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Case Report

Value of Liu's stain in rapid diagnosis of *Plasmodium ovale* infection

Abstract

In this article, we report a 38-year-old man who presented to our emergency department with an infection of *Plasmodium ovale* malaria. Accurate diagnosis of the *Plasmodium* species is essential for adequate treatment of malaria. Peripheral smear examination for malarial parasite is the gold standard in confirming the diagnosis of malaria. Thick and thin smears prepared from the peripheral blood are used for this purpose. To identify *Plasmodium* species, microscopic examination of Wright-Giemsa stain blood smears has been the diagnostic method of choice. Interestingly, we revealed that Schuffner's dots appeared in erythrocytes with Liu's stain but not with Wright-Giemsa stain. Wright-Giemsa stain failed to demonstrate Schuffner's dots in erythrocytes. In this study, we reveal that Liu's stain is a more reliable staining method than Wright-Giemsa stain for observing Schuffner's dots in erythrocytes infected with *P ovale*.

In this article, we report a 38-year-old man who presented to our emergency department. He had intermittent fever with chills and denied any systemic disease before. According to his description, he experienced chills every night before the fever attacked, which was followed by cold sweating, on February 7, 2008. The day before, he traveled to Taitung, eastern Taiwan. He also mentioned that he had traveled to Republic of Guinea, Africa, for a month (October 2007 to November 2007). During that period, he had febrile while he stayed in Republic of Guinea, Africa, and he took an antimalarial agent for 3 to 4 days. Admission laboratory results revealed a white blood cell count of 5400/μL (reference range, 4000-10 000/μL), hemoglobin level of 14.9 g/dL (reference range, 14.1-18.1 g/dL), hematocrit level of 0.419 (reference range, 0.435-0.537), and platelet count of 122 000/μL (reference range, 150 000-45 000/μL). The serum chemistry showed a total bilirubin level of 1.01 mg/dL (reference range, 1.0-1.3 mg/dL), AST of 35 U/L (reference range, 20-50 U/L), ALT of 49 IU/L (reference range, 20-40 U/L), and C-reactive protein of 76.09 mg/L (reference range, <5 mg/L). Urine routine analysis was unremarkable. Antibiotics with unasyn plus doxycyclin were given first, and then he was immediately transferred to our ward. Under the impression of malarial infection, peripheral blood smears (thick and thin) were ordered by the on-duty physician.

Accurate diagnosis of the *Plasmodium* species is essential for proper treatment of malarial infection. Peripheral smear examination for malarial parasite is the gold standard in confirming the diagnosis of malaria. Thick and thin smears prepared from the peripheral blood are used for this purpose. To identify *Plasmodium* species, microscopic examination of Wright-Giemsa stain blood smears has been the diagnostic method of choice [1]. However, it is not easy to identify *Plasmodium ovale* on a blood smear, particularly when parasite numbers are low and mixed species infections are present. [2]. Schuffner's dots are fine, round, uniform red or yellow dots characteristically observed in erythrocytes infected with *Plasmodium vivax* and *P ovale*, but not ordinarily in *Plasmodium malariae* and *Plasmodium falciparum* infection. Liu's stain takes only 2 minutes and is one of the rapid staining methods of the Romanowsky series which was used for immediate interpretation. We compared different staining methods interestingly, we found that Schuffner's dots appeared in erythrocytes with Liu's stain (TONYAR Biotech, Taipei, Taiwan) (refer to Fig. 1A and B, red arrow) but not Wright-Giemsa stain (refer to Fig. 1C and D, black arrow) at our laboratory. The Wright-Giemsa-stain failed to demonstrate Schuffner's dots in erythrocytes. The results was further confirmed by the experienced physician of infectious diseases. From this study, we revealed that Liu's stain is a more reliable staining method than Wright-Giemsa stain for observing Schuffner's dots in erythrocytes infected with *P ovale*. Confirmation by the Center for Disease Control (CDC) through polymerase chain reaction indicated that the man had been infected with *P ovale*. Taiwan CDC (center for disease control) confirmed this case with *P ovale* infection by polymerase chain reaction (PCR).

Malaria is documented to have been prevalent throughout much of Taiwan in the 19th and 20th centuries. The maximum estimated number of cases was 1.2 million in 1952 [3]. The World Health Organization declared Taiwan to be free of malaria on December 4, 1965. Furthermore, after 1973, almost all of the reported cases of malaria in Taiwan (22-83 cases per year) were acquired from other countries [4,5]. Importantly, under the real-time surveillance and proper management of those imported malarial cases, our government successfully prevent the reintroduction of malaria into

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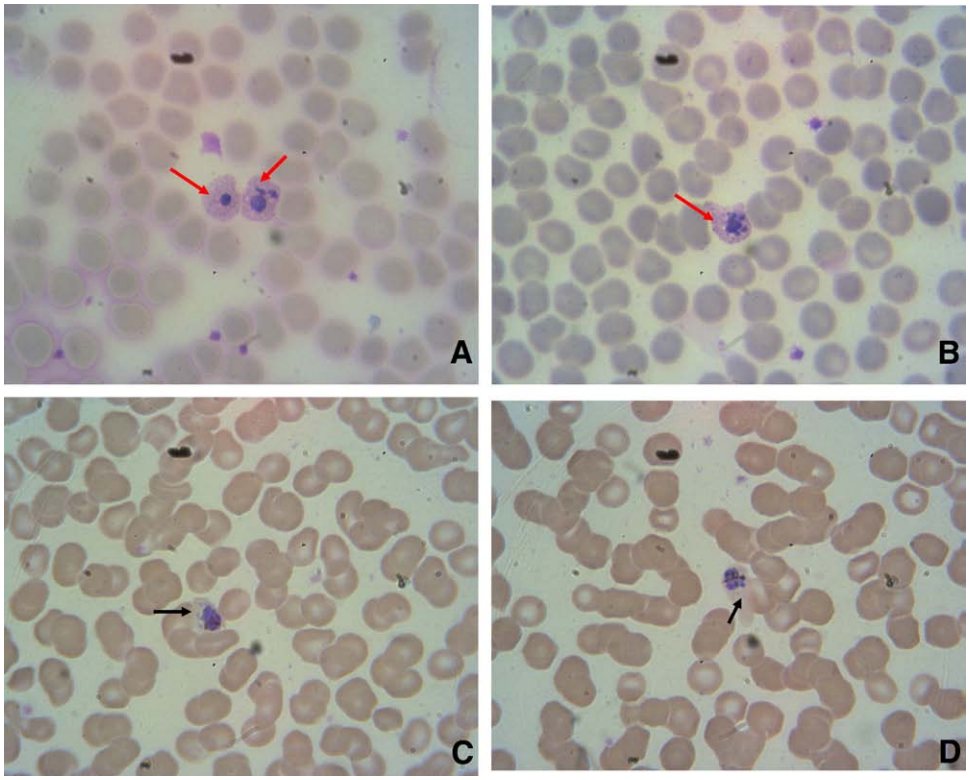

Fig. 1 (A and B) Enlarged infected erythrocytes of oval shape with trophozoites (1A) and schizont (1B). The presence of Schuffner's dots in erythrocytes using Liu's stain in the peripheral blood smear. (magnification ×1000). (C and D) Infected erythrocytes of oval shape with schizonts (1C and 1D). The absence of Schuffner's dots in erythrocytes using Wright-Giemsa-stain in the peripheral blood smear. (magnification ×1000).

Taiwan [6]. According to statistical results from the CDC, Taiwan, there are 14 cases of malaria from January to September 2008 that were acquired from other countries, and 3 cases occurred in the area of Kaohsiung-Pingtung region, southern Taiwan [7]. Notably, the consideration should be given to provide presumptive anti-relapse therapy in those individuals being treated for *P. ovale* or *P. vivax* malarial infections, especially for those returning from endemic areas who have potential exposure to these parasites.

Malaria acquired from other places remains a difficult problem in nonendemic areas of the world. It occasionally happens that patients are not suspected to have malaria and thus delayed diagnosed by the ED physicians. Obtaining a thorough travel history on all patients with clinical features suggesting an infectious origin and considering this diagnosis in any patient with a history of travel to or migration from malaria-endemic areas must be done by emergency physicians. If the travel history is not carefully taken, the diagnosis and treatment of malaria acquired from other places can be delayed. Somehow, junior emergency physicians are still not knowledgeable regarding malaria. Even one case of malarial infection will cause a big problem if it begins to spread to more people in the area with proper and efficient vectors. Actually, in Taiwan, the vector, *Anopheles minimus*, still exists with the potential of transmitting malaria.

This is the first report to illustrate that Liu's stain is more reliable than Wright-Giemsa stain to show Schuffner's dots in the erythrocytes of *P ovale*–infected patients. Our finding highlights that it may be useful to endemic areas for rapid diagnosis of the *Plasmodium* species and provides a prompt initiation of appropriate anti-malarial treatment.

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