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Primaquine-induced hemoglobinuria: a case report of a G6PD deficient malaria patient with Mahidol trait from Bandarban, Bangladesh

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We report a case of Primaquine (PQ) induced hemoglobinuria in a patient with the glucose-6-phosphate dehydrogenase (G6PD) Mahidol variant from Bandarban, Bangladesh. The patient presented with mixed *Plasmodium falciparum* and *Plasmodium vivax* malaria and was recommended to be treated according to national guidelines with Artemether-Lumefantrine for three days and PQ for 14 days. Ten days later, the patient developed a fever and jaundice, followed by hemoglobinuria twelve days after the initial diagnosis. This highlighted the need for G6PD testing, which was subsequently confirmed by both Point-of-Care (POC) testing and spectrophotometry. The POC test showed a G6PD activity of 2.6 IU/g Hb, while spectrophotometry measured 1.47 IU/g Hb, both indicating G6PD deficiency (<30% activity). As a result, PQ was discontinued, and the patient received four units of blood transfusion. Additionally, genotyping was carried out, confirming the Mahidol variant. This case highlights the importance of routine G6PD screening before PQ administration, especially in malaria-endemic regions with different G6PD variants.

KEYWORDS

malaria, G6PD deficiency, primaquine, hemoglobinuria, Mahidol variant

Introduction

Malaria is a potentially fatal disease caused by the *Plasmodium* parasite. The World Malaria Report 2024 documented approximately 263 million reported cases along with an estimated 597,000 fatalities in 2023, marking an increase of approximately 11 million cases from the previous year (World Health Organization, 2024). Recently, the most

geographically prevalent malaria parasite is *Plasmodium vivax* (*P. vivax*), accounting for approximately 45% of malaria cases in the WHO South-East Asia Region leading to a significant global increase in associated morbidity and mortality (Anwar et al., 2024). However, *Plasmodium falciparum* (*P. falciparum*) continues to be the main contributor to the majority of fatalities (World Health Organization, 2024). The coexistence of these two species in varying proportions and geographical areas is also occasionally observed (Kumar et al., 2024).

Although Bangladesh has reduced malaria by 93% between 2008 and 2020, approximately 19 million people remain at risk of infection. Three districts in Chittagong Hill Tracts (CHT) remain high-transmission areas: Bandarban, Rangamati, and Khagrachari, a region where 90% of malaria cases occur. Bandarban accounts for more than 50% of these cases. Three sub-districts of Bandarban - Thanchi, Alikadam, and Rowangachari - typically have the highest annual parasitological index (API), although the overall malaria burden is decreasing. *P. falciparum* is predominant in Bangladesh, accounting for 73% of cases, with *P. vivax* accounting for the remainder. A few documented cases of *P. malariae* and *P. ovale* have also been reported (National Malaria Control Programme, 2021; Haldar et al., 2023).

Currently, the only medication that has gametocytocidal and hypnozoitocidal activity against *P. falciparum* and *P. vivax* is an 8-aminoquinoline compound (Primaquine- PQ). Although PQ is generally well tolerated by recipients, in individuals with glucose-6-phosphate dehydrogenase (G6PD) deficiency, it can lead to severe hemolysis (Phru et al., 2020). The degree of hemolysis depends on the level of G6PD deficiency and the dose and duration of exposure to PQ (Beutler, 1994). The level of induced hemolysis associated with G6PD deficiency is influenced by the specific genetic variant, with over 230 clinically relevant variants reported to date, although not all of them are polymorphic and of clinical relevance. The clinical presentation of G6PD variants varies from no clinical manifestations to very mild with essentially no symptoms to severe acute (Howes et al., 2013; Geck et al., 2023)hemolytic anemia (Yi et al., 2019). The majority of variants are asymptomatic but can cause neonatal jaundice and acute hemolytic anemia when exposed to primaquine or tafenoquine, which are class B of the WHO G6PD variants. WHO recommends G6PD testing before administering these antimalarial drugs for certain G6PD variants (Luzzatto et al., 2024). G6PD variants are widespread worldwide. In Southeast Asia, numerous G6PD variants have been identified in different populations, with G6PD Viangchan and G6PD Mahidol being the most common (Phompradit et al., 2011a). Thus far, the Orissa, Mediterranean, Kerala-Kalyan, and Mahidol G6PD variants have been identified in Bangladesh (Phru et al., 2020).

G6PD deficiency, a genetic disorder that affects approximately 500 million people globally (Li et al., 2024), is particularly common in regions where malaria is endemic (Dechyotin et al., 2021). G6PD is a crucial enzyme in the pentose phosphate pathway that produces NADPH, which is in turn required for reactive oxygen species homeostasis and cellular biosynthesis are crucial for red blood cells (Shah et al., 2024). G6PD is an essential enzyme in the pentose phosphate pathway, producing NADPH. NADPH is necessary for maintaining reactive oxygen species homeostasis and regulating cellular biosynthesis, both of which are crucial for red blood cell function. In the majority of cases, G6PD deficiency is asymptomatic, but under certain circumstances such as exposure to fava beans, infections, or medications, it can also present as mild-to-severe hemolytic anemia (Gupta, 2024).

Case presentation

A 16-year-old man weighing 50 kg from Rowangchhari Upazila, Bandarban presented with a fever on 27 October 2023 and was diagnosed with a mixed malaria infection by *P. falciparum* and *P. vivax* by an NGO health worker using a rapid diagnostic test (RDT). The patient was started on antimalarial treatment with Artemether-Lumefantrine (AL) 20/120 mg, four tablets twice daily for three days (total of 24 tablets), and PQ 15 mg, one tablet daily for 14 days, according to National Guidelines (Directorate General of Health Services (DGHS), 2019). Despite 10 days of treatment, the patient's fever persisted. He presented to the Rowangchhari Upazila Health Complex on 5 November 2023 with a temperature of 102°F and a blood pressure of 90/60 mmHg. A repeat RDT was negative for malaria. Given the unresolved fever and at the patient's request, he was referred to Bandarban Sadar Hospital, the district referral hospital. Before referral, the patient received oral Paracetamol 500 mg and a single intramuscular dose of Ceftriaxone 1 gram.

On admission to Bandarban Sadar Hospital the same day, the patient's vital signs were stable, with a body temperature of 99°F and blood pressure of 120/80 mmHg. However, the attending physician noted clinical jaundice. Initial management included intravenous dextrose 5% DNS (1 liter) and oral paracetamol for fever, intravenous ondansetron 8 mg for nausea, and intravenous Ceftriaxone 1 gram every 12 hours. PQ 15 mg was continued for 4 days according to National Guidelines.

Routine diagnostic tests were ordered on admission and the following day. Despite two additional negative malaria RDTs, the patient's fever persisted at a moderate level (100-102°F) with fluctuating blood pressure (100/60 to 120/70 mmHg). The decision was made to complete the full course of PQ in accordance with the guidelines while continuing supportive care and the Ceftriaxone antibiotic.

On 7 November 2023, a urine examination revealed hemoglobinuria. The recorded temperature was 100°F and the BP level was 100/50 mmHg. The patient's temperature was 100°F, and blood pressure dropped to 100/50 mmHg. A two-unit blood transfusion was administered. Given the suspicion of PQ-induced hemolysis, all quinine derivatives were withheld pending further evaluation. Baseline investigations and fever tests were unremarkable but unfortunately, the data were not documented

in the patient's medical file. A request for G6PD level testing was urgently sent to the local icddr,b (International Centre for Diarrhoeal Disease Research, Bangladesh) field office in Bandarban.

The patient's legal guardian provided written, informed consent for retrieving his medical records and G6PD activity and genotyping testing. A total of 3 mL of venous blood was collected in a BD Vacutainer® EDTA Tubes (Becton, Dickinson and Company, Franklin Lakes, NJ) and transported to the Emerging Infections and Parasitology Laboratory of the icddr,b in Dhaka. Initially, the hematological history was evaluated before G6PD testing. The patient's hematological parameters showed significant anomalies, which included severe anemia, as indicated by low hemoglobin (5.6 g/dL), hematocrit (19.2%), and RBC count (2.45 $*10^{12}$/L), along with reduced MCV, MCH, and MCHC (Khan et al., 2013). To confirm the root cause of the hemolytic event, G6PD activity was measured using both a gold standard spectrophotometry assay (kits from Pointe Scientific, Canton, MI on a Shimadzu UV-1800 Spectrophotometer, Shimadzu Scientific Instruments, Kyoto, Japan) (Alam et al., 2018) and a point-of-care (POC) test (STANDARD G6PD Test, SD BIOSENSOR, ROK). The POC test showed G6PD activity levels of 2.6 IU/g Hb, which is considered deficient according to the IFU (Instructions for Use). Using the Adjusted Male Median (AMM) the G6PD level for spectrophotometry was defined as 100% G6PD activity, whereas G6PD deficiency was classified as <30% activity. A study conducted on 1,002 participants from the ethnic groups of CHT, Bangladesh previously set the AMM at 7.03 U/g Hb (Ley et al., 2020). The patient's spectrophotometry result showed a G6PD value of 1.47 IU/g Hb, indicating <30% activity and defined as G6PD deficiency. A single unit of fresh blood was transfused on the same day following a rapid point-of-care G6PD testing and CBC testing. Vital signs were monitored closely. On 9 November, after confirmation of G6PD deficiency using reference laboratory data, a second blood transfusion was administered. The patient's condition improved significantly during this period, with resolution of jaundice and stable vital signs (pulse 76 bpm, blood pressure 110/80 mmHg). There were no signs of recurrent hemoglobinuria post-transfusion. The patient remained hospitalized for an additional three days for observation before being discharged from Bandarban Sadar Hospital on 13 November 2023. A summarized timeline of the case history and treatment process described in Figure 1.

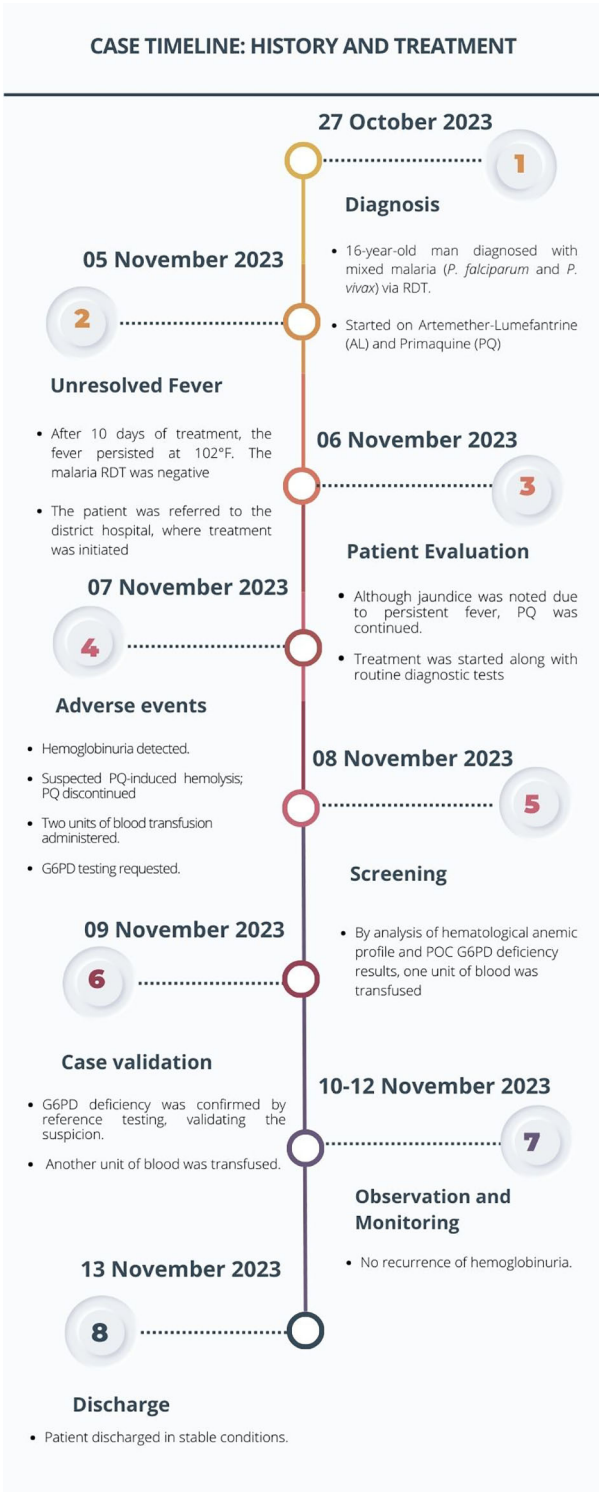

FIGURE 1
Timeline of the summarized case history and treatment process.

Genomic DNA was extracted from a whole blood sample using the QIAamp® DNA Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's protocol. To exclude malaria as a potential cause of hemolysis, a real-time qPCR assay targeting five human malaria species was performed as previously described (Sazed et al., 2021). The results were negative confirming the absence of malaria parasites at the time of hemolysis.

To confirm the host G6PD variants we targeted known variants circulating in this region (Mahidol, Mediterranean, Orissa and Kalyan-Kerala) by PCR RFLP (Restriction fragment length polymorphism) using Primer and restriction enzyme sets for the corresponding variants reported elsewhere. The thermal cycling profile was followed as described in the reference literature (Ley et al., 2020). PCR was performed in a 20 µL reaction volume containing 10µL DreamTaq™ PCR Master Mix (2X) (Thermo

Fisher Scientific, Massachusetts, United States), 1μL forward and reverse primer (10μM), 3 μL DNA and the rest of the volume filled with nuclease-free water. After thermal cycling, we performed enzyme digestion for the Mahidol, Mediterranean, and Orissa variants. Sequencing was required for the Kalyan-Kerala variant. A total of 5 μL of the PCR product was digested by specific restriction enzymes (Except for Kalyan-Kerala) in a 50 μL reaction by the manufacturer's instruction (New England Biolabs, Massachusetts, United States). The DNA bands were then separated on a 3% agarose gel and visualized by the FastGene® FAS-V Imaging System. The sample was identified as a Mahidol variant. Mahidol (487 G > A) digestion should have resulted in the visualization of two distinct bands at 82 bp and 22 bp (Phompradit et al., 2011b). Our gel run showed two separate bands (82 + 22 bp) for the sample and confirmed it as a Mahidol variant (Figure 2). Enzyme digestion did not appear in the gel run for the two other variants, confirming them as negative. Since the sample tested positive for the Mahidol variant, we did not proceed with sequencing, which is necessary only for the Kalyan-Kerala variant.

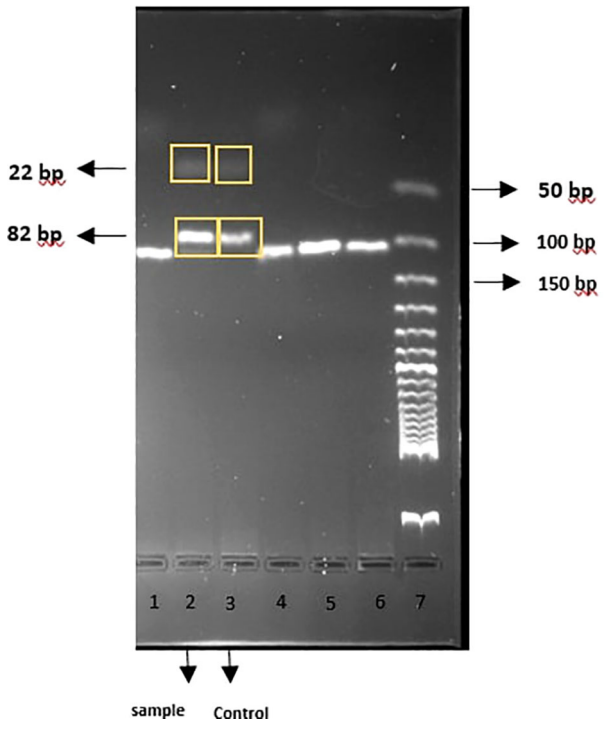

FIGURE 2
PCR-RFLP products visualized by 3% gel electrophoresis; Lane 2: Sample, Lane3: G6PD Mahidol Control.

Discussion

This patient experienced severe hemolysis as a consequence of PQ treatment for presumed *P. vivax* infection in the setting of significant G6PD deficiency. The World Health Organization (WHO) recommends routine G6PD screening before PQ administration. While individuals with regular G6PD activity (≥30%) can receive daily PQ, those with G6PD deficiency require a modified regimen of 0.75 mg/kg PQ weekly for eight weeks instead of the standard 14-day course (WHO, 2022).

Bangladesh lacks a surveillance system for G6PD deficiency. The CHT region is known to have a high prevalence of G6PD deficiency, with significant variation among ethnic groups. This region is permanently inhabited by at least 12 major indigenous populations and a Bengali subpopulation (Hussain et al, 2015). A study conducted on 999 participants from both Bengali and ethnic groups of CHT showed that 9% of the individuals had G6PD deficiency, with 93.9% of those affected belonging to different ethnic groups. The Chak tribe had the highest prevalence (26%), while the Tripura tribe showed the lowest prevalence (2%) (Ley et al., 2020). Widespread and reliable testing remains a challenge due to budgetary and logistical constraints. However, this is likely an underestimate due to the lack of routine G6PD testing before PQ administration for malaria. A previous case with the Mediterranean G6PD variant resulted in severe PQ-induced hemolysis. This case was also from CHT with *P. vivax* mono-infection and was associated with severe hemolysis. The patient's hemoglobin level dropped to 6.0 g/dL, and serum bilirubin was measured at 5.6 mg/dL, despite no evidence of malaria in laboratory tests. G6PD levels were subsequently determined using a spectrophotometer, which confirmed a deficiency, indicating that the hemolysis was induced by PQ in the context of G6PD deficiency. The patient recovered after receiving two units of blood transfusion. Subsequent whole genome sequencing identified the G6PD Mediterranean variant (Phru et al., 2020).

We documented a case of PQ-induced hemoglobinuria associated with the G6PD Mahidol variant in the CHT tribal population (Tripura). The Mahidol variant, commonly found in Myanmar and Thailand, exhibits enzyme activity ranging from 5 to 32% of normal levels. Its susceptibility to induced hemolysis falls between that of the G6PD A− and Mediterranean variants (Howes et al., 2013). Both cases highlight the adverse impact of PQ in individuals with G6PD deficiency. In contrast, the previous case involved an individual of Bengali origin with the G6PD Mediterranean variant and a *P. vivax* mono-infection. Our case underscores the potential for underdiagnosed severe hemolytic events due to misdiagnosis or insufficient G6PD testing. PQ should be administered with extra precautions to prevent serious

adverse events by identifying early signs of hemolysis in settings where testing is unavailable. Most importantly, implementing routine G6PD screening before PQ administration in all healthcare facilities nationwide is essential to prevent such adverse outcomes.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author/s.

Ethics statement

The studies involving humans were approved by Ethical Review Committee (ERC) of icddr,b. The studies were conducted in accordance with the local legislation and institutional requirements. Written informed consent for participation in this study was provided by the participants' legal guardians/next of kin. Written informed consent was obtained from the individual(s), and minor(s)' legal guardian/next of kin, for the publication of any potentially identifiable images or data included in this article.

Author contributions

MZ: Data curation, Formal analysis, Investigation, Methodology, Resources, Software, Validation, Writing – original draft, Writing – review & editing. CP: Formal analysis, Investigation, Methodology, Project administration, Resources, Supervision, Writing – review & editing. AH: Investigation, Methodology, Project administration, Supervision, Validation, Writing – review & editing. AT: Investigation, Methodology, Project administration, Resources, Validation, Writing – review & editing. MS: Investigation, Project administration, Resources, Supervision, Validation, Writing – review & editing. SA: Investigation, Project administration, Resources, Supervision, Validation, Writing – review & editing. MH: Data curation, Project administration, Resources, Supervision, Visualization, Writing – review & editing. MA: Conceptualization, Funding acquisition, Project administration, Resources, Supervision, Writing – original draft, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Generative AI statement

The author(s) declare that no Generative AI was used in the creation of this manuscript.

Publisher's note

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