Case Report: A Case of Primaquine-Induced Hemoglobinuria in Glucose-6-Phosphate Dehydrogenase Deficient Malaria Patient in Southeastern Bangladesh

Ching Swe Phru,¹ Mohammad Golam Kibria,¹ Kamala Thriemer,² Mahtab Uddin Chowdhury,³ Nusrat Jahan,¹ M. M. Aktaruzzaman,⁴ Hisni Rahmat,⁵ Ari Winasti Satyagraha,⁵ Aung Swi Prue,³ Wasif Ali Khan,¹ Benedikt Ley,² and Mohammad Shafiul Alam^{1*}

¹International Centre for Diarrhoeal Disease Research, Bangladesh (icddr,b), Dhaka, Bangladesh; ²Global and Tropical Health Divisions, Menzies School of Health Research and Charles Darwin University, Darwin, Australia; ³Civil Surgeon's Office, Bandarban, Bangladesh; ⁴National Malaria Elimination Program, Directorate General of Health Services, Dhaka, Bangladesh; ⁵Eijkman Institute for Molecular Biology, Jakarta, Indonesia

Abstract. We herein report the first case of Mediterranean glucose-6-phosphate dehydrogenase (G6PD) variant from Bangladesh. A boy had been admitted to hospital and was diagnosed with uncomplicated *Plasmodium vivax* infection and treated with 30 mg/kg body weight (BW) chloroquine for 3 days and 4.8 mg/kg BW primaquine (PQ) to be taken over 14 days. The boy was discharged but represented 4 days later with severe hemoglobinuria and fatigue. Hemoglobin was measured at 6.0 g/dL and serum bilirubin was at 5.6 mg/dL, although malaria microscopy was negative. The boy had taken the 4-fold recommended daily dose of PQ and was treated with two fresh blood transfusions. Subsequent molecular analysis showed the boy to have the Mediterranean G6PD variant and a G6PD activity of 0.93 U/gHb.

Bangladesh has seen a significant decline in malaria cases over the last decade, dropping from 55,873 cases in 2010 to 10,523 cases in 2018.¹ Although many areas of the country are in a pre-elimination phase, the Chittagong Hill tracts in the southeast of the country on the border to Myanmar continue to show high numbers of both *Plasmodium falciparum* and *Plasmodium vivax* cases.

First-line treatment for uncomplicated *P. falciparum* malaria according to the national treatment guidelines² is artemether-lumefantrine (AL) given twice daily over 3 days (adult dose for patients with body weight [BW] ≥ 35 kg is four tablets per dose for a total of six doses with each tablets containing 20 mg artemether and 120 mg lumefantrine) plus a single dose of primaquine (PQ; total dose: 0.25 mg/kg BW) as a gametocidal agent. For *P. vivax*, the recommended treatment is 3 days of chloroquine (CQ; total dose: 25 mg/kg BW) and 14 days of PQ; (total dose: 3.5 mg/kg BW) for radical cure. Although well tolerated in most recipients, PQ can cause severe and potentially fatal hemolysis in patients with low activities of the enzyme glucose-6-phosphate dehydrogenase (G6PD deficiency).^{3,4} Accordingly, the WHO suggests routine testing for G6PD deficiency before PQ-based radical cure;^{5,6} however, this is currently not reflected in the National Malaria Treatment Guidelines of Bangladesh.² We herein report the case of a 9-year-old Bengali boy who presented with hemolysis following PQ overdosing.

The patient presented at the emergency medical department of Alikadam Upazila Health Complex in Bandarban district on June 28, 2018. The child had proper physical development and weighing 22 kg. According to the legal guardian, the boy had low-grade continuous fever for two days with no other symptoms associated. At time of presentation, the axillary body temperature was 37.8°C. Blood slide microscopy confirmed *P. vivax* monoinfection with a parasitemia of 680 parasites per μ L and no gametocytes present.

Following the Bangladesh National Treatment Guidelines,² the patient received four and half tablets of CQ (250 mg = 150 mg base/tablet) to be taken on days 0, 1, and 2 (1.5 tablets/day, total dose: 30 mg/kg BW) as well as seven tablets of PQ (15 mg/tablet) to be taken over the following 14 days (0.5 tablets/day, total dose: 4.8 mg/kg BW). Instructions on dosage and administration of the treatment course were provided to the legal guardian verbally and in written form, and the boy was discharged. Locally available PQ tablets can be halved; however, no further division was allowed. Accordingly, the boy received a slightly higher dose of 7.5 mg/day PQ (corresponding to 1/2 tablets) rather than the optimal dose of 5.5 mg/day (corresponding to roughly 1/3 of a tablet).

Four days later, on July 2, 2018, the patient revisited the hospital with ongoing fever. At presentation, the boy was icteric, body temperature was 37.7°C, Hb was 6 g/dL, serum bilirubin 5.6 mg/dL, and microscopy showed no malaria blood stream infection, but the boy complained of fatigue and dark-colored urine. On enquiry, it became apparent that the guardian had misinterpreted the treatment directions; the boy had been taking two PQ tablets each day, which was the 4-fold the daily dose prescribed. A subsequent urine analysis showed the presence of red blood cells, and the dark coloration of the urine was confirmed. The boy was diagnosed with hemoglobinuria following drug-induced hemolysis. No baseline Hb measurement was available before initiation of treatment to calculate the fall of Hb caused by the hemolysis. On admission, PQ treatment was terminated. The patient was treated conservatively with injection of tranexamic acid (500 mg) twice, a single injection of dextrose aqua (500 mL), paracetamol suppository (250 mg/day) as needed for three days, injection of ceftriaxone 1 g daily for 6 days, ursodeoxycholic acid tablets (150 mg) once in 12 hours, and 2 units of fresh blood transfusion. The patient was discharged from hospital 6 days later, on July 8, 2018, after becoming afebrile with normal-colored urine output. Hemoglobin was not measured at discharge as the boy showed satisfactory clinical improvement. Although the attending clinicians classified the event as a drug-induced hemolytic event, the local resources and infrastructure did not allow any further testing to confirm this diagnosis.

* Address correspondence to Mohammad Shafiul Alam, International Centre for Diarrhoeal Disease Research, Bangladesh (icddr,b), 68 Shaheed Tajuddin Ahmed Sarani, Mohakhali, Dhaka 1212, Bangladesh. E-mail: shafiul@icddr.org

Two weeks later, on July 26, 2018, the patient revisited the outpatient department with complaints of high-grade continuous fever and an Hb of 8.4 g/dL. Malaria microscopy confirmed a *P. falciparum* monoinfection with a parasitemia of 28,300 per μ L but no gametocytes present. The boy's previous hemolytic history was known by the attending clinician; thus, instead of treating him as an outpatient case, he was admitted for close observation during his treatment. The patient was readmitted to hospital and treated with six doses of AL given over 3 days (total dosage of artemether: 10.9 mg/kg BW, total dosage of lumefantrine: 65.45/kg BW) and a single dose of PQ (total dose: 0.25 mg/kg BW) according to the national guidelines²; in addition, he was given 1.0 g intravenous ceftriaxone and intravenous ondansetron injection (4 mg) once in 8 hours for nausea (0.36 mg/kg BW in total). Following the single PQ dose, the patient again developed dark urine but visually less dark than during the previous visit and no clinical signs of hemolysis. Hemoglobin was not measured again. After uneventful completion of AL treatment and daily monitoring of urine color for six days, he was discharged from the hospital on July 31, 2018, with clear urine and referred to icddr,b Bandarban field office for G6PD activity testing. Incidentally, during the child's second malaria episode, icddr,b research activities on G6PD were ongoing in a nearby area, which was an option for the referral.

The boy presented to the icddr,b field office in Bandarban two weeks later, on August 14, 2018, with an Hb of 10.1 g/dL (measured by HemoCue[®] Hb 301, HemoCue AB, Ängelholm, Sweden). The legal guardian provided written informed consent to collect the patient history of the boy from the local hospital and for a quantitative measurement of G6PD activity and G6PD genotyping. A total of 2 mL of venous blood was collected in an EDTA vacutainer (Becton, Dickinson and Company, Franklin Lakes, NJ) and shipped to the Emerging Infections and Parasitology Laboratory of icddr,b in Dhaka for G6PD activity measurement by a gold standard spectrophotometry assay (kits from Pointe Scientific, Canton, MI on a Shimadzu UV-1800 Spectrophotometer, Shimadzu Scientific Instruments, Kyoto, Japan).⁷ Spectrophotometry showed the child to be severely G6PD-deficient with a total activity of 0.93 U/gHb.

The boy and the legal guardian were provided with background information on G6PD deficiency and associated risks. This information was provided verbally and in written form. Both were advised to present this report for any future medical visit.

Genomic DNA was extracted from 1.0 mL of the collected blood using a QIAamp DNA Blood Midi kit (QIAGEN, Hilden, Germany) as per the manufacturer's instruction. The DNA sample was sent to the Eijkman Institute for Molecular Biology, Jakarta, Indonesia, for sequencing of the G6PD gene. The extracted DNA was amplified using primers as described earlier.⁸ Sequencing and data analysis were performed using PuTTY Software and SPAdE algorithm (open source software distributed under the MIT license) with NG_009015.2 as the G6PD reference sequence. The patient was shown to have G6PD Mediterranean variant, a severe G6PD deficiency with less than 10% activity and categorized as class II according to the WHO enzyme classification.⁹ The whole gene sequence was submitted to GenBank with accession number MK281402.

To date, Orissa, Kerala-Kalyan, and Mahidol G6PD variants have been identified in Bangladesh.¹⁰ Although this is the first report from Bangladesh describing the presence of the Mediterranean variant, the Mediterranean variant had been

described frequently from neighboring India and a number of Southeast Asian countries.¹¹ This variant confers severe, non-self-limiting G6PD deficiency (under oxidative stress), and affected patients are at significant risk of severe hemolysis following treatment with PQ and other oxidants.¹²

The described patient suffered from severe PQ-induced hemolysis due to overdosing of PQ in the presence of severe G6PD deficiency. High daily PQ doses of up to 1 mg/kg have been shown to be safe in G6PD-normal patients¹³ but are clearly not indicated in patients with reduced G6PD activity.

Little is known on the true prevalence of G6PD deficiency within Bangladesh, specifically the malaria-endemic areas of the country. Whereas numbers based on malaria patients suggest very low rates of severe deficiency of less than 1%,¹⁴ reports on prevalence of severe deficiency among the healthy population ranged from 4.7%¹¹ to 6.4%,¹⁵ and unpublished data from a cross-sectional survey suggest a prevalence of more than 8.0% (Ley et al., unpublished data).

Routine G6PD testing has not been considered in Bangladesh and many countries throughout Asia,¹⁶ among others, because of fears of additional costs and an assumed very low incidence of PQ-induced severe hemolysis for a low-dose PQ regimen provided over an extended period of time.¹⁷ This results either in a very limited use of radical cure, exposing patients to repeated episodes of vivax malaria, or to treatment without testing, exposing G6PD-deficient patients to an increased risk of hemolysis. Whereas the extent of PQ-induced hemolysis remains unclear in many settings, there is no doubt that the wide-scale introduction of radical cure is necessary if the ambitious goal of malaria elimination by 2030 from the Asia-Pacific region will be met.¹⁸

With wider use of fairly complex and long treatment, erroneous self-medication will increase, requiring better patient counseling for radical cure, including clearer instructions on dosing and how to detect signs of hemolysis. This is particularly important in the absence of routine testing to guide treatment.

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Authors' addresses: Ching Swe Phru, Mohammad Golam Kibria, Nusrat Jahan, Wasif Ali Khan, and Mohammad Shafiul Alam, International Centre for Diarrhoeal Disease Research, Bangladesh (icddr,b), Dhaka, Bangladesh, E-mails: ching.swe@icddr.org, golam.kibria@icddr.org, n.jahan@icddr.org, wakhan@icddr.org, and shafiul@icddr.org. Kamala Thriemer and Benedikt Ley, Global and Tropical Health Divisions, Menzies School of Health Research and Charles Darwin University, Darwin, Australia, E-mails: kamala.ley-thriemer@menzies.edu.au and benedikt.ley@menzies.edu.au. Mah-tab Uddin Chowdhury and Aung Swi Prue, Civil Surgeon's Office, Bandarban, Bangladesh, E-mails: chowdhurymahatab43@gmail.com and draungsprue@yahoo.com. M. M. Aktaruzzaman, National Malaria Elimination Program, Directorate General of Health Services, Dhaka, Bangladesh, E-mail: mmaktaruzzaman93@gmail.com. Hisni Rahmat and Ari Winasti Satyagraha, Red Blood Cells Membrane and Enzyme Disorders, Eijkman Institute for Molecular Biology, Jakarta, Indonesia, E-mails: hisni@eijkman.go.id and ari@eijkman.go.id.

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