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Letter to the Editor

No evidence that chloroquine or hydroxychloroquine induce hemolysis in G6PD deficiency☆

To the Editor,

Hydroxychloroquine and chloroquine have come under intense scrutiny over the past six months as they have been proposed as treatments for COVID-19. It is widely quoted and stated that the 4-aminoquinolines chloroquine and hydroxychloroquine cause oxidant hemolysis in glucose-6-phosphate dehydrogenase (G6PD) deficiency, yet there is no convincing evidence for this claim, and there is substantial evidence against it. X-linked G6PD deficiency is the most common human enzymopathy. It affects an estimated 400 million people worldwide with average allelic frequencies of 8–10% in populations living in or originating from tropical areas [1]. Billions of chloroquine treatments have been given in these malaria endemic areas, and millions have received hydroxychloroquine for rheumatological conditions. As a result, hundreds of millions of people with G6PD deficiency have received these drugs. Chloroquine has been used safely, and without evidence of iatrogenic hemolysis for over six decades in both the prevention and the treatment of malaria, even in countries with the most severe G6PD deficiency variants.

The 4-aminoquinolines were developed as antimalarials because they were more effective and less toxic than their predecessors- the 8-aminoquinolines. Notably they lacked hemolytic toxicity. The oxidative and hemolytic effects of the 8-aminoquinolines were apparent in early clinical testing nearly 100 years ago [2,3]. Investigation of primaquine "sensitivity" ultimately led to the discovery of G6PD deficiency in 1956 [4]. This provided a common mechanism for hemolytic reactions to different drugs, chemicals and oxidant foods [1,5,6]. Both the dose of the stressor and the genetic background determined the final hemolytic outcome. Today, over 200 different polymorphic genetic variants causing G6PD enzymatic deficiency have been described. The A- genotype prevalent across Africa confers generally less severe deficiency than the variants prevalent across Southern Europe and Asia, but severe hemolysis may occur in any of the variants. Being X-linked, males are either normal or fully deficient whereas women can be phenotypically normal, fully deficient or have intermediate levels of enzymatic activity. Hemolysis in G6PD deficiency may be provoked by oxidant drugs (e.g. primaquine), chemical exposures (e.g. naphthalene moth balls), dyes (e.g. henna), foods (e.g. fava beans), or febrile illnesses (e.g. malaria or COVID-19). A decade after the discovery of G6PD deficiency as the cause of drug-induced hemolysis, a survey of 73 episodes of hemolysis in a hospital in New York City suggested that infection was the most common precipitating factor of hemolytic anemia among G6PD-deficient patients [7]. Hemolysis in G6PD deficiency sufficient to cause black urine (blackwater) may occur occasionally without an identified precipitant.

Chloroquine and hydroxychloroquine have been shown to exert some antioxidant and oxidant effects *in vivo* [8]. As with other redox active compounds the redox activity of these drugs may reflect the intracellular or extracellular redox potential. In normal individuals chloroquine does not cause clinically significant methemoglobinemia. A clinically insignificant approximately three-fold rise in methemoglobin levels (compared with 15-fold rise following primaquine) was observed in individuals with genetic deficiency of the main methemoglobin reductase (NADH-cytochrome *b*5-methemoglobin reductase) [9]. In the original detailed investigations of G6PD deficiency conducted in the late 1950s and 1960s, summarized by Beutler [10], chloroquine was included in the list of drugs that could be administered safely to individuals who were G6PD deficient. By this time hundreds of millions of prevention and treatment regimens were being given to populations with G6PD deficiency allele frequencies up to 30%.

There are very few case reports suggesting that chloroquine might cause hemolysis in G6PD deficiency. The letter by Sicard in 1978 describing a partially completed study from Laos (where G6PD deficiency prevalence was estimated to be around 13%) is unusual. "When a single high dose of chloroquine (600 mg) was given routinely to soldiers for malaria prophylaxis, some of them had very severe hemolytic anaemia, with 'coca-cola' urine and acute renal failure. 50 such cases were thoroughly investigated. All were G6PD deficient and the alleged diagnosis of blackwater fever could be ruled out" [11]. Nothing else like this has ever been reported since. It may be relevant that at that time a combined chloroquine-primaquine medication was commonly used by militaries and that later reports of chloroquine-induced hemolysis might have used the same combination [12]. A recent meta-analysis of 3421 chloroquine treated patients with vivax malaria found no evidence for hemolysis above that associated with malaria itself [13]. It is highly unlikely that significant oxidant hemolysis following treatment with chloroquine alone would remained unnoticed in the hundreds of millions of occasions that chloroquine has been given to prevent or treat malaria in patients who were G6PD deficient. Similarly, use of hydroxychloroquine in rheumatological conditions is not associated with hemolysis in G6PD deficient individuals [14]. Furthermore, hemolysis and methemoglobinemia are not features of chloroquine overdose, even though chloroquine was commonly used in self-poisoning in malaria endemic regions where G6PD deficiency prevalences are high.

Hemolysis in G6PD deficient patients treated with chloroquine for malaria is most likely explained by the febrile illness. This same applies to COVID-19. Beauverd et al. recently reported a case of a severe hemolysis in G6PD deficiency attributed partly to hydroxychloroquine use [15]. But the hemolysis clearly pre-dated the addition of hydroxychloroquine [16]. Kuipers et al. report a 2.5 g fall in hemoglobin con-

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centration within 12 h of starting chloroquine in a man with the A- variant of G6PD deficiency [17]. This is not the pattern seen in primaquine or dapsone induced hemolysis in G6PD deficiency in which the fall in hemoglobin is typically delayed by more than one day as residual erythrocytic stores of antioxidants are depleted. The other case reports are also satisfactorily explained by disease rather than drug induced hemolysis [18]. In conclusion, there is no evidence that chloroquine or hydroxychloroquine provoke oxidant hemolysis in G6PD deficient individuals.

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