Case Report: Histopathology of Fatal Respiratory Distress Caused by Plasmodium vivax Malaria


National Institute of Malaria Research, Delhi, India; Departments of Pathology, Microbiology, Medicine and Forensic Medicine, Goa Medical College and Hospital, Bambolim, Goa, India; Nuffield Department of Clinical Laboratory Sciences, John Radcliffe Hospital, University of Oxford, Oxford, United Kingdom; National Institute of Malaria Research Field Unit, Goa, India; Southeast Asian Infectious Diseases Clinical Research Network, Eijkman-Oxford Clinical Research Unit, Jakarta, Indonesia; Centre for Tropical Medicine, Nuffield Department of Clinical Medicine, Oxford University, Oxford, United Kingdom

Abstract. An otherwise healthy 20-year-old woman in Goa, India, received antibiotics after a diagnosis of upper respiratory tract infection. One week later, vivax malaria was diagnosed at a health center, but the patient developed respiratory distress and lost consciousness. She arrived at emergency department in shock, breathless, and comatose. She died within minutes. Two independent laboratories later confirmed Plasmodium vivax by microscopy (140,000/μL) and by nested and real-time polymerase chain reaction methods. Post-mortem examination showed congestion of alveolar capillaries by heavy monocytic infiltrates, along with diffuse damage to alveolar membranes consistent with acute respiratory distress syndrome. Parasites seen in lung tissue were roughly proportionate to both peripheral hyperparasitemia and those seen in other organs without lesions. In this patient, vivax malaria caused a rapidly fatal respiratory distress.

INTRODUCTION

Plasmodium vivax causes malaria in 70–390 million people annually, with an estimated 2.6 billion living at risk.1–3 Grassi and Feletti3 named this parasite in 1890, then known as benign tertian malaria. The pernicious course of the other tertian malaria, Plasmodium falciparum, prompted the name malignant tertian malaria. Contemporary medical texts describe vivax malaria as rarely causing fatal complications. Recent case reports,4,6 case series,7,8 and hospital-based studies9–11 challenge the notion of vivax malaria as rarely serious, complicated, or threatening to life. Among patients with vivax malaria having definitively ruled out infection by P. falciparum, there has been severe malarial anemia, cerebral malaria, acute respiratory distress syndrome (ARDS), renal failure, hepatic dysfunction, sepsis, and shock.12,13 Autopsy of a patient with a diagnosis of vivax malaria confirmed by molecular biologic methods has not been reported. Autopsies are rarely performed in South and Southeast Asian countries for a variety of social and economic reasons. However, to discourage dowry-motivated homicide,14 India enacted legislation requiring autopsy on death of recently married women. Here we report a post-mortem examination of a recently married young woman who died in respiratory distress caused by vivax malaria.

CASE REPORT

In June 2008, a woman 20 years of age died in hospital at Goa in southwestern India. Family members reported that in the week before death, private practitioners had treated the patient with ampiroxcillin, diclofenac, and antipyrines for an upper respiratory tract infection. At that time she had intermittent fever and chills with body aches. On the day of death she reported to a primary health care center with abdominal pain and giddiness a few hours duration. Routine microscopic examination of Giemsa-stained thick blood films at the health center identified many trophozoites of P. vivax. Before any therapy could be administered, the patient developed respiratory distress and lost consciousness. She was transferred to the hospital emergency room. On arrival, she was in shock, gasping for breath, febrile (38.3°C), and comatose with no sign of neck rigidity. Her blood pressure was not measurable. Chest sounds were clear, and heart sounds were normal. She was jaundiced, and the abdomen was distended with liver palpable 4 cm below costal margin. The spleen was not palpable. The patient was being transferred to the intensive care unit for mechanical ventilation when she suffered cardiopulmonary arrest. Attempts to resuscitate her were not successful.

Subsequent laboratory evaluations of samples taken at admission showed normal hemoglobin (15.0 g/dL), blood urea (25 mg/dL), blood urea nitrogen (BUN) (8 mg/dL), and total leukocyte count (7,700/μL with 50% neutrophils). Blood sugar (65 mg/dL) was slightly below the normal range. Commercially available rapid diagnostics for leptospirosis (Leptocheck IgM capture), dengue fever (IgM ELISA), enteric fever (blood culture and Widal agglutination test), hepatitis (rapid test for surface antigen), and HIV 1 and 2 (ELISA) were all negative. Microscopic examination of Giemsa-stained thick films showed parasitemia of trophozoites of P. vivax numbering ~140,000/μL blood (Figure 1). A rapid antigen (HRP2) detection kit for falciparum malaria was negative. Chest roentgenograms were not possible because of the rapidly fatal course.

In the months after death, polymerase chain reaction (PCR) was performed at two separate laboratories to confirm the microscopic diagnosis and rule out co-infection with P. falciparum. At the National Institute for Malaria Research (NIMR) in India, DNA was isolated from fixed and stained thick and thin blood smears taken in the emergency department immediately before death using the Qiagen DNA isolation kit. PCR analysis was done by nested PCR assay along with various Plasmodium species as control.15 The US Naval Medical Research Unit No. 2 (NAMRU-2) in Jakarta, Indonesia, blindly read blood films by microscopy and also applied both standard nested and real-time PCR methods from DNA extracted from those slides.16,17 At both laboratories, the microscopic and PCR diagnoses were consistently positive for P. vivax and negative for all other species evaluated.

*Address correspondence to Neena Valecha, National Institute of Malaria Research, 22-Sham Nath Marg, Delhi 110 054, India, E-mail: neenavalecha@gmail.com; and Kevin Baird, Eijkman-Oxford Clinical Research Unit, Jalan Diponegoro No.69, Jakarta 10430, Indonesia, E-mail: k Baird@eocru.org.
Pathologic features of cerebral malaria caused by parasitized erythrocytes in the brain, with none of the characteristic examinations confirmed an absence of sequestration of parasitized erythrocytes (upward arrows) and the distinctive ameboid trophozoites of *P* *vivax* (downward arrows). This figure appears in color at www.ajtmh.org.

At autopsy, the congested and edematous lungs weighed 480 and 460 g, ~35% heavier than normal lungs in Indian women. The brain and liver were congested but within normal weight ranges at 1,080 and 1,350 g, respectively. The spleen weighed 250 g (well above normal weight of ~130 g), and it was enlarged with a smooth surface without evidence of rupture. Microscopic examination of post-mortem tissue samples from major organs was done using standard histologic stains including hematoxylin and eosin, Martius scarlet blue (MSB), and elastin von Gieson (EVG) performed by two independent pathologists (one experienced in the diagnosis of fatal falciparum malaria). The examinations confirmed an absence of sequestration of parasitized erythrocytes in the brain, with none of the characteristic pathologic features of cerebral malaria caused by *P* *falciparum* infection. Sections of the spleen showed marked dilation and congestion of sinusoids, increased hemozoin pigment, and scanty malarial parasites. Sections of the liver showed dilation and congestion of sinusoids, mild steatosis, increased hemozoin pigment deposition in Kupffer cells, and occasional malaria parasites. Sections of the kidney showed congestion and parasites in the glomeruli, with significant post-mortem autolysis but no clear evidence of acute tubular necrosis or background glomerular disease. Examination of the gastrointestinal and reproductive tract tissues showed no lesions.

Sections from the lung showed focal pulmonary edema (Figure 3A) and variable but focally marked congestion of the alveolar capillaries and larger vessels by mononuclear cells, predominantly monocytes and lymphocytes, with only scattered neutrophils (Figure 3A and B). No intra-alveolar consolidative pneumonia was seen. There were early signs of diffuse alveolar damage with focal hyaline membrane formation positive on MSB staining (Figure 3C). Sections from the lung were immunostained, again using standard protocols, for the leukocyte markers CD3 (T cell), CD19 (B cell), and CD68 (monocyte/macrophage). The inflammatory infiltrate, forming an early pneumonitis like picture, was predominantly monocytic on immunophenotyping, many of which contained phagocytosed pigment. Scanty surviving parasitized erythrocytes were also present, and some lymphocytes, with only small numbers of neutrophils (Figure 3D). No pre-existing granulomatous or interstitial lung disease was seen, and background interstitial connective tissue appeared normal on EVG staining.

There was no evidence of illness other than vivax malaria. Initial misdiagnosis as an upper respiratory tract infection led to inappropriate therapy and a significant delay in making the correct diagnosis. The patient was neither anemic nor malnourished. Evidence of active co-infections was not found. Although in agonal coma caused by shock, she had no clinical evidence of encephalopathy, and histopathologic examination of the brain showed no lesions. Antibiotic therapy during the week before death, negative blood cultures, and normal leukocyte counts at admission all argued against bacterial sepsis. In summary, laboratory studies showed conclusively that this patient had vivax malaria without evidence of falciparum malaria or any other infection. Post-mortem histopathologic analysis of her organs showed pulmonary pathology consistent with early ARDS and no other significant findings in other organs that could account for death. She was a native of Goa, where in 2008, the annual incidence of malaria was 48/1,000 residents, with about one half of those caused by *P* *vivax*.

**DISCUSSION**

Survey of published reports showed thrombocytopenia as the most common serious complication of vivax malaria, followed by pulmonary, hepatic, renal, and cerebral complications. 18 In some endemic settings, severe malarial anemia represents the majority of severe complications. 10,11 Several dozen cases of ARDS caused by vivax malaria have been documented, including cases with PCR confirmation of diagnosis. 15 We found only three reports of histopathologic examination of fatal pulmonary complications allegedly caused by vivax malaria. Those cases were reported in the early 1900s and the diagnoses lacked PCR confirmation of *P* *vivax* and exclusion of *P* *falciparum*. 15,20 Autopsy of this case, made possible by legislation aimed at discouraging homicide, permitted histopathologic examination of all organs. Only the lungs
showed lesions consistent with the clinical course and outcome in this patient, and those were consistent with early ARDS.

The post-treatment inflammatory response may exacerbate lung injury in vivax malaria, but in this case and others, no antimalarial treatment was given. The true pathophysiologic significance of lung disease may involve soluble mediators and endothelial damage, hypoxia, and systemic shock, leading to a common pathway of diffuse alveolar-capillary damage presenting with the clinical syndrome of ARDS. Sequestration of mature stages of *P. vivax*-infected erythrocytes in the pulmonary microvasculature has been hypothesized based on uncoupling of the pretreatment pulmonary capillary vascular and the alveolar-capillary membranous components of gas transfer. In this case, the degree of parasite accumulation in the lung tissue appeared mild compared with the host leukocyte response in the same organ, and similar features have been seen histologically in fatal cases of *P. falciparum* infection with pulmonary complications (G. Turner and others, unpublished data). A better measure of the extent of true parasite “sequestration” is the sequestration index, comparing the tissue burden of parasites with circulating forms. This allows comparison of parasite numbers and stages with the peripheral blood parasitemia to determine whether a disproportionate collection of parasitized red blood cells has occurred in tissues relative to that expected in a free-mixing model. The burden of parasitized red blood cells seen in the lung tissues in this case appeared low. The scanty parasites observed in tissue sections were proportionate to the peripheral hyperparasitemia documented immediately before death. This raises the possibility that end organ damage in fatal malaria caused by *P. vivax* may be unrelated to parasite load within failing organs. Pulmonary phagocytic activity is increased and gas exchange impaired during acute uncomplicated vivax malaria. Our histopathologic findings in this single fatal case of vivax malaria showed heavy intravascular monocytic infiltrates provoking inflammatory lesions to the endothelium of the lungs, but without marked sequestration of parasitized red blood cells in the pulmonary microvasculature.

**Figure 3.** Histologic and immunohistochemical examination of post-mortem lung tissues. **A**, Low-power micrograph of a lung section showing focal edema and congestion of alveolar capillaries with mononuclear cells, many of which contain phagocytosed pigment (magnification, ×200; hematoxylin and cosin). **B**, Higher-power view of lung showing occasional parasitized erythrocytes within alveolar capillaries, numerous mononuclear cells, and early hyaline membrane formation (magnification, ×400, hematoxylin and cosin). **C**, Martius Scarlet Blue staining shows early hyaline membrane formation in alveolar walls, consistent with ARDS (magnification, ×200). **D**, Immunohistochemistry for CD68 showing numerous monocyte/macrophages within alveolar capillaries, some of which contain phagocytosed pigment (DAB immunostain with hematoxylin counterstain; magnification, ×400). This figure appears in color at www.ajtmh.org.
The number of parasites found in peripheral circulation immediately before death represents a very high count relative to those ordinarily seen in acute vivax malaria. Among 221 Thai patients with *P. vivax*, counts ranged from 45/μL to 108,000/μL, with a geometric mean of 10,381/μL, for example.\(^1\)
\(^{24}\) Among 101 Korean patients, parasitemias ranged from 32/μL to 52,127/μL (median, 1,287/μL).\(^3\) Among 109 human subjects experimentally challenged with the St. Elizabeth strain of *P. vivax*, parasitemia before therapy was <10,000/μL in 96% of cases, and <20,000/μL among 99%.\(^4\) Even among 40 severely ill patients with *P. vivax* in India,\(^5\) including two fatalities, no parasitemia exceeded 60,000/μL. Counts substantially exceeding 100,000/μL, although exceptional, are known among naturally infected people.\(^6\) The same was true in at least one series of experimental challenge with Chesson strain *P. vivax*, where parasitemias before therapy among seven subjects reached levels ranging from 74,160/μL to 126,000/μL before therapy was administered (Collins WE, personal communication). The high level of parasitemia occurring in our patient may bear on events leading to death (e.g., through provocation of the soluble inflammatory mediators that seem responsible for lesions observed in the lungs). Hyperparasitemia by *P. vivax* (perhaps >100,000/μL) may be a risk factor for severe and fatal disease, but the two fatalities in the series of 11 patients described from India\(^7\) had parasitemias of only 6,000/μL and 15,000/μL. Also, in the hospital study from Indonesia by Barcus and others,\(^8\) five of nine fatalities caused by *P. vivax* had relatively low parasitemia levels.

The infection by *P. vivax* described here caused ARDS and precipitous death within 1 week of onset of illness in an otherwise healthy young woman. Contemporary medical texts and training do not ascribe fatal pulmonary complications to *P. vivax* but to *P. falciparum*. With few exceptions (e.g., the Korean peninsula), *P. falciparum* occurs where there is endemic *P. vivax*. This case raises the possibility that severe, complicated, and life-threatening vivax malaria may be confused for falciparum malaria and highlights the importance of applying PCR diagnostics in severely ill patients.

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Authors’ addresses: Neena Valecha, National Institute of Malaria Research, 22 Sham Nath Marg, Delhi 110054, India. E-mail: neenava. lecha@gmail.com. Rock G. W. Pinto, Department of Pathology, Goa Medical College and Hospital, Bambolim-403202, Goa, India. Gareth D. H. Turner, Department of Cellular Pathology, Level 1, John Radcliffe Hospital, Oxford OX3 9DU, UK. E-mail: gareth.turner@orh.mhs.uk. Ashwani Kumar, National Institute of Malaria Research, Field Unit, Directorate of Health Services Building, Campal, Panaji-403001. Savio Rodrigues, Department of Microbiology, Goa Medical College and Hospital, Bambolim-403202, Goa, India. Nagesh G. Dubhashi, Department of Medicine, Goa Medical College and Hospital, Bambolim-403202, Goa, India. Edmond Rodrigues, Department of Forensic Medicine, Goa Medical College and Hospital, Bambolim-403202, Goa, India. Ruchi Singh, National Institute of Malaria Research, 22 Sham Nath Marg, Delhi 110054, India. Aditya P. Dash, National Institute of Malaria Research, 22 Sham Nath Marg, Delhi 110054, India. Kevin Baird, Eijkman-Oxford Clinical Research Unit, Jalan Diponegoro No.69, Jakarta 10430, Indonesia. E-mail: kbaird@eocru.org.

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