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Evaluation of hematological changes in *Plasmodium falciparum*-infected patients before and after artesunate-mefloquine treatment in Dak Lak province, Vietnam

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Abstract

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 Dak Lak province, located in Central highlands 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.

Keywords: Malaria; *Plasmodium falciparum*; Treatment; Hematological recovery

1. 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 leucopenia 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 drug combination 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 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.

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2. Material and methods

2.1. 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 100/200 mg at a dose of two tablets daily for 3 days and were required to undergo regular clinical reassessment.

2.2. 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).

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

2.3. 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 count were 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.

2.4. 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-sample 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).

3. Results and discussion

3.1. Results

3.1.1. 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=80) |
|---|---------------------------|
| Age (mean \pm SD) (year) | 33 (± 10) |
| Sex (male/female ratio) | 77:3 |
| Height (mean \pm SD) (cm) | 163 (± 5) |
| Weight (mean \pm SD) (kg) | 58 (± 5) |
| Temperature (mean \pm SD) ($^{\circ}\text{C}$) | 39 (± 0.6) |
| Median parasite density (IQR) (no./ μl) | 11058 (IQR: 2389 – 34211) |

3.1.2. 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 ($150 \times 10^3/\mu\text{l}$) ($p = 0.018$).

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

| Parameters | Day 0 (Mean \pm SD) | Normal values | P-value |
|-----------------------------------|------------------------|---------------|-----------------------|
| RBC ($\times 10^6/\mu\text{l}$) | 4.87 (± 0.68) | | |
| HGB (g/l) | 128.65 (± 15.40) | ≥ 110 | < 0.001 |
| WBC ($\times 10^3/\mu\text{l}$) | 5.84 (± 1.63) | 4 - 11 | < 0.001 - < 0.001 |
| PLT ($\times 10^3/\mu\text{l}$) | 132.59 (± 64.19) | ≥ 150 | 0.018 |

P-value by one simple T-test

3.1.3. 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 \pm SD) | Day 7 (Mean \pm SD) | P-value |
|-----------------------------------|------------------------|------------------------|-----------|
| RBC ($\times 10^6/\mu\text{l}$) | 4.87 (± 0.68) | 4.83 (± 0.71) | 0.314 |
| HGB (g/l) | 128.65 (± 15.40) | 126.29 (± 16.49) | 0.050 |
| WBC ($\times 10^3/\mu\text{l}$) | 5.84 (± 1.63) | 7.47 (± 1.92) | < 0.001 |
| PLT ($\times 10^3/\mu\text{l}$) | 132.59 (± 64.19) | 275.73 (± 89.45) | < 0.001 |

P-value by Wilcoxon signed rank test

3.1.4. 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 \pm SD) | Day 42 (Mean \pm SD) | P-value |
|-----------------------------------|------------------------|------------------------|-----------|
| RBC ($\times 10^6/\mu\text{l}$) | 4.87 (± 0.68) | 5.07 (± 0.57) | 0.016 |
| HGB (g/l) | 128.65 (± 15.40) | 138.09 (± 15.72) | < 0.001 |
| WBC ($\times 10^3/\mu\text{l}$) | 5.84 (± 1.63) | 6.60 (± 2.34) | 0.037 |
| PLT ($\times 10^3/\mu\text{l}$) | 132.59 (± 64.19) | 231.65 (± 70.50) | < 0.001 |

P-value by Wilcoxon signed rank test

3.1.5. 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 counts 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 ($\times 10^6/\mu\text{l}$) | 4.83 (± 0.71) | 5.07 (± 0.57) | 0.003 |
| HGB (g/l) | 126.29 (± 16.49) | 138.09 (± 15.72) | <0.001 |
| WBC ($\times 10^3/\mu\text{l}$) | 7.47 (± 1.92) | 6.60 (± 2.34) | 0.003 |
| PLT ($\times 10^3/\mu\text{l}$) | 275.73 (± 89.45) | 231.65 (± 70.50) | <0.001 |

P-value by Wilcoxon signed rank test

3.2. 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 ($4\text{--}11 \times 10^3/\mu\text{l}$) ($p < 0.001$), 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 $1\text{--}2 \times 10^3/\mu\text{l}$. [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 [12]. And 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 [13]. Results from other previous studies showed that thrombocytopenia seems to occur through peripheral destruction [14]. Immune-mediated destruction of circulating platelets may be a cause of thrombocytopenia in malaria infections, especially those caused by *P. falciparum* [15].

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 ($231.65 \pm 70.50 \times 10^3/\mu\text{l}$) compared to PLT count at Day 7 ($275.73 \pm 89.45 \times 10^3/\mu\text{l}$) ($p < 0.001$). This finding is correlated to previous study which showed a decrease in platelet count at D42 following treatment [10].

4. Conclusion

This study can conclude that 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 standards

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Disclosure of conflict of interest

The authors declared that they have no conflicts of interest.

Statement of ethical approval

The research protocol was reviewed by the Institutional Review Board of the Institute of Malariology, Parasitology and Entomology Quy Nhon.

Statement of informed consent

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

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