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Associations between COVID-19 and Glucose-6-Phosphate Dehydrogenase Activity in Brazil

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Abstract. Glucose-6 phosphate dehydrogenase deficiency (G6PDd) was suggested as a risk factor for severe disease in patients with COVID-19. We evaluated clinical outcomes and glucose-6 phosphate dehydrogenase (G6PD) activity during and after illness in patients with COVID-19. This prospective cohort study included adult participants (≥ 18 years old) who had clinical and/or radiological COVID-19 findings or positive reverse transcription–polymerase chain reaction results. Epidemiological and clinical data were extracted from electronic medical records. Glucose-6 phosphate dehydrogenase activity was measured using SD Biosensor STANDARD G6PD® equipment on admission and 1 year after discharge. Samples were genotyped for the three most common single nucleotide polymorphisms for G6PDd in the Brazilian Amazon. Seven hundred fifty-three patients were included, of whom 123 (16.3%) were G6PD deficient. There was no difference between groups regarding the risks of hospitalization ($P = 0.740$) or invasive mechanical ventilation ($P = 0.31$), but the risk of death was greater in patients with normal G6PD levels ($P = 0.022$). Only 29 of 116 participants (25%) carried the African G6PDd genotype. Of 30 participants tested as G6PD deficient during disease, only 11 (36.7%) results agreed 1 year after discharge. In conclusion, this study does not demonstrate an association of G6PDd with severity of COVID-19. Limitations of the test for detecting enzyme levels during COVID-19 illness were demonstrated by genotyping and retesting after the disease period. Care must be taken when screening for G6PDd in patients with acute COVID-19.

INTRODUCTION

COVID-19, a disease caused by SARS-CoV-2, is the most critical public health problem in the world, and its impact is evident by its rapid spread.[1] The infection is asymptomatic in most cases, but it can progress to severe illness, which is characterized by pneumonia, SARS, and multiorgan failure, with high mortality rates.[2–4] Older people and those with chronic diseases are more likely to be susceptible to severe disease and death.[5–10] Biochemical factors that may predispose individuals to the development of severe disease are unclear.[11] Some reports[12–18] of glucose-6-phosphate dehydrogenase enzyme deficiency (G6PDd), an important enzyme involved in the hexose monophosphate pathway associated with COVID-19, are found in the literature.

Glucose-6-phosphate dehydrogenase deficiency is an X-linked genetic condition that affects approximately 400 million people worldwide.[19–21] In erythrocytes, glucose-6-phosphate dehydrogenase (G6PD) is the only source of enzyme activity that protects cells against oxidative damage.[22] Glucose-6-phosphate dehydrogenase deficiency is related to neonatal hyperbilirubinemia, chronic hemolytic anemia, acute hemolysis, and risk of acute hemolytic anemia.[23–25] Although most affected individuals are asymptomatic during their lifetime, exposure to oxidative stressors such as certain drugs and other substances or infections can elicit acute hemolytic anemia.[26,27] Studies suggest that the pathogenesis of SARS-CoV-2 involves the overproduction of reactive oxygen species (ROS).[28,29] The production of excessive ROS is critical in individuals with G6PDd because the antioxidant system is unbalanced,[30] and this can contribute to the progression and severity of respiratory disease.[29,31–33] Thus, G6PDd was suggested as being a means to increase the severity and mortality in people with COVID-19.[34,35]

Observational studies report that patients with G6PDd are more hospitalized than patients with normal G6PD (G6PDn) levels.[14,36] In addition, among those hospitalized requiring supplemental oxygen, those with G6PDd had worse hematological indices, and the group with G6PDd had a prolonged partial pressure of arterial oxygen-to-fractional inspired oxygen ratio and more days spent on mechanical ventilation, indicating the severity of pneumonia.[37] Vick[38] suggests that COVID-19 infection may be a trigger for hemolysis and coagulopathy in patients with G6PDd, explaining stroke symptoms in severe cases.[39,40] Kumar et al.,[41] however, showed that the occurrence of noninvasive ventilation, intubation, or death—all of which are indicative of severe COVID-19—are not significantly different in hospitalized patients with COVID-19 with and without G6PDd. We evaluated G6PD activity during and after COVID-19 and the frequency of clinical outcomes in patients with COVID-19 based on G6PD activity.

MATERIALS AND METHODS

Study design and participants. This cohort study included adult (≥18 years old) patients admitted to the Hospital e Pronto-Socorro Delphina Rinaldi Abdel Aziz and Unidade de Pronto Atendimento Campos Sales (Manaus, Amazonas, Brazil) from March 23 to May 24, 2020. Manaus is the most populous municipality in Amazonas, with an estimated population of 2.2 million inhabitants, and has become an important transmission hotspot for SARS-CoV-2 in Brazil.[42] Participants

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in our study were included if they had clinical and/or radiological suspicion of COVID-19 (history of fever and any respiratory symptom, such as cough or dyspnea and/or ground-glass opacity or pulmonary consolidation visible on computed tomographic scan) or were reverse transcription–polymerase chain reaction (RT-PCR) positive. Those who met the inclusion criteria, survived 1 year after inclusion, and were willing to have a new blood sample collected had their G6PD status assessed.

Data collection. Epidemiological, demographic, clinical, laboratory, treatment, and outcome data were extracted from electronic medical records using a standardized data collection form and then transferred to an electronic data-base (REDCap). Study data were collected and managed using REDCap electronic data capture tools hosted at Fundação de Medicina Tropical Dr Heitor Vieira Dourado.[43,44] Clinical parameters were measured daily by the hospital's clinical staff from day 1 until discharge or death, and daily patient information from the first visit to 28 days or until death was collected. One year later, patients who survived were contacted; those who agreed to have a new blood sample collected were evaluated for G6PD enzymatic activity.

Laboratory procedures. Laboratory tests were carried out according to the hospital's routine and at the doctor's discretion. A diagnosis of COVID-19 was acquired from nasopharyngeal and oropharyngeal swab specimens, and was determined using molecular techniques according to the protocol developed by the U.S. CDC, updated March 15, 2020.[45] Swab specimens were collected on days 1, 5, and 7. On day 1 and 1 year from infection, the measurement of G6PD enzyme activity, and G6PD and hemoglobin occurred using SD Biosensor STANDARD G6PD® equipment (Gyeonggi-do, Republic of Korea).[46] According to the activity of the G6PD enzyme, patients were divided into two groups: G6PDd (activity <4) and G6PDn (activity ≥ 4).[47]

Samples were genotyped for the three most common single nucleotide polymorphisms for G6PDd in the Brazilian Amazon[48,49] (s1050828, rs1050829 and rs5030868), corresponding to G6PD African A– (G202A, A376G), G6PD African A+ (A376G), and G6PD Mediterranean (C563T), respectively.[50]

Definitions. The lowest values of hemoglobin and lymphocytes were considered laboratory parameters of severity, as well as the maximum values of C-reactive protein; total, direct, and indirect bilirubin; aspartate aminotransferase, alanine aminotransferase, lactate dehydrogenase, and D-dimer at the follow-up. Clinical evolution and outcomes of COVID-19 according to G6PD status (exposition) included need for hospitalization and intensive care unit (ICU) admission, need for invasive and noninvasive mechanical ventilation, length of hospital and ICU stay, time to ICU admission, and death.

Sample size. The sample size calculation considered the formula from Pourhoseingholi et al.,[51] implemented in Epitools. For a precision of 0.02, the estimated minimum sample size was 457. However, because of ethical concerns, we decided to test all patients included in the cohort ($N = 753$), which gave us a precision of 0.01 and an estimated prevalence of 0.02.

STATISTICAL ANALYSES

Proportions between groups were compared using the χ^2 test for categorical variables. The mean and SD, as well as the median and interquartile range were calculated for continuous variables, and the comparison between groups was performed using analysis of variance and the Wilcoxon rank-sum test, accordingly. The Wilcoxon rank-sum test was performed to compare laboratory parameters between the groups. Comparison of G6PD activity during and after COVID-19 was performed using the Wilcoxon matched-pair signed classification test. All analyzes were performed using the Stata statistical package (version 17, StataCorp LLC, College Station, TX).

RESULTS

A total of 753 participants were included, of whom 123 (16.3%; 95% CI, 0.14–0.19) had G6PDd based on a G6PD quantitative test. Demographic and clinical characteristics on admission are shown in Table 1. Hemoglobin concentration was significantly greater for the G6PDd group (13.2 g/dL, $P = 0.001$). The G6PDn group had greater values of C-reactive protein (79.5 mg/L, $P = 0.009$) and leukocytes (11,443.1 mm_3, $P < 0.001$) on admission. A greater proportion of patients with G6PDn levels was subjected to invasive mechanical ventilation (25.9%, $P = 0.004$) and hospitalized (70.4%, $P = 0.002$) on admission. Approximately 45% of patients used chloroquine combined with antibiotics. The frequency of medication used previously or during hospitalization by the group is shown in Supplemental Tables 1 and 2, respectively. The use of insulin (31.2%, $P = 0.027$) and vasoactive amines (30.6%, $P = 0.012$) was significantly greater in the G6PDn group at follow-up. Regarding clinical outcomes, 29.8% of the study population died, with the proportion of death significantly greater in the G6PDn group ($P = 0.022$). Other outcomes showed similar proportions between groups (Table 2).

The laboratory markers throughout the follow-up are shown in Supplemental Figure 1. The G6PDd group presented greater values, especially on D14, although SDs overlap, considering direct, indirect, and total bilirubin. These values are similar between groups up to day 28. The lowest values of hemoglobin ($z = –3.931$, $P < 0.001$) and lymphocytes ($z = –3.011$, $P = 0.003$) were statistically different between groups (Figure 1A and C). Maximum direct bilirubin was greater in the G6PDn group ($z = 2.098$, $P = 0.036$) (Figure 1F). The other parameters evaluated showed no significance between groups (Figure 1B, D, E, and G–J).

Of the total number of participants with G6PDd, 116 were genotyped, with 29 (25%) presenting a known deficiency variant. Fifteen of 116 (12.9%) were classified as African+, 14 as African– (12.1%), and 87 as wild genotypes (75%). One year after infection, 30 selected participants, classified previously as G6PD deficient by phenotype were retested, of whom only 11 (36.7%) were confirmed as G6PD deficient. Such participants had higher post-COVID-19 G6PD activity ($P = 0.001$) (Figure 2).

DISCUSSION

Glucose-6-phosphate dehydrogenase deficiency (G6PDd) is the most prevalent enzyme deficiency globally and the most common form of red blood cell enzymopathy. The overall G6PDd allelic frequency across malaria-endemic countries is estimated to be 8%.[52] In the Amazon region, prevalence ranges from 4% in the southern areas to 10% on the borders of Guyana.[48] In Manaus, the frequency of G6PDd is about 4%.[49,53] In Latin America, a G6PD A–202A mutation was the most broadly distributed variant identified in 81.1% of the deficient individuals surveyed.[48] Santana et al.[54] showed the estimated frequency of Mediterranean

TABLE 1
Demographic and clinical characteristics of the patients evaluated

Variable	Total (N = 753)	G6PDn (n = 630)	G6PDd (n = 123)	P-Value
Female gender, *n/N* (%)	297/753 (39.4)	248/630 (39.4)	49/123 (39.8)	0.920
Age, years; mean (SD)	51.1 (15.6)	51.3 (15.8)	50.0 (14.5)	0.400
Race, *n/N* (%)				
White	114/753 (15.1)	96/630 (15.2)	18/123 (14.6)	
Mixed*	562/753 (74.6)	470/630 (74.6)	92/123 (74.8)	
Black	42/753 (5.6)	32/630 (5.1)	10/123 (8.1)	0.500
Asian	15/753 (2.0)	14/630 (2.2)	1/123 (0.8)	
Indigenous	20/753 (2.7)	18/630 (2.9)	2/123 (1.6)	
Hemoglobin, g/dL; mean (SD)	12.7 (1.9)	12.5 (1.9)	13.2 (1.9)	**<0.001**
Hematocrit, %; mean (SD)	40.4 (8.1)	40.1 (8.2)	41.7 (7.3)	0.059
Platelet count, $10^3/mm^3$; mean (SD)	280.1 (113.1)	283.4 (112.1)	263.8 (117.1)	0.081
Creatinine, mg/dL; median (IQR)	0.9 (0.8–1.3)	0.9 (0.8–1.4)	0.9 (0.8–1.1)	0.320
Total bilirubin, mg/dL; median (IQR)	0.4 (0.3–0.6)	0.4 (0.3–0.6)	0.3 (0.3–0.6)	0.180
Indirect bilirubin, mg/dL; median (IQR)	0.2 (0.1–0.3)	0.2 (0.1–0.4)	0.2 (0.2–0.3)	0.880
Direct bilirubin, mg/dL; median (IQR)	0.2 (0.1–0.3)	0.2 (0.1–0.3)	0.2 (0.1–0.3)	0.075
Blood glucose, mg/dL; median (IQR)	174.5 (134.0–232.0)	174.0 (135.0–234.5)	176.0 (123.0–217.0)	0.220
Lactate dehydrogenase, U/L; median (IQR)	717.0 (418.0–1,035.0)	727.5 (421.0–1046.0)	662.0 (398.0–929.0)	0.330
IL-6, pg/mL; median (IQR)	74.6 (19.7–184.8)	73.4 (19.7–185.8)	83.1 (20.9–183.9)	0.860
Ferritin blood level, ng/mL; median (IQR)	936.5 (528.0–1,650.0)	936.5 (528.0–1,680.0)	901.5 (529.0–1,255.0)	0.730
C-reactive protein, mg/L; median (IQR)	78.3 (58.0–99.8)	79.5 (60.6–107.6)	74.7 (38.5–84.1)	**0.009**
Leukocytes, mm^3; mean (SD)	10,848.7 (8,795.1)	11,443.1 (9,316.9)	7,891.6 (4,479.3)	**<0.001**
Lymphocytes, mm^3; mean (SD)	1,412.0 (4,821.5)	1,417.8 (5,271.6)	1,383.2 (850.0)	0.940
Alanine aminotransferase, U/L; median (IQR)	48.6 (30.5–78.4)	49.3 (30.1–78.4)	44.8 (33.3–74.8)	0.430
Aspartate aminotransferase, U/L; median (IQR)	41.3 (27.2–64.2)	42.7 (27.1–66.8)	38.3 (28.2–62.6)	0.450
D-dimer, ng/mL; median (IQR)	934.6 (449.2–3,734.2)	1,003.9 (440.6–3,663.4)	710.9 (519.0–4,066.0)	0.990
Acute renal failure, *n/N* (%)	249/436 (57.1)	225/383 (58.7)	24/53 (45.3)	0.063
Transfusion, *n/N* (%)	2/750 (0.3)	1/627 (0.2)	1/123 (0.8)	0.200
Diabetes, *n/N* (%)	175/674 (26.0)	148/562 (26.3)	27/112 (24.1)	0.620
Insulin use, *n/N* (%)	99/544 (18.2)	91/470 (19.4)	8/74 (10.8)	0.076
Noninvasive mechanical ventilation, *n/N* (%)	3/753 (0.4)	3/630 (0.5)	0/123 (0.0)	0.440
Invasive mechanical ventilation, *n/N* (%)	180/753 (23.9)	163/630 (25.9)	17/123 (13.8)	**0.004**
Intensive care unit admission, *n/N* (%)	166/512 (32.4)	148/443 (33.4)	18/69 (26.1)	0.230
Hospitalized, *n/N* (%)	512/752 (68.1)	443/629 (70.4)	69/123 (56.1)	**0.002**
SARS-CoV-2 positive PCR result, *n/N* (%)	574/753 (76.2)	481/630 (76.3)	93/123 (75.6)	0.860
BMI, kg/m^2; median (IQR)	28.7 (25.6–32.9)	28.7 (25.7–32.9)	29.3 (25.4–32.8)	0.820

BMI = body mass index; G6PDd = glucose-6 phosphate dehydrogenase deficiency; G6PDn = normal glucose-6 phosphate dehydrogenase; IQR = interquartile range; PCR = polymerase chain reaction. For some variables, patients' unconsciousness did not allow for complete personal history data collection. *P*-values in bold type are statistically significant.
* Mixed population refers to subjects with different ethnic backgrounds.

G6PDd (1%), and G6PDd A– (4%) variants in rural communities in Manaus. The G6PD A– variant is associated with mild-to-moderate enzyme deficiency (class III), with residual enzyme activity (10–60%), and is recognized predominately in black population.[55,56] Deficiency phenotypes are classified according to the quantification of G6PD activity, which affects the severity of the hemolysis directly.[57] In our study, G6PD activity was determined using validated system SD Biosensor STANDARD G6PD® equipment, discriminating G6PDd from G6PDn, and defining the enzyme activity quantitatively.[46] We evidenced a much greater prevalence of G6PDd (16.3%) in patients with COVID-19 when compared with another study carried out in Manaus.[54]

High prevalence was also observed in a study conducted in Sardinia, Italy, showing that the frequency of patients with G6PDd was greater among patients with COVID-19 compared with the health population.[36] Although suggesting a greater susceptibility to SARS-CoV-2 infection in patients with G6PDd, which was demonstrated previously in vitro[58] and in an observational study,[36] and based on expert opinion,[14] our results, as well as those of Kumar et al.[41] did not demonstrate an association of G6PDd with COVID-19 severity in the study population. The frequency of patients with G6PDd hospitalized or requiring invasive mechanical ventilation was less than that seen in the G6PDn group. A recent study, however, demonstrated that younger black veterans

TABLE 2
Clinical characteristics throughout the follow-up

Variable	Total (N = 753)	G6PDn (n = 630)	G6PDd (n = 123)	P-Value
Death, *n/N* (%)	224/753 (29.8)	198/630 (31.4)	26/123 (21.1)	**0.022**
Hospitalization after D1, *n/N* (%)	6/233 (2.6)	5/181 (2.8)	1/52 (1.9)	0.740
ICU admission after D1, *n/N* (%)	54/196 (27.6)	50/171 (29.2)	4/25 (16.0)	0.170
Noninvasive mechanical ventilation after D1, *n/N* (%)	7/725 (1.0)	6/605 (1.0)	1/120 (0.8)	0.870
Invasive mechanical ventilation after D1, *n/N* (%)	77/568 (13.6)	66/463 (14.3)	11/105 (10.5)	0.310
Length of hospitalization, days; mean (SD)	10.6 (5.9)	10.5 (5.9)	11.0 (6.1)	0.650
Length of ICU stay, days; mean (SD)	10.7 (7.0)	11.3 (7.2)	6.3 (2.5)	0.260
Time to ICU admission, days; mean (SD)	3.4 (2.7)	3.4 (2.7)	3.5 (2.1)	0.950

D1 = day of inclusion in the study; G6PDd = glucose-6 phosphate dehydrogenase deficiency; G6PDn = normal glucose-6 phosphate dehydrogenase; ICU = intensive care unit. *P*-values in bold type are statistically significant.

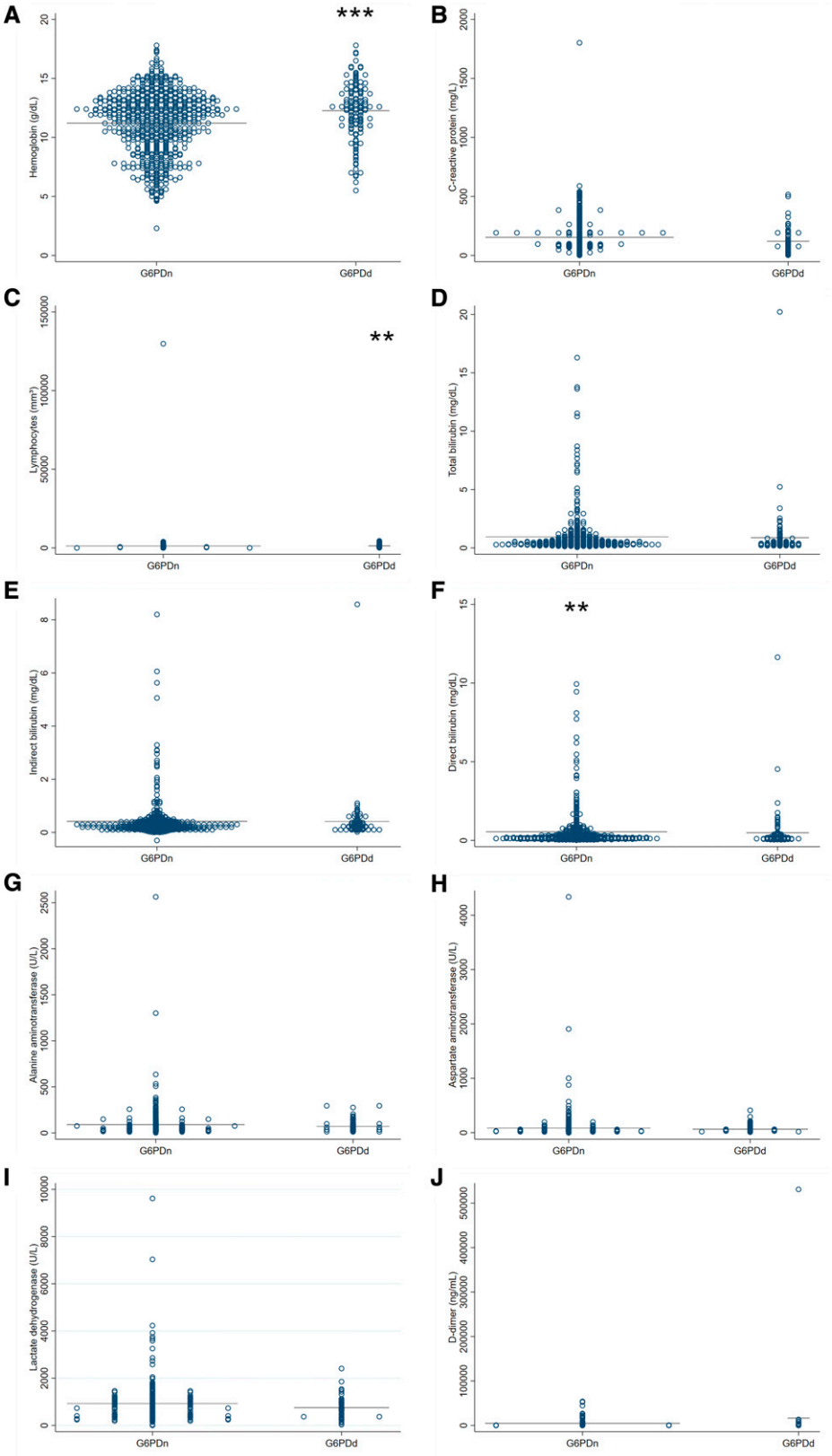

FIGURE 1. Mean of the severity laboratory parameters evaluated: (**A**) hemoglobin, (**B**) C-reactive protein, (**C**) lymphocytes, (**D**) total bilirubin, (**E**) indirect bilirubin, (**F**) direct bilirubin, (**G**) alanine aminotransferase, (**H**) aspartate aminotransferase, (**I**) lactate dehydrogenase, (**J**) D-dimer. Groups were compared using the Wilcoxon rank-sum test, with ***$P < 0.001$ and **$P < 0.05$. G6PDd = glucose-6 phosphate dehydrogenase deficiency; G6PDn = normal glucose-6 phosphate dehydrogenase.

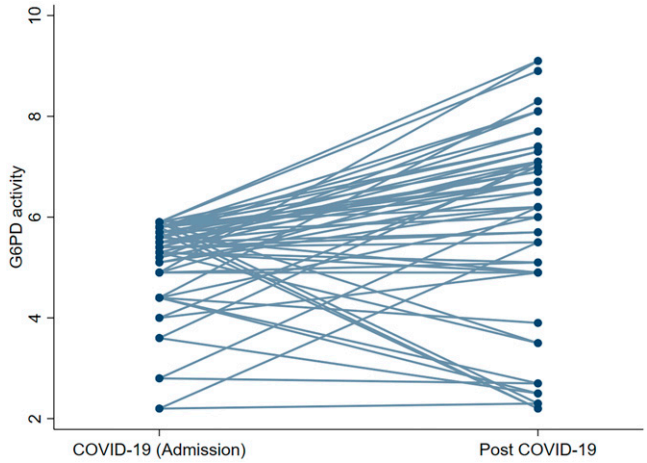

FIGURE 2. Glucose-6 phosphate dehydrogenase (G6PD) activity during and after COVID-19.

with G6PDd were more likely to have worse clinical outcomes resulting from SARS-CoV-2 infection.[59]

On admission, patients with G6PDd had increased hemoglobin levels and decreased leukocytes. At the follow-up, the minimum hemoglobin values were also greater in the G6PDd group, in contrast to maximum direct bilirubin values, which were lower than those in the G6PDn group. These findings diverge from previous studies,[37,60,61] which have reported a decreased hemoglobin level, increased blood neutrophils, and increased bilirubin in patients with G6PDd and COVID-19. Analyzing a selected subgroup 1 year later, we showed that only one third of those participants classified previously as deficient were classified correctly, suggesting a G6PD activity modulation during the illness. Influenza virus in vitro assays downregulate the expression and activity of G6PD during infection, inducing high levels of oxidative stress that favor viral replication.[62] After acute malaria, a change in G6PDd activity is also demonstrated, reinforcing that enzyme activity is influenced in acute infections.[63] Our results suggest that SARS-CoV-2 may exert an influenza virus-like mechanism to increase oxidative stress and, therefore, that activity is influenced by the virus.

In our study, the classification of G6PDd during COVID-19 illness was performed by quantifying enzyme activity. This diagnostic method may have skewed the results that assessed the relationship between G6PDd and the severity of COVID-19. After disease resolution, participants' enzyme activity increased, suggesting that caution should be exercised when classifying G6PDd by enzyme activity during SARS-CoV-2 infection.

Our study has limitations. Approximately 20% of the participants were not confirmed by RT-PCR because laboratory confirmation was not available for all patients at that time. Manaus was facing greater levels of COVID-19 transmission, which allowed us to assume that SARS cases were caused primarily by SARS-CoV-2. Also, because of high mortality, we were not allowed to reach all participants to reassess G6PD enzyme levels after infection. This was accomplished only in those who survived or allowed the procedure. Furthermore, we only reassessed those participants who were classified previously as deficient. In our study, vitamin D levels were not assessed. Previous studies using mouse models show that those who are deficient in vitamin D tend to have lower G6PD activity in intestinal epithelial cells compared with those who are not deficient.[64] This would be a potential variable related to the reduction in G6PDd levels in patients evaluated during SARS-CoV-2 infection; however, better studies must be carried out.[65]

CONCLUSION

In conclusion, our study does not demonstrate an association of G6PDd with severity of COVID-19. Limitations of the test for detecting enzyme levels during COVID-19 illness was demonstrated by results from both genotyping and retesting carried out after the disease period, showing that care must be taken when screening for G6PDd in patients with acute COVID-19, and that the clinicians must evaluate the risks and benefits of different interventions, especially medications.

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