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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

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Glucose-6-phosphate dehydrogenase deficiency-associated hemolysis and methemoglobinemia in a COVID-19 patient treated with chloroquine

To the Editor:

Novel coronavirus disease (COVID-19) is spreading around the world and although clinical data are limited, immunomodulatory agents such as chloroquine and hydroxychloroquine are used as off-label treatment.[1] While these medications have an established clinical safety profile for their common use, (eg, malaria) their efficacy and safety in COVID-19 pneumonia remains unclear.[1] This is as most of the evidence to support use of chloroquine, or the less toxic hydroxychloroquine against the disease, comes from a small single arm trial.[2] As we demonstrate in this correspondence, the use of chloroquine for treatment of patients with COVID-19 infection is not without risks.

A 56-year-old man, with a medical history of diabetes mellitus type 2, presented to the emergency department with a 6-day history of myalgia and a dry cough. Oxygen saturation was 94% with room air and respiratory rate 24/min. There was no fever and his pulse and blood pressure were normal. So, COVID-19 was suspected which was confirmed by a real-time-PCR assay. A chest CT scan showed bilateral ground glass opacities. The patient was admitted for observation on a COVID-19 ward. During the following 2 days, his condition deteriorated with increasing need for oxygen administration. On the third day of admission his peripheral oxygen saturation dropped to 83% despite the use of a non-rebreathing mask with 15 L/min of oxygen. His respiratory rate was 30/minute. He was admitted to the intensive care unit (ICU) for initiation of mechanical ventilation. Treatment with chloroquine was started consisting of a first dose of 600 mg, followed by 300 mg twice a day (for 5 days).[1] Initial ICU laboratory results demonstrated a hemoglobin level of 11.4 g/dL (reference 13.7-17.7 g/dL), 12 hours later his hemoglobin level dropped to 8.9 g/dL and additional laboratory investigations demonstrated signs of severe hemolysis. A peripheral blood smear revealed findings consistent with hemolysis (Figure 1). Arterial blood gas results demonstrated increased levels of methemoglobin (9.1%; reference <1.5%). Given his ethnic background (African-Caribbean), glucose-6-phospate dehydrogenase (G6PD) deficiency was suspected and chloroquine was stopped.[3] He received 3 units of packed red blood cells in the following 48 hours. Although his methemoglobin level was relatively low, 1000 mg ascorbic acid (vitamin C) was administered intravenously four times a day for 2 days, to help optimize his oxygenation. His methemoglobin normalized within 6 days and laboratory testing for G6PD deficiency confirmed very low G6PD activity in the patient's red blood cells (Figure 1A,B). Genetic analysis demonstrated variant G6PD A- (the African variant).

Note, G6PD deficiency is an X-linked disease that affects 400 million people worldwide.[3] During a period of oxidative stress, intracellular levels of the reduced form of the nicotinamide adenine dinucleotide phosphate (NADPH) in these patients are depleted. This leads to the accumulation of oxidative damaged proteins and lipids in their red blood cells, resulting in hemolysis of deficient red blood cells. Chloroquine is on the list of oxidative drugs known to cause hemolysis in patients with G6PD deficiency.[3] The G6PD A- variant results in a moderate enzyme deficiency and clinically insignificant hemolysis. A short duration of chloroquine treatment at the above mentioned dose usually does not result in severe hemolysis, unless the antioxidant reserves are depleted by another (pre-existing) trigger, such as intensive systemic inflammation. In our patient, the ongoing inflammation due to the COVID-19 pneumonia had probably resulted in excessive consumption of intracellular antioxidants and thus NADPH. Under these circumstances, chloroquine possibly triggered a complete depletion of NADPH resulting in severe hemolysis. Our patient also suffered from a functional anemia due to methemoglobinemia. Hemoglobin is transformed to methemoglobin once ferrous iron (Fe^{2+}) of the heme group is oxidized to ferric iron (Fe^{3+}). Methemoglobin has such a high oxygen affinity that it virtually cannot release its oxygen in the tissues and this usually becomes clinically apparent at a level of 15% or more.[4] However, patients with severe anemia may have symptoms at lower levels. The most frequent cause of methemoglobinemia is exposure to oxidative drugs such as chloroquine.[5,6] Under normal circumstances methemoglobin is rapidly converted back to hemoglobin by the NADH-dependent cytochrome-b_5

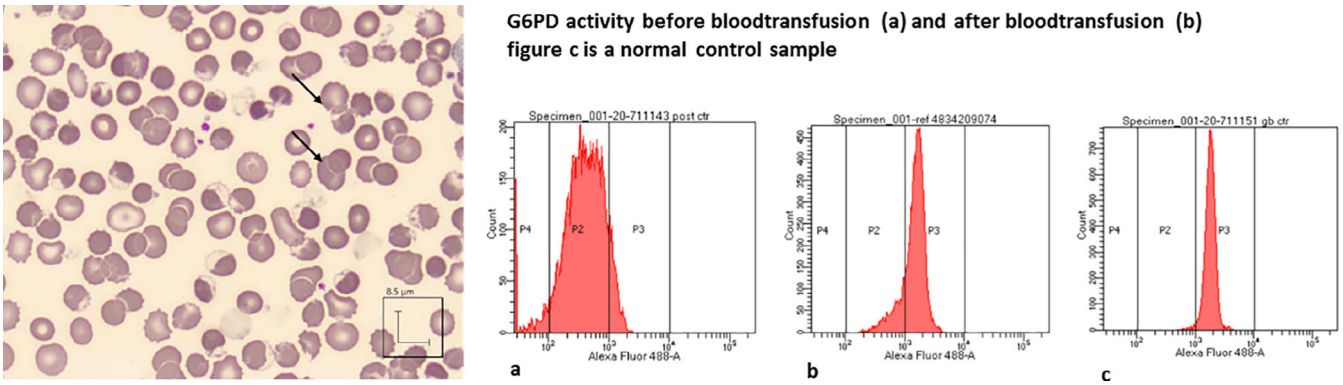

FIGURE 1 Signs of hemolysis and low G6PD activity. Glucose-6-phosphate dehydrogenase (G6PD) deficiency in red blood cells was suspected by the presence of blister cells on peripheral blood smear (left) and was confirmed by a low G6PD enzyme activity assay (right, x-axis G6PD activity; fluorescence intensity 490/525 nm in arbitrary units (ferryl Hb as measure for G6PD activity), y-axis number of red blood cells).[5] A, demonstrates the lack of G6PD activity in red blood cells 12 hours after chloroquine. Mean G6PD activity of 0.1 IE/gHb (reference 3.8-5.9). B, demonstrates that after ongoing hemolysis and a blood transfusion the main fraction of red blood cells in the circulation had normal G6PD activity (mean 4.0 IE/gHb) with only a minor fraction deficient cells. C, demonstrates G6PD activity in a healthy control (mean 5.0 IE/gHb).

methemoglobin reductase (CYB5R) enzyme. Oxidizing agents may overwhelm this reducing system and cause methemoglobin levels to rise. Mutations affecting the activity of CYB5R enzyme can lead to congenital methemoglobinemia.[5] Molecular analysis of the CYB5R enzyme in our patient, however, did not show any pathogenic variants. Based on this result together with the normalization of the methemoglobin level after chloroquine discontinuation and no medical history of methemoglobinemia, the reducing capacity of the deficient G6PD in our patient was probably overwhelmed by the antioxidant consumption during COVID-19 in combination with chloroquine use.

Widespread off-label use of chloroquine harbors potential benefit, but also a risk of harm. Monitoring of well-known side effects such as QT prolongation, bone marrow suppression, and mental disturbances is recommended.[1] This case-report illustrates that hemolytic anemia due to G6PD deficiency is another complication that can occur and exacerbate an already compromised oxygenation in the setting of COVID-19 pneumonia.

Pending results from well-designed clinical trials, we recommend caution with using chloroquine as treatment of COVID-19. If feasible, G6PD deficiency should be ruled out before administering chloroquine in patients with COVID-19.

CONFLICT OF INTEREST

The authors declare no potential conflict of interest.

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The emergence of methemoglobinemia amidst the COVID-19 pandemic

To the Editor:

Coronavirus disease 2019 (COVID-19) has been associated with a range of hematologic findings and complications [1]. We have encountered three cases of significant methemoglobinemia, and five cases of relatively mild methemoglobinemia, among patients being treated for COVID-19 in our health system during a 4 week period in April 2020. For comparison, there was only one case of mild acquired methemoglobinemia of any cause documented in our health system during the preceding year. Below we describe the three cases of significant methemoglobinemia, including their presentations, treatments, and outcomes.

Case 1: A 50 year-old man with no medical history presented with acute hypoxic respiratory failure (AHRF) due to COVID-19. He received hydroxychloroquine on hospital day (HD) 1, and continued through HD5. He also received brief courses of azithromycin and ceftriaxone. He deteriorated quickly, requiring intubation and mechanical ventilation on HD2, initiation of vasopressors on HD3, and renal replacement therapy on HD4. Persistently low oxygen saturation on pulse oximetry, despite mechanical ventilation with 100% FIO_2, prompted blood co-oximetry. This demonstrated steadily increasing levels of methemoglobin (Met-Hb) which peaked at 10.6% on HD6. He received 1mg/kg of methylene blue, however demonstrated minimal response with respect to oxygenation and Met-Hb level, and therefore received another 1 mg/kg on HD7, and 2 mg/kg on HD8. The Met-Hb levels began to decrease gradually thereafter although did not normalize, and the patient was treated with ascorbic acid 500 mg thrice daily on HD8 – HD10, with normalization of Met-Hb level by HD11. The patient's clinical status improved thereafter, and although he no longer requires mechanical ventilation or dialysis, he remains hospitalized.

Case 2: A 52 year-old man with history of morbid obesity and uncomplicated diabetes mellitus was admitted for AHRF in the setting of COVID-19 infection. He required initiation of mechanical ventilation and vasopressors on HD2, and renal replacement therapy on HD7. He received a 5 day course of hydroxychloroquine on HD2-6. Other medications received included azithromycin, cefepime, vancomycin, and apixaban. On HD6, persistently low oxygen saturation on pulse oximetry despite mechanical ventilation with 100% FIO_2 prompted blood co-oximetry, which demonstrated a Met-Hb level of 22%. The patient received methylene blue 1 mg/kg and then 2 mg/kg as well as concurrent ascorbic acid, however his Met-Hb level increased to > 30%. He therefore received red cell exchange on HD9 with prompt improvement in his Met-Hb level (to 2.9%) and his oxygenation. He was continued on ascorbic acid for several days thereafter until complete normalization of his Met-Hb level. The patient remains critically ill and although he is no longer requiring vasopressors, he remains intubated and dialysis dependent.

Case 3: A 54 year-old man with a history of uncomplicated diabetes mellitus was admitted for AHRF due to COVID-19 infection. He was started on azithromycin on HD1 and hydroxychloroquine on HD2. He required intubation for worsening respiratory failure on HD4, however demonstrated persistent hypoxia despite mechanical ventilation with FIO_2 of 100%. This prompted blood co-oximetery which demonstrated a Met-Hb level of 13.7%. Concurrently, he was noted to have evidence of Coombs-negative hemolysis. He received 1.8 mg/kg of methylene blue however his Met-Hb increased further to 18.8%, and laboratory studies indicated worsening hemolysis as well as a new diagnosis of G6PD deficiency. The patient passed away shortly thereafter.

Methemoglobinemia occurs when iron in the porphyrin group of heme is oxidized from the ferrous (Fe^{2+}) to the ferric (Fe^{3+}) form [2]. Ferric heme binds oxygen irreversibly resulting in a left shift of the oxygen-hemoglobin dissociation curve. Methemoglobinemia is most often acquired, predominantly due to oxidizing medications. Antimalarial agents including chloroquine have been associated with methemoglobinemia, albeit rarely [3]. Although seemingly of less oxidizing potential than chloroquine, it is possible that the dramatic increase in use of hydroxychloroquine amidst the COVID-19 pandemic is now making this complication more common. This is likely exacerbated by the degree of critical illness among many COVID-19 patients, which puts them under greater oxidative stress, leaving them more susceptible to medication-induced methemoglobinemia. All three patients with significant methemoglobinemia discussed above received hydroxychloroquine, as did all five patients with relatively mild Met-Hb elevations (3% - 7%) encountered during this time period. The only other drug frequently used among this patient cohort was azithromycin, which is not known to be an oxidizing agent. Additionally, all patients in this series (including the three with significant Met-Hb elevation presented above, and the five with milder elevations) were already severely ill prior to initiation of hydroxychloroquine, and thus likely under oxidative stress even before the initiation of any medications. It is unclear if any additional properties of COVID-19, besides inducing oxidative stress during severe illness, might predispose to