Turkiye Parazitol Derg 2024;48(4):256-60
DOI: 10.4274/tpd.galenos.2024.25633

Two Imported Malaria Cases with Delayed Response to Treatment in Hatay

Hatay'da Yurt Dışı Kaynaklı Tedaviye Geç Yanıt Veren İki Sıtma Olgusu

Tuğba Kaya[1], Mehmet Çabalak[2], Gülnaz Çulha[1], Ceren Ünal[1]
[1]Hatay Mustafa Kemal University Faculty of Medicine, Department of Parasitology, Hatay, Türkiye
[2]Hatay Mustafa Kemal University Faculty of Medicine, Department of Infectious Diseases and Clinical Microbiology, Hatay, Türkiye

Cite this article as: Kaya T, Çabalak M, Çulha G, Ünal C. Two Imported Malaria Cases with Delayed Response to Treatment in Hatay. Turkiye Parazitol Derg. 2024;48(4):256-60.

ABSTRACT

The study presents two imported malaria cases with a history of travel to malaria-endemic areas and replied late response to treatment. In the blood preparations of the first case, dot-shaped nucleus structures were identified in the erythrocytes, which looked different from the classical erythrocytic forms. In the SD-Pf/Pan test, bands were obtained for both P.f and Pan; while in the SD-Pf/Pv test, a band was obtained for P.f. The *P. falciparum 18S rRNA* gene was detected using real-time polymerase chain reaction. Artemether-lumefantrine treatment protocol was started. Due to deterioration in general condition on the third day, artemether-lumefantrine treatment was extended to six days, and primaquine phosphate was added. Discharge was on the 16th day of treatment. In the second case, young trophozoites were identified in blood smears. Bands in P.f were obtained in both the SD-Pf/Pan and SD-Pf/Pv tests. Artemether-lumefantrine treatment protocol was started. On the third day of treatment, banana-like gametocytes were observed in blood smears. The patient was discharged at his own request and two days later, upon follow-up, gametocytes were still observed in blood smears. Artemether-lumefantrine treatment was restarted. Gametocytes continued to be observed in the following days. Primaquine phosphate was added to the treatment protocol. The patient was discharged after a 3-week follow-up. The study is presented to draw attention to the increasing cases of imported malaria in Hatay and the increase of malaria cases that respond late to treatment in recent years.
Keywords: Imported malaria, *Plasmodium falciparum*, Hatay

ÖZ

Çalışmada, sıtma endemik bölgeye seyahat öyküsü bulunan ve tedaviye geç yanıt veren iki importe sıtma olgusu sunulmaktadır. Birinci olgunun kan preparatlarında, eritrositlerde klasik eritrositer formlardan farklı görünümde nokta tarzında nükleus yapıları saptandı. SD Bioline Malaria Ag P.f/Pan (SD-Pf/Pan) testinde P.f ve Pan'da; SD Bioline Malaria Ag P.f/P.v (SD-Pf/Pv) testinde P.f'da band elde edildi. Gerçek zamanlı polimeraz zincir reaksiyonunda *P. falciparum 18S rRNA* geni saptandı. Artemether-lumefantrine tedavi protokolü başlanıldı. Genel durumunun üçüncü gününde kötüleşmesi nedeniyle artemether-lumefantrine tedavisi altı güne uzatıldı ve primaquine phosphate eklendi. Tedavisinin 16. gününde taburcu edildi. İkinci olgunun kan preparatlarında genç trofozoitler saptandı. SD-Pf/Pan ve SD-Pf/Pv testlerinde P.f'da band elde edildi. Artemether-lumefantrine tedavi protokolü başlanıldı. Tedavinin üçüncü gününde, kan preparatlarında muz şeklinde gametositler görüldü. Hasta kendi isteği ile taburcu oldu ve iki gün sonra kontrole geldiğinde kan preparatlarında gametositler görülmeye devam etti. Artemether-lumefantrine tedavisi tekrar başlanıldı. Sonraki günlerde gametositler görülmeye devam etti. Tedavi protokolüne primaquine phosphate eklendi. Üç haftalık takip sonunda hasta taburcu edildi. Çalışma Hatay'da artan importe sıtma olgularına ve tedaviye geç cevap veren sıtma olgularının son yıllardaki artışına dikkat çekmek amacıyla sunulmuştur.
Anahtar Kelimeler: İmporte sıtma, *Plasmodium falciparum*, Hatay

INTRODUCTION

Malaria is a disease transmitted to humans by female Anopheles mosquitoes infected with parasites of the *Plasmodium* genus. According to World Health Organization data, approximately half of the world's population is at risk, with approximately 247 million people infected with malaria in 85 countries in 2021, resulting in approximately 619,000 deaths. (1) Since 2010, no indigenous malaria cases have been reported in Türkiye, but imported cases are observed (2).

Received/Geliş Tarihi: 07.05.2024 **Accepted/Kabul Tarihi:** 13.12.2024 **Publication Date/Yayınlanma Tarihi:** 22.01.2025

Address for Correspondence/Yazar Adresi: Tuğba Kaya, Hatay Mustafa Kemal University Faculty of Medicine, Department of Parasitology, Hatay, Türkiye
E-mail/E-Posta: tugbakaya42@yahoo.com **ORCID ID:** orcid.org/0000-0001-7612-5414

***This case presentation was presented at the 23rd Parasitology Congress held in Antalya between October 30th and November 3rd.**

Türkiye PARAZİTOLOJİ Dergisi

Malaria diagnosis is made by observing the parasite under a microscope during the examination of blood preparations prepared from patient samples. Additionally, rapid diagnostic tests prepared to detect *Plasmodium* antigens and serological and molecular tests are also used for diagnosis (3).

Quinine is the first drug used in malaria treatment. Resistance developed 278 years after its use as an antimalarial agent, leading to the use of chloroquine in malaria treatment. After resistance also developed against chloroquine 12 years later, proguanil, sulfadoxine-pyrimethamine, mefloquine, and atovaquone began to be preferred in malaria treatment as antimalarial drugs. However, resistance to these agents has been reported (4).

The aim of the study was to draw attention to the diagnosis, effective treatment protocol, and importance of patient follow-up in malaria, following the identification of two cases of malaria from foreign sources traveling to malaria-endemic areas and showing delayed response to treatment.

CASE REPORT

Case 1

A 50-year-old patient who presented to the Infectious Diseases Outpatient Clinic of Hatay Mustafa Kemal University Faculty of Medicine with complaints of fever, chills, and tremors for the past three days revealed, upon history-taking, that he had traveled to Ghana for work and returned to Hatay three days ago. It was learned that he had received malaria treatment in Ghana but sought medical attention due to ongoing complaints.

On physical examination, the patient's general condition was moderate, vital signs were stable, with a temperature of 38 °C, and hepatosplenomegaly was present. Laboratory findings showed: white blood cell (WBC); 13230 µ/L, hemoglobin (Hgb); 13.5 g/dL, platelets; 90,000 µ/L, aspartate transaminase (AST): 214 U/L, alanine transaminase (ALT): 92 U/L, T. bil: 2.17 mg/dL, D. bil: 1.23 mg/dL, lactate dehydrogenase: 1086 U/L, C-reactive protein (CRP): 126 mg/dL, prothrombin time-international normalized ratio (PTZ-INR): 1.16.

Since the clinical symptoms were consistent with malaria and due to the history of international travel, blood was drawn from the patient and sent to the parasitology department. Thick and thin blood smears were prepared and stained with Giemsa staining method. During the examination of thin blood smears under the immersion objective, nucleus structures resembling dot-like formations, different from the classic erythrocytic forms, were observed in erythrocytes. Nucleus structures were also detected in the examination of thick blood smear preparations (Figure 1). Two rapid diagnostic tests recommended by the manufacturer's protocol were used to distinguish *P. falciparum* and other *Plasmodium* species from the peripheral blood sample taken from the patient: SD Bioline Malaria Ag P.f/Pan (SD-Pf/Pan) and SD Bioline Malaria Ag P.f/P.v (SD-Pf/Pv) (Standard Diagnostics, Inc; Suwon City, Republic of Korea). In the SD-Pf/Pan rapid diagnostic test, positive bands were obtained in both P.f and Pan, while in the SD-Pf/Pv test, a positive band was only obtained in P.f (Figure 2). DNA isolation was performed from the blood sample obtained from the patient using a commercial kit (QIAamp DNA Isolation kit, Qiagen, Germany), and species-specific real-time polymerase chain reaction (RT-PCR) was performed using a commercially prepared kit (Genesig® Std Real-time PCR detection kit for *P. falciparum, P.vivax, P. ovale*, *P. malaria*, Primerdesign™ Ltd., Chandler's Ford, UK). The PCR mixture and amplification protocol were applied according to the manufacturer's protocol. The *P. falciparum 18S rRNA* gene was detected.

The patient was treated with Artemether-lumefantrine therapy according to the protocol: Four tablets every 8 hours on the first day, followed by 4 tablets every 12 hours for the next two days. Due to shortness of breath observed during patient follow-ups, consultations with chest diseases and cardiology were sought, and their recommendations were followed. Additionally, on the third day of treatment, as the patient's condition worsened significantly and the presence of atypical morphology with dot-like nucleus structures continued to be observed in blood smears, the Artemether-lumefantrine treatment was extended to six days, and primaquine phosphate was added to the treatment protocol.

Peripheral blood samples were taken from the patient daily, and thin and thick blood smears were prepared and examined. On the third day of treatment, dot-like nucleus structures were still observed intensely. However, on the 10th day, there was a decrease in the atypical morphology observed, and the patient was discharged on the 16th day of follow-up (Figure 3, 4). The patient, whose condition improved clinically, was discharged with a follow-up appointment recommended 10 days later.

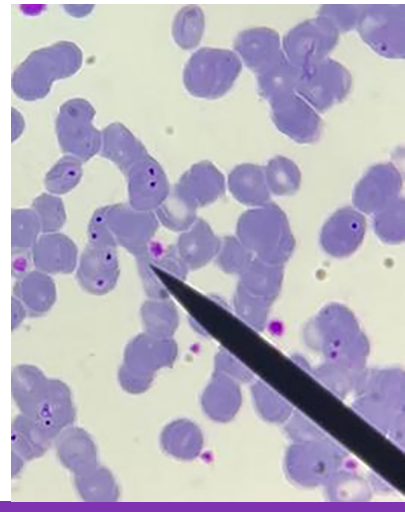

Figure 1. Point-like nuclear structures in the thin blood smear (100X magnification)

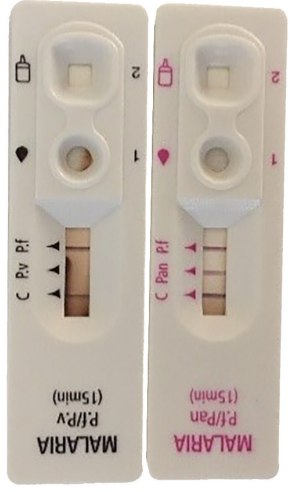

Figure 2. Rapid diagnostic tests for case 1

Case 2

A 54-year-old male patient presented to the Infectious Diseases Outpatient Clinic of Hatay Mustafa Kemal University Faculty of Medicine with complaints of high fever, chills, and tremors for the past 20 days. It was learned that the patient had been working in Sudan, had come to Hatay on leave, and had received malaria treatment in Sudan.

On physical examination, the patient's general condition was moderate, vital signs were stable, with a temperature of 39 °C, and hepatosplenomegaly was present. Laboratory findings showed: WBC; 624 µ/L, Hgb; 11.4 g/dL, platelets; 339,000 µ/L, AST: 29 U/L, ALT: 25 U/L, T. bil: 1.73 mg/dL, D. bil: 0.66 mg/dL, LDH: 345 U/L, CRP: 148 mg/dL, PTZ-INR: 1.12.

Due to clinical suspicion of malaria based on the patient's symptoms, laboratory findings, and history of working abroad, blood was drawn from the patient and referred to the parasitology department. Young trophozoites were detected in erythrocytes in thin smear blood preparations every 3-4 fields (Figure 5). Two rapid diagnostic tests, SD-Pf/Pan (Standard Diagnostics, Inc; Suwon City, Republic of Korea) and SD-Pf/Pv (Standard Diagnostics, Inc; Suwon City, Republic of Korea), were performed, and positive bands were obtained in P.f in both rapid diagnostic tests (Figure 6). The patient was admitted to the infectious diseases ward. Upon admission, the patient, who was in good general condition, was treated with the Artemether-lumefantrine therapy protocol.

Blood was drawn from the patient daily, and thick drop and thin smear preparations were examined in the parasitology department. On the third day of treatment, 1-2 banana-like gametocytes were detected in each preparation of the blood sample taken from the patient (Figure 7). The patient was advised to continue treatment. However, against medical advice, the patient chose to discharge themselves. Two days later, when the patient returned for a follow-up, gametocytes were still observed in preparations made from their blood samples. The Artemether-lumefantrine treatment protocol was restarted. Due to shortness of breath and low saturation observed during patient follow-ups, the patient was admitted to the intensive care unit and closely monitored. As gametocytes continued to be observed in subsequent blood samples, primaquine phosphate 1x1 was added to the treatment protocol for 14 days.

After approximately three weeks of follow-up, as gametocytes were no longer observed in blood preparations and the patient's clinical condition improved, they were discharged (Figure 8).

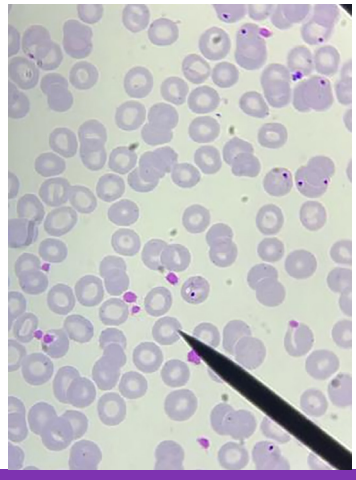

Figure 3. Point-like nuclear structures in the thin blood smear prepared on day 10 (100X magnification)

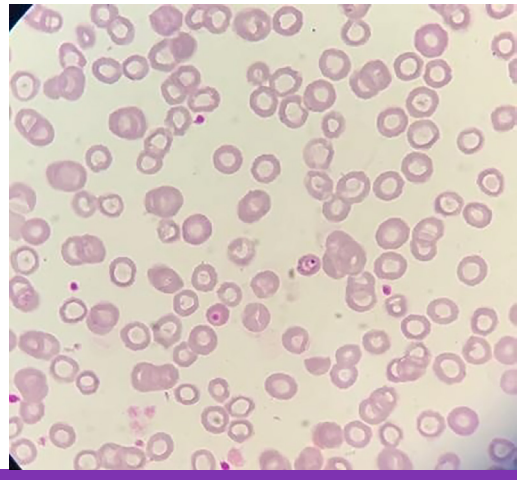

Figure 5. Young trophozoites in the thin blood smear (100X magnification)

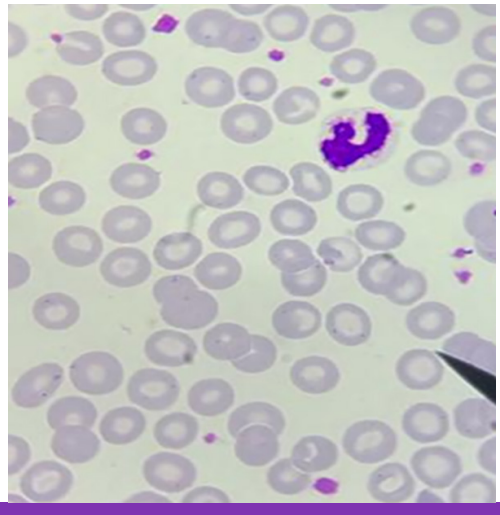

Figure 4. Thin blood smear prepared on day 16 (100X magnification)

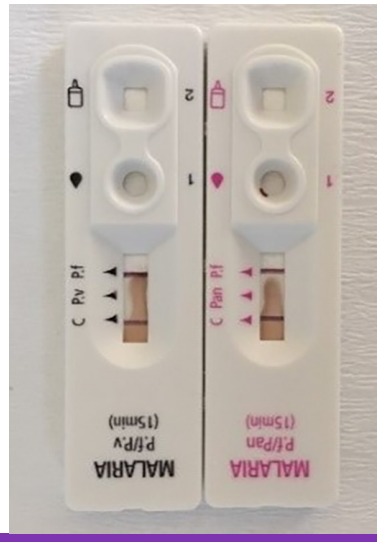

Figure 6. Rapid diagnostic tests for case 2

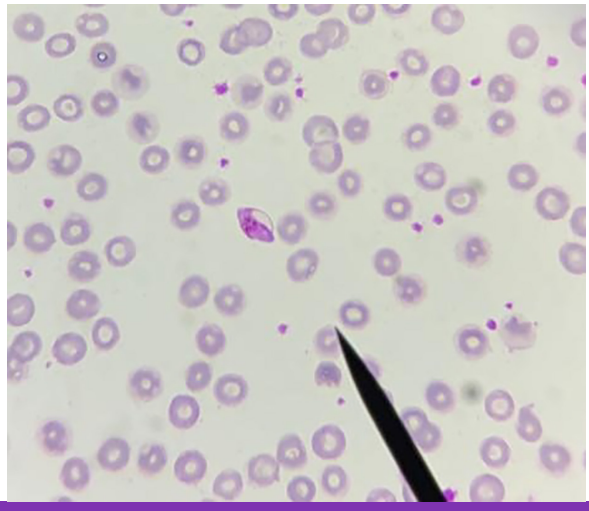

Figure 7. Gametocyte in the thin blood smear (100X magnification)

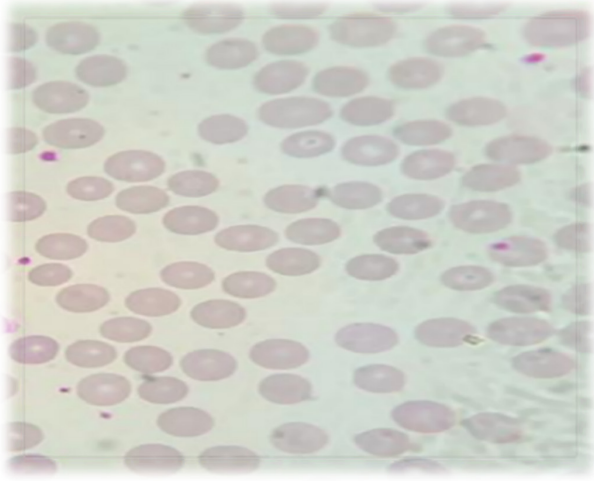

Figure 8. Thin blood smear after treatment (100X magnification)

In both cases, the causative species was identified as *P. falciparum*. However, as the artemether-lumefantrine combination therapy protocol did not yield sufficient response in both cases, primaquine phosphate was added to the treatment protocols to address possible mixed infections.

DISCUSSION

Malaria is a treatable disease when diagnosed. However, reports indicate resistance to drugs used in malaria treatment over the past decade. Resistance has been reported in three of the five malaria species that cause disease in humans, namely *P. falciparum, P. vivax*, and *P. malaria* (1).

Quinoline derivatives (quinine, chloroquine, mefloquine, primaquine), antifolates (sulfadoxine/pyrimethamine), artemisinin, and atovaquone are agents used in malaria treatment (4). Chloroquine is the preferred first-line antimalarial agent for treatment. However, in cases of resistance, other quinoline derivatives, along with doxycycline, atovaquone/proguanil, artemether/lumefantrine, artesunate, and sulfadoxine/pyrimethamine combinations, are used in treatment (4-7). Resistance to these agents varies according to geographical regions. In some areas where malaria is endemic, *P. vivax* infection has shown resistance to chloroquine and primaquine, while *P. falciparum* infection has developed resistance to many of the currently used antimalarial drugs (6).

Artemether and other artemisinin derivatives act on both the asexual and sexual stages of the parasite. However, when used alone in treatment, the rate of recurrence is high. Therefore, combination therapy with agents like lumefantrine is recommended (8,9). In the combination of artemisinin and lumefantrine, the different half-lives of these agents are utilized to achieve effective treatment (8). In a study by Ural et al. (10), a case of a patient who traveled to cameroon for a business trip and presented with symptoms of high fever, chills, tremors, and weakness a week after returning to Türkiye was reported. Upon examination of thin and thick blood smears, the patient was diagnosed as *P. falciparum* and treated with artemether 20 mg/lumefantrine 120 mg tablets. The study reported that artemisinin-based combination therapies are the best treatment option for *P. falciparum* cases (10). In the study, two cases returning from business trips to Ghana and Sudan to Hatay were diagnosed with malaria and typed as *P. falciparum*. Both cases were treated with Artemether-lumefantrine combination therapy, but due to inadequate response, primaquine phosphate was added to the treatment protocols.

Due to the fact that some of the population living in Hatay work abroad, especially in African countries and Saudi Arabia, where malaria is endemic, imported malaria cases are frequently encountered. Şahin et al. (11) reported 75 imported malaria cases between January 2008 and December 2017 and emphasized the significance of malaria in our region. In the study, two cases of imported malaria, which were caused by *P. falciparum* and showed delayed response to treatment, were presented.

CONCLUSION

It is believed that rapid diagnosis of suspected malaria cases, species identification, implementation of effective treatment protocols, and elimination of transmission, along with providing education on malaria disease and vector, individual control methods, and chemoprophylaxis before traveling to malaria-endemic countries, would contribute to reducing resistant parasites. In addition, the study aimed to draw attention to the importance of learning the region where the patients come from in imported malaria cases and applying the appropriate treatment protocol by looking at the antimalarial drug resistance of that region.

*Ethics

Informed Consent: In this cases, written consent of the patient has been obtained.

Footnotes

*Authorship Contributions

Data Collection or Processing: T.K., M.Ç., G.Ç., C.Ü., Analysis or Interpretation: T.K., M.Ç., G.Ç., C.Ü., Literature Search: T.K., G.Ç., C.Ü., Writing: T.K., M.Ç., G.Ç.

Conflict of Interest: No conflict of interest was declared by the authors.

Financial Disclosure: The authors declared that this study received no financial support.

REFERENCES

1. World Health Organization. World Malaria Report; WHO: Geneva, Switzerland; Available from: https://www.who.int/news-room/questions-and-answers/item/malaria.
2. World Health Organization. World Malaria Report; WHO: Geneva, Switzerland, 2020; Available from: https://www.who.int/ publications/i/item/9789240015791.
3. Zorbozan O, Pullukçu H, Atalay Şahar E, Karakavuk M, Can H, Tunali V, et al. Mikroskopide atipik görünümlü dış kaynaklı iki sıtma olgusunda hızlı test, serolojik ve moleküler yöntemlerin tanıya katkısının önemi [The importance of the contribution of rapid test, serological and molecular methods in the diagnosis of two imported malaria cases with atypical microscopy]. Mikrobiyol Bul. 2017; 51: 396-403. Turkish.
4. Salman Ö, Erbaydar T. Drug resistant malaria. TAF Preventive Medicine Bulletin. 2016; 15: 368-75.
5. Parlak E, Ertürk A, Cayır Y, Parlak M. Sporadik bir bölgede: dört import sıtma olgusu [Four malaria-import patterns: sporadic region]. Turkiye Parazitol Derg. 2013; 37: 161-4. Turkish.
6. Bloland PB. Drug resistance in malaria. A background document forthe WHO global strategy for containment of antimicrobial resistance. 2001. p. 5-15.
7. World Health Organization. Guidelines for the treatment of malaria. Geneva: WHO, 2006: 64.
8. Ehrhardt S, Meyer CG. Artemether-lumefantrine in the treatment of uncomplicated *Plasmodium falciparum* malaria. Ther Clin Risk Manag. 2009; 5: 805-15.
9. Mehra N, Bhasin VK. *In vitro* gametocytocidal activity of artemisinin and its derivatives on *Plasmodium falciparum*. Jpn J Med Sci Biol. 1993; 46: 37-43.
10. Ural S, Aslan S, Kaptan F, El S, Sezak N, Demirdal T. *Plasmodium falciparum* malaria: A case of cameroonian origin treated with artemether/lumefantrine. Klimik Derg. 2015; 28: 35-7.
11. Şahin Sİ, Çabalak M, Bal T, Ocak S, Önlen Y, Çulha G. Retrospective analysis of cases with ımported malaria in Hatay province of Turkey: Seventy-five cases in ten years. Türkiye Parazitol Derg. 2019; 43: 60-4. Turkish.