Original Research

Paraoxonase 1 rs662 polymorphism, its related variables, and COVID-19 intensity: Considering gender and post-COVID complications

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Impact statement

COVID-19 became a pandemic in 2020, and many individuals suffered from its irreversible complications after contracting severe forms of the disease, which resulted in countless recorded deaths. Thus far, the reason why patients experience different intensities of the disease is not precisely known, and there is no comprehensive information available about the long-term complications of this disease. Therefore, the investigation of the risk factors can be very important for disease management. PON1 is one of the key antioxidant enzymes that play a critical role in inflammatory diseases. Accordingly, we aimed to evaluate the effect of the rs662 polymorphism, the arylesterase (ARE) activity of PON1, and the lipid profile of patients on disease severity and the mortality rate three months after the infection. We found that PON1 rs662 polymorphism affects disease severity and mortality in COVID-19 patients. Furthermore, decreased ARE activity and lipid profile are directly related to the severity of COVID-19 and have an upward trend after recovery.

Abstract

In this study, we aimed to investigate the effect of paraoxonase 1 (PON1) rs662 polymorphism, arylesterase (ARE) activity, and the serum lipid profile in patients with coronavirus disease 2019 (COVID-19) in different stages of the disease considering post-COVID outcomes. A total of 470 COVID-19 patients (235 female and 235 male patients) were recruited into the study, and based on the World Health Organization (WHO) criteria, the patients were divided into three groups: moderate, severe, and critical. PON1 rs662 polymorphism was determined by the Alw 1 enzyme followed by agarose gel electrophoresis. Moreover, serum levels of triglycerides (TG), cholesterol (Chol), high-density lipoprotein–cholesterol (HDL-c), and low-density lipoprotein–cholesterol (LDL-c), as well as the level of the ARE activity of PON1 in the sera of patients were measured at the time of infection and one and three months after hospitalization. There was a significant relationship between the G allele and the severity of the disease. In addition, the probability of death in homozygous individuals (GG) was higher than in heterozygous patients (GA), and it was higher in heterozygous patients than in wild-type individuals (AA). There was also a significant relationship between the decrease in serum lipids and the intensity of COVID-19. On the contrary, at the onset of the disease, the HDL-c level and serum ARE activity were reduced compared to one and three months after COVID-19 infection. The findings of this study indicated the significant impact of PON1 rs662 polymorphism on ARE activity, lipid profiles, disease severity, and mortality in COVID-19 patients.

Keywords: COVID-19, PON1, rs662, serum lipids, ARE activity, post-COVID

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Introduction

Coronavirus disease 2019 (COVID-19) has spread to all countries, and the incidence of COVID-19 infection, as well as its mortality rate, varies in different parts of the world, with no clear reason for this disparity.[1,2]

Paraoxonases (PONs) are a family of three enzymes, namely, PON1, PON2, and PON3,[3] which are involved in

various biochemical pathways such as reducing oxidative damage and lipid peroxidation, promotion of innate immunity, detoxification of active molecules, bioactivation of drugs, modulation of endoplasmic reticulum stress, and regulation of cell proliferation/apoptosis. Because they are able to perform several independent and often unrelated functions, PONs are referred to as "moon proteins."[4]

Further studies have been performed on the PON1 enzyme, a calcium-dependent glycoprotein with a molecular weight of 43–47 kDa, and the *PON1* gene located on the long arm of chromosome 7.[5] The PON1 enzyme is produced in the liver and hydrolyzes organophosphate compounds and fatty acid lactones. It is abundantly found in the bloodstream bound to high-density lipoprotein (HDL) and has antioxidant properties owing to its arylesterase (ARE) and lactonase activities. PON1 hydrolyzes phospholipids and hydroperoxides in oxidized low-density lipoproteins (LDLs) and destroys proinflammatory molecules. PON1 is also shown to hydrolyze hydrogen peroxide (H_2O_2), a major reactive oxygen species produced under oxidative stress.[6]

Manipulation of host cell function by viral pathogens is essential for the infection and creating an environment that favors virus replication. Viruses depend on the metabolic sources of the host cell to reproduce, such as nucleic acids, proteins, and membranes.[7] Most viruses alter host cell metabolism to optimize virus biosynthetic needs through proviral metabolic modifications. On the contrary, host cells use metabolic strategies to inhibit virus replication through antiviral metabolic changes.[7,8] Oxidative stress produced by infectious processes may impair cell function, which may in turn lead to a further increase in the production of free radicals.[9,10]

The innate immune system uses several mechanisms to overcome oxidative stress. One of the factors involved in these mechanisms is the antioxidant enzyme PON1.[11] Serum PON1 activity is lower in HIV-infected patients than in the general population, and this change is associated with the immune status of patients and their degree of inflammation.[12] Increased oxidative stress and decreased serum PON1 activity have also been reported in other viral infections, including influenza, hepatitis B, and hepatitis C. Studies have indicated that infectious diseases including COVID-19 are often associated with oxidative stress and inflammation.[13,14] The enzymatic activity of PON1 depends on various factors, including PON1 polymorphisms, ethnicity, sex, age, and a number of environmental variables. PON1 has a common functional polymorphism, Q192R (rs662).[14]

Possible associations have been observed between the Q192R polymorphism genotypes of the *PON1* gene and the chronicity of hepatitis B virus (HBV) infection.[15] It has also been suggested that PON1 rs662 polymorphism is associated with a higher frequency of spontaneous hepatitis C virus (HCV) clearance (defined as undetectable concentrations of HCV RNA in the blood).[16] Moreover, a relationship has been found between the prevalence and mortality of COVID-19 and another functional PON1 polymorphism, namely, L55M (rs854560).[17] On the contrary, the prevalence and mortality rate of COVID-19 exhibit significant geographical variations. Since no viral mutations that could explain the significant geographical changes have been reported, the question arises as to whether it is possible that the genetic diversity of the host PON1 may affect the outcome of COVID-19 infection.[18] Therefore, in this study, we investigated changes in the lipid profile and ARE activity of PON1 and its rs662 polymorphism in both male and female patients with COVID-19 based on the severity of the disease during the infection and also one and three months after the infection.

Materials and methods

Study design

This research is a cohort and analytical observational study. We investigated the lipid profile and ARE activity of PON1 and its rs662 polymorphism based on the severity of COVID-19 during the disease and also one and three months after the infection.

Patients and samples

We collected 6 mL of blood (3 mL in a complete blood count [CBC] tube containing the ethylenediamine tetraacetic acid [EDTA] anticoagulant and 3 mL for serum separation) from 470 patients with COVID-19 (including 235 female and 235 male patients) who were hospitalized in Imam Khomeini Hospital in Jiroft, Kerman Province, Iran. Patients under 20 years, over 80 years, and pregnant women were not included in the study.

COVID-19 was diagnosed by a positive severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) quantitative reverse-transcription polymerase chain reaction (RT-qPCR) test on nasopharyngeal and throat swabs. Whole blood was separated by centrifugation, and small aliquots of leukocytes were stored at −70°C until the measurements.

Sera were isolated and stored at −20°C for lipid assays and measurement of the ARE activity of PON1.

All 470 patients were followed up one and three months after the first sampling, and any fatalities were recorded. Blood samples were taken from 270 patients one month after the infection and from 198 patients three months after the infection. The remaining subjects either did not participate in the study or died before the one-month and three-month samplings.

The selected patients (men: 235 and women: 235) were divided into three groups according to the World Health Organization (WHO)[19] criteria, including moderate (pneumonia with fever, cough, dyspnoea, fast breathing, and $SpO_2 \geqslant 90\%$), severe (severe pneumonia with fever, cough, dyspnoea, fast breathing, respiratory rate > 30 breaths/min, severe respiratory distress, or $SpO_2 < 90\%$), and critical (acute respiratory distress syndrome [ARDS], chest imaging with bilateral opacities, lobar or lung collapse or nodules, and oxygenation impairment) groups (Supplemental Table 3S).

DNA analysis

Using the Favorgen kit (Cat No. FABGK 001-1), the patients' DNAs were extracted from whole blood samples and kept at −70°C until further assessment.

To amplify the target region, which included the two sides of the SNP (rs662), PCR was performed using the forward (5′-GAAGGCTCCATCCCACATCTT-3′) and reverse (5′-TTCACATACTTGCCATCGGGT-3′) primers.

The rs662 polymorphism (CAA converted to CAG at position 192), which results in the glutamine to arginine

Table 1. General and outcome of COVID-19 patients based on gender.

	Gender	Group	*n*	Mean	95% confidence interval for mean		*P* value
Age	Male	Moderate	100	41 ± 1.02	38.992	43.008	<0.0001
		Severe	96	47.645 ± 0.88	45.882	49.410	
		Critical	39	55.692 ± 1.47	52.708	58.676	
	Female	Moderate	135	42.77 ± 0.96	40.862	44.679	<0.0001
		Severe	70	49.2 ± 1.14	46.907	51.493	
		Critical	30	58.333 ± 1.74	54.770	61.897	
	Gender		*n*	Mean	95% confidence interval for mean		*P* value
					Lower bound	Upper bound	
BMI	Male	Moderate	100	24.796 ± 0.314	24.1729	25.4191	<0.0001
		Severe	96	25.365 ± 0.303	24.7635	25.9677	
		Critical	39	27.3410 ± 0.460	26.4095	28.2725	
	Female	Moderate	135	25.573 ± 0.304	24.9712	26.1755	0.04
		Severe	70	26.95 ± 0.465	26.0222	27.8778	
		Critical	30	25.48 ± 0.731	23.9844	26.9756	
	Gender			Group			
				Moderate	Severe	Critical	
Smoking	Male	No		94 (47.2%)	78 (39.2%)	27 (13.6%)	<0.01
		Yes		6 (16.7%)	18 (50.0%)	12 (33.3%)	
	Female	No		131 (58.5%)	65 (29.0%)	28 (12.5%)	0.348
		Yes		4 (36.4%)	5 (45.5%)	2 (18.2%)	

n: number; BMI: body mass index.
Kruskal–Wallis test was used to examine the relationship between age and BMI with disease severity, and chi-square test was used to examine the relationship between smoking and disease severity. *P* value < 0.05 was considered as significant level.

substitution, at PON1 was detected after PCR amplification followed by digestion with Alw 1 restriction endonuclease. The digested products were transferred onto 2% agarose gel containing Green Viewer and examined for the presence of the homozygous genotype (GG), heterozygous genotype (GA), or the wild-type genotype (AA).

Serum lipid assay

The levels of total cholesterol (Chol), high-density lipoprotein–cholesterol (HDL-c), and triglycerides (TG) in the serum of patients at the time of infection and one and three months after the infection were measured using Pars Azmoon kits and the Mindray BS480 autoanalyzer. The LDL-c levels were evaluated using the Friedewald formula.[20]

Serum ARE activity assay

The ARE activity of PON1 was measured in the sera of patients with early COVID-19 infection and the one-month and three-month samples.

The assay mixture contained 4.0 mM/L phenylacetate and 1 mM/L $CaCl_2$ dissolved in 20 mM/L Tris HCl buffer with a pH of 8.0 at 25°C. The activity is expressed as µmol/min/mL based on the extinction coefficient of phenol (1310 $M^{-1} cm^{-1}$) at 270 nm, pH 8.0, and 25°C, after correction for non-enzymatic hydrolysis.[21]

Statistical analysis

SPSS 26 software was used for the statistical analysis, while GraphPad Prism 8 software was utilized for drawing graphs. The 5% alpha error was considered the limit for the rejection or confirmation of the null hypothesis. All mean comparison tests were performed by using two-sided tests. In this study, two-way analysis of variance (ANOVA), one-way ANOVA, and the post hoc Sidak test were used.

Results

Baseline characteristics of the study participants

According to the analyses (Table 1), there was a significant relationship between age and disease severity, and the mean age of women and men in the critical group was higher than the mean age of those in the severe and moderate groups. In male patients, we observed a higher body mass index (BMI) in the critical group than in the severe and moderate groups, whereas in female patients, there was no significant relationship between BMI and the disease severity. Moreover, smoking was not significantly associated with the severity of the disease in women, but it showed a significant relationship with the disease severity in men.

Investigating the relationship between PON1 rs662 polymorphism and morbidity and mortality rates

The chi-square test was used to assess the relationship between disease severity and types of genotypes resulting from rs662 polymorphism (Table 2). According to the results, no significant relationship was observed between disease severity and genotype. However, the severity of the disease in various alleles of the rs662 polymorphism was significantly different ($P = 0.03$).

In addition, the Cox regression test was employed to evaluate the association of gender, disease severity, and the

Table 2. Comparison of the frequency of different disease severities in various genotypes of rs662 polymorphism.

		Group			OR	OR	*P* value
		Moderate	Severe	Critical			
Genotype	AA	212 (90.2%)	134 (80.7%)	59 (85.5%)	0.632075	1.100746	0.101
	AG	18 (7.7%)	27 (16.3%)	8 (11.6%)	1.5	0.740741	
	GG	5 (2.1%)	5 (3.0%)	2 (2.9%)	1	1	
Allele	A	442	295	126	0.505075	0.665158	0.03
	G	28	37	12	1	1	

OR: odds ratio; AA: wild type; AG: heterozygote; GG: homozygote.
Chi-square test was used to investigate the relationship between disease severity and types of genotypes resulting from rs662 polymorphism. There was no significant relationship between the genotypes obtained from the rs662 polymorphism and the severity of COVID-19 (*P* value = 0.101). However, the G allele was associated with the increased severity of COVID-19 (*P* value = 0.03). *P* value < 0.05 was considered as significant level.

Table 3. Regression coefficients and the significance of different variables and risk of death.

Variable		*P* value	Crude HR	95.0% CI for HR		Variable		*P* value	Adjusted HR	95.0% CI for HR	
				Lower	Upper					Lower	Upper
Group	Moderate	–	1	–	–	Group	Moderate	–	1	–	–
	Severe	<0.0001	9.835	2.922	33.099		Severe	<0.01	8.210	2.419	27.865
	Critical	<0.0001	17.293	4.927	60.693		Critical	<0.0001	19.336	5.456	68.521
Genotype	AA	–	–	–	–	Genotype	AA	–	1	–	–
	AG	<0.0001	8.381	4.095	17.156		AG	<0.0001	7.098	3.435	14.667
	GG	<0.0001	16.598	6.429	42.853		GG	<0.0001	20.728	7.793	55.137

HR: hazard ratio, CI: confidence interval; AA: wild type; AG: heterozygote; GG: homozygote.
Cox Regression test was used to evaluate the relationship between severity and genotypes of rs662 polymorphism on death rate. *P* value < 0.05 was considered as significant level.

genotypes of rs662 polymorphism with the mortality rate. According to the findings (Table 3), there was no significant relationship between gender and the mortality rate, but the mortality rate was directly related to disease severity.

As shown in Table 3, the risk of death in the severe group was 9.8 times higher than that in the moderate group (95.0% confidence interval [CI] for hazard ratio [HR] 2.922–33.099; $P < 0.0001$), and in the critical group, it was 17.293 times higher than that in the moderate group (95.0% CI for HR 4.927–60.693; $P < 0.0001$).

It is noteworthy that there was a significant relationship between the different genotypes resulting from the rs662 polymorphism and the mortality rate, such that the risk of death in individuals with the AG genotype was 8.381 times higher than that in individuals with the AA genotype (95.0% CI for HR 6.429–17.156; $P < 0.0001$). In addition, the risk of death in patients with the GG genotype was 16.598 times higher than that in subjects with the AA genotype (95.0% CI for HR 6.429–42.853; $P < 0.0001$). Moreover, the genotype of dead patients is displayed in Supplemental Table 1S.

In order to better show the percentage of survival in different genotypes resulting from the rs662 polymorphism and different severities of COVID-19, the corresponding Kaplan–Meier curves are shown in Figure 1.

Assessment of ARE activity and lipid profile in patients with different intensities of COVID-19 and different genotypes of PON1 rs662 polymorphism

The Kruskal–Wallis test was used to evaluate the relationship between ARE activity and lipid profile in patients with different intensities of COVID-19 and different genotypes of rs662 polymorphism. According to the results (Table 4), it can be stated that the ARE activity in individuals with the AA genotype (wild type) was higher than that in heterozygous (AG) individuals while heterozygous individuals had higher ARE activity than homozygous (GG) individuals. However, there was no statistically significant relationship between lipid levels and different genotypes of the rs662 polymorphism.

ARE activity of PON1 in patients with COVID-19 at the time of infection and one and three months after the infection

The Kruskal–Wallis test was used to investigate the relationship between ARE activity and disease severity. According to the results (Figure 2(A) to (C)), ARE activity in women (Figure 2(A)) and men (Figure 2(B)) in the critical and severe groups was significantly lower than that in the moderate group ($P < 0.0001$).

The one-way repeated-measures ANOVA test was used to examine the association of disease severity and time with the level of ARE activity, and the post hoc Sidak test was employed to compare binaries between different times. The results indicated that in women, except for the one- and three-month stages in female patients in the moderate group, the level of ARE activity increased over time and was improved (Figure 2(D)). In men, except for the one-month stage, compared to the time of onset in the severe group, the level of ARE activity increased over time in other groups (Figure 2(E)).

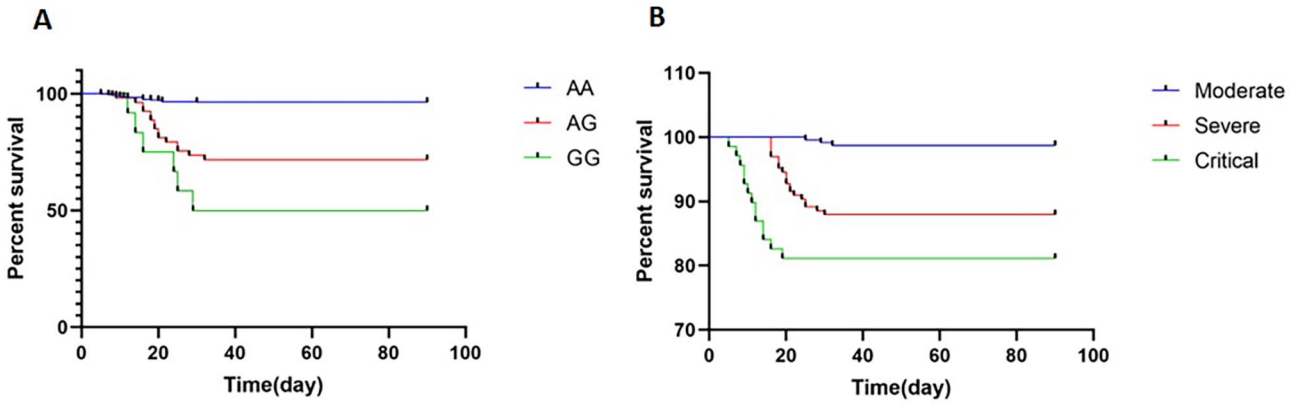

Figure 1. Kaplan–Meier curves showing the percentage of survival in different genotypes resulting from rs662 polymorphism (A) and the percentage of survival in different severities of COVID-19 (B). (A color version of this figure is available in the online journal.)

Table 4. Differences in the lipid profile and ARE activity between various genotypes of rs662 polymorphism in COVID-19 patients with different intensities at the time of infection.

Time	Severity	ARE activity	HDL-c	LDL-c	TG	Chol
COVID-19 patients	Moderate	AA > AG > GG $P < 0.001$	GG > AA > AG $P = 0.470$	GG > AA > AG $P = 0.669$	GG > AA > AG $P = 0.520$	GG > AG > AA $P = 0.662$
	Severe	AA > AG > GG $P = 0.067$	GG > AA > AG $P = 0.352$	GG > GA > AA $P = 0.362$	GG > GA > AA $P = 0.245$	GG > AG > AA $P = 0.108$
	Critical	AA > AG $P = 0.177$	GG > AG > AA $P = 0.234$	GG > GA& AA $P = 0.941$	AA > AG > GG $P = 0.457$	AG > AA > GG $P = 0.995$

ARE: arylesterase; TG: triglycerides; COVID-19: coronavirus disease 2019; AA: wild type; AG: heterozygote; GG: homozygote; HDL-c: high-density lipoprotein–cholesterol; LDL-c: low-density lipoprotein–cholesterol; TG: triglycerides; Chol: cholesterol.
Kruskal–Wallis test was used to investigate the relationship between aryl esterase activity and lipid profile of patients with different intensities of COVID-19 in different genotypes of rs662 polymorphism. *P* value < 0.05 was considered as significant level.

Two-way repeated-measures ANOVA was used to evaluate the difference in the level of ARE activity between men and women over time. The results revealed no significant difference in the level of ARE activity between men and women over time. In general, in men and women, the level of ARE activity increased over time (Figure 2(F)) (Supplemental Table 2S).

Lipid profile of male and female patients with COVID-19 at the time of infection

The Kruskal–Wallis test was used to evaluate the levels of HDL, LDL, total Chol, and TG in moderate, severe, and critical groups. According to the results, the level of HDL was significantly lower in men (Figure 3(E)) and women (Figure 3(A)) in the critical group than in those in the moderate group ($P < 0.01$). There was no significant difference in the level of LDL between women (Figure 3(B)) ($P = 0.48$) and men (Figure 3(F)) ($P = 0.08$) in different groups.

There was no significant difference in TG ($P = 0.3$) and Chol ($P = 0.4$) levels in women (Figure 3(C) and (D)) in different groups. In men, TG (Figure 3(G)) ($P < 0.01$) and Chol (Figure 3(H)) ($P = 0.037$) levels in the critical group were significantly higher than in the moderate group.

Lipid profile one and three months after COVID-19 infection in women and men

The one-way repeated-measures ANOVA test was used to investigate the association of disease severity and time with the level of HDL, LDL, TG, and Chol, and post hoc Sidak test was employed to compare binaries between different times.

According to our results, over time, HDL levels in women (Figure 4(A)) and men (Figure 5(A)) in the three groups of moderate, severe, and critical increased significantly compared to the levels at the time of onset ($P < 0.0001$). Comparing the trend of changes in HDL between men and women after the infection shows that HDL levels in women were visually higher in all three stages of sampling, but there was no statistically significant difference ($P = 0.3$) (Figure 6(A)).

LDL levels in men did not differ significantly between the three stages of sampling at various disease intensities (Figure 5(B)) ($P = 0.2$).

LDL levels in women did not differ significantly between the second and third stages of sampling ($P = 0.08$), but in the second stage, they were significantly less than the levels at the time of infection ($P < 0.002$) (Figure 4(B)).

Over time, TG levels in both women (Figure 4(C)) ($P = 0.02$) and men (Figure 5(C)) ($P < 0.0001$) increased compared to their levels at the time of infection (Figures 4 and 5). Moreover, the mean TG levels in both male and female patients were not significantly different ($P = 0.08$) (Figure 6(C)).

The mean Chol in the three stages of sampling was significantly different between women (Figure 4(D)) and men (Figure 5(D)) and had an increasing trend ($P < 0.0001$).

The mean Chol in the three stages of sampling was significantly different between the two genders and was higher in

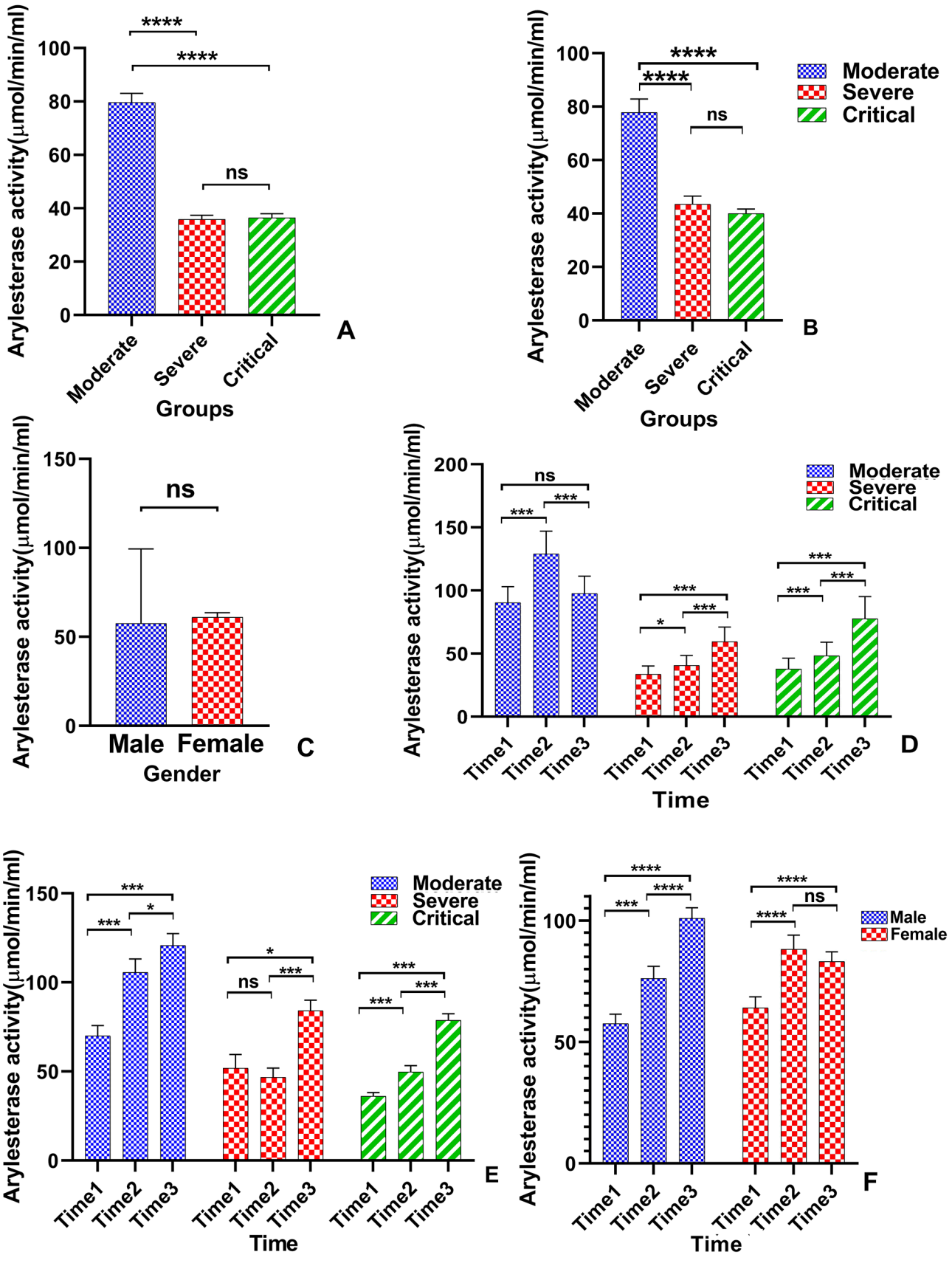

Figure 2. Mean and standard error mean of aryl esterase activity in women (A) and men (B) separated by disease severity. Aryl esterase activity between all male and female patients (C). Mean and standard error mean of aryl esterase activity in women (D) and men (E) at different times and severity. Mean and standard error mean of aryl esterase activity in women and men at different times (F). ARE: arylesterase; Time1: at the time of infection; Time2: one month after infection; Time3: three months after infection. ns: not significant, $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, $^{****}P < 0.0001$. (A color version of this figure is available in the online journal.)

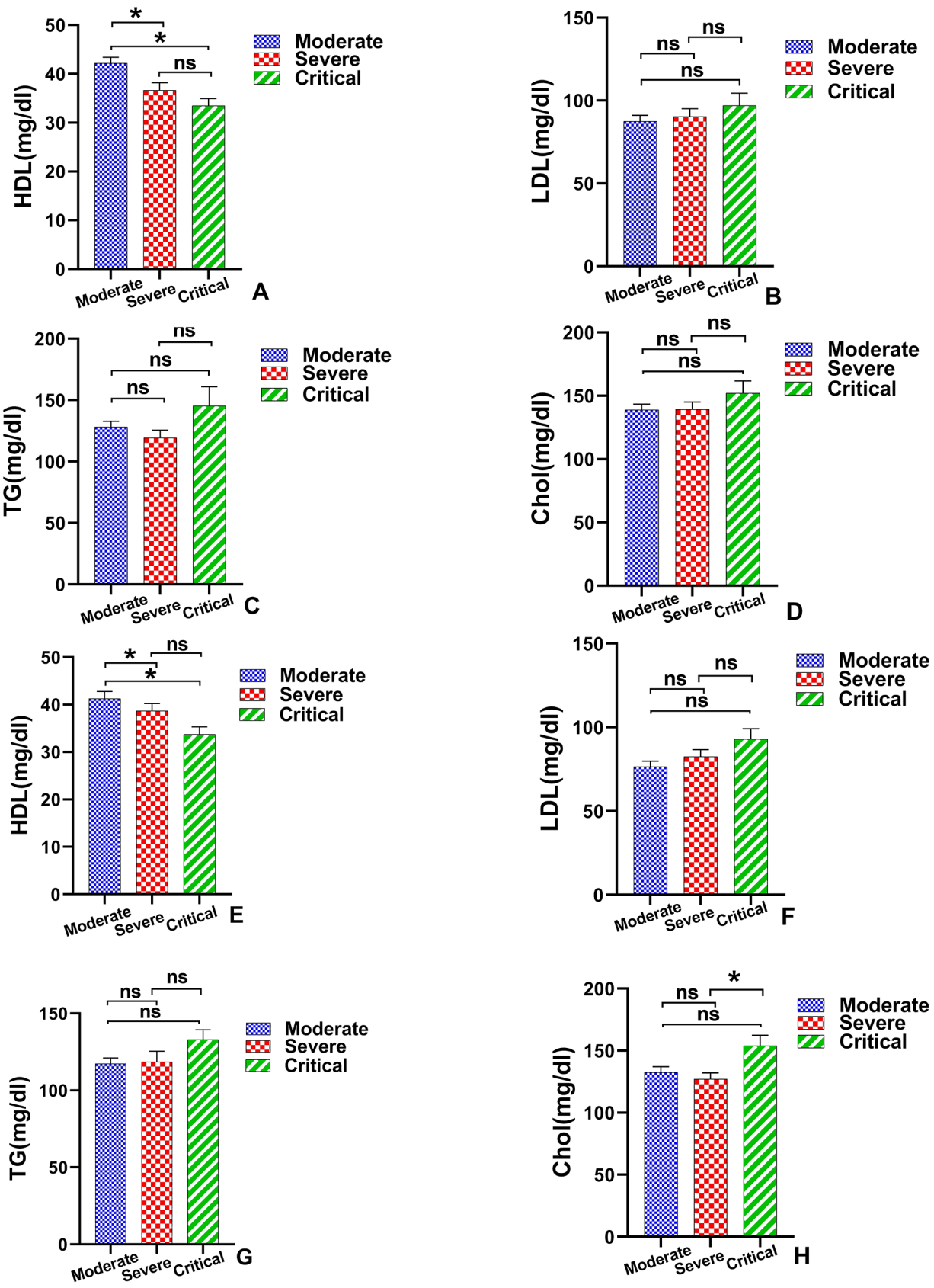

Figure 3. Mean and standard error mean of HDL, LDL, TG, and Chol in women (A, B, C, D) and men (E, F, G, H) separated by disease severity. ns: not significant, $^{*}P < 0.05$, $^{**}P < 0.01$. (A color version of this figure is available in the online journal.)

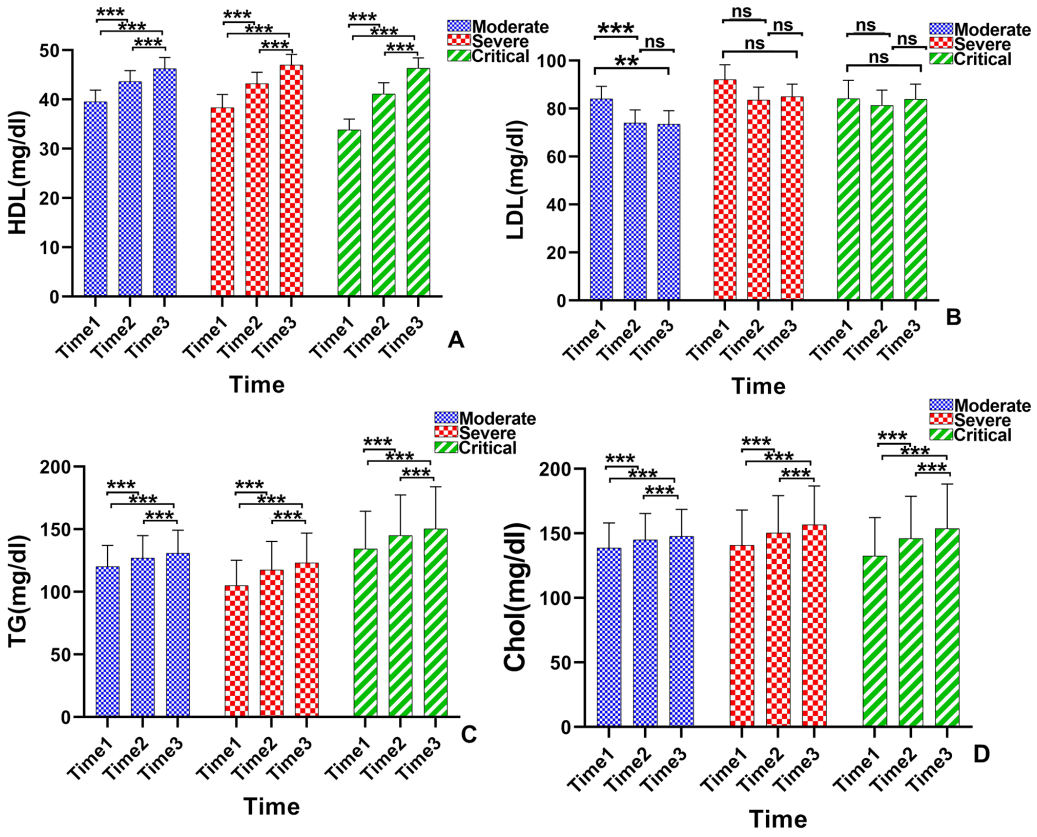

Figure 4. Mean and standard error mean of women HDL, LDL, TG, and Chol at different measurement times separated by disease severity. Time1: at the time of infection; Time2: one month after infection; Time3: three months after infection. ns: not significant, $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$. (A color version of this figure is available in the online journal.)

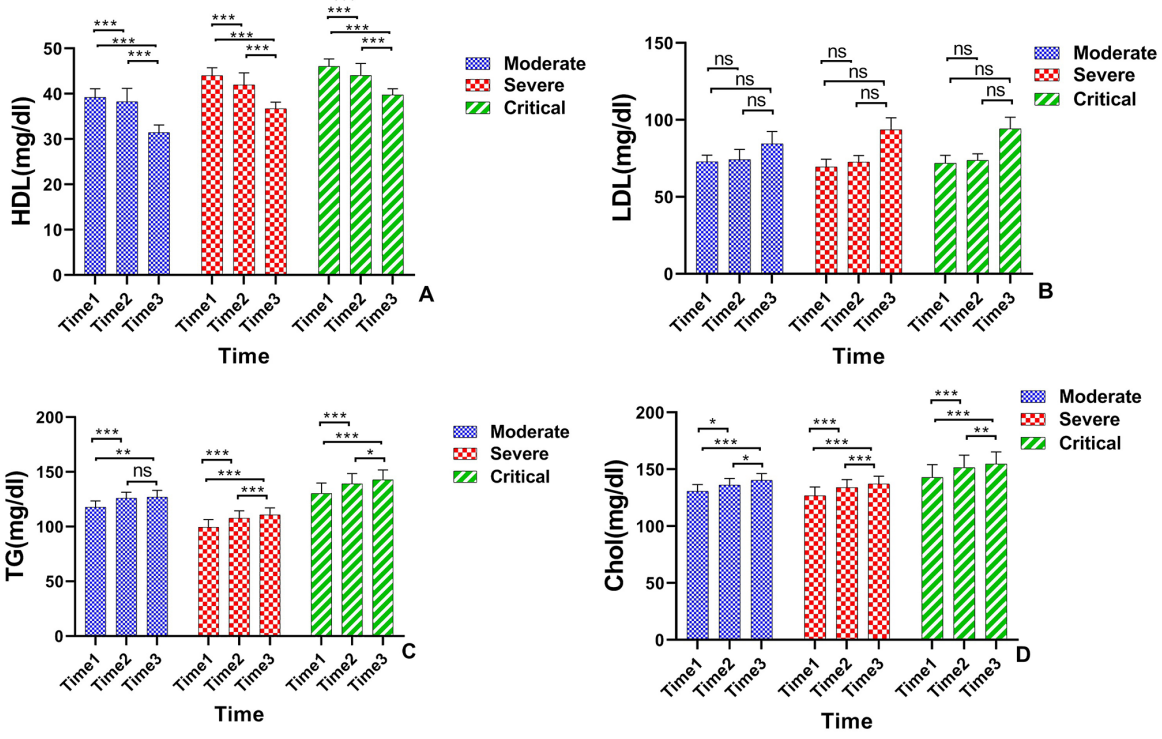

Figure 5. Mean and standard error mean of men HDL, LDL, TG, and Chol at different measurement times separated by disease severity. Time1: at the time of infection; Time2: one month after infection; Time3: three months after infection. ns: not significant, $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$. (A color version of this figure is available in the online journal.)

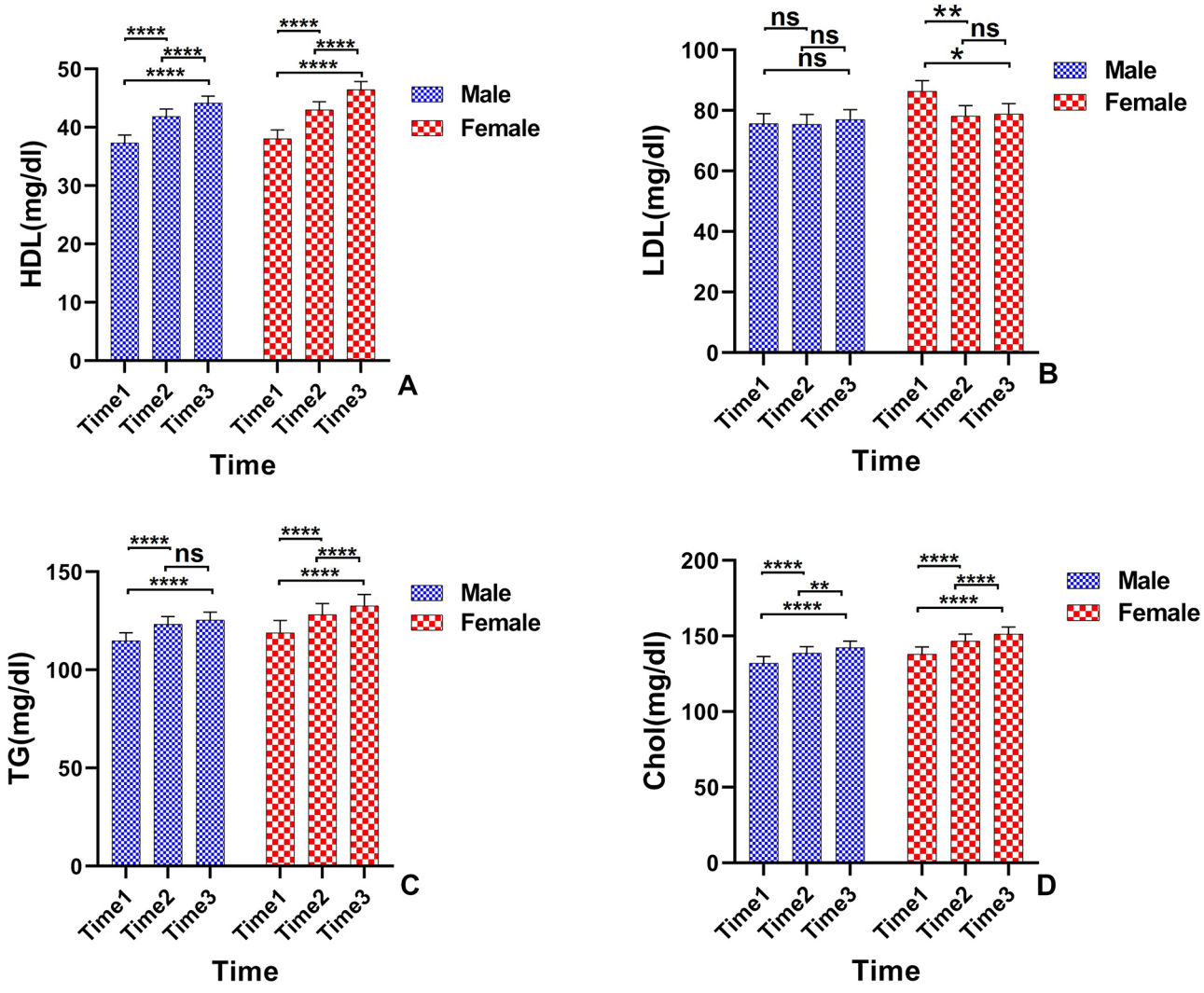

Figure 6. Mean and standard error mean of HDL, LDL, TG, and Chol at different measurement times disaggregated by gender. Time1: at the time of infection; Time2: one month after infection; Time3: three months after infection. ns: not significant, $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$, $^{****}P<0.0001$. (A color version of this figure is available in the online journal.)

women than in men ($P<0.01$) (Figure 6(D)) (Supplemental Table 2S).

Discussion

The COVID-19 pandemic has changed the normal lives of people around the world.[22] Serious health threats, significant complications, and the mortality rates from SARS-CoV-2 have prompted many researchers from diverse backgrounds to investigate potential protective factors against this global health problem.[23]

In this study, the effect of one of the most common polymorphisms of PON1 (rs662) on disease severity and mortality in COVID-19 patients as well as the ARE activity of PON1 and lipid profile in different severities of the disease (three groups including moderate, severe, and critical) at the time of infection and one and three months after the infection were investigated.

According to our results, there was no significant difference in the frequency of various genotypes related to rs662 (AA/AG/GG) between different intensities of the disease. However, the severity of the disease in varying alleles of the rs662 polymorphism was significantly different ($P=0.03$) such that patients with the G allele had a higher risk of developing the more severe form of COVID-19 (Table 2).

In addition, assessing the risk of mortality in different genotypes of rs662 indicated that the risk of death in heterozygous individuals (AG) was 8.3 times higher than that in wild-type individuals (AA), and in homozygous patients (GG), it was 16.5 times higher than that in individuals with the AA genotype ($P<0.0001$) (Table 3) (Figure 1(A)). On the contrary, this polymorphism also led to a reduction in ARE activity (Table 4).

In previous epidemics, as well as the current COVID-19 pandemic, ethnicity has been implicated in viral infectious diseases. Ethnicity is very complex in nature and ethnic groups are remarkably different in terms of the gene pool and environmental factors such as socioeconomic factors and cultural behaviors.[24] Changes in PON1 activity attributed to the Q192R polymorphism can have a significant

effect on oxidative conditions and disease exacerbation in individuals.[25]

A study by Ferré *et al*. showed that PON-192 polymorphism, along with other polymorphisms, contributes to changes in the host response to HCV infection.[26] Taken together, allelic frequency as well as the effects of ethnicity-related factors on the PON1 activity may, at least in part, explain the differences in mortality and the prevalence of COVID-19 between various countries.[17] According to previous studies, the ARE activity of the PON enzyme is reduced by rs662 polymorphism.[27] In order to understand the importance of ARE activity in different intensities of COVID-19 and to evaluate the changes in the activity levels after recovery, we examined the level of PON1 ARE activity in the serum of COVID-19 patients at the time of infection and one and three months after the infection. Our results revealed a decline in ARE activity at the beginning of COVID-19 infection. ARE activity in critical and severe groups was lower than in the moderate group (Figure 2), and after improvement in one-month and three-month periods, we observed an increase in this activity compared to the time of infection in men and women (Figure 2).

There was no significant difference in changes in ARE activity levels between men and women (Supplemental Table 2S). According to several studies conducted so far, decreased antioxidant activity may play a role in the severity of COVID-19, and it has been observed that the level of antioxidant activity in patients with COVID-19 is lower than in the control group.[28]

A study by Novak *et al*.[29] on the level of ARE activity of PON1 in patients with sepsis showed that there was a significant inverse relationship between the severity of the infection and the level of ARE activity. A study by Parra *et al*.[12] on HIV-infected patients found that PON1 activity was affected by HIV infection and was associated with changes in HDL composition and patients' immune status.

As it is well known, PON1 is an HDL-associated enzyme that inhibits the oxidation of LDL-c and HDL-c and promotes Chol efflux from macrophage foam cells.[30]

A number of studies have reported abnormalities in the lipid profile of individuals with COVID-19.[31] In this part of the study, we examined the level of serum lipids in COVID-19 patients at the time of infection, and one and three months after the infection.

According to our results, HDL-c levels in the critical and severe groups were significantly lower than in the moderate group (in both women and men) (Figure 3). While monitoring the HDL-c levels one and three months after the infection, we detected an increasing trend in these levels (within the normal range) (Figures 4 to 6).

These findings are controversial from two perspectives: first, in severe forms of COVID-19, HDL-c levels decrease and return to baseline after recovery, and second, low HDL-c levels are a risk factor for more intense COVID-19 symptoms. Information on the HDL levels of the study subjects before they were admitted to the hospital were not available to us, and it is not entirely clear whether individuals with lower HDL are more susceptible to more severe forms of COVID-19 than others; however, our study revealed that the increase in the severity of COVID-19 was effective in reducing the level of HDL, and after the recovery, the HDL level had an upward trend.

In addition to their function as a reverse transporter of Chol from peripheral tissues back to the liver, HDLs display endothelial protective properties as well.[32] HDL-c has anti-inflammatory activities and is able to fight apoptosis induced by tumor necrosis factor–alpha 2 (TNFα2).[33] The "cytokine storm" underlying COVID-19 infection appears to induce immune-mediated inflammatory dyslipoproteinemia, leading to low HDL-c levels. High levels of interleukin-6 and other cytokines in COVID-19 infection may inhibit the synthesis of apolipoprotein AI, resulting in a decrease in HDL-c levels. In addition, the "cytokine storm" increases the activity of secretory phospholipase A2 (sPLA2) and endothelial cell lipase, which are enzymes that metabolize the main components of HDL, and ultimately leads to a decrease in HDL-c.[34] Importantly, HDLs are part of the innate immune system and can buffer toxins from infection and inflammation by modulating the inflammatory burst.[35] According to a study by Begue *et al*.,[31] HDL-c levels in COVID-19 patients were lower than in healthy individuals. Based on what has been mentioned and the results of our study, it seems that the increase in the severity of COVID-19 reduced the level of HDL.

LDL-c levels in men and women in our study were not significantly different in the three moderate, severe, and critical groups (Figure 3). On the contrary, there was no significant difference in LDL-c levels in men after one and three months (Figure 5), but in female patients in the moderate stage, a decrease in LDL-c levels was observed in the second and third stages compared to the first stage (Figure 4).

There was no significant difference in TG and Chol levels in women with COVID-19 between different study groups (moderate, severe, and critical); however, in men, Chol levels in the critical group were significantly higher than in the moderate group (Figure 2).

In the one- and three-month monitoring of women and men with COVID-19, we detected an increase in TG and Chol levels over time (Figures 4 to 6). To interpret these results, it can be stated that high levels of Chol may be a risk factor for more severe symptoms of COVID-19. On the contrary, during the acute stage of the disease, the level of these lipids was slightly reduced and then increased to its previous level after recovery.

A study by Wei *et al*.[36] on patients with COVID-19 concluded that hypolipidemia occurs in patients with mild symptoms and gradually worsens with the severity of the disease.

According to a study by Masana *et al*.,[37] decreased HDL-c levels and elevated TG levels may be indicative of a poor prognosis of COVID-19. On the contrary, a study by Roccaforte *et al*.[38] reported that plasma lipid levels rise after recovery from the acute phase of infection. Moreover, a study by Wu *et al*.[39] on individuals infected with SARS revealed that after 12 years, some of their lipid levels were still higher than those in normal controls. The reduction in lipid levels during COVID-19 infection can be explained by the fact that lipids are essential in viral infection since they provide the

structural and energy sources for the formation of viral cell membranes and organs. RNA viruses target lipid synthesis to modulate intercellular and intracellular signals in favor of the processes needed to generate the particles necessary for them to enter the host cell, infect it, and hide from the immune system.[40]

In addition, similar to other RNA-positive viruses, SARS-CoV-2 modifies the host cell membrane for the production of viral replication organs (ROs), RNA synthesis, and replication plants. Electron microscopy has shown that SARS-CoV-2 produces a variety of bilayer structures called double-membrane vesicles (DMVs) that consist of the space between plasma membranes with different organs and also uses endosomes as ROs.[41]

Conclusions

In general, the results of this study indicated the significant impact of PON1 rs662 polymorphism on ARE activity of PON1, COVID-19 severity, and mortality rate.

It was reported that homozygous individuals are more likely to die from COVID-19 than heterozygous individuals, and heterozygous individuals are more likely to die from this disease than wild-type individuals (AA). These findings may predict, at least in part, the different prevalence/mortality rate of COVID-19 in various ethnicities. Low ARE activity indicates a poor prognosis for COVID-19. High levels of interleukin-6 and other cytokines in COVID-19 infection may inhibit the synthesis of apolipoprotein AI and lead to a decrease in HDL-c levels.[34] On the contrary, HDLs are part of the innate immune system. Therefore, a decrease in HDL levels is related to an increase in the severity of COVID-19, and after recovery, the HDL levels rise due to the decrease in inflammatory cytokines. In addition, it can be stated that high levels of Chol may be a risk factor for more severe COVID-19 symptoms. On the contrary, during the acute stage of the disease, the level of these lipids (HDL-c, Chol, and TG) is slightly reduced and often returns to normal after recovery. Therefore, the lipid profile of patients with COVID-19 should be periodically followed up during the infection and after treatment. Our accumulation data suggest that in order to generalize the findings of the present study at the individual level, further studies may be needed to better assess the effect of rs662 polymorphism on susceptibility to COVID-19 and other viral infections and their outcomes.

AUTHORS' CONTRIBUTIONS

All authors participated in the design, interpretation of the studies, analysis of the data, and review of the manuscript; GA: conceptualization, methodology, supervision. ZG: performing experiments, writing the original draft, statistical analysis. MS: performing experiments, statistical analysis. MA: sample collection. FS, MKH, MZ: resources, data curation, scientific and technical support. All authors read and approved the final manuscript.

DECLARATION OF CONFLICTING INTERESTS

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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SUPPLEMENTAL MATERIAL

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