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Article

Single nucleotide polymorphisms in apolipoprotein A-I (rs670) and B (rs693) associated with serum lipoproteins and endothelial activation in COVID-19 outpatients

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This cross-sectional study investigated the association of SNPs rs670 (C>T) and rs693 (G>A) with parameters of lipid metabolism and endothelial activation in 167 subjects, including 86 COVID-19 outpatients and 81 healthy subjects (control group) matched for sex and age. Serum levels of total cholesterol (TC), high-density lipoprotein cholesterol (HDL-c), low-density lipoprotein cholesterol (LDL-c), non-high-density lipoprotein cholesterol (non-HDL-c), triglycerides (TG), apolipoproteins (Apo A-I and B), and vascular cell adhesion molecule-1 (VCAM-1) were determined. The SNPs were genotyped by quantitative polymerase chain reaction (qPCR). A p-value<0.05 was assumed. COVID-19 outpatients showed increases in TC (187.0±48.3 *vs*. 160.8±32.0 mg/dL), LDL-c (110.3±41.5 *vs*. 98.8±28.2 mg/dL), non-HDL-c (138.3±45.8 *vs*. 115.6±31.6 mg/dL), TG (139.7±80.3 *vs*. 84.9±35.1 mg/dL), and Apo A-I (149.5±40.0 *vs*. 133.3±20.9 mg/dL), along with a high frequency of hypercholesterolemia and hypertriglyceridemia. VCAM-1 levels were double those of the control (682.7±231.8 vs. 299.3±102.9 ng/mL) and a strong predictor of COVID-19 (AUC=0.946). Moreover, VCAM-1 correlated with TC (r = -0.223) and HDL-c (r = -0.225). The decrease in TG and VCAM-1 is predicted by the dominance of the T allele (rs670) and its codominance with the A (rs693) allele in COVID-19 outpatients. COVID-19 has been shown to be associated with dyslipidemias and endothelial activation, and apolipoprotein polymorphisms may influence this.

Keywords: SARS-CoV-2. Single nucleotide polymorphism. Lipid metabolism. Vascular cell adhesion molecule-1.

INTRODUCTION

COVID-19 is characterized by an inflammatory and pro-thrombotic nature, driven by the exaggerated immune response triggered by cytotoxicity and the immune escape mechanisms of SARS-CoV-2 (Silva *et al*., 2021). Complications involving the cardiovascular system, such as myocarditis, cardiopulmonary stress, pulmonary embolism, heart failure, and acute myocardial infarction, were associated with a worse prognosis and death. These complications predominantly affected older patients (over 70) with metabolic syndromes, which directly impact endothelial function (Zhao *et al*., 2023).

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Among metabolic syndromes, dyslipidemias are characterized by causing endothelial dysfunction, oxidative stress, and atherosclerosis (Faludi *et al.*, 2017). Endothelial dysfunction caused by changes in serum lipoprotein levels is associated with the formation of foam cells in the intimal layer of the vessel and leads to chronic and silent inflammation caused by the increase in nuclear factor kappa B (NF-κB), IL-1β, IL-6, IL-18, tumor necrosis factor alpha (TNF-α), and vascular cell adhesion molecule-1 (VCAM-1) expression, which play a fundamental role in the mechanisms of cardiovascular events (Medina-Leyte *et al.*, 2021).

SARS-CoV-2 directly affects endothelial cells and leads to apoptosis and pyroptosis mechanisms such as activation of the NLRP3 (NOD-, LRR-, and pyrin domain-containing protein 3) inflammasome (López-Reyes *et al.*, 2020). The virus's presence within cells and the upregulation of molecular patterns associated with damage result in activation of the immune response, leading to a cytokine storm, including IL-1β, IL-6, IL-18, TNF-α, and interferon gamma (IFN-γ) (Nasab *et al.*, 2023). COVID-19 has also been linked to dyslipidemia, with serum cholesterol levels being associated with the severity of the disease, particularly in relation to low-density lipoproteins (LDL-c) and high-density lipoproteins (HDL-c) (Surma, Banach, Lewek, 2021). SARS-CoV-2 may negatively regulate scavenger receptor B type 1 (SR-BI), resulting in greater macrophage activation and possibly increasing the synthesis of cholesterol and pro-inflammatory cytokines through activation of sterol regulatory element binding protein-2 (Alkazmi *et al.*, 2023; Lee *et al.*, 2020).

Dyslipidemias can result from a combination of environmental and genetic factors. Polygenic dyslipidemias, in particular, often stem from the interplay between single nucleotide polymorphisms (SNP) and environmental factors like infections and lifestyle (Faludi *et al.*, 2017). Considering the associations of SNP rs670 (apolipoprotein A-I) and rs693 (apolipoprotein B) with serum lipoprotein disorders, abdominal circumference, and cardiovascular events in some populations, and the potential role of genetic factors in explaining the heterogeneity of COVID-19, this study takes an innovative role by investigating the association of SNP rs670 and rs693 with serum lipoproteins and endothelial activation in COVID-19 outpatients from a Brazilian population (Alves *et al.*, 2020; Hsu *et al.*, 2013; De Luis *et al.*, 2019).

MATERIAL AND METHODS

Study design, population, period, and ethical aspects

This was an observational, analytical and cross-sectional study in which 167 subjects were analyzed, including 86 COVID-19 outpatients and 81 healthy subjects (the control group) matched for sex and age. The control group consisted of healthy subjects recruited before the COVID-19 pandemic. The COVID-19 outpatients were between 18 and 60 years old and had been suffering from severe acute respiratory syndrome for four to seven days when they sought treatment at a reference ambulatory unit in Fortaleza City (Fortaleza, 2022).

Patients were recruited from February to July 2021, when the second wave of COVID-19 cases occurred in Brazil and no vaccines were available for the population. COVID-19 outpatients were approached and interviewed when nasopharyngeal swabs were collected to identify SARS-CoV-2 by reverse transcriptase followed by quantitative polymerase chain reaction (RT-qPCR). Serum and whole blood samples were collected from both groups without fasting in 5-mL vacuum tubes and stored at -80 °C in the Laboratory for Clinical and Toxicological Analyses Prof. Dr. Eurico Litton Pinheiro de Freitas (LACT).

Exclusion criteria were a history of hypertension, diabetes, dyslipidemia, COVID-19, and use of glucocorticoids or triglycerides ≥ 400 mg/dL. This study was approved by the Research Ethics Committee of the Federal University of Ceará under No. 4505911.

Laboratory parameters

Serum samples were used to measure total cholesterol (TC), high-density lipoprotein cholesterol (HDL-c), and triglycerides (TG) by the colorimetric method (Bioclin®, Belo Horizonte, Brazil); Apolipoproteins A-I and B (Apo

A-I and Apo B) by turbidimetric immunoassay (Randox®, Crumlin, UK); and soluble vascular cell adhesion molecule-1 (VCAM-1) by enzyme-linked immunosorbent assay (ELISA) using the Human VCAM-1/CD106 kit (R&D Systems®, Minneapolis, USA). Low-density lipoprotein cholesterol (LDL-c) was calculated using the Friedewald formula (TC = HDL-c + LDL-c + VLDL-c; where VLDL-c = TG/5 if TG < 400 mg/dL) (Friedewald *et al.*, 1972). Non-high-density lipoprotein cholesterol (non-HDL-c) was determined mathematically (non-HDL-c = TC - HDL-c) (Faludi *et al.*, 2017). Colorimetric and turbidimetric analyses were performed using the BM-200 automatic spectrophotometer (Vyttra®, São Paulo, Brazil) and the reference values for TC, HDL-c, non-HDL-c, LDL-c, and TG established by the Brazilian Society of Cardiology Guidelines were adopted as parameters for interpretation (Faludi *et al.*, 2017).

Genotyping of rs670 and rs693 polymorphisms

Genomic DNA (deoxyribonucleic acid - gDNA) samples were obtained from leukocytes in whole blood (EDTA) using the PureLink™ Genomic DNA Kit (Thermo Fisher Scientific, USA). Polymorphisms were genotyped by quantitative polymerase chain reaction (qPCR) using fluorescent hydrolysis probes (VIC: 6-carboxyfluorescein and FAM: 2'-chloro-7'-phenyl-1,4-dichloro-6-carboxyfluorescein) from the TaqMan™ SNP Genotyping Assays (Thermo Fisher Scientific, USA). For SNP rs670, VIC fluorescence was used to identify the C wild allele and FAM fluorescence was used to identify T polymorphic allele, whereas for SNP rs693, FAM fluorescence was used to identify G wild allele and VIC fluorescence was used to identify A polymorphic allele.

To each reaction, 5 µL of TaqMan™ Genotyping Master Mix (containing primers, d-nucleotides, TaqMan polymerase enzyme, and cofactors), 2.5 µL of DNA-free ultrapure water, 0.5 µL of TaqMan™ SNP Genotyping Assays (containing the probes for SNP rs670 or rs693), and 2.0 µL of gDNA (20 ng/µL) were added, resulting in a total volume of 10 µL for each reaction. The qPCR protocol used in the CFX 96™ Real Time System Thermocycler (BioRad) was: 1st cycle of 2 minutes at 50 ºC, followed by 10 minutes at 95 ºC; then 40 cycles of 15 seconds at 95 ºC for denaturation and 1 minute at 60 ºC for annealing and extension. The results were qualitatively evaluated based on the type of fluorescence emitted at the end of the 40 cycles. To verify the accuracy of the assay, 5% of the samples were repeated with 100% agreement.

Statistical and genetic analyzes

Statistical analyses were performed using IBM-SPSS® v.22 software (IBM, USA) and GraphPad Prism® v.6. Normality of continuous variables was tested using the Kolmogorov-Smirnov test. The distribution of categorical variables was analyzed using the chi-square test (X2). The continuous variables of up to two groups were analyzed with the Student's t test, and the results were expressed as mean ± standard deviation. Correlation between two independent continuous variables was assessed with Pearson correlation. Prediction analyses were performed with linear regression tests and receiver operator characteristic (ROC) curves. HW_TEST_v1.1 software was used to check whether the genotype distributions of each polymorphism were in Hardy-Weinberg (HW) equilibrium by applying Pearson's chi-square test (X2) (Santos *et al.*, 2020). A p-value < 0.05 was adopted as the criterion for statistical significance.

RESULTS

This study analyzed 86 COVID-19 outpatients and 81 healthy subjects. Patient sociodemographic data showed a prevalence of females (n = 61; 70.9%) compared with males (n = 25; 29.1%) and a mean age of 39.7 ± 13.9 years, with no difference from the control group. It was found that, regardless of genotype, COVID-19 was associated with higher levels of lipid metabolism parameters, such as TC, LDL-c, non-HDL-c, TG, and Apo A-I, as well as a higher frequency of TC, non-HDL-c, and TG parameters outside the reference values established by the Brazilian Society of Cardiology Guidelines, indicating hypercholesterolemia and hypertriglyceridemia. Notably, COVID-19 outpatients were found to have VCAM-1 levels, on average, twice those of the control group, as shown in Table I.

TABLE I – Comparison of laboratory parameters and distribution of conventional lipid metabolism parameters in terms of reference values

Parameters	Control ± SD	COVID-19 ± SD	p^a	RV	Control N = 81 (%)	COVID-19 N = 86 (%)	p^b
TC (mg/dL)	160.8 ± 32.0	187.0 ± 48.3	**< 0.001**	<190 mg/dL	69 (85.2%)	48 (55.8%)	**< 0.001**
				>190 mg/dL	12 (14.8%)	38 (44.2%)	
HDL-c (mg/dL)	45.2 ± 12.6	48.8 ± 13.6	0.074	>40 mg/dL	53 (65.4%)	56 (65.1%)	0.966
				<40 mg/dL	28 (34.6%)	30 (34.9%)	
non-HDL-c (mg/dL)	115.6 ± 31.6	138.3 ± 45.8	**< 0.001**	<130 mg/dL	56 (69.1%)	38 (44.2%)	**0.001**
				>130 mg/dL	25 (30.9%)	48 (55.8%)	
LDL-c (mg/dL)	98.8 ± 28.2	110.3 ± 41.5	**0.036**	<130 mg/dL	70 (86.4%)	66 (76.7%)	0.108
				>130 mg/dL	11 (13.6%)	20 (23.3%)	
TG (mg/dL)	84.9 ± 35.1	139.7 ± 80.3	**< 0.001**	<175 mg/dL	77 (95.1%)	62 (72.1%)	**< 0.001**
				>175 mg/dL	4 (4.9%)	24 (27.9%)	
Apo A-I (mg/dL)	133.3 ± 20.9	149.5 ± 40.0	**0.001**				
Apo B (mg/dL)	85.4 ± 18.9	80.3 ± 21.7	0.107				
VCAM-1 (ng/mL)	299.3 ± 102.9	682.7 ± 231.8	**< 0.001**				

[a] Student's t-test; [b] Chi-square test (X^2); RV – reference value according to the Brazilian Society of Cardiology Guidelines; SD – standard deviation; TC – total cholesterol; HDL-c – high-density lipoprotein cholesterol; LDL-c – low-density lipoprotein cholesterol; non-HDL-c – non-high-density lipoprotein cholesterol; TG – triglycerides; Apo A-I – apolipoprotein A-I; Apo B – apolipoprotein B; VCAM-1 – vascular cell adhesion molecule-1; Significance of $p < 0.05$

Given the difference in the VCAM-1, ROC (receiver operator characteristic) curve analysis showed that this endothelial activation parameter had an excellent ability to distinguish COVID-19 outpatients from healthy subjects, with an area under the curve value of 0.946, sensitivity of 87.2%, and specificity of 87.7%, as illustrated in Figure 1 and shown in Table II.

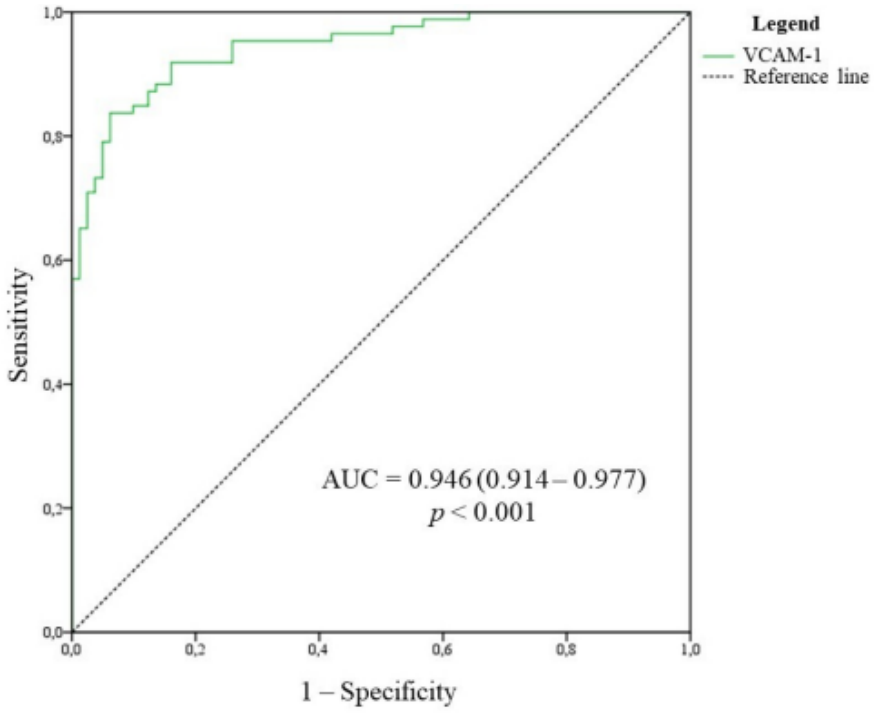

FIGURE 1 – ROC curve of VCAM-1 as a predictor of COVID-19.

AUC – area under the curve (95% confidence interval); ROC - receiver operator characteristic; VCAM-1 - vascular cell adhesion molecule-1; Significance of $p < 0.05$.

TABLE II - ROC curve analysis of VCAM-1 as a predictor of COVID-19

Parameter	AUC	*p*	CI 95%	Sensitivity	Specificity
VCAM-1	0.946	**< 0.001**	0.914 - 0.977	87.2%	87.7%

ROC - receiver operator characteristic; AUC - area under the curve; 95% CI - 95% confidence interval; VCAM-1 - vascular cell adhesion molecule-1; Significance of $p < 0.05$.

When checking the correlation between lipid metabolism parameters and endothelial activation in COVID-19 outpatients, VCAM-1 showed a negative correlation with TC and HDL-c, as shown in Table III.

TABLE III – Correlation between VCAM-1 and lipid metabolism parameters

Parameters	Control		COVID-19	
	r	*p*	r	*p*
TC (mg/dL)	-0.083	0.463	-0.223	**0.039**
HDL-c (mg/dL)	-0.108	0.337	-0.225	**0.037**
non-HDL-c (mg/dL)	-0.040	0.720	-0.169	0.121

TABLE III – Correlation between VCAM-1 and lipid metabolism parameters

Parameters	Control		COVID-19	
	r	*p*	**r**	*p*
LDL-c (mg/dL)	-0.047	0.678	-0.206	0.057
TG (mg/dL)	-0.012	0.914	0.052	0.636
Apo A-I (mg/dL)	-0.166	0.138	0.001	0.994
Apo B (mg/dL)	-0.101	0.370	-0.004	0.970

Pearson's correlation; TC - total cholesterol; HDL-c - high-density lipoprotein cholesterol; LDL-c - low-density lipoprotein cholesterol; non-HDL-c - non-high-density lipoprotein cholesterol; TG - triglycerides; Apo A-I - apolipoprotein A-I; Apo B - apolipoprotein B; VCAM-1 - vascular cell adhesion molecule-1; Significance of $p < 0.05$

Genotype distributions of SNP rs670 and rs693 were as follows: in the COVID-19 outpatients, rs670 had 54 CC, 31 CT, and 1 TT individuals, while the control group had 48 CC, 27 CT, and 6 TT individuals. Regarding rs693, COVID-19 outpatients included 31 GG, 43 GA, and 12 AA individuals, while the control group had 28 GG, 40 GA, and 13 AA individuals. A comparison of genotype frequencies between the groups revealed no differences, as shown in Table IV.

TABLE IV – Allelic and genotypic frequencies of SNP rs670 and rs693

SNP	Alleles and Genotypes	Control N = 81 (%)	COVID-19 N = 86 (%)	*p*
rs670	C	75.93%	80.81%	0.148
	T	24.07%	19.19%	
	CC	48 (59.3%)	54 (62.8%)	
	CT	27 (33.3%)	31 (36.0%)	
	TT	6 (7.4%)	1 (1.2%)	
rs693	G	59.26%	61.05%	0.954
	A	40.74%	38.95%	
	GG	28 (34.6%)	31 (36.0%)	
	GA	40 (49.4%)	43 (50.0%)	
	AA	13 (16.0%)	12 (14.0%)	

Chi-square test (X^2); Significance of $p < 0.05$

When checking whether the genotype distributions followed the Mendelian segregation, it was observed that both groups adhered to Hardy-Weinberg equilibrium, since no differences ($p > 0.05$) were observed between observed genotype distributions (black points) and expected genotype distributions (red points), as illustrated in Figure 2.

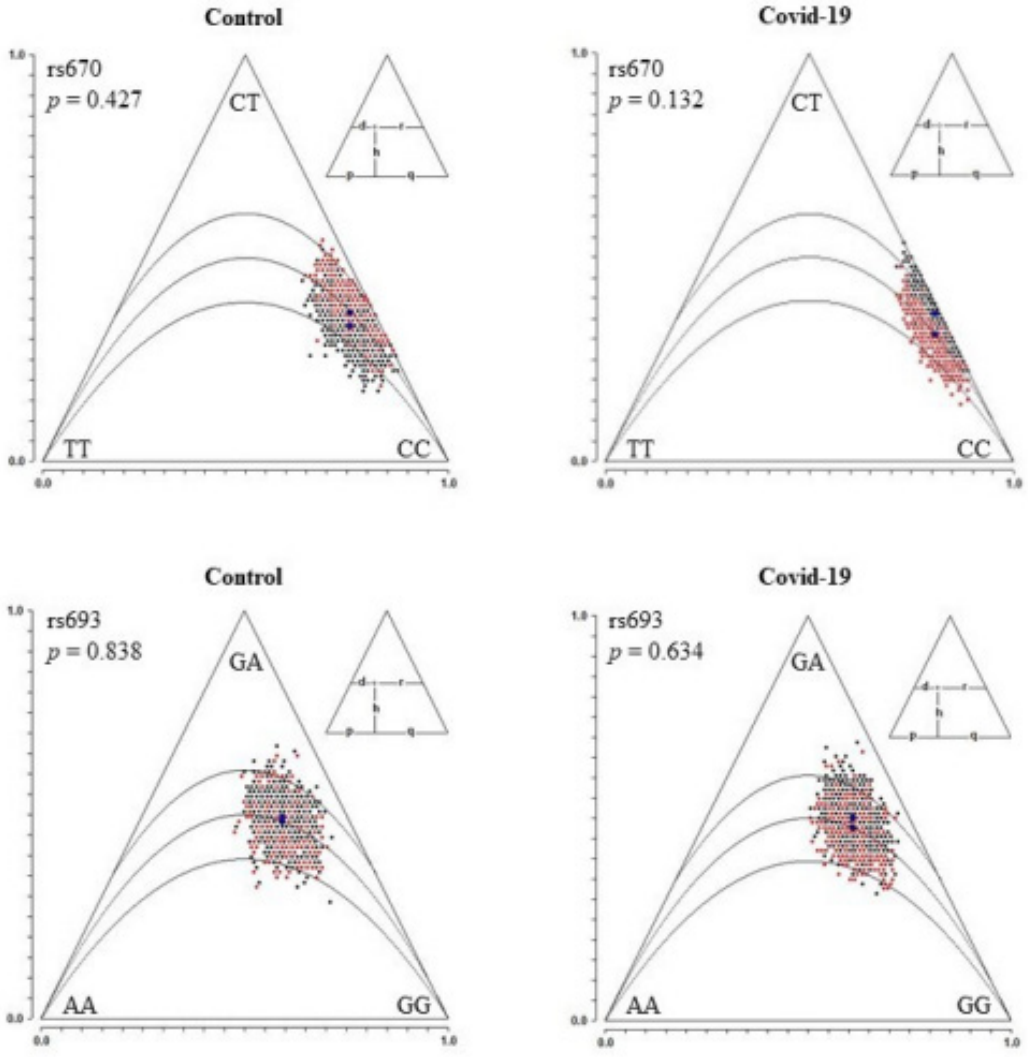

FIGURE 2 – Hardy-Weinberg equilibrium ternary graphs for the SNP rs670 and rs693.

Hardy-Weinberg equilibrium verified by Pearson's chi-square (X^2) test; Expected (red points) and observed (black points) genotype distribution; Significance of $p < 0.05$.

Next, in order to enable comparative analyses of laboratory parameters according to genotype, it was adopted a classification based on the dominance and codominance of polymorphic alleles, since only one individual with the TT genotype was observed for SNP rs670 in the group of COVID-19 outpatients. The new classification is based on the presence or absence of the polymorphic allele of each SNP separately and simultaneously, as shown in Table V.

TABLE V – Classification according to dominance and codominance of polymorphic alleles of SNP rs670 and rs693

SNP	Classifications	Genotypes	Control N = 81 (%)	COVID-19 N = 86 (%)
rs670	Without T allele	CC	48 (59.3%)	54 (62.8%)
	With T allele	CT/TT	33 (40.7%)	32 (37.2%)
rs693	Without A allele	GG	28 (34.6%)	31 (36.0%)
	With A allele	GA/AA	53 (65.4%)	55 (64.0%)
rs670 and rs693	Without T and A alleles	CC and GG	13 (16.0%)	17 (19.8%)
	With T and A alleles	CT/TT and GA/AA	18 (22.2%)	18 (20.9%)

When the laboratory parameters were compared according to the new genotypic categorization, it was found that the dominance of the T allele of rs670 was associated with lower levels of TG and VCAM-1 in COVID-19 outpatients, as shown in Table VI. In contrast, the dominance of the A allele of SNP rs693 showed no association with any parameter, as shown in Table VII. Moreover, codominance of the T (rs670) and A (rs693) alleles was associated with a marked reduction in TG and VCAM-1 in COVID-19 outpatients, as shown in Table VIII.

TABLE VI – Comparison of laboratory parameters according to the dominance of the T polymorphic allele of SNP rs670

Parameters	Control ± SD			COVID-19 ± SD		
	Without T allele N = 48	With T allele N = 33	*p*	Without T allele N = 54	With T allele N = 32	*p*
TC (mg/dL)	160.5 ± 35.3	161.1 ± 26.9	0.937	187.6 ± 52.0	186.1 ± 42.1	0.887
HDL-c (mg/dL)	44.4 ± 12.8	46.3 ± 12.3	0.499	47.0 ± 11.0	51.9 ± 16.8	0.146
non-HDL-c (mg/dL)	116.2 ± 34.0	114.8 ± 28.3	0.843	140.6 ± 50.2	134.3 ± 37.4	0.504
LDL-c (mg/dL)	99.3 ± 30.6	98.0 ± 24.9	0.825	109.7 ± 44.2	111.5 ± 37.1	0.843
TG (mg/dL)	85.4 ± 36.3	84.1 ± 33.8	0.865	155.0 ± 86.8	114.0 ± 61.1	**0.012**
Apo A-I (mg/dL)	131.6 ± 22.2	135.8 ± 18.9	0.357	146.4 ± 36.1	154.8 ± 45.9	0.376
Apo B (mg/dL)	86.2 ± 19.8	84.2 ± 17.7	0.636	82.8 ± 22.5	76.1 ± 19.9	0.160
VCAM-1 (ng/mL)	281.2 ± 92.2	325.5 ± 113.1	0.068	719.6 ± 238.7	620.5 ± 208.6	**0.048**

Student's t-test; SD – standard deviation; TC – total cholesterol; HDL-c – high-density lipoprotein cholesterol; LDL-c – low-density lipoprotein cholesterol; non-HDL-c – non-high-density lipoprotein cholesterol; TG – triglycerides; Apo A-I – apolipoprotein A-I; Apo B – apolipoprotein B; VCAM-1 – vascular cell adhesion molecule-1; Significance of $p < 0.05$.

TABLE VII - Comparison of laboratory parameters according to the dominance of the A polymorphic allele of SNP rs693

Parameters	Control ± SD			COVID-19 ± SD		
	Without A allele N = 28	With A allele N = 53	*p*	Without A allele N = 31	Without A allele N = 55	*p*
TC (mg/dL)	157.3 ± 27.4	162.6 ± 34.2	0.454	183.2 ± 49.5	189.2 ± 48.0	0.589
HDL-c (mg/dL)	44.8 ± 13.2	45.3 ± 12.4	0.864	46.5 ± 13.8	50.1 ± 13.4	0.237
non-HDL-c (mg/dL)	112.5 ± 26.9	117.3 ± 34.0	0.494	136.8 ± 46.2	139.1 ± 45.9	0.824
LDL-c (mg/dL)	96.6 ± 24.3	99.9 ± 30.2	0.598	107.8 ± 42.4	111.8 ± 41.3	0.674
TG (mg/dL)	79.4 ± 32.5	87.8 ± 36.3	0.292	144.9 ± 74.5	136.8 ± 84.0	0.644
Apo A-I (mg/dL)	130.7 ± 20.3	134.7 ± 21.2	0.404	148.2 ± 38.9	150.2 ± 40.9	0.821
Apo B (mg/dL)	82.9 ± 17.9	86.7 ± 19.4	0.386	82.4 ± 23.6	79.1 ± 20.6	0.513
VCAM-1 (ng/mL)	309.3 ± 105.9	294.0 ± 101.9	0.533	724.6 ± 241.0	659.1 ± 225.2	0.220

Student's t-test; SD – standard deviation; TC – total cholesterol; HDL-c – high-density lipoprotein cholesterol; LDL-c – low-density lipoprotein cholesterol; non-HDL-c – non-high-density lipoprotein cholesterol; TG – triglycerides; Apo A-I – apolipoprotein A-I; Apo B – apolipoprotein B; VCAM-1 – vascular cell adhesion molecule-1; Significance of $p < 0.05$.

TABLE VIII – Comparison of laboratory parameters according to the codominance of the T (rs670) and A (rs693) polymorphic alleles

Parameters	Control ± SD			COVID-19 ± SD		
	Without T and A alleles N = 13	With T and A alleles N = 18	*p*	Without T and A alleles N = 17	With T and A alleles N = 18	*p*
TC (mg/dL)	156.5 ± 25.5	163.6 ± 24.7	0.442	191.4 ± 60.6	196.1 ± 47.8	0.801
HDL-c (mg/dL)	41.5 ± 11.3	45.1 ± 10.6	0.371	41.6 ± 10.0	51.5 ± 18.1	0.053
non-HDL-c (mg/dL)	115.0 ± 24.1	118.5 ± 27.2	0.709	149.8 ± 57.6	144.6 ± 45.0	0.768
LDL-c (mg/dL)	99.7 ± 22.1	101.3 ± 23.6	0.849	114.8 ± 53.4	120.9 ± 43.9	0.715
TG (mg/dL)	76.4 ± 22.2	85.8 ± 28.8	0.311	175.3 ± 75.1	118.7 ± 65.6	**0.024**
Apo A-I (mg/dL)	129.0 ± 23.4	139.0 ± 19.6	0.225	142.0 ± 39.2	154.1 ± 52.0	0.441
Apo B (mg/dL)	81.9 ± 16.5	84.5 ± 16.6	0.666	90.1 ± 27.4	78.5 ± 23.7	0.191
VCAM-1 (ng/mL)	279.2 ± 77.5	317.3 ± 108.0	0.263	756.2 ± 247.5	569.3 ± 174.3	**0.016**

Student's t-test; SD – standard deviation; TC – total cholesterol; HDL-c – high-density lipoprotein cholesterol; LDL-c – low-density lipoprotein cholesterol; non-HDL-c – non-high-density lipoprotein cholesterol; TG – triglycerides; Apo A-I – apolipoprotein A-I; Apo B – apolipoprotein B; VCAM-1 – vascular cell adhesion molecule-1; Significance of $p < 0.05$.

Finally, a linear regression analysis was carried out to see if the dominance or codominance of the T and A polymorphic alleles could predict TG and VCAM-1 parameters in COVID-19 outpatients. The prediction of TG was significant only in the codominance model [Z (1, 33) = 5.656; p = 0.023; R^2 = 0.146], indicating that the concomitant presence of the alleles is associated with a reduction in TG of 56.63 mg/dL. The prediction of VCAM-1 was significant in both models, where in the dominance model [Z (2, 83) = 3.119; p = 0.049; R^2 = 0.070], only the dominance of the T allele predicts a reduction in VCAM-1 of 108.78 ng/mL and, when there is codominance [Z (1, 33) = 6.733; p = 0.014; R^2 = 0.169], the reduction in VCAM-1 is more pronounced, around 186.86 ng/mL, as shown in Table IX.

TABLE IX – Prediction of TG and VCAM-1 from the dominance and codominance of the T (rs670) and A (rs693) polymorphic alleles

Predicted variables	Models	COVID-19			
		β	SE	$\beta_{standardised}$	*p*
TG	T allele	-42.57	17.65	-0.258	**0.018**
	A allele	-13.43	17.77	-0.081	0.452
	T and A alleles	-56.63	23.81	-0.383	**0.023**
VCAM-1	T allele	-108.78	50.86	-0.228	**0.035**
	A allele	-79.06	51.20	-0.165	0.126
	T and A alleles	-186.86	72.01	-0.412	**0.014**

Multiple Linear Regression; SE - standard error; TG - triglycerides; VCAM-1 - vascular cell adhesion molecule-1; Significance of $p < 0.05$

DISCUSSION

This study is the first to investigate the association of SNP rs670 and rs693 with parameters of lipid metabolism and VCAM-1 in COVID-19 outpatients from a Brazilian population. COVID-19 is caused by SARS-CoV-2, and the clinical course is very heterogeneous. The exacerbated immune response to SARS-CoV-2 was the main cause of clinical complications characterized by cytokine storms and organ dysfunction. Among the organ systems, the cardiovascular system is the main one involved in complications and worse outcomes, since it is a system susceptible to the cytokine storm and the pro-thrombotic state (Zhao *et al.*, 2023).

In Brazil, cardiovascular disease mortality increased from 1.58% to 13.3% in 2020, further substantiating studies that link COVID-19 to acute myocardial infarction, heart failure, cardiac inflammation, arrhythmias, and coagulation (Makarova *et al.*, 2023). Moreover, individual factors such as advanced age, chronic diseases, and immunosuppression have been associated with fatal outcomes, although deaths have also occurred among patients without pre-existing conditions (Makarova *et al.*, 2023; Nasab *et al.*, 2023). In light of this, research to understand the pathophysiological mechanisms and genetic and environmental factors that may influence the prognosis of COVID-19, has gained prominence.

SARS-CoV-2 directly affects endothelial cells because its immune escape mechanisms cause the endothelial cell to develop more effective mechanisms of innate immunity, such as activation of the NLRP3 inflammasome, which not only increases the production of pro-inflammatory cytokines such as IL-1β and IL-18, but also causes macrophage pyroptosis (López-Reyes *et al.*, 2020). Hyperactivation of the immune response causes increased levels of reactive oxygen species (ROS),

the expression of adhesion molecules such as VCAM-1, and a storm of pro-inflammatory cytokines such as IL-1β, IL-6, IL-18, TNF-α, and IFN-γ (Nasab *et al.*, 2023).

The elevation of pro-inflammatory cytokines and ROS in the intimal layer contributes to the development and expansion of atherosclerotic plaques. This is because ROS promote the oxidation of LDL cholesterol (LDL-ox), which is subsequently taken up by macrophages, resulting in their differentiation into foam cells rich in oxidized cholesterol and an increased production of pro-inflammatory cytokines (Lin, 2020). COVID-19, in addition to elevating these cytokines, has been linked to lipid metabolism disorders, with an increase in TC, non-HDL-c, LDL-c, and TG being progressively reported, as well as observed in this study. This profile provides for endothelial dysfunction and, depending on the patient's criticality at the time of infection, may be enough to cause plaque rupture, disseminated intravascular coagulation crisis, and multiple organ dysfunction (Surma *et al.*, 2021).

Lipid metabolism disorders in COVID-19 may arise from viral-induced liver damage, leading to reduced mitochondrial activity and potentially affecting lipoprotein uptake. Furthermore, in addition to ACE2, SARS-CoV-2 can utilize scavenger receptor B type 1 (SR-BI) to infect hepatocytes and macrophages, and can have an impact on the expression of these receptors. SR-BI receptors are responsible for binding to apolipoprotein A-I (Apo A-I) and mediating the delivery of cholesterol to HDL particles, a process by which the HDL particle plays anti-atherogenic and immunomodulatory roles, since it removes oxidized cholesterol from macrophages (Alkazmi *et al.*, 2023). Moreover, the decrease in HDL-c associated with higher LDL-c was related to a worse outcome and a greater cytokine storm in COVID-19, which may reflect the activation of macrophages by the increase in oxidized cholesterol and the activation of sterol regulatory element binding protein-2, which, in addition to increasing cholesterol synthesis, is associated with an increase in C-reactive protein (CRP), IL-1β, TNF-α, NF-κB, and activation of the NLRP3 inflammasome (Lee *et al.*, 2020).

In the context of COVID-19, the evaluation of various inflammatory markers has revealed that VCAM-1 levels stand out as a reliable indicator of the inflammatory response and endothelial activation (Medina-Leyte *et al.*, 2021). Elevated VCAM-1 levels have shown an association with severe COVID-19 cases, displaying positive correlations with the coagulation marker d-dimer and several other inflammatory markers, including CRP, amyloid A protein, IL-6, TNF-α, and progranulin (Liu *et al.*, 2022). This study's findings are consistent with previous research, affirming the robust predictive potential of VCAM-1 in COVID-19 outpatient settings (Fernández *et al.*, 2022; Yao *et al.*, 2021). Nevertheless, the utility of VCAM-1 as a prognostic indicator for disease severity appears to diminish, hinting at its suitability for identifying infections but raising questions regarding its reliability in assessing prognosis (Tufa *et al.*, 2022).

VCAM-1, in response to the binding of oxidized LDL-c (LDL-ox) to Toll-like receptor 4 (TLR4), is produced concomitantly with the NF-κB, triggered by various signaling pathways (Miller *et al.*, 2012). This dynamic interaction between the increase in LDL-ox and the presence of SARS-CoV-2 highlights the antioxidant and immunomodulatory functions of HDL particles. Among the main constituents of HDL, Apo A-I and the enzyme paraoxonase-1 (PON-1) play key roles in attenuating oxidative stress. PON-1 mainly exerts its antioxidant activity by hydrolyzing oxidized cholesterol and lipid peroxidation products (Mackness; Mackness, 2015). Notably, Apo A-I emerges as a central player in the anti-inflammatory properties of HDL particles, since in vitro research has shown that Apo A-I, in association with the ATP-binding cassette protein subfamily A, attenuates signaling via TLR4 and CD40 receptors, thus inhibiting activation of the MyD88/IRAK-1/TRAF-6/NF-κB and mitogen-activated protein kinase (MAPK/p38MAPK/JNK) pathways (Sultana *et al.*, 2016; Yin *et al.*, 2012; Zhang *et al.*, 2018). Furthermore, glycation of Apo A-I and cleavage of its carboxyl terminus by mast cell chymase were associated with attenuation of its anti-inflammatory properties, indicating that this protein domain is important for this role (Maarfi *et al.*, 2023; Nguyen *et al.*, 2016).

Considering the observed association between decreased levels of HDL-c and severe COVID-19 outcomes, researchers proposed the potential therapeutic utility of HDL particles enriched with recombinant Apo

A-I and its mimetic peptides for inflammatory disorders and acute coronary syndrome (Guo *et al.*, 2021; Kalayci *et al.*, 2022). A pilot study demonstrated substantial clinical improvement in hospitalized COVID-19 patients following the administration of pre-β HDL particles containing recombinant Apo A-I (Faguer *et al.*, 2022). In the present study, although HDL-c levels did not differ from the control group, COVID-19 outpatients had elevated Apo A-I levels. This finding may help explain the negative correlation observed between HDL-c and VCAM-1 levels among these patients. It is possible that the greater presence of Apo A-I associated with HDL particles played a modulating role in VCAM-1 expression, potentially influencing the results.

Genetic components may be involved in lipid metabolism disorders, apolipoprotein gene expression, and the inflammatory response to COVID-19. Among the gene alterations of interest, single nucleotide polymorphisms (SNP) are the main ones involved in polygenic dyslipidemias, which are difficult to identify clinically due to their heterogeneity and association with environmental factors. In this sense, SNP rs670 (Apo A-I) and rs693 (Apo B) are widely associated with changes in LDL-c, TG, HDL-c, Apo A-I, and Apo B concentrations, as well as the occurrence of metabolic syndromes and cardiovascular events (Alves *et al.*, 2020; Hsu *et al.*, 2013; De Luis *et al.*, 2019). In this study, the dominance of the T polymorphic allele (rs670) was associated with lower levels of TG and VCAM-1 in COVID-19 outpatients, while the codominance of the T (rs670) and A (rs693) polymorphic alleles amplified this decrease.

These findings suggest that the SNP rs670, in the context of COVID-19, may exert a more pronounced influence on lipid metabolism and immune response modulation, potentially leading to enhanced endothelial protection. The presence of the T polymorphic allele has previously been linked to elevated levels of Apo A-I and a more favorable profile concerning insulin resistance, insulin, TC, LDL-c, and HDL-c (De Luis *et al.*, 2019; Swanson *et al.*, 2015). Nonetheless, it is noteworthy that several studies have reported conflicting results, associating the presence of the T allele with increased waist circumference, metabolic syndromes, and higher TC and LDL-c levels (Magray *et al.*, 2021; Wu *et al.*, 2016).

Considering that rs670 occurs in an intronic region of the Apo A-I gene, its presence may affect messenger RNA (mRNA) maturation and generate alternative splicing, the main problems being the occurrence of aberrant splicing or the formation of a stop code. It is important to emphasize that the expression of the APOA1 gene can also be regulated by the expression of its antisense RNA (APOA1-AS). This is a long non-coding RNA (LncRNA) that can regulate gene transcription, stability, and translation and is increasingly being investigated as a biomarker and therapeutic target for coronary atherosclerotic disease (Kimura, 2020; Wang *et al.*, 2021).

Expression of APOA1-AS was found to be linked with reduced HDL-c levels and elevated levels of TC, LDL-c, LDL-ox, endothelial selectin, and VCAM-1 in individuals with systemic lupus erythematosus complicated by atherosclerosis. It was further observed that the expression of APOA1-AS can recruit histone modifiers, resulting in diminished transcription of genes such as Apo A-I, as well as neighboring genes like Apo C-III, A-IV, and A-V (Abd-Elmawla *et al.*, 2018). Experimental evidence supporting APOA1-AS involvement in these pro-atherogenic mechanisms is highlighted by the reduction in TNF-α and VCAM-1 levels, and the concurrent increase in Apo A-I, following its silencing through small interfering RNA in both in vitro and in vivo models (Halley *et al.*, 2014; Yang; Jiang, 2022).

The SNP rs670 causes changes in the intronic sequence of the APOA1 gene, as well as the sequence of APOA1-AS, potentially leading to alternative splicing and alterations in APOA1-AS activity (NCBI, 2022). In the present study, COVID-19 outpatients had higher levels of Apo A-I, and those carrying the T allele had lower levels of TG and VCAM-1. This observation suggests that the SNP rs670 may either modify Apo A-I mRNA, resulting in a protein with enhanced anti-inflammatory capabilities, or that alterations in APOA1-AS might suffice to downregulate pro-inflammatory cytokine genes, thereby reducing VCAM-1 production. The reduction in TG levels, on the other hand, might be attributed to the potential regulation of Apo C-III expression by APOA1-AS, rendering lipoprotein lipase more active in TG hydrolysis (Halley *et al.*, 2014).

This study highlights the need for further investigations into the role of apolipoproteins polymorphisms in lipid metabolism disorders and cardiovascular risk in subjects with COVID-19. Although the study did not longitudinally monitor the laboratory and clinical outcomes of COVID-19 outpatients, it was able to analyze the distribution of SNP rs670 and rs693 and their association with lipid metabolism parameters and VCAM-1. The population sample used in the study had genotype frequencies that adhered to Hardy-Weinberg equilibrium and followed Mendelian segregation, a result of Brazil's rich ethnic miscegenation. Therefore, this study presents a pioneering role in establishing the correlation between the SNP rs670 and rs693 and parameters of lipid metabolism and endothelial activation in COVID-19 outpatients treated in a reference unit.

CONCLUSION

In summary, the present work revealed that COVID-19 outpatients exhibited elevated levels of TC, LDL-c, non-HDL-c, TG, and Apo A-I, along with a high frequency of hypercholesterolemia and hypertriglyceridemia. Notably, elevated levels of VCAM-1 were associated with COVID-19 and demonstrated high predictive power for disease. Moreover, VCAM-1 exhibited a negative correlation with TC and HDL-c parameters. In terms of the genetic component, the dominance of the T polymorphic allele (rs670) was associated with reduced TG and VCAM-1 levels in COVID-19 outpatients, and this reduction was further intensified when both the T (rs670) and A (rs693) polymorphic alleles were found in codominance.

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CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

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