EVALUATION OF HEMATOLOGYCAL CHANGES IN *Plasmodium falciparum* - *INFECTED PATIENTS BEFORE AND AFTER* artesunate - mefloquine TREATMENT IN DAKLAK PROVINCE OF VIETNAM

Quang Huynh Hong[1], Khanh Van Chau[1], Hien Thanh Pham[1], Hien Tinh Tran[2], Nhien Thuy Thanh Nguyen[2], Hong Duc Nguyen[1].

Summary

The hematological changes following treatment have been poorly understood. This study was designed to determine the hematological alterations and recovery in *plasmodium falciparum* - infected patients, treated with Artesunate/Mefloquin. A retrospective study was conducted in Daklak province, located in central highland Vietnam, between August 2019 and April 2020. All data from 80 patients who were diagnosed with *P. falciparum* infection - including clinical characteristics, and hematological parameters in 42 days follow up - were reviewed and analyzed.The results showed that there were no anemia ($p < 0.001$), no leukopenia ($p < 0.001$), no leukocytosis ($p < 0.001$), but thrombocytopenia ($p = 0.018$) in 80 patients before treatment. There was a slightly decrease in hemoglobin (HGB) at day 7 ($p = 0.05$), then HGB significantly increased at day 42 after treatment ($p < 0.001$). White blood cell (WBC) and platelet (PLT) counts rapidly recovered at day 7 after treatment ($p < 0.001$ and $p < 0.001$, respectively). This study indicated that malaria patients treated with Artesunate/Mefloquin exhibit important changes in hematological parameters with HGB and PLT counts being the two most important changes before and after treatment. This data could be useful for detection, treatment and prevention of malaria in Vietnam.

Key words: malaria; *plasmodium falciparum;* treatment; hematological recovery.

INTRODUCTION

Changes in blood cell counts are a well - known features of malarial infections. These changes involve major cell lines including red blood cell (RBC), leukocyte and thrombocyte. Hematological changes in the course of a malaria infection, such as anemia, thrombocytopenia and leukocytosis or leucopoenia are well recognized. These alterations vary with the level of malarial endemicity, background hemoglobinopathy, nutritional status, demographic factors, and also malaria immunity[1,2,3].

The World Health Organization (WHO) protocol for the evaluation of an antimalarial drug or drugcombination includes hematological recovery as an efficacy end - point[4,5]. Although the treatment of uncomplicated *P. falciparum* malaria aims at clearing parasite, relieving symptoms and permitting hematological recovery, data on the impact of antimalarial treatment on hematological recovery are few. Further research is needed to assess the factors related to hematological recovery in order to exploit

(1)Institute of Malariology, Parasitology and Entomology Quynhon, Binhdinh, Vietnam. (2)Oxford University Clinical Research Unit (OUCRU), Vietnam
Date of receipt: *October 16, 2020.*
Date of reviewed completions: *October 23, 2020.*
Accepted date for publication: *November 11, 2020.*
Responsibility for the scientific content of the article: Quang Huynh Hong, Institute of Malariology, Parasitology and Entomology Quynhon, Binhdinh, Vietnam.
Tel: 0905103496. E-mail: huynhquangimpe@yahoo.com

the underlying mechanisms.

Therefore, this study was designed to determine hematological changes in *P. falciparum* infected - patients before and after treatment with Artesunate/Mefloquin. An understanding of hematological response and recovery following the treatment of uncomplicated malaria is crucial if one is to quantify the risks and benefits of treatment.

MATERIAL AND METHODS

Study population: We retrospectively reviewed the results of peripheral blood counts and parasite density of 80 patients admitted to the hospital for treatment of *P. falciparum* malaria between August 2019 and April 2020. The inclusion criteria included following: mono - infection with *P. falciparum* confirmed by positive blood smear (i.e. no mixed infection); parasitemia of minimum 1000 per µl asexual forms. Patients, who consented for participation in study, were treated with Artesunate/Mefloquin 100/200mg at a dose of two tablets daily for 3 days and were required to undergo regular clinical reassessment.

Baseline evaluation

Demographic and clinical characteristics of patient were recorded. A complete blood count, including red blood cell, hemoglobin, white blood cell and platelet count, was determined by an automated cell counter (Abacus 380, Hungary).

Parasite density, expressed as the number of asexual parasite per µl of blood, will be calculated by dividing the number of parasite by the number of white blood cell counted and then multiplying by an assumed white blood cell density (8000 per µl).

$$\text{Parasite density (per µl)} = \frac{\text{Number of parasites counted} \times (8000)}{\text{Number of leukocytes counted}}$$

Follow-up study over 42 days

The day a patient was enrolled and received the first dose was designated day 0. Blood films for parasite countwere made every 12h on days 1, 2, 3 until clear, then weekly for the remainder of the follow - up period; i.e. on days 7, 14, 21, 28, 35 and 42. Complete blood count was performed at days 0 (i.e. before patient received the first dose), 7 and 42.

Statistical analysis

Complete blood count at day 0 followed a normal distribution, therefore, the differences in hematological parameters at day 0 compared to normal values were analyzed by using One - simple T - test. In contrast, complete blood count at day 7 and 42 followed a skewed distribution, therefore, the differences in hematological parameters before and after treatment were analyzed by using the Wilcoxon signed rank test. Data analysis was performed using IBM SPSS Statistics 20.0 (SPSS Inc., Chicago, IL, USA).

RESULTS

Patient's characteristics:

Between August 2019 and April 2020, 80 patients who were infected with *P. falciparum,* with all parameters available, were recruited to this study. The mean age was 33 (± 10) years. There was a male predominance, with a male to female ratio of 77:3. Most patients represented at the hospital with fever, the mean temperature was 39 (± 0.6) degrees Celsius. The median parasite density was 10200 per µl (IQR: 2523 - 34266). All the characteristics of patient are shown in Table 1.

Table 1. Patient's demographic and characteristics

Characteristics	All patients (n = 8)
Age (mean ± SD) (year)	33 (± 0)
Sex (male/female ratio)	77:3
Height (mean ± SD) (cm)	163 (± 5)
Weight (mean ± SD) (kg)	58 (± 5)
Temperature (mean ± SD) (oC)	39 (± 0.6)
Median parasite density (IQR) (no./µl)	11058 (IQR: 2389 34211)

Differences in hematological parameters at day 0 and normal values.

The results are shown in table 2. HGB and WBC counts at day 0 were in normal range ($p < 0.001$) PLT count was significantly lower than the normal lowest point (150 x 103µl) (p 0.018).

Table 2. Differences in hematological parameters at day 0 and normal values

Parameters	Day 0 (Mean ± SD)	Normal values	P - value
RBC ($\times 10^6$/µl)	4.87 (± 0.68)		
HGB (g/l)	128.65 (± 15.40)	>= 110	< 0.001
WBC ($\times 10^3$/µl)	5.84 (± 1.63)	4 – 11	< 0.001 - < 0.001
PLT ($\times 10^3$/µl)	132.59 (± 64.19)	>= 150	0.018

P - value by One simple T - test

Differences in hematological parameters before and 7 days after treatment

Hematological alterations at day 7 following treatment have been investigated, and the results are shown in table 3. There was a slightly decrease in RBC count but this decrease was not statistically significant (p = 0.314). HGB was statistically lower at day 7 after treatment compared to before treatment (p = 0.05). Both WBC and PLT counts were significantly higher at day 7 after treatment compared to before treatment (p < 0.001).

Table 3. Differences in hematological parameters before and 7 days after treatment

Parameters	Day 0 (Mean ± SD)	Day 7 (Mean ± SD)	P - value
RBC (× 10^6/µl)	4.87 (± 0.68)	4.83 (± 0.71)	0.314
HGB (g/l)	128.65 (± 15.40)	126.29 (± 16.49)	0.050
WBC (× 10^3/µl)	5.84 (± 1.63)	7.47 (± 1.92)	< 0.001
PLT (× 10^3/µl)	132.59 (± 64.19)	275.73 (± 89.45)	< 0.001

P - value by Wilcoxon signed rank test

Differences in hematological parameters before and 42 days after treatment

Hematological alterations at day 42 after treatment have been investigated, and the results are shown in table 4. RBC count and HGB were significantly higher at day 42 after treatment compared to before treatment (p < 0.05). Similarly, both WBC and PLT counts were significantly higher at day 42 after treatment compared to before treatment (p < 0.05).

Table 4. Differences in hematological parameters before and 42 days after treatment

Parameters	Day 0 (Mean ± SD)	Day 42 (Mean ± SD)	P - value
RBC (× 10^6/µl)	4.87 (± 0.68)	5.07 (± 0.57)	0.016
HGB (g/l)	128.65 (± 15.40)	138.09 (± 15.72)	< 0.001
WBC (× 10^3/µl)	5.84 (± 1.63)	6.60 (± 2.34)	0.037
PLT (× 10^3/µl)	132.59 (± 64.19)	231.65 (± 70.50)	< 0.001

P - value by Wilcoxon signed rank test

Differences in hematological parameters between day 7 and day 42 after treatment

The results are shown in table 5. There was a significantly increase in RBC and HGB count at day 42 after treatment compared to its level at day 7 (p = 0.003 and p < 0.001 respectively). Both WBC and PLT count were significantly lower at day 42 after treatment compared to the figures at day 7 (p = 0.003, p < 0.001).

Table 5. Differences in hematological parameters between day 7 and day 42 after treatment

Parameters	Day 7 (Mean ± SD)	Day 42 (Mean ± SD)	P - value
RBC (× 10^6/µl)	4.83 (± 0.71)	5.07 (± 0.57)	0.003
HGB (g/l)	126.29 (± 16.49)	138.09 (± 15.72)	<0.001
WBC (× 10^3/µl)	7.47 (± 1.92)	6.60 (± 2.34)	0.003
PLT (× 10^3/µl)	275.73 (± 89.45)	231.65 (± 70.50)	<0.001

P - value by Wilcoxon signed rank test

DISCUSSION

This study found that there was no anemia in 80 patients who participated (p < 0.001). A previous study found that although the pathogenesis of anemia in malaria is complex and poorly understood, it is commonly seen in patients with *P. falciparum* malaria[6]. In addition, previous studies demonstrated a significant increase in the prevalence of anemia with increase in parasite density[7]. Similarly, a previous study reported that anemia was observed in 72.4% of patients with a high parasite count, as well as an inverse relationship between parasite densities and hemoglobin levels[8]. While patients in this study had low parasite density (Median: 11058, IQR: 2389 - 34211), which could explain this study's finding about hemoglobin at baseline.

This study found that hemoglobin decreased within the first week following treatment (p = 0.05) but then significantly increased again at day 42 (p < 0.001). A previous study indicated that hemoglobin concentration is significantly lower following the initial drug regimen compared to before treatment[9]. Then hemoglobin level increased progressively through day 42. This was correlated with a previous study, which showed that between day 0 and day 42, there was a significant difference in the drop in prevalence of anemia between treated and untreated patients (p = 0.04)[10].

In this study, WBC count was in normal range (4 – 11 × 10^3/µl) (p < 0.001), unlike some studies which showed that leukopenia appears to be a common finding in patients with *P. falciparum* malaria, where may be as low as 1 – 2 × 10^3/µl.[11] WBC count increased within 7 days after treatment (p < 0.001). A previous study also indicated that the total leukocyte is improved following standard treatment. It was correlated with another study showed that patients who were treated with antimalarial drugs

exhibited leukocyte change following the initial drug regimen[9].

This study found that there was thrombocytopenia in patients before treatment ($p = 0.018$). This is correlated with the previous study indicating that a reduction in the number of platelets is one of the most well - known hematological changes observed in patients with malaria. Results from other previous studies showed that thrombocytopenia seems to occur through peripheral destruction. Immune - mediated destruction of circulating platelets may be a cause of thrombocytopenia in malaria infections, especially those caused by *P. falciparum*.

PLT count significantly increased within 7 days after treatment ($p < 0.001$). A previous study showed that platelet count is significantly higher following the initial drug regimen compared to before treatment[9]. This suggests that PLT response after completion of antimalarial therapy is a good early predictor of a good recovery. A decrease in PLT count was observed at day 42 ($231.65 \pm 70.50 \times 10^3/\mu l$) compared to PLT count at Day 7 ($275.73 \pm 89.45 \times 10^3/\mu l$) ($p < 0.001$). This finding is correlated to previous study which showed a decrease in platelet count at D42 following treatment[10].

CONCLUSIONS

In conclusion, patients treated with Artesunate/Mefloquin exhibit important changes in hematological parameters with hemoglobin and platelet count being the two most important changes before and after treatment. This data could be useful for detection, treatment and prevention of malaria in Vietnam.

Compliance with ethical standard

Acknowledgements. The authors would like to appreciate all colleagues in tropical diseases research and treatment department in the sentinel sites, especially focal points of malaria in Daklak province, Vietnam.

Conflict of interest. The authors declared that they have no conflicts of interest.

Statement of ethical approval. The research protocol was reviewed by…

Statement of informed consent. Informed consent was obtained from all individual participants included in the study.

REFERENCES

1. Erhart LM, Yingyuen K, Chuanak N, Buathong N, Laoboonchai A, Miller RS, et al. Hematologic and clinical indices of malaria in a semi-immune population of western Thailand. The American journal of tropical medicine and hygiene. 2004;70(1):8-14.

2. Price RN, Simpson JA, Nosten F, Luxemburger C, Hkirjaroen L, ter Kuile F, et al. Factors contributing to anemia after uncomplicated falciparum malaria. The American journal of tropical medicine and hygiene. 2001;65(5):614-622.

3. Wickramasinghe SN, Abdalla SH. Blood and bone marrow changes in malaria. Bailliere's best practice & research. Clinical haematology. 2000 Jun;13(2):277-299.

4. World Health Organization. Assessment of therapeutic efficacy of antimalarial drugs for uncomplicated falciparum malaria in areas with intense transmission. Division of Control of Tropical Diseases□. 1996. Available from: https://apps.who.int/iris/handle/10665/63295.

5. World Health Organization. Monitoring antimalarial drug resistance: report of a WHO consultation, Geneva, Switzerland, 3-5 December 2001. Cluster on Communicable Diseases & WHO Consultation on Monitoring Antimalarial Drug Resistance. 2002. Available from: https://apps.who.int/iris/handle/10665/67590.

6. Bashawri LA, Mandil AA, Bahnassy AA, Ahmed MA. Malaria: hematological aspects. Annals of Saudi medicine. 2002;22(5-6):372-376.

7. Kitua AY, Smith TA, Alonso PL, Urassa H, Masanja H, Kimario J, et al. The role of low level Plasmodium falciparum parasitaemia in anaemia among infants living in an area of intense and perennial transmission. Tropical medicine & international health. 1997;2(4):325-333.

8. Ali H, Ahsan T, Mahmood T, Bakht SF, Farooq MU, Ahmed N. Parasite density and the spectrum of clinical illness in falciparum malaria. Journal of the College of Physicians and Surgeons – Pakistan. 2008;18(6):362-368.

9. Kotepui M, Punsawad C, Kotepui KU, Somsak V, Phiwklam N, PhunPhuech B. Prevalence of malarial recurrence and hematological alteration following the initial drug regimen: a retrospective study in Western Thailand. BMC Public Health. 2019 Oct 15;19(1):1294.

10. Sumbele I, Theresa NA, Samje M, Thomas Ndzeize, Manka N, Titanji V.Haematological changes and recovery associated with treated and untreated Plasmodium falciparum infection in children in the Mount Cameroon Region. Journal of Clinical Medicine and Research. 2010;2:143-151.

11. Facer CA. Hematological aspects of malaria: Infection and Hematology. Oxford: Butterworth Heinmann Ltd. 1994:259-294.