REDUCED EFFICACY OF PYRONARIDINE-ARTESUNATE (PYRAMAX®) FOR THE TREATMENT OF UNCOMPLICATED *PLASMODIUM FALCIPARUM* MALARIA IN CHILDREN IN GIA LAI PROVINCE, VIETNAM (2022-2023)

Nguyen Van Thanh[1, 2,✉], Huynh Hong Quang[3], Nguyen Ngoc San[1]
Nguyen Kien Cuong[2], Trinh Xuan Phu[2], Chau Van Khanh[3]
Dang Van Hoa[3], Geoffrey W. Birrell[5], Kimberly A. Edgel[4]
Andrew G. Letizia[4], Huy C. Nguyen[4], Nicholas J. Martin[4]
Michael D. Edstein[5], Marina Chavchich[5]

[1]Hanoi Medical University
[2]Military Institute of Preventive Medicine (MIPM)
[3]Institute of Malariology, Parasitology, and Entomology Quy Nhon
[4]US Naval Medical Research Unit (NAMRU) INDO PACIFIC
[5]Australian Defence Force Malaria and Infectious Disease Institute (ADFMIDI)

There are limited data on the tolerability and efficacy of pyronaridine-artesunate (Pyramax®) for the first-line treatment of Plasmodium falciparum malaria in children. A three-day course of Pyramax® plus one dose of primaquine was administered to 50 children and 70 adults (mean age of 13.8 and 31.6 years old, respectively) infected with P. falciparum in Krong Pa district, Gia Lai province in 2022 and 2023. The follow-up period was 42 days after commencement of treatment. Pyramax® was well-tolerated, with no serious adverse event reported. The PCR-adjusted adequate clinical and parasitological response at Day 42 in children was lower than in adults (86.7%, 39/45 vs 96.7%, 59/61; P=0.069). 44.9% (22/49) children and 54.3% (38/70) adults were detected with asexual parasites on Day 3, suggestive of partial artemisinin resistance. The six children, who experienced recrudescent malaria, had significantly higher median parasitemia on Day 0 (27,693 parasites/μL vs 13,395 parasites/μL; P=0.047) and lower median blood pyronaridine concentrations on Day 7 (33.0 ng/mL vs 49.2 ng/mL; P=0.106) compared to those who remained malaria-free by Day 42. The lower efficacy of Pyramax® in children is concerning and requires monitoring.

Keywords:Pyronaridine-artesunate, Pyramax®, *Plasmodium falciparum*, children, Gia Lai, Viet Nam.

I. INTRODUCTION

Malaria remains a serious health threat to children, who lack immunity against the disease. In 2024, the World Health Organization (WHO) estimated that there were an estimated 282 million malaria cases in 80 malaria endemic countries and 610 000 malaria deaths (primarily among children under 5 years old).[1] Artemisinin-based combination therapies (ACTs) continue to be the first-line treatment of *Plasmodium falciparum* in both children and adults. Since pharmacokinetics, pharmacodynamics and drug metabolism in children differ from those in adults, therapeutic efficacy trials need to include pediatric populations.[2] Additionally, the development of resistance significantly affects

Corresponding author: Nguyen Van Thanh
Hanoi Medical University
Email: dr.thanh1981@gmail.com
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parasite pharmacodynamics.[2] Lacking malaria immunity, especially in children, is associated with a rapid onset and more severe disease.[3] For these populations, it may be necessary to increase drug exposure to achieve complete parasite clearance.[2] Thus, it is important to evaluate and monitor the treatment efficacy in different epidemiological settings, as well as in various target populations, especially in children since the treatment outcomes are often not generalizable.

In response to increasing drug resistance and malaria treatment failures, pyronaridine-artesunate (Pyramax®) was used as a replacement for artesunate-mefloquine, artemether-lumefantrine, and dihydroartemisinin-piperaquine (DHA-PPQ) in Cambodia, Laos and Viet Nam, respectively.[4] In 2020, the Vietnamese Ministry of Health recommended the use of Pyramax® to replace DHA-PPQ in the treatment of uncomplicated *P. falciparum* malaria in areas with high levels of drug resistance.[5] As yet, there are no confirmed report of resistance against Pyramax®, although partial artemisinin resistance is prevalent in the countries of the Greater Mekong Sub-region (GMS) and resistance against pyronaridine monotherapy has been previously documented in *P. falciparum* isolates from China and Myanmar.[6]

Between 2017 and 2019, Pyramax® was evaluated in Viet Nam for the treatment of uncomplicated *P. falciparum* malaria, showing overall high safety and efficacy (>95%) in both children and adults combined.[7,8] However, there are limited data on the efficacy, tolerability and drug exposure of Pyramax® for the treatment of uncomplicated *P. falciparum* malaria in children in Viet Nam. Studies conducted in African children (6 months to 12 years) have shown Pyramax® to be well tolerated, with PCR-adjusted adequate clinical and parasitological response (ACPR) rates at Day 28 exceeding 97%.[9,10]

In accordance with the WHO recommendation that the efficacy of malaria treatment regimens be evaluated at least every two years,[11] we re-evaluated Pyramax® for the treatment of uncomplicated *P. falciparum* malaria in 2022 to 2023 in Gia Lai province Central Highlands of Viet Nam.[12] As the pharmacokinetic/pharmacodynamics of drugs can vary between children and adults, we further analysed the data from our Pyramax® study in Gai Lai[12] to determine the efficacy and tolerability of Pyramax®, as well as blood pyronaridine concentration exposure in the pediatric patients and compared the findings to those in adults.

II. SUBJECTS AND METHODS

1. Subjects

Children, aged 5 to <18 years old, weighing at least 20 kg, and adults (≥18 years old) with uncomplicated *P. falciparum* malaria, with asexual parasite densities ranging from 500 to 100,000 parasites/μL, who were able to provide clinical data and blood samples were included in this study. Informed assent/consent was obtained from both children and their guardians. All adults provided informed consent. The exclusion criteria were unwillingness to provide consent, information and blood sample; inability to communicate with the study staff; pregnancy or lactation, as well as any condition that in the judgment of the investigators would make participation in the study unsafe for the potential participant.

2. Methods

Study design

This study conducted in accordance with the guidelines for monitoring antimalarial drug efficacy[11] and was a part of a prospective, open-

label, observational trial evaluating the efficacy of Pyramax® for the treatment of uncomplicated *P. falciparum* malaria in Krong Pa district, Gia Lai province, Vietnam, carried out from March 2022 to December 2023. The primary aim of the study was to evaluate the therapeutic efficacy of a 3-day course of Pyramax® plus a single dose of primaquine for the treatment of patients with *P. falciparum* malaria. The secondary aim was to determine the blood drug concentrations in children and adult patients to ensure adequate drug exposure in patients following treatment.

Sample size

For single-arm efficacy studies of antimalarial drugs against *P. falciparum* to detect treatment failure rates greater than 10%, a minimum of 50 patients is required for each age group.[11] The sample size for this study was 50 children and 70 adults.

Drug treatment

Pyramax® (180 mg pyronaridine tetraphosphate plus 60 mg artesunate per tablet; Shin Poong Pharmaceutical Co. Ltd., South Korea) was administered daily for three days according to body weight: from 20 to <24 kg, one tablet; from 24 to <45 kg, two tablets; from 45 to <65 kg, three tablets; and over 65 kg, four tablets. A single dose of primaquine (film-coated tablet of 7.5 mg; Remedica Ltd., Cyprus) (0.25 mg/kg) was administered on Day 0. The drug dosing was observed by the study team and commune health station staff within one hour of administration.

Follow-up of Pyramax® treatment

The primary treatment efficacy of Pyramax® was monitored in patients for 42 days after starting treatment to determine the ACPR of the ACT. Finger-prick blood samples were collected before treatment (Day 0) for the detection of malaria parasites and *Plasmodium* subspecies by microscopy by two malaria microscopists and PCR. Blood films and body temperature were collected twice daily (about 12 hours apart) until two consecutive blood films were negative and the axillary temperature was below 37.5°C for over 24 hours. Patients were asked to return for follow-up on Days 7, 14, 21, 28, 35 and 42, or when symptoms occurred, to monitor disease progression and the possibility of recurrence infection.

Tolerability to Pyramax®

Clinical symptoms were recorded by the study doctors before each drug administration, as well as 24 h after the last Pyramax® dose and weekly for six weeks after starting drug treatment.

Genotyping of recurrent infections

For patients with treatment failure, genotyping of *P. falciparum* was performed using *msp1 and msp2* markers as previously described, and two microsatellite markers (*PfPK2* and *PolyA*) from samples collected at Day 0 and the day of recurrence. Recrudescence and reinfection were defined according to WHO criteria.[13]

Pyronaridine blood concentrations

Drug exposure after Pyramax® treatment was determined by measuring pyronaridine (PRN) concentrations in the patient's blood on Day 7 after treatment initiation using liquid chromatography-mass spectrometry (LCMS), with a quantification limit of 1 ng/mL, as previously described.[7] The precision between tests (% coefficient of variation) for the quality control samples was <10%, and the imprecision was <2.1%.

Data analysis

Treatment efficacy was estimated as the proportion of patients with *P. falciparum* malaria who achieved PCR-adjusted ACPR on Day 42, with a 95% confidence interval (95% CI) determined by Kaplan-Meier analysis (StataMP

17, Stata Statistical Software: Release 17. TX, USA), with PCR adjustment to exclude reinfection. Descriptive statistics were provided with mean, median, and interquartile range (IQR) values. The Mann-Whitney *U* test (GraphPad version 10) was used to compare median values. A P <0.05 was considered statistically significant. Blood PRN concentration data were analysed by GraphPad version 10 software (MA, USA).

3. Ethics

The study protocol was approved by: Institute of Malariology, Parasitology, and Entomology, Quy Nhon (171/QD-VSR), Ministry of Health (242/CN-HDDD and 37/CN-HDDD), and the Australian Departments of Defence and Veterans' Affairs Human Research Ethics Committee (DDVA HREC 407-22). The study was registered with the Australian New Zealand Clinical Trials Registry (identifier number ACTRN12621001085864).

III. RESULTS

1. Demographic and clinical characteristics of study participants

Fifty children and 70 adults with *P. falciparum* malaria were enrolled from two study sites (Chu Rcam and Ia Dreh commune health stations). The majority of children were male (70%, 35/50), with a mean age of 13.8 years old (range: 7 to 17 years old). Most adults were also males (91.4%, 64/70), with a mean age of 31.6 years old (range: 18 to 54 years old). On Day 0, before Pyramax® treatment, the children's mean body temperature was 38.9°C and mean parasite density was 23,809 parasites/μL (range: 600 to 98,736). Corresponding values for adults were 38.8°C and 25,270 parasites/μL (range: 599 to 94,250). Among the 50 enrolled pediatric patients (intention-to-treat population), 45 completed the study (as per-protocol), one withdrew after the first Pyramax® dose, and four were lost to follow-up before Day 42, as shown in Fig. 1A. For adults, 64 completed the study and six were lost to follow-up before Day 42, as shown in Fig. 1B.

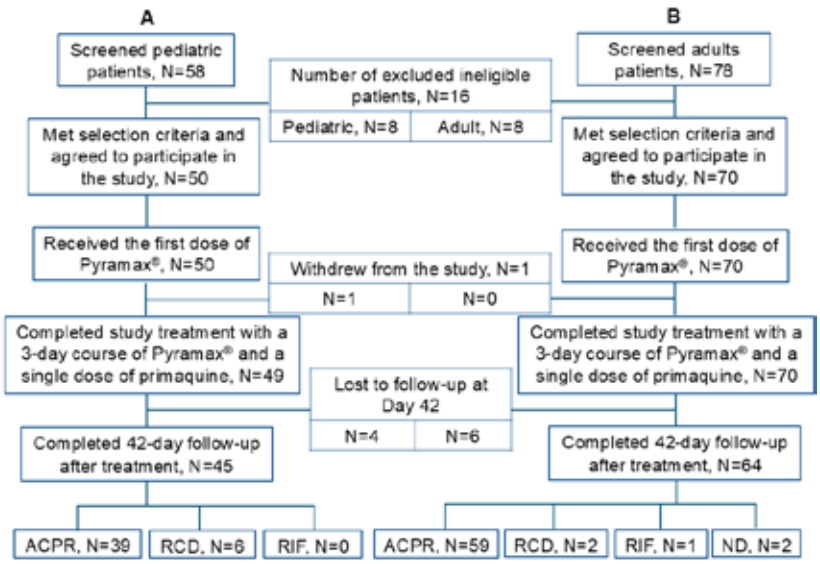

Figure 1. Flow-chart of children (A) and adult (B) participants including efficacy indicators: clinical and parasitological response (ACPR) at Day 42, recrudescence (RCD), reinfection (RIF), and not determined (ND) infection after treatment

2. Efficacy of Pyramax® in treating *P. falciparum* malaria

The median parasite clearance time (PCT) was 72 hours in children and 84 hours in adults (Table 1). The time to 50% parasite clearance (PC_{50}) in children was 10.6 hours and the time to parasite clearance half-life ($PC_{1/2}$) was 7.2 hours. Of the 40 children and 60 adult patients included in the parasite clearance estimator analysis,[15] 95.0% (38/40) of children and 95.0% (57/60) of adults had a $PC_{1/2} \geq 5$ hours. The median PCT, PC_{50}, and $PC_{1/2}$ parameters were faster in children than in adults. However, the median FCT in children was slower than in adults (36 hours *vs* 24 hours). None of the differences in efficacy parameters between children and adults were significant (Table 1).

Table 1. Demographic characteristics for pediatric and adult patients infected with *Plasmodium falciparum* malaria (intention-to-treat population) and efficacy parameters following treatment with pyronaridine-artesunate (Pyramax®)

Parameter	Patients		
	Children	Adults	*P**
Median parasite clearance time (PCT) (h), [IQR] (range)	72 [72-84] (24-120); n=49	84 [72-96] (36-132); n=70	0.220
Median fever clearance time (FCT) (h), [IQR] (range)	36 [24-48] (12-108); n=49	24 [24-36] (12-72); n=70	0.092
Median time to 50% parasite clearance**(PC_{50}) (h), [IQR], (range)	10.6 [8.1-15.2] (1.0-34.3); n=40	11.6 [7.6-16.5] (1.5-37.0); n=60	0.701
Median parasite clearance half-life** ($PC_{1/2}$) (h), [IQR], (range)	7.2 [6.1-8.5] (4.5-9.8); n=40	7.5 [6.5-8.2] (3.1-11.9); n=60	0.439

** Estimated by Mann-Whitney U test; **Determined using the WWARN parasite clearance estimator[14] based on data from 40 children and 60 adults, with at least three post-treatment parasite density measurements after initiating Pyramax® treatment. Range = (minimum, maximum)*

A high proportion of pediatric patients (44.9%; 95% CI: 30.8 to 59.0, n=22/49) had asexual parasites in their blood at Day 3 (72 hours after starting Pyramax® treatment), and this proportion was lower (*P*=0.313) than in adults of 54.3% (95% CI: 42.5 to 66.0; n=38/70). Day 0 parasitemia density in both children and adults was significantly correlated with PCT (Spearman correlation coefficient, r = 0.584, *P* <0.001; Fig 2A). Day 0 parasitemia density was significantly correlated with Day 3 parasite density (r =0.525, *P* <0.001; Fig. 2D).

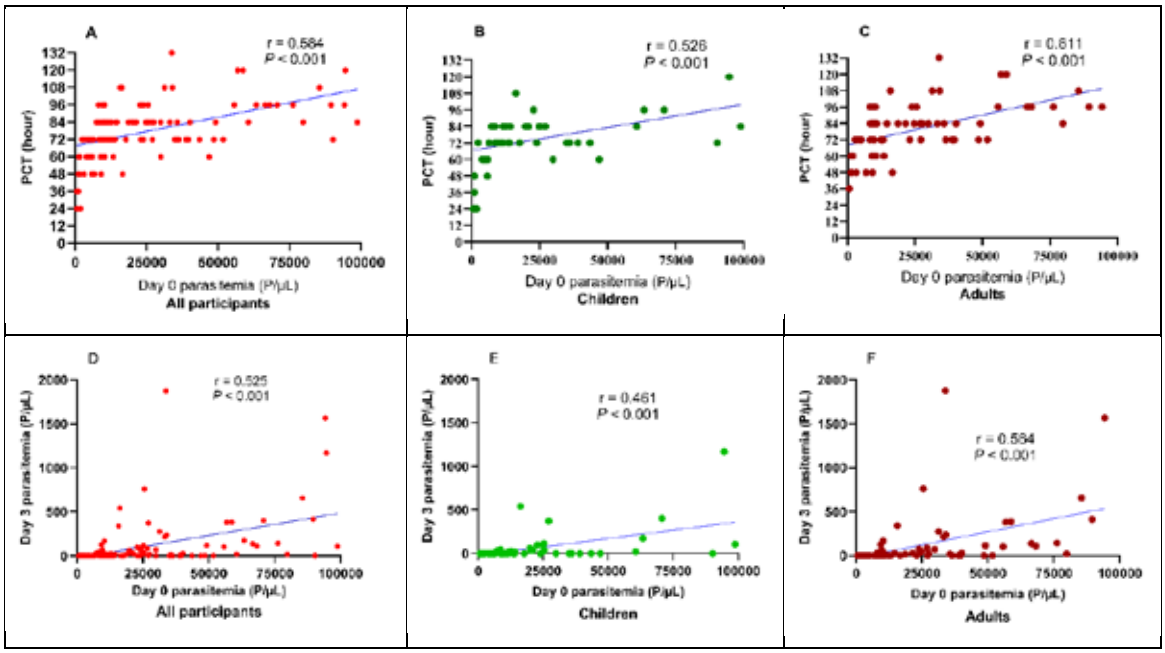

Figure 2. Correlation between Day 0 parasite density and parasite-free time

(A. Total participants, B. Children, and C. Adults). Correlation between Day 0 and Day 3 parasite densities (D. All participants, E. Children and F. Adults)

Kaplan-Meier survival analysis for children showed PCR-adjusted ACPR rates of 95.9% (47/49) on Day 28 and 86.7% (39/45) on Day 42 (Table 2 and Fig. 3). Six children had recrudescence of infection determined by genotyping of *P. falciparum* during the 42-day follow-up period (four late parasitological failures and two late clinical failures) (Fig. 1A and Table 2). For adults, PCR-adjusted ACPR rates of 95.9% (66/67) on Day 28 and 96.7% (59/61) on Day 42 (Table 2 and Fig. 2). The PCR-adjusted ACPR at Day 42 in children was lower than in adults (*P*=0.069).

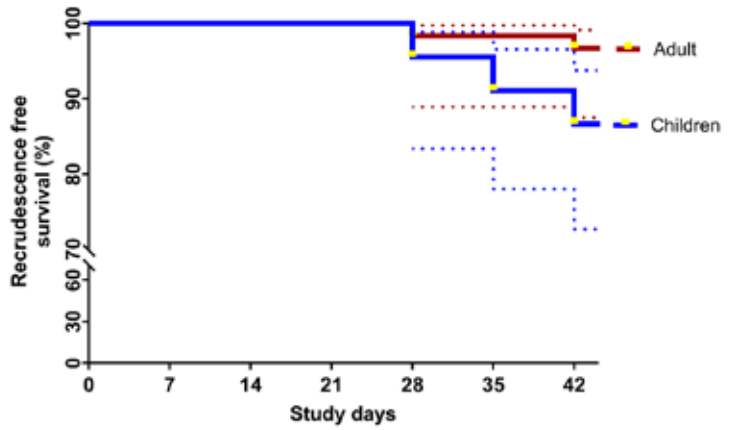

Figure 3. Kaplan-Meier survival analysis with 95% confidence intervals (dotted red and blue lines) showing PCR-adjusted ACPR over 42 days following treatment of *P. falciparum* malaria in children and adults with Pyramax®

The median parasite density in six children on Day 0, who had recrudescence (27,693 parasites/μL, 95% CI: 13,986 to 90,250) was significantly higher *(P*=0.047) than the 43 children who did not have parasites in their blood on Day 42 (13,395 parasites/μL, 95% CI: 8,613 to 21,006) (Fig. 4). Median parasite density on Day 0 between the two adults who had a recrudescence and the 65 adults who were malaria-free on Day 42 (19,299 parasites/μL, 95% CI: 13,098 to 25,500 *vs* 13,225 parasites/μL, 95% CI: 10,194 to 24,557).

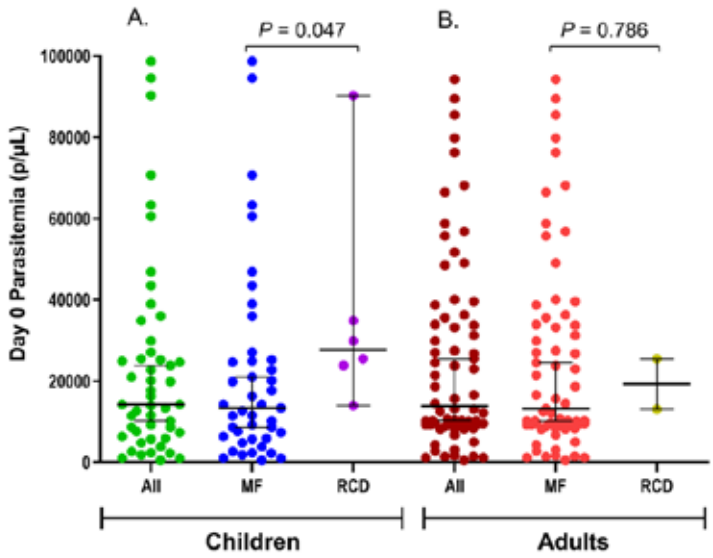

Figure 4. A. Median parasitemia (95% CI) on Day 0 for all pediatric patients (n=49) and those who were malaria-free (MF) on Day 42 (n=43) and those who had a recrudescence (RCD) by Day 42 (n=6). B. Median parasitemia in all adults (n=70); adults, who were MF on Day 42 (n=65), and adult patients, who had RCD by Day 42 (n=2)

Table 2. PCR-unadjusted and PCR-adjusted ACPR for uncomplicated *P. falciparum* malaria treated with Pyramax® in children and adults by Kaplan-Meier (KM) analysis

Treatment outcome	Day 28		Day 42	
	Children	Adult	Children	Adult
PCR-unadjusted				
Intention-to-treat (N)	50	70	50	70
Withdrawn/lost to follow-up (N)	1	3	5	6
Total patients per protocol (N)	49	67	45	64
ETF[a] (N)	0	0	0	0
LCF[b] (N)	1	0	2	2
LPF[c] (N)	1	1	4	3

Treatment outcome	Day 28		Day 42	
	Children	Adult	Children	Adult
Total failures (n)	2	1	6	5
ACPR (N)	47	66	39	59
ACPR (%)	95.9%	99.0%	86.7%	92.2%
PCR-adjusted				
Intention-to-treat (N)	50	70	50	70
Withdrawn/lost to follow-up (N)	1	3	5	6
Total patients per protocol (N)	49	67	45	64
ETF[a] (n)	0	0	0	0
LCF[b] (n)	1	0	2	0
LPF[c] (n)	1	1	4	2
Total failures (n)	2	1	6	2
New infections (n)	0	0	0	1
Non-determinant (n)	0	0	0	2
Total evaluable (N)	49	67	45	61
ACPR, n	47	66	39	59
ACPR, %	95.9%	98.5%	86.7%	96.7%
KM: cumulative cure rate (95% CI)	95.9% (84.7-99.0)	98.5% (89.9-99.8)	86.7% (72.7-93.8)	96.7% (87.5-99.2)

[a]ETF-early treatment failure; [b]LCF-late clinical failure, [c]LPF-late parasitological failure as defined by WHO.[14]

3. Tolerability of Pyramax®

Pyramax® was well tolerated in 49 pediatric patients with *P. falciparum* malaria who completed the 3-day regimen. No serious adverse event was reported by the investigators. Most pediatric patients presented with common malaria symptoms on Day 0 before receiving Pyramax® (Table 3). Clinical symptoms (including vomiting) subsided rapidly within 2 days of Pyramax® administration and by Day 3, only a few patients still had headache (20.4%, 10/49) or fatigue (4.1%, 2/49). The incidence of malaria symptoms and adverse events on Days 1, 2 and 3 after starting treatment in children appeared to be higher than in adults (Table 3).

Table 3. Malaria symptoms and adverse events before treatment with Pyramax® in children (n = 49) and adults (n = 70) infected with mono-infection of P. falciparum malaria

Adverse events		Day 0 % (n/N)	Day 1 % (n/N)	Day 2 % (n/N)	Day 3 % (n/N)
Rigors/ Chills	Children	65.3 (32/49)	34.7 (17/49)	6.1 (3/49)	2.0 (1/49)
	Adult	65.7(46/70)	37.1 (26/70)	4.3 (3/70)	1.4 (1/70)
Sweating	Children	87.8 (43/49)	44.9 (22/49)	6.1 (3/49)	2.0 (1/49)
	Adult	82.9 (58/70)	35.7 (25/70)	2.9 (2/70)	1.4 (1/70)
Headache	Children	98.0 (48/49)	91.8 (45/49)	57.1 (28/49)	20.4 (10/49)
	Adult	100 (70/70)	84.3 (59/70)	42.9 (30/70)	15.7 (11/70)
Cough	Children	4.1 (2/49)	2.0 (1/49)	0.0 (0/49)	0.0 (0/49)
	Adult	2.9 (2/70)	0.0 (0/70)	0.0 (0/70)	0.0 (0/70)
Nausea	Children	8.2 (4/49)	8.2 (4/49)	2.0 (1/49)	0.0 (0/49)
	Adult	8.6 (6/70)	5.7 (4/70)	1.4 (1/70)	0.0 (0/70)
Abdominal pain	Children	22.4 (11/49)	16.3 (8/49)	4.1 (2/49)	0.0 (0/49)
	Adult	11.4 (8/70)	2.9 (2/70)	2.9 (2/70)	1.4 (1/70)
Vomiting	Children	2.0 (1/49)	2.0 (1/49)	2.0 (1/49)	0.0 (0/49)
	Adult	4.3 (3/70)	1.4 (1/70)	0.0 (0/70)	0.0 (0/70)
Loss of appetite	Children	73.5 (36/49)	32.7 (16/49)	8.2 (4/49)	2.0 (1/49)
	Adult	70.0 (49/70)	25.7 (18/70)	7.1 (5/70)	1.4 (1/70)
Fatigue	Children	85.7 (42/49)	73.5 (36/49)	24.5 (12/49)	4.1 (2/49)
	Adult	85.7 (60/70)	65.7 (46/70)	15.7 (11/70)	2.9 (2/70)
Myalgia	Children	65.3 (32/49)	38.8 (19/49)	8.2 (4/49)	0.0 (0/49)
	Adult	71.4 (50/70)	42.9 (30/70)	8.6 (6/70)	2.9 (2/70)
Jaundice	Children	2.0 (1/49)	0.0 (0/49)	0.0 (0/49)	0.0 (0/49)
	Adult	1.4 (1/70)	1.4 (1/70)	0.0 (0/70)	0.0 (0/70)

4. Pyronaridine blood concentrations

Forty-seven children provided a blood sample with measurable PRN concentrations on Day 7, with a median concentration of 49.2 ng/mL (IQR: 33.9 to 63.8; range: 10.6 to 123.0) among the 49 children who completed the 3-day Pyramax® regimen (Fig. 5). There was no significant difference (P=0.709) in the median blood PRN concentration between children and adults (49.2 ng/mL *vs* 46.5 ng/mL, Fig. 5A). The median concentration among the six children who experienced a recrudescence was 33.0 ng/mL (IQR: 26.1 to 50.4; range: 12.4

to 90.5) which was lower than the 37 children who remained malaria-free by Day 42 (49.2 ng/mL; IQR: 36.8 to 62.4; range: 10.6 to 123.0), although this difference was not statistically significant (*P*=0.106. Mann-Whitney U-test; Fig. 5B). There were two adults who also had a lower median PRN concentration (40.0 ng/mL) than the median blood PRN concentration in the 58 adults who were malaria-free on Day 42 (46.5 ng/mL; IQR: 35.5 to 60.1; range: 16.6 to 118.7).

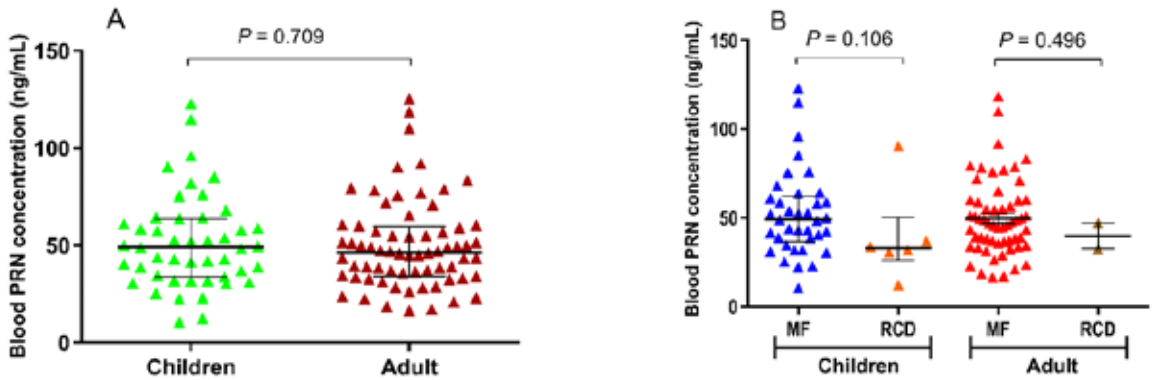

Figure 5. A. Blood pyronaridine (PRN) concentrations in 47 children and 68 adults on Day 7 after starting Pyramax® treatment, with median value and interquartile range. B. Comparison of PRN concentrations in six children with malaria recrudescence (RCD) and 37 children who were malaria-free (MF) on Day 42.

IV. DISCUSSION

In this therapeutic efficacy study of Pyramax®, we report reduced efficacy in treating children with uncomplicated *P. falciparum* malaria in Gia Lai province, Central Highlands of Viet Nam. The PCR-adjusted ACPR of Pyramax® in children at Day 42 was 86.7%, which is lower the 90% efficacy threshold recommended by WHO.[15] However, the efficacy of Pyramax® in treating adults with uncomplicated *P. falciparum* malaria at the same study sites achieved a high efficacy of 96.7%.

Little information is available on the effectiveness of Pyramax® in pediatric patients in Viet Nam and other GMS countries in treating *P. falciparum*. The efficacy observed in this study was lower than was reported in previous studies involving both children and adults conducted by Bui Quang Phuc *et al*. in Gia Lai (2017 to 2018)[8] and by Nguyen Duc Manh *et al*. in Dak Nong (2018 to 2019),[7] with Day-42 PCR-adjusted ACPR rates of 98.4% and 95.2%, respectively. In this study, the Day-28 PCR-adjusted ACPR of 95.6% was also lower than in two recent studies from Africa conducted in pediatric patients, which reported cure rates of 98.9% (93/94) in Kenya (2018)[9] and 97.4% (76/78) in Nigeria (2023).[10] The lower efficacy of Pyramax® in children compared to adults is of concern and bears vigilance as Viet Nam and other countries move toward the goal of malaria elimination or eradication.

The reasons for this apparent decline in the efficacy of Pyramax® in children with falciparum malaria after its recent introduction in Viet Nam are unclear. With malaria becoming increasingly rare in Viet Nam, one plausible explanation for the reduced efficacy of Pyramax® in children is their lower protective immunity due to a lack

of previous exposure to malaria compared to adults.[3] In the two previous therapeutic efficacy studies of Pyramax® in Viet Nam,[7,8] the number of children participating was small. In the study by Nguyen Duc Manh *et al*. only three children out of 50 patients with uncomplicated *P. falciparum* malaria participated, with a mean age of 12 years old (range: 10-13).[7] In the study by Bui Quang Phuc *et al*. in Gia Lai province, only two children aged between 5 and 15 years old out of 69 patients with *P. falciparum* were assessed for Pyramax® efficacy.[8] There was no report of treatment failures in children in the two aforementioned studies.[7,8]

In this study, a high proportion of participants were children (41.7%, 50/120), with *P. falciparum* malaria. Of note, the mean age of children, mostly males, was older (13.8 years old), with no children younger than 7 years old. The prevalence of clinical malaria in this age group is likely to be linked to the current malaria epidemiology in Central Viet Nam, with older children more likely to be exposed to mosquito vectors when accompanying adults to forested areas rather than younger children, who typically stay back with their mothers in the commune. It follows that in similar settings this age group should be considered as high malaria risk and need to be included in malaria elimination interventions. More studies, with younger children included, would be highly informative in assessing the efficacy of Pyramax®.

In addition to the reduced treatment efficacy of Pyramax® in pediatric malaria, another concern is the delayed parasite clearance, with 44.9% of children having asexual parasites in their blood on Day 3 after initiating Pyramax® treatment, exceeding the WHO threshold of ≥10%, indicating partial artemisinin resistance.[16] Data from previous studies in South and Central Viet Nam of 24.0% (40/167)[8] and in Dak Nong province of 44.9% (22/49)[7]a single-arm trial of pyronaridine-artesunate (Pyramax, PA also showed a high proportion of patients (both adults and children) with delayed parasite clearance on Day 3. Furthermore, $PC_{1/2}$ of 7.2 hours is above the WHO threshold of ≥5 hours, with 95.0% of children and adults having a $PC_{1/2}$ ≥5 hours, far exceeding the ≥10% criterion for suspected artemisinin partial resistance.[16] Prolonged $PC_{1/2}$ was also reported in our previous study in Dak Nong province.[7] Partial artemisinin resistance still remains a serious concern.

This study showed a significant positive correlation (P<0.001) between Day 0 and Day 3 parasite densities with asexual parasites surviving 72 hours after commencement of Pyramax® treatment. This is consistent with previous studies, where the initial parasitemia was significantly higher in children who had parasites on Day 3 compared to those who did not.[17] In addition, this study also showed that median parasite clearance parameters (PCT, PC_{50} and $PC_{1/2}$) in children were faster than in adults, while FCT was slower after Pyramax® treatment. Although, at present there is no report of parasite resistance to Pyramax®, the likelihood of drug tolerant and resistant parasites emerging is high as resistance against pyronaridine monotherapy has been previously documented[6] and multidrug-resistant parasites have been present in this area over the past decades.[4] Future studies are required to ensure that Pyramax® remains an effective antimalarial treatment for both children and adults.

Although this study demonstrated lower efficacy of Pyramax® in treating uncomplicated *P. falciparum* malaria in children, the ACT was well-tolerated, with no serious adverse event reported. The frequency of adverse events in this study was similar to those reported in

African children when comparing pyronaridine-artesunate and artemether-lumefantrine in Kenya (41.6% *vs* 34.4%)[9] and in Nigeria (30.6% *vs* 31.0%).[10]declining responsiveness to artemether-lumefantrine (AL The clinical symptoms experienced by children, such as fever, headache, and fatigue were typical of acute malaria and appeared unrelated to the ACT. These symptoms rapidly subsided within two days after starting Pyramax® treatment, and all patients were symptom-free within seven days, consistent with previous studies.[7,8]

There is limited information available on drug exposure to PRN in children following treatment with Pyramax®. For long half-life antimalarial drugs, the Day 7 drug concentration is a surrogate for the area under the curve from Day 7 to infinity, and is increasingly being measured as a correlate for drug exposure after ACT administration.[2] Similar Day 7 blood PRN concentrations were observed in both children and adults who were malaria-free at Day 42 of follow-up after Pyramax® treatment. Noteworthy, blood PRN concentrations on Day 7 in the six recrudescence pediatric patients were lower (although not significantly) than those of malaria-free patients on Day 42, which is a point of concern. All six children recrudesced after Day 28. Of note, one recrudescence child (IDKPGL57) classified as a late parasitological failure on Day 42, had a higher PRN concentration (90.5 ng/mL, *P*=0.207) than the five other recrudescent patients, which is of concern as it may indicate reduced parasite susceptibility or tolerance to PRN. In our previous study in Dak Nong province, Day 7 PRN concentrations in two adult patients with recrudescent malaria were similar to those who remained malaria-free on Day 42 after Pyramax® treatment.[7]

Blood concentrations on Day 7 of long-acting antimalarial, such as mefloquine and PPQ have been identified as pharmacological determinants of therapeutic responses to artesunate-mefloquine,[2,18] and DHA-PPQ,[19] respectively. Pyronaridine is also a long acting antimalarial with an elimination half-life of about two weeks.[20] Given that Pyramax® dosing was body weight-based and the drug administration was directly observed, the lower PRN concentrations among recrudescent cases may have resulted from pharmacokinetic variabilities such as reduced absorption or individual differences in metabolism and/or clearance leading to reduce therapeutic action in children. However, due to the limited number of recrudescent cases, this study lacked the statistical power to detect an association between treatment success and failure based on Day 7 blood PRN concentrations. This is a limitation of this study and future studies should aim to assess the efficacy of Pyramax® in larger numbers of children and adults, preferably at different sentinel sites in Vietnam and elsewhere. Notably, six children with recurrent malaria had significantly higher parasitemia on Day 0, than those who were malaria free. Furthermore, consistent with previous reports,[17] this study identified a significant correlation between parasite density on Day 0 and parasite density on Day 3 and with PCT. Further studies are needed to determine whether PRN concentration on Day 7 may be a clinical predictor of treatment response to Pyramax® and whether baseline parasite density may contribute to treatment failure. Adjusting the Pyramax® dose based on baseline parasite density may require further investigation.

V. CONCLUSIONS

Pyramax® was well tolerated, with no serious adverse events reported in children treated for uncomplicated *P. falciparum* malaria. The efficacy of Pyramax® remained high in adults

(96.7%), but the efficacy of 86.7% in treating *P. falciparum* in children was less than the WHO efficacy threshold of 90% for consideration to change drug policy. The lower PRN concentrations in children with recrudescent malaria, although not statistically significant likely due to a lack of power, is of concern and warrants further studies to determine whether blood PRN concentrations on Day 7 can be predictive of treatment response to Pyramax®.

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Transparency declarations

The views expressed in this article are those of the authors and do not necessarily reflect the official policy or position of the Australian Defense Force Joint Health Command or any extant Australian Defense Force policy and the Department of the Navy, the Department of Defense or the U.S. government. All other authors have no conflicts of interest to disclose.

Authors' contributions

N.V.T., H.H.Q., N.N.S., K.A.E., N.J.M., M.D.E., and M.C. conceived and designed the study. N.V.T., H.H.Q., N.K.C., T.X.P., and C.V.K. executed the study including sample collection and transportation. N.T.M.T. and M.C. performed molecular analysis. G.W.B. oversaw LC-MS/MS analysis. N.V.T., H.H.Q., N.N.S., M.D.E., and M.C. were responsible for interpretation of the data. N.V.T. performed statistical analysis. N.V.T., M.D.E., and M.C. wrote the first draft and A.G.L. edited the first draft. All authors reviewed and approved the final manuscript.

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