done on Days 0, 1, 2, 3, 7, 14, 21, 28, 35, 42, 49, 56 and 63 or on any other day if the patient returns.

Two to three drops of blood were collected on a 3 mm filter paper (Whatman, U.K.) from each patient at inclusion. A second specimen was collected only in case of reappearance of parasites as evidenced by a positive microscopy slide. All filter papers were then transferred to NIMR for genotyping. In order to differentiate recrudescence from reinfection, three genetic markers, merozoite surface proteins (*mSP1* and *mSP2*) and glutamate rich protein (*glurp*) were used.

Blood samples were drawn for haematological

(haemoglobin level, leucocyte count and haematocrit) and biochemical (alanine aminotransferase, aspartate aminotransferase, bilirubin and creatinine) assessments on Days 0, 7, 28, 63 and on the day of recurrent parasitaemia.

Samples for assessment of population pharmacokinetic (PK) parameters were collected for AS and dihydroartemisinin (DHA) (before 1st dose, within 8 h post 1st and 3rd dose); while samples for MQ were collected before the 1st dose, within 72 h of 1st dose, on Day 7 and on Days 28, 35 or 42.

Ethical issues

The study was conducted in accordance with the local laws and regulations including the schedule Y, Indian Good Clinical Practices, Ethical guidelines on biomedical research issued by the Indian Council of Medical Research and the International Conference on Harmonisation-Good Clinical Practices (ICH-GCP). The protocol was reviewed and approved by the Ethics Committee of the National Institute of Malaria Research, New Delhi, Goa Medical College and Hospitals, Goa and Kasturba Medical College, Mangalore. Written informed consent was obtained from participants. In case of an illiterate patient, his/her thumb impression and signature of an independent witness were obtained.

Initially two sites (Goa and Guwahati) were approved for the study. Since one site (Guwahati) was unable to recruit patients, another site (Mangalore) was later added and Guwahati site was closed.

Sample size calculation

The sample size calculation was based on the precision method. With an expected ASMQ failure rate of 5%, a sample size of 73 patients would be needed for a precision of 5% (i.e. failure rate 0 to 10%), using a two sided α -0.05 and a power of 80%. Allowing for a 15% loss to follow up, the target sample size was 84. Samples for PK analysis were collected from all the patients enrolled in the study. A minimum of 50 patients were necessary for the population PK analysis.

Analysis populations

Three patient populations were evaluated. The safety population comprised all patients who were randomized and received at least one dose of study drug. The safety analysis was done on the safety population. The intent to treat (ITT) population comprised of all patients who were randomized and received at least one dose of the study drug and underwent at least one efficacy assessment. The secondary efficacy analysis was performed on ITT population. The per protocol (PP) population comprised of all

patients who completed the study as per the protocol, i.e. only patients with a protocol defined falciparum efficacy end point. The primary and secondary efficacy analyses were performed on PP population.

Statistical analysis

The PCR adjusted and crude cure rates (ITT and PP populations) are presented as percentages (%) with 95% CI (confidence interval). The cumulative probability of subjects remaining parasite free by Day 63 was determined by Kaplan Meier analysis on the ITT population.

The time to fever clearance, parasite clearance and development of recurrent parasitaemia was summarized using Kaplan Meier survival analysis as mean, standard error (SE), median, percentiles (P25 and P75) and 95% CI of median. Safety analyses included summaries of AEs and SAEs, clinical signs and symptoms, physical examination findings, vital signs and changes from baseline for laboratory values.

The population PK analysis was done using NONMEM programme to calculate estimates of: (i) PK parameters and their inter subject variances; (ii) residual random intra subject error; and (iii) possible relationships between the covariates (body weight, age, hemoglobin, hepatic enzymes and serum creatinine) and inter individual variability of the PK parameters. The goodness of fit of each model was determined by the precision of the parameter estimates and an examination of the scatter plot of residuals vs. predicted levels. AS and DHA concentrations are expressed as DHA equivalents (defined as the sum of AS and DHA concentrations corrected for molecular weight).

RESULTS

A total of 77 patients (42 at Goa and 35 at Mangalore) were screened and all met the inclusion criteria; there were no screen failures. The ITT population comprised of 77 subjects. The demographic and baseline characteristics of the patients enrolled are shown in Table 1.

Table 1. Baseline and demographic characteristics of enrolled patients

Gender	Sample size (n)
Male n (%)	74 (96.1)
Female n (%)	3 (3.9)
Age (Mean \pm SD)	28.2 (\pm 8.84)
Weight (Mean \pm SD)	53.23 (\pm 7.26)
Asexual parasitaemia (Mean \pm SD)	517.8 (\pm 625.8)
Gametocyaemia (Mean \pm SD)	131.6 (\pm 774.9)

n = No. of sample size; SD— Standard deviation.

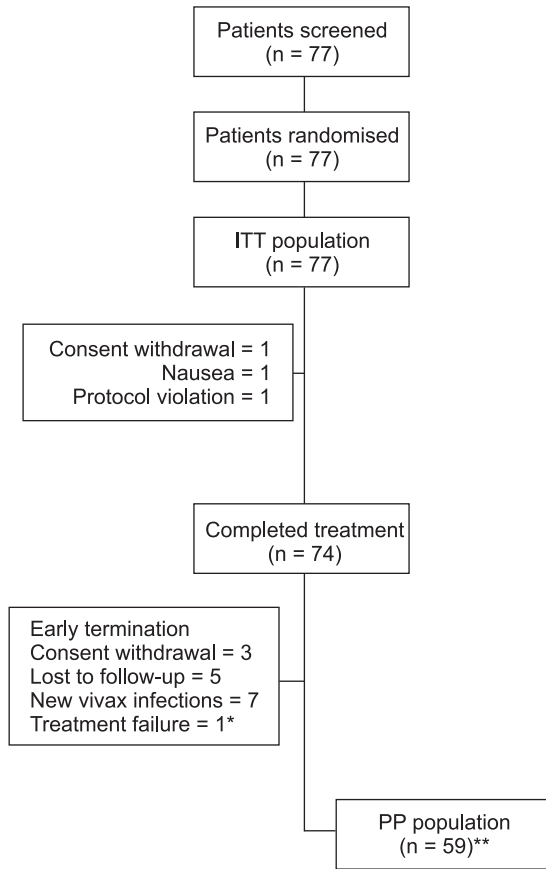


Fig. 1: Participants flow chart. *Treatment failure by ACPR. Classified as a new infection by PCR; **PCR uncorrected, after PCR correction, n = 58.

Three patients did not complete treatment: (i) one because of nausea (an AE requiring rescue treatment); (ii) one treated epileptic patient, who did not declare his illness, absconded after one dose of ASMQ; and (iii) one who withdrew consent. By Day 63, 59 patients completed the study with a protocol defined parasitological endpoint (Fig. 1).

Efficacy results

Primary efficacy endpoint: By PP analysis, patients with ACPR numbered 58 for an ACPR rate of 98.3% [95% CI 90.9–99.9%]. Only one patient with recurrent parasitaemia on D53 was a PCR proven new infection. Therefore, the PCR adjusted D63 cure rate was 100% [95% CI 93.8–100%] and cent percent by KM analysis. By ITT, the ACPR rate was 75.3% [95% CI 64.1–84.4%].

Secondary efficacy endpoints: There was no patient with treatment failure by D28, for an ACPR rate of 64/64 [100%; 95% CI 94.4–100%] by PP and 64/77 [83.1%; CI 72.9–90.7%] by ITT. By 24 and 48 h, the proportions of

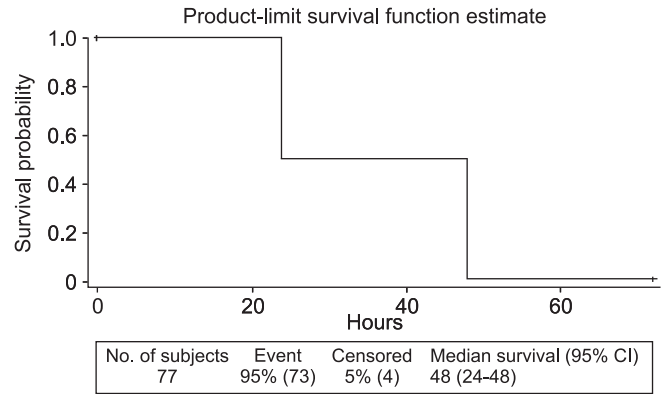


Fig. 2: Survival curve (Kaplan Meier Plot) of parasite clearance time for ASMQ-ITT population.

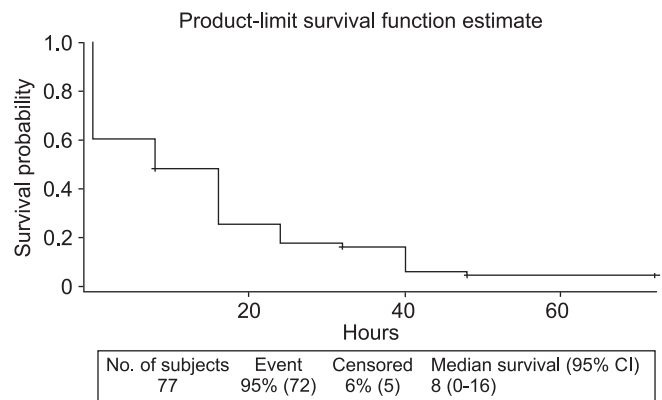


Fig. 3: Survival curve (Kaplan Meier Plot) of fever clearance time for ASMQ-ITT population.

patients without parasites were 38/75 (50.6%) and 73/74 (98.6%), respectively, so a PRR 48 h could not be calculated. Patient proportions with a measured fever at D0, 1 and 2 were 62.3% (48/77), 17.1% (13/76) and 1.3% (1/75), respectively (Figs. 2 & 3).

Gametocyte carriage: Patients with baseline gametocytaemia (n = 7, 9.1%) cleared their gametocytes by Day 21; five had cleared by Day 7. Out of the 70 patients without D0 gametocytes, gametocytaemia was present in 2/68 [2.9%; 95% CI 0.4–10.2%] on Day 1 and 2/67 [2.9%; CI 0.4–10.4%] on Day 2 and 2/66 [3%; CI 0.4–10.5%] on Day 3. Thereafter, none had gametocytes.

Recurrent vivax parasitaemia: Patients with a new vivax infection during the follow up numbered 7/66 of the PP population for a rate of 10.6% (4.4–20.6); these occurred between D42 and D59 (median D56).

Safety endpoints: ASMQ was well-tolerated. Overall, 36 out of 77 (46.8%) patients reported 39 AEs at least once (Table 2) of which 36 (92.3%) were not considered ASMQ

Table 2. Adverse events by system organ class according to relatedness to the study medication – ITT population (n=77)

System organ class Preferred term	Relation to study drug				
	None	Remote	Possible	Probable	Definite
At least one adverse event	36 (46.8)	1 (1.3)	2 (2.6)	0 (0)	0 (0)
Blood and lymphatic system disorders	6 (7.8)	0 (0)	0 (0)	0 (0)	0 (0)
Anaemia	5 (6.5)	0 (0)	0 (0)	0 (0)	0 (0)
Hemolysis	1 (1.3)	0 (0)	0 (0)	0 (0)	0 (0)
Gastrointestinal disorders	6 (7.8)	1 (1.3)	2 (2.6)	0 (0)	0 (0)
Diarrhoea	3 (3.9)	0 (0)	1 (1.3)	0 (0)	0 (0)
Gastritis	0 (0)	0 (0)	1 (1.3)	0 (0)	0 (0)
Nausea	2 (2.6)	1 (1.3)	0 (0)	0 (0)	0 (0)
General disorders and administration site conditions	28 (36.4)	0 (0)	0 (0)	0 (0)	0 (0)
Fatigue	3 (3.9)	0 (0)	0 (0)	0 (0)	0 (0)
Pyrexia	26 (33.8)	0 (0)	0 (0)	0 (0)	0 (0)
Toothache	1 (1.3)	0 (0)	0 (0)	0 (0)	0 (0)
Musculoskeletal and connective tissue disorders	1 (1.3)	0 (0)	0 (0)	0 (0)	0 (0)
Myalgia	1 (1.3)	0 (0)	0 (0)	0 (0)	0 (0)
Nervous system disorders	5 (6.5)	0 (0)	0 (0)	0 (0)	0 (0)
Headache	5 (6.5)	0 (0)	0 (0)	0 (0)	0 (0)
Vascular disorders	1 (1.3)	0 (0)	0 (0)	0 (0)	0 (0)
Pallor	1 (1.3)	0 (0)	0 (0)	0 (0)	0 (0)

Figures in parentheses indicate percentages.

related. Only two AEs, gastritis and diarrhoea, were considered possibly ASMQ related. There were no SAEs.

The mean Hb fell by Day 7, increasing thereafter but by Day 63, it was still significantly lower compared to baseline. The lowest recorded Hb concentration was 5.8 g/dl on Day 7 (meeting the WHO definition of severe anaemia for adults), a grade 4 AE. It improved to 8.8 g/dl by D63 without the need for a blood transfusion. The median total white cell counts and the absolute neutrophil counts showed similar changes over time, increasing initially to Day 7 and

declining thereafter. Three patients had moderate neutropenia (<1500/ μ l) at baseline ranging from 1060 to 1400/ μ l; all returned to normal by Day 28 or 63. During follow-up, the lowest absolute neutrophil counts were 1680 to 1763/ μ l in three patients. All the mean biochemical parameters were in the normal range at baseline (Table 3) and declined significantly by D63, except for ALT whose decline was not quite statistically significant. There was one male patient with a D63 total bilirubin of 3 mg/dl which had been normal at all other time points.

Table 3. Mean (\pm standard deviation) laboratory parameters overtime

Parameter	D0 (n = 77)	D7 (n = 67)	D28 (n = 64)	D63 (n = 58)
Hemoglobin (g/dl)	13.11 \pm 2.13	12.31 \pm 2.01	12.46 \pm 1.85	12.52 \pm 1.78
Hematocrit (%)	37.57 \pm 5.47	34.9 \pm 5.33	35.77 \pm 4.7	35.88 \pm 4.86
Red blood cells ($\times 10^6$ /mm ³)	4.44 \pm 0.72	4.12 \pm 0.68	4.28 \pm 0.61	4.34 \pm 0.65
White blood cells (/mm ³)	5588 \pm 1741	8235 \pm 2513	7347 \pm 2069	7441 \pm 1834
Neutrophils (%)	64.4 \pm 14.1	53.4 \pm 7.3	52 \pm 6.2	53 \pm 8.4
Lymphocytes (%)	31.9 \pm 13.2	40.2 \pm 7.4	41.5 \pm 6.2	41.8 \pm 8.2
Monocytes (%)	1.01 \pm 1.28	1.16 \pm 1.25	0.75 \pm 0.89	0.68 \pm 1.03
Eosinophils (%)	2.2 \pm 2.67	5 \pm 3.95	5.7 \pm 5.79	4.2 \pm 5.19
Basophils (%)	0.13 \pm 0.33	0.16 \pm 0.51	0.25 \pm 0.56	0.34 \pm 0.54
Total bilirubin (mg/dl)	0.87 \pm 0.69	0.15 \pm 0.35	0.28 \pm 0.48	0.45 \pm 0.62
ALT (U/L)	34.4 \pm 14.09	30.5 \pm 15.94	27.9 \pm 8.58	27.4 \pm 7.12
AST (U/L)	26.38 \pm 16.94	26.46 \pm 19.94	22.67 \pm 8.29	22.67 \pm 6.9
Creatinine (mg/dl)	0.96 \pm 0.18	0.92 \pm 0.17	0.88 \pm 0.13	0.87 \pm 0.15

n = Sample size.

Pharmacokinetic parameters

PK results have been summarized here as more comprehensive report will be published elsewhere. A population PK model was constructed for DHA equivalents (DHAe) using data from 70 patients. The DHAe disposition was a one compartment model with linear absorption and elimination, with a proportional residual error. The estimated mean [standard deviation (SD) on the estimated parameter] parameters for AS were: (i) absorption rate constant (k_a) 2.49 (1.37); (ii) apparent oral clearance (CL/F) 31.5 (2.74) L/h; (iii) apparent volume of distribution (Vc/F) of the central compartment 65.5 (7.63) L; and (iv) proportional residual error 0.314 (0.0638). No residual trend was observed between the weighted residuals and the different covariates, indicating the lack of relationship with the PK parameters.

The population PK model pharmacokinetics of MQ (n=77) was a two compartment model with first order absorption. The main, mean (SD) PK population estimates from the final model were: (i) K_a 0.163 (0.0263); (ii) CL/F 1.13 (0.05) L/h; (iii) Vc/F 271 (14.1) L; (iv) half life 21.6 (8–41) days; and (v) proportional residual error 0.0692 (0.015). Body weight had a significant positive relationship with the central and peripheral distribution volumes.

DISCUSSION

The combination of AS and MQ was highly efficacious in the chloroquine resistant areas of Goa and Karnataka. The efficacy of the combination of ASMQ exceeded the WHO threshold of 95% for introducing a new ACT. The combination of ASMQ has been extensively studied and has shown good efficacy and tolerability. A systematic review of relevant studies on the treatment of uncomplicated *P. falciparum* malaria conducted over the past ten years in Southeast Asia showed that MQ was significantly more efficacious than CQ in clearing of parasites, with a tendency for faster clinical recovery. This difference was also observed in areas with considerable CQ resistance^{15–17}.

A Cochrane review published in 2009 systematically reviewed eight clinical trials comparing ASMQ versus artemether-lumefantrine. Both the combinations showed a PCR corrected cure rate of >95%¹⁸. ASMQ has been highly effective for many years in treating uncomplicated falciparum malaria in western Thailand and Cambodia. However, efficacy has declined in recent years in parallel with increasing prevalence of the *pfmdr1* gene copy number and artemisinin resistance^{19–21}. This calls for continued vigilance for the development of drug resistance with any deployed ACT.

India has switched over to artesunate+SP for the treatment of falciparum malaria all over the country. It is likely that the life of AS+SP may be relatively short lived and an alternative ACT needs to be deployed. In addition, SP may not be the optimal partner drug for drug resistant *P. vivax* malaria²². ASMQ had a D63 cure rate of 98.3% and a PCR adjusted cure rate of 100%. The results from this study are comparable to a number of African and South Asian studies. In a study carried out in Mali, ASMQ was compared to artemether-lumefantrine in 470 patients (235 in each arm). After correction for re-infection, the Day 28 cure rates were similar in the two groups (96.04 versus 96.93%)²³.

Although there are no MQ efficacy data in India, MQ was highly effective against CQ resistant *P. vivax* in Papua New Guinea, and Indonesia, an added bonus that is shared by other ACTs^{24–25}.

Our patients reported few drug related adverse events and FDC ASMQ was generally well-tolerated, perhaps in part related to the common prescribing of an antiemetic. One patient developed severe nausea before completing his ASMQ and was rescued with intravenous artesunate. The side-effects of mefloquine are well-known and, not surprisingly, our study has not shown anything unexpected. One unexpected finding was the lower mean D63 Hb compared to baseline. This is difficult to explain given that Hb recovery accompanies highly effective antimalarial treatment; this could be due to a chance finding. The PK parameters of ASMQ FDCs studied in the Indian population were similar to the results reported from previously published studies done on ASMQ loose combinations in other populations^{26–28}. The study suggests that no pharmacogenetic or food induced differences between Indian and other patients²⁸.

To conclude, the present study provides evidence that the new, fixed dose combination of AS and MQ over three days was well-tolerated and highly efficacious, and is a viable option for the National Vector Borne Disease Control Programme in India as a treatment for acute uncomplicated *P. falciparum* malaria.

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