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BRIEF REPORT

Clinical Spectrum of Primaquine-induced Hemolysis in Glucose-6-Phosphate Dehydrogenase Deficiency: A 9-Year Hospitalization-based Study From the Brazilian Amazon

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(See the Editorial Commentary by Grobusch, et al. on pages 1443-5).

Despite glucose-6-phosphate dehydrogenase (G6PD) deficiency prevalence of 5% in the Amazon, primaquine is administered without G6PD screening. This is an important cause of hospitalization among *Plasmodium vivax*–infected individuals, leading to life-threatening anemia and acute renal failure across endemic areas. In Manaus, the frequency of primaquine-induced hemolysis was 85.2 cases per 100 000 primaquine users.

Keywords. G6PD deficiency; acute renal failure; severe anemia; elimination; primaquine.

Acute hemolytic anemia (AHA) is a common complication in vivax malaria cases due to primaquine (PQ) use in glucose-6-phosphate dehydrogenase (G6PD) deficiency [1]. Under PQ challenge, cells are prone to exacerbated oxidative stress, leading to an early erythrocyte destruction, which is directly dependent on the enzyme variant class and antimalarial dose. More than 185 clinically relevant variants of the enzyme have been described in 8% of the world's population [2]. This condition is not systematically screened in hospitals prior to antimalarial treatment. It is usually detected during PQ therapy, when patients return to healthcare units presenting classical signs of AHA. Hemolysis in the most common G6PD variant, African A(−), is usually thought to be self-limiting and is solved when the oxidant agent is withdrawn [3].

Although described decades ago, G6PD deficiency greatly impacts the treatment of vivax malaria, and the true extent of PQ-induced hemolytic anemia is limited to scarce case reports. A critical issue in malaria elimination programs today is how radical cure regimens should be routinely used. As most malaria is treated on an outpatient basis in rural clinics, estimating the burden of PQ-induced hemolysis at the population level may aid in defining the real danger of PQ use. This study aims to describe the morbidity associated with PQ-induced hemolysis in *P. vivax* malaria patients with G6PD deficiency admitted to a tertiary health unit in the Brazilian Amazon.

METHODS

The Fundação de Medicina Tropical Doutor Heitor Vieira Dourado (FMT-HVD) is a specialist referral unit for diagnosis and treatment of tropical diseases in the Western Brazilian Amazon. Patients can seek medical assistance directly or be referred from primary-level healthcare units. After vivax malaria diagnosis, a short 7-day course of PQ (0.5 mg/kg/day) is given [4]. A retrospective collection of medical records was conducted to identify all vivax malaria patients hospitalized between 2009 and 2017. Inclusion criteria were (1) *P. vivax* malaria infection diagnosed at primary or transferring unit; (2) history of PQ treatment for that episode; (3) G6PD deficiency confirmed by qualitative or molecular test; and (4) hospitalization. All laboratory, demographic, and clinical data from included patients were extracted. Although the number of PQ-induced hemolysis cases is probably higher than presented here, moderate to severe cases requiring PQ interruption are referred to FMT-HVD, thus becoming a good proxy of the proportion of patients for whom the drug is actually contraindicated.

Information on pretreatment parasitemia was retrieved from the Epidemiological Surveillance Information System for Malaria database, on a semiquantitative basis of parasites per cubic millimeter: 1/2+ (200–300 parasites/mm^3), 1+ (301–500 parasites/mm^3), 2+ (501–10 000 parasites/mm^3), 3+ (10 001–100 000 parasites/mm^3), and 4+ (≥100 001 parasites/mm^3) [4]. Polymerase chain reaction–restriction fragment length polymorphism was performed to detect common G6PD mutations in 29 patients (30.8%) in a background study. Severe anemia was classified according to World Health Organization guidelines [5]. The onset and severity of acute kidney injury (AKI) were established according to Acute Kidney Injury Network guidelines [6], using the difference between the highest recorded measure during hospitalization (peak) and the last recorded measure at time of patient discharge (baseline) [7].

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Descriptive statistics were used for demographic and clinical data. Independent *t* test, Wilcoxon Mann-Whitney test, or χ^2 test was used to compare variables stratified by severe anemia and AKI. Receiver operating characteristic (ROC) curves were used to assess diagnostic ability of continuous laboratory parameters using AKI and intensive care support as endpoints. Fractional polynomials were used to assess the relationship between hemoglobin and days of ongoing PQ treatment. All statistical analyses were performed using Stata version 14 (StataCorp, College Station, Texas). This study was approved by the institutional Ethics Review Board (approval 1943/2008) and the National Brazilian Committee of Ethics (approval 343/2009).

RESULTS

From 2009 to 2017, 28 095 vivax malaria cases were recorded, among which a total of 672 patients (2.4%) required further hospitalization (Supplementary Figure 1). Among those, 94 cases (13.9%) had G6PD deficiency and received PQ treatment for that infection. The frequency of PQ-induced hemolysis was 85.2 cases per 100 000 PQ users (Figure 1). Age varied greatly, from 4 to 84 years (mean 25.0, standard deviation [SD] 14.7 years). Most patients were 16–30 years of age (n = 36 [38.3%]), followed by age 4–15 years (n = 30 [31.9%]). Pretreatment parasitemias ranged mostly between 200 and 300 parasites/mm^3 (n = 35), followed by 501–10 000 parasites/mm^3 (n = 16), 301–500 parasites/mm^3 (n = 15), and 10 001–100 000 parasites/mm^3 (n = 7), most of them already negative at hospital admission (n = 56 [67.5%]). Patients were mostly hospitalized following their third (n = 23), fourth (n = 20), and fifth (n = 12) PQ doses, when symptoms of AHA became clinically significant. The average duration of hospitalization was 6 days (SD, 6.3 days).

Twenty-eight (29.8%) patients had the G6PD A(–)$^{202A/376G}$ variant, and 1 male (1.1%) had G6PD A(+)^{376G}. Of the 6 G6PD A(–)$^{202A/376G}$ females genotyped, 3 were heterozygotes and 3 homozygotes. Laboratory results showed severe anemia (n = 55 [58.5%]) as the most common finding, followed by moderate (n = 29 [30.8%]) and mild cases (n = 7 [7.4%]). Urine examination, when available, showed that 48.3% (n = 29) of cases presented some level of hemoglobinuria, 58.2% presented proteinuria (n = 32), and 17.8% (n = 10) presented urobilinogenuria.

Thirty patients presented signs of bleeding, mostly as hematuria (n = 25 [83.3%]), followed by melena (n = 3 [10.0%]) and epistaxis (n = 2 [6.6%]). Thirty patients presented clinical complications mostly as prostration (n = 18 [60.0%]) and respiratory impairment (n = 3 [10.0%]). Additionally, 26 patients (27.6%) developed AKI categorized as stage I (n = 11 [42.3%]), stage II (n = 3 [11.5%]), or stage III (n = 12 [46.2%]), of whom 7 required renal replacement therapy. Blood transfusion was necessary in 46 patients (48.9%). In total, 4 patients required admission to the intensive care unit (ICU), and 1 hemizygous male, genotyped as G6PD A(–)$^{202A/376G}$, died (Supplementary Table 1).

Compared to the opposite group, the group with AKI was characterized by a higher proportion of males (26 vs 0; $P = .004$), an increased need for ICU support (4 vs 0; $P < .001$), and longer duration of hospitalization (10.9 days vs 4.1 days; $P < .001$), as well as increased serum creatinine (2.2 mg/dl vs 0.9 mg/dl; $P < .001$), urea (97 mg/dl vs 37 mg/dl; $P < .001$), and urine heme (16 positives vs 13 positives; $P < .001$). Regarding severe anemia, only hemoglobin (6.4 g/dl vs 9.9 g/dl; $P < .001$) and reticulocytes (5.9% vs 2.4%; $P = .013$) showed significant differences compared to the group without severe anemia. When using ROC curves for AKI as outcome, creatinine (area under the curve [AUC], 0.87; 95% confidence interval [CI], .77–.97) and urea (AUC, 0.83; 95% CI, .73–.93) presented fair discriminative performance. When using ICU admission as endpoint, creatinine (AUC, 0.90; 95% CI, .84–.96), urea (AUC, 0.95; 95% CI, .91–1.0), unconjugated bilirubin (AUC, 0.72; 95% CI, .41–1.0), and aspartate aminotransferase (AUC,

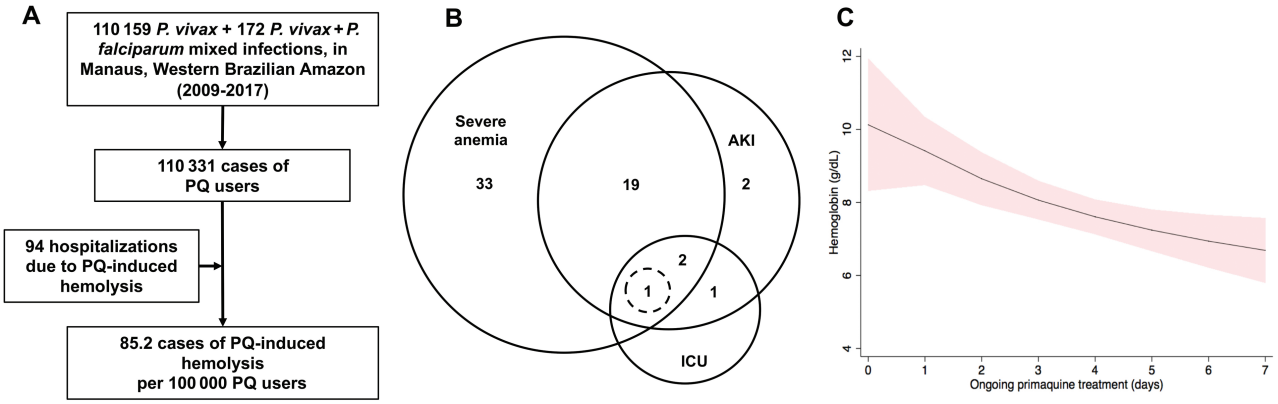

Figure 1. *A*, Flowchart of primaquine-induced hemolysis cases per 100 000 primaquine users in Manaus from 2009 to 2017. *B*, Venn diagram summarizing the overlap in clinical syndromes, where death is represented by the dashed circle. *C*, Univariate fractional polynomial regression curve for the prediction of hemoglobin fall through days of ongoing primaquine treatment, with 95% confidence intervals. Abbreviations: AKI, acute kidney injury; ICU, intensive care unit; *P. falciparum*, *Plasmodium falciparum*; PQ, primaquine; *P. vivax*, *Plasmodium vivax*.

0.74; 95% CI, .35–1.0) presented fair discriminative performances (Supplementary Table 2).

DISCUSSION

Primaquine intake is the main cause of hemolysis in Latin America [8]. In total, 55 patients presented severe anemia (46 patients received transfusion) and 26 developed mostly severe stages of AKI, irrespective of age. It confirms that, although the African variant is considered to cause self-limiting mild to moderate hemolysis, the clinical course can be severe and life-threatening.

The pathogenesis of acute renal failure in G6PD deficiency, as in other hemolytic syndromes, is believed to be caused by the effect of free heme on renal cells, mostly due to decreased renal perfusion, direct toxicity, and intratubular casts formed from the interaction with Tamm-Horsfall protein [9]. Therefore, cases of exacerbated hemolysis must be closely monitored to either avoid or promptly treat AKI. Standard markers of renal function can aid in the diagnosis of AKI and serve as early predictors for the need for intensive care.

Four patients required ICU support and 1 patient died as a result of severe anemia and complications. In a previous study on deaths associated with vivax malaria in Brazil, 2 cases were found to be a direct result of AHA in G6PD-deficient patients [10, 11]. Due to lack of testing at primary care, this number may be an underestimate of the true mortality in vivax malaria infections.

Despite G6PD deficiency prevalence of 5% in the Amazon region, PQ is still administered without G6PD screening. In Brazil, the treatment of choice after diagnosis relies on a 12-week-long weekly chloroquine regimen to steadily clear out blood stage forms, although no studies have proven the efficacy of this approach [4]. Alternatively, weekly PQ has shown good efficacy against relapse, but its safety in more severe variants still requires further addressing and close monitoring [12].

Our study has certain limitations. Lack of prospective and detailed investigation, such as G6PD activity quantification and preplanned assessment of laboratory markers of hemolysis at different times, resulted in incomplete data. Furthermore, testing during a hemolytic crisis may falsely normalize G6PD status. Although in some cases hemolysis was clearly evident and highly suggestive of G6PD deficiency, the qualitative method employed may falsely lead to normal results, because testing was performed in the midst of the hemolytic event.

Last, results show high morbidity in an important fraction of patients undergoing a short-course PQ treatment. However, many clinicians focus entirely on the rare adverse events and never use PQ, condemning the population to a continued cycle of relapse and infection. Malaria elimination will require the use of PQ, but in safer regimens. Therefore, the current practice must be changed and, despite all of the difficulties involved, G6PD deficiency must systematically be tested for, especially with the implementation of tafenoquine, a substitute drug for which no safety net exists as it is given as a single dose.

Supplementary Data

Supplementary materials are available at *Clinical Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

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