Infection and Drug Resistance

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ORIGINAL RESEARCH

Impact of the *ABCB1* Drug Resistance Gene on the Risk Factors of Patients with COVID-19 and Its Relationship with the Drugs Used

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Objective: In the last two years progress was made in molecular, physio pathological understanding and the form of transmission of COVID-19, and different therapeutic strategies have been explored to deal with the situation of the pandemic. However, the evaluation of certain genes that participate in the metabolism and transport of these drugs has not been fully explored. A lack of response to treatment and a lower survival have been observed that may be due to the presence of the *ABCB1* drug resistance gene. Our research group analyzed whether the expression levels of the *ABCB1* gene are associated with comorbidities, treatments, overall survival and risk of death in patients with severe COVID-19.

Methods: The expression levels of the *ABCB1* gene were analyzed by RT-qPCR in 61 patients diagnosed with COVID-19. The association between the levels of expression, the risk variables and different treatments were determined by the Chi-Square test and the Fisher's exact test. Global Survival (GS) was determined by the Kaplan–Meier method. The impact of high levels of expression and the risk of death was performed by odds ratio.

Results: The different risk variables showed that patients with either high or absent levels of *ABCB1* gene expression presented a greater risk of death (OR 3.08, 95%, CI 1.02–9.26) as well as need for ventilatory support (OR 2.8, 95%, CI 0.98 −8.5). Patients with diabetes and COVID-19, treated with metformin, were associated with a lower risk of death (OR 1.11, 95%, CI 0.38–3.22). OS with respect to high or absent levels of expression of the *ABCB1* gene was lower.

Conclusion: High levels or null expression of the *ABCB1* gene are associated with a higher risk of death or progression of the disease, the use of metformin in patients with COVID-19 confers a lower risk of death.

Keywords: *ABCB1*, SARS-CoV2, drug transporter genes

Background

The Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) is the virus that causes the disease by Coronavirus 2019 (COVID-19).[1] SARS-CoV-2 was reported for the first time in the Chinese city of Wuhan and has spread rapidly around the world through human interactions. WHO declared the COVID-19 outbreak as a pandemic on March 12, 2020, as a result of the increase in the infection rate outside of China.[2]

SARS-CoV-2 belongs taxonomically to the family of the Coronavirus, and the Sarbecovirus subgenus, which contains other species that cause human diseases from mild to severe.[3]

The mortality rate of COVID-19 is lower than that of severe acute respiratory syndrome (SARS) and the Middle East respiratory syndrome (MERS), both by coronaviruses.[4] However, COVID-19 is highly contagious and spreads rapidly, as it can be transmitted by respiratory droplets. The common symptoms observed in patients with COVID-19 are fever, cough, severe headache, myalgia and fatigue.[5,6]

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The incubation period of a COVID-19 infection is usually 1 to 14 days and can be extended up to 24 days.[7] The health conditions of most patients with COVID-19 are mild, but they can become serious, especially among the elderly or with underlying diseases, such as chronic or cardiovascular pulmonary disease.[8] Various factors such as male gender, diabetes, hypertension, alterations in blood counts and hypoalbuminemia have been associated with a rapid progression towards respiratory failure.[9,10]

Infection by other viruses such as influenza A or B can cause symptoms similar to those of COVID-19, which can complicate the distinction between this and other diseases, especially during the flu season.[11,12]

The current gold standard for the diagnosis of COVID-19 is based on the detection of the virus RNA in respiratory samples of nasopharyngeal swabs or bronchial aspirates by means of a molecular test by quantitative reverse transcription polymerase chain reaction (RT-qPCR).[13] This is a sensitive and specific method for detecting SARS-CoV-2, with different diagnostic protocols, including target primer sequences available in the WHO public database.[14]

The test has its limitations since false negatives can be presented if the amount of viral genome is insufficient; also, it is estimated that the incubation period of COVID-19 is 5 days, and false negative results are common within 7 days afterwards the infection. In addition, the analyzes and studies of imaging and artificial intelligence (AI) have helped achieve a more accurate diagnosis, as well as the evaluation of patients during the monitoring of the therapy and treatment of COVID-19.[15,16]

Treatments in patients with COVID-19 are based on different combinations of medicines and treatment schemes (antiviral and/or immunotherapy and/or steroids) with a high potential for interactions,[17,18] and one of the least evaluated effects is either the expression or the blocking of different drug-resistance genes. Some examples are the families of *ABC* membrane transporters (ATP-Binding Cassette) that include 49 different types of transporters, including the P-glycoprotein (P-gp).[19,20] Their main function is cell detoxification, and are distributed along the hemato-encephalic barrier, placenta, kidney and liver.[21–23] The role of the expression of *ABC* genes in COVID-19 is still unknown, and various drugs used for COVID-19 may show some interaction with the *ABC* family, such as azithromycin (*ABCB1*), lopinavir/ritonavir (*ABCC2*) or antimalarial agents like chloroquine.[24,25] In Mexico, the mortality associated with COVID-19 in severely ill patients is higher than expected due to a large extent by related risk factors.[26] Our working group has evaluated the impact of *ABCB1* overexpression in patients with acute leukemia, which causes a decrease in survival.[27] The main objective of this study is to establish the impact of comorbidities, drugs used and the expression of *ABCB1* gene on the overall survival and risk of death in COVID-19 patients.

Methods

Study Design

This was an exploratory type study, performed during the second wave of COVID-19 in our country. All patients were in severe condition of intensive therapy, treated with different treatment schemes. Sixty-one patients with a diagnosis of COVID-19 admitted to the Intensive Care Unit of the General Hospital of Mexico ¨Dr. Eduardo Liceaga¨ were included, and their informed consent was obtained for the molecular analysis of the *ABCB1* gene. The study was conducted in accordance with the Declaration of Helsinki. The project was approved by the Research, Ethics and Biosecurity Committees of the General Hospital of Mexico "Dr. Eduardo Liceaga" D1/15/103/03/57.

Isolation of Peripheral Blood Mononuclear Cells by Density Gradient

EDTA-Anticoagulated peripheral blood was obtained by venopunction. Mononuclear cells were isolated by density gradient centrifugation of an identical volume of peripheral blood and Lymphoprep ™ (density 1.077 ± 0.001 g/mL, Axis-Shield PoC AS, Oslo, Norway). The preparations were centrifuged in a Sorvall ST8 centrifuge (Thermo Fisher Scientific, Leicestershire, UK) at 300 xg for 15 minutes, the cell fractions were homogenized in TRIzol reagent and stored at −80°C until RNA extraction.

RNA Extraction and cDNA Synthesis

Total RNA isolation was performed according to the manufacturer's protocol (Invitrogen corporation, Carlsband, CA). The isolated RNA was dissolved in nuclease free water (Promega, Madison, WI). Complementary DNA (cDNA) was synthesized from 2 µg of RNA using Oligo (dT) and with MML-V RT enzyme (Promega Biotech AB).

Levels of Expression of *ABCB1* Gene

The expression levels of the *ABCB1* gene were determined by RT-qPCR using gene-specific TaqMan™ hydrolysis probe (Hs01069047, Applied Biosystems, Inc. Foster City, CA, USA). Normalization was made with the endogen *GUSB* gene (Hs00939627). Each sample was analyzed in triplicate.

Relative expression levels were calculated using method $2^{-\Delta\Delta}$Ct. The high and low expression cut-points were determined according to the average values observed in a previous study.[27]

Criterion and Type of Treatment

The treatment of COVID-19 included the combination of high-flow oxygen with the best available therapy, according to the local recommendations and the time of recruitment. 8.2% (n = 5) initiated treatment with hydroxychloroquine, 44.3% (n = 27) with metformin hydrochloride, 36.1% (n = 22) with azithromycin, 55.7% (n = 34) with atorvastatin and 95.2% (n = 58) with low molecular weight heparin, all during the hospital period. 49.2% (n = 30) required ventilatory support, which modified the treatment according to each particular case, being mainly vasopressor drugs, sedation, analgesia, parenteral nutrition and anticoagulation therapy. No patient received Tocilizumab, convalescent plasma, nor plasma replacement. Only an individual in critical condition received Baricitinib for a short period of time. The average of hospital stay was 38.78 days, 57.4% died due to complications associated with COVID-19.

Statistical Analysis

The association between the levels of expression, the risk variables and different treatments were determined by the Chi-Square test and the Fisher exact test. Global survival (GS) was determined by the Kaplan–Meier method. The impact of high levels of expression and the risk of death was performed through the odds ratio. The statistical software used was SPSS version 22.0.

Results

A total of 61 patients diagnosed with COVID-19 were included, 59% corresponded to the male gender (n = 36), obesity was the most frequent comorbidity (45.9%, n = 28), followed by non-insulin-dependent diabetes (36.1%, n = 22) and hypertension (29.5%, n = 18). One-third of the patients had a history of smoking (32.8%) and about half of alcoholism (42.6%). The average of leukocytes when admitted was 13.1 × 10^3 /μL (3.1–55.4x10^3/μL), 54.1% (n = 33) had leukocytosis at the time of diagnosis. Neutrophilia was reported by 77% (n = 47) and 65.6% (n = 40) counted with lymphopenia. Eosinopenia was constant, unlike thrombocytopenia (minor counts of 100x10^3/μL) which was infrequent (3.3%). 74.3% (n = 52) had a neutrophil-lymphocyte index greater than 7 with an average of 18.1 (2 −123). The average of bilirubin was 0.42 mg/dL, 15.7% (n = 11) had bilirubin levels greater than 1.5 mg/dL. The average of albumin was 3.3 g/dL (1.7–4.1 g/dL), with 57.1% (n = 40) of individuals with hypoalbuminemia. No patient presented renal failure when admitted (Table 1). When we analyzed the expression levels of the *ABCB1* gene in patients with COVID-19, we found that 37.7% (n = 23) had high levels, 36% (n = 22) low levels and for 26.2% (n = 16) It was absent. When evaluating the different risk factors such as diabetes, hypertension, smoking, alcohol consumption and overweight with respect to the expression levels of the *ABCB1* gene, the results showed that alcohol consuming patients were associated with groups of patients expressing either high levels or absence of the *ABCB1* gene ($p \leq 0.05$, 95% CI). No association of risk factors was observed with respect to low levels of gene expression (Table 2).

When analyzing the effect of the treatments used in patients with metformin, atorvastatin, hydroxychloroquine, azithromycin and anticoagulants, the results showed that groups of patients with high and negative levels of expression of the *ABCB1* gene were associated with the use of atorvastatin and metformin ($p \leq 0.05$, 95% CI), unlike low levels of gene expression (Table 3).

The different risk variables showed that patients with high levels and with absence of expression of the *ABCB1* gene presented greater risk of death (Odds ratio [OR] 2.8, 95% CI 0.98–8.5) and need for ventilatory support (OR 3.08, 95% CI 1.02–9.26). Other risk factors such as male gender (OR 2.54, 95% CI 0.89–7.27) and age (OR 5.19, 95% CI 1.48–18.2) were associated with risk of death.

Table 1 General Characteristics in COVID-19 Patients

	Mean	Range
Age, years	49	16–80
Sex; %		
Male, 59%	36	–
Female, 41%	25	–
Hemoglobin, g/dL	14.02	2.90–18.60
Hematocrit,%	38.89	8.90–55.30
WBC count, 10^3/µL	13.19	3.10–55.40
Neutrophils, 10^3/µL	19.90	1.80–98.00
Lymphocytes, 10^3/µL	4.14	0.10–31.12
Monocytes, 10^3/µL	0.51	0.10–6.20
Platelets, 10^3/µL	272	13–652
MPV, fL	7.93	5.10–10.80
DB, mg/dL	0.46	0–1.10
TB, mg/dL	0.43	0–3.80
PT, 10^3/µL	6.41	4.30–8.20
ALB, g/dL	3.32	1.75–4.10
ALT, u/L	75	9–265
AST, u/L	96	12–788
LDH, u/L	542	214–1518

Abbreviations: MPV, mean platelet volume; DB, direct bilirubin; TB, total bilirubin; PT, prothrombin; ALB, albumin; ALT, alanine transferase; AST, aspartate transferase; LDH, lactate dehydrogenase.

Table 2 ABCB1 Levels Associated with Risk Factors in COVID-19 Patients

Risk Factors		ABCB1 Levels	
		Low n (%)	High and Absent n (%)
DM	No	15 (24.6)	24 (39.3)
	Yes	7 (11.5)	15 (24.6)
SAH	No	18 (29.5)	25 (41.0)
	Yes	4 (6.6)	14 (23.0)
Smoking	No	12 (19.7)	29 (47.5)
	Yes	10 (16.4)	10 (16.4)
Alcoholism	No	8 (13.1)	27 (44.3)
	Yes	14 (23.0)	12 (19.7)*
Overwight	No	11 (18.0)	22 (36.1)
	Yes	11 (18.0)	17 (27.9)

Note: Chi-square test, *p<0.05.
Abbreviations: DM, diabetes mellitus; SAH, systemic arterial hypertension.

It is important to note that patients with diabetes which were treated with metformin due to their condition were associated with a lower risk of death (OR 1.11.95% CI 0.38–3.22), as well as patients who presented low levels of expression of the *ABCB1* gene (OR 0.34, 95% CI 011–1.08) Table 4.

Finally, in the overall survival (OS) at 78 days of follow-up with respect to the expression levels of the *ABCB1* gene, it was found that the high and absent expression groups showed an average of 11.18 days (9–13.37) and an OS of 33.3%, while those who presented low levels had an average of 67.42 days (56.3–78.7) and an OS of 59.1% (p = 0.00, Log Rank Test). Figure 1.

Table 3 Treatment in COVID-19 Patients Associated with *ABCB1* Levels

Treatment		ABCB1 Levels	
		Low n (%)	High and Absent n (%)
Metformin	No	8 (13.1)	26 (42.6)
	Yes	14 (23.0)	13 (21.3)*
Atorvastatin	No	5 (8.2)	22 (36.1)
	Yes	17 (27.9)	17 (27.9)*
Hydroxychloroquine	No	19 (31.1)	37 (60.7)
	Yes	3 (4.9)	2 (3.3)
Azithromycin	No	13 (21.3)	26 (42.6)
	Yes	9 (14.8)	13 (21.3)
Anticoagulant	No	2 (3.3)	1 (1.6)
	Yes	20 (32.8)	38 (62.3)

Note: Chi-square test, *p<0.05.

Table 4 Factors Associated with Mortality and Respiratory Support

	Mechanical Ventilation			Death		
	OR	(95% CI)	*p*	OR	(95% CI)	*p*
Male genre	3.33	(1.139, 9.782)	0.027*	2.54	(0.890, 7.278)	0.081
DM	2.51	(0.856, 7.391)	0.093	1.11	(0.386, 3.220)	0.838
Age over 55 years	1.62	(0.561, 4.729)	0.369	5.194	(1.481, 18.216)	0.010*
Hypertension	1.43	(0.475, 4.344)	0.520	1.11	(0.366, 03.370)	0.852
ABCB1 levels						
High /Absent	3.08	(1.024, 9.262)	0.045	2.88	(0.981, 8.503)	0.054
Low	0.32	(0.108, 0.976)	0.045	0.34	(0.117, 1.018)	0.054

Note: Chi-square test, *p<0.05.
Abbreviations: DM, diabetes mellitus; OR, odds ratio; CI, confidence interval.

Discussion

The COVID-19 has currently become the leading cause of death worldwide, causing a great economic and social crisis. The WHO has reported more than 462 million confirmed cases of SARS-CoV2 infection around the world with more than ~6 million deaths.[28] Adverse drug reactions (ADR) associated with current treatments for COVID-19 are one of the main causes of death in patients with multiple comorbidities.[29,30] In a recent pharmacovigilance study conducted in Spain, an incidence 4.75 times higher to present ADR in severe patients with COVID-19 compared to patients with some other condition and treated with azithromycin (8.4%), dexamethasone (7.6%), lopinavir-ritonavir (7.4%) and chloroquine (CQ)/hydroxychloroquine (HCQ) (6.9%) was reported.[31] Another study conducted in Brazil with 402 patients with COVID-19 indicated that chloroquine (CQ) (OR = 5.4, 95% CI: 1.9–15.6) and hydroxychloroquine (HCQ) (OR = 2.1 95% CI: 1.2–3.6) were the only drugs associated with severe ADR.[32]

At present there are no specific therapeutic recommendations for the treatment of COVID-19.[29,33] The treatment based on chloroquine, a drug used as immunosuppressor in lupus and rheumatoid arthritis, has shown an in vitro antiviral effect against influenza and different types of coronavirus, including COVID-19.[34,35] When analyzing the available evidence, its use did not impact mortality, but it did increase the risk of complications, mainly cardiovascular.[36] Also, a drug widely used is azithromycin, which contributes to preventing the entry of the virus to the cell via CD147 with potential antiviral activity, and it is generally used in combination with hydroxychloroquine.[37,38] Other drugs with immunomodulatory effect are statins and metformin hydrochloride, its use became a daily practice in our country due to the high prevalence of diabetes and obesity.[39,40] It has been shown that a group of patients with COVID-19 suffering

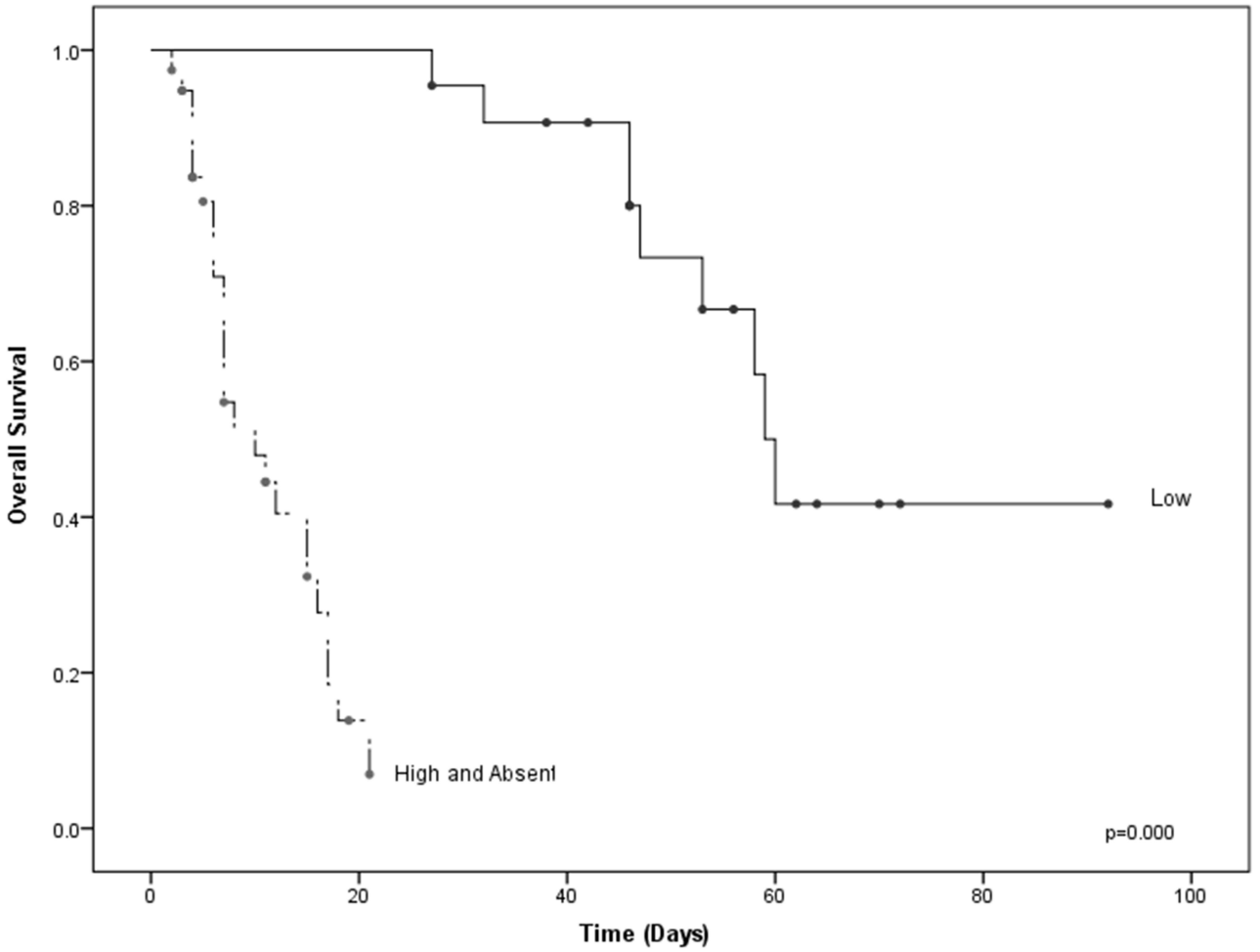

Figure 1 Overall survival at 78 days of follow up with respect to the expression levels of the *ABCB1* gene.

from chronic type 2 diabetes, being treated with metformin, increased their survival, which was associated with a lower risk of death.[41–43] In conjunction with anticoagulation and various anti-inflammatory drugs, the combination of these drugs was a fundamental part of the treatment for COVID-19 during the first months of pandem*ic* in Mexico.

We evaluated the expression levels of *ABCB1* genes in COVID-19 patients and found high levels of expression, 37.7%. Several studies have shown that the expression levels of the mRNA are increased by the response to substrates of the *ABCB1* pump, and within the substrates described are azithromycin and atorvastatin, as well as self-medicated antibiotics.[44] The use of these drugs would explain the increase in levels, unlike the healthy population. It is important to highlight that patients who presented high levels of expression mostly had associated comorbidities such as diabetes and hypertension, and most of them were of the male gender.

It has been previously demonstrated that the expression of the *ABCB1* gene is altered in different metabolic diseases including diabetes.[45]

When analyzing the risk factors of patients with COVID-19 associated with high levels of expression and absence of expression of *ABCB1* gene, alcohol consumption was significant, this is consistent with the work of Haaman et al 2019, in which prolonged exposure to drug abuse, ethanol and cocaine alters the expression of *ABCB1* mRNA.[46] When the impact of the different treatments was evaluated, we observed increased expression levels in patients who did not consume metformin. Metformin inhibits the expression of the NF-kB nuclear factor, inducing the apoptosis of hepatocytes and inhibiting the expression of the *ABCB1* gene.[47,48]

Finally, the group of patients with either high levels or absence of expression of the *ABCB1* gene showed a lower survival rate, unlike the group of patients with low levels. These results are very similar to other studies that explain that the increased expression of these genes is related to the expulsion of the drugs used in the treatments, and, therefore, the failure thereof and the absence of expression would be causing an intoxication with the drugs by not protecting against xenobiotic agents in the different tissues, which leads to an organic failure, and this agrees with the reported in other ailments.[49,50]

Conclusion

The alteration in the expression of the *ABCB1* gene is associated with an increased risk of death or progression of the disease; the failure of previous treatments can be explained by the interaction of the drugs with the flow pump, modifying its distribution in different tissues.

Abbreviations

SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; COVID-19, coronavirus disease 2019; ABCB1, ATP-binding cassette B1; P-gp, P-glycoprotein; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; *NF-kB*, nuclear factor *kappa* B; OS, overall survival.

Data Sharing Statement

The datasets used and/or analysed during the current study available from the corresponding author on reasonable request.

Ethics Approval and Consent to Participate

Written informed consent was obtained from all subjects and the study was approved by the Ethics Committee of the Hospital General Mexico "Dr Eduardo Liceaga".

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Author contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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Disclosure

The authors declare that they have no competing interests.

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