High triglyceride to HDL-cholesterol ratio as a biochemical marker of severe outcomes in COVID-19 patients

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Accepted Version

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Estefanía Alcántara-Alonso[a*], Fernando Molinar-Ramos[b], Jesús A González-López[a], Viridiana Alcántara-Alonso[c], Marco A Muñoz-Pérez[a], José Juan Lozano-Nuevo[a], Daniel Rabindranath Benítez-Maldonado[a], Elizabeth Mendoza-Portillo[b*]

[a] Hospital General de Ticomán, Ciudad de México, México.

[b] Hospital General "Dr. Rubén Leñero," Ciudad de México, México.

[c] School of Biological Sciences, University of Reading, Reading RG6 6AS, United Kingdom.

* Corresponding authors

AAE:estefania.alcantara@hotmail.com Hospital General de Ticomán, Servicios de Salud Pública de la Ciudad de México, Plan de San Luis S/N, Col. Ticomán, Gustavo A. Madero, 07330, Ciudad de México, México.

MPE:elizabeth.mendozap@imss.gob.mx Hospital General "Dr. Rubén Leñero," Plan de San Luis -Salvador Díaz Mirón, Col. Santo Tomás, Miguel Hidalgo. 11340, Ciudad de México, México.

Abstract

Background & aims: Coronavirus disease 2019 (COVID-19) patients with severe complications have shown comorbidities with cardiovascular-disease, hypertension and type 2 diabetes mellitus; clinical disorders that share the common metabolic alterations of insulin resistance and dyslipidaemia. A high triglyceride to high density lipoprotein cholesterol (Tg/HDL c) ratio has been associated with reduced insulin sensitivity, metabolic syndrome and adverse cardiovascular events. Our aim in this study was to determine the association between different components of the lipid profile and particularly the Tg/HDL c ratio with severe complications like the requirement of invasive mechanical ventilation in COVID-19 patients.

Methods: We collected demographic, clinical and biochemical data to conduct a cohort study in 43 adult patients with confirmed COVID-19 diagnosis by quantitative polymerase chain reaction (qPCR) at baseline and in the subsequent 15 days. Patients were subjected to a very similar treatment scheme. Descriptive statistics, variable association and logistic regression were applied to identify predictors of disease severity among elements and calculations from the lipid profile.

Results: Patients were aged 57±14 years; 55.8% were male from which 75% required hospitalization and 44.2% were female who 58% were hospitalized. The most common comorbidities were type 2 diabetes mellitus (58%) and hypertension (40%). Hospitalized and critical care patients showed lower HDL c blood levels and increased Tg/HDL c ratio than those with outpatient management and mild/asymptomatic COVID-19. Tg/HDL c ratio correlated with variables of disease severity such as lactate dehydrogenase (LDH) levels (r=0.356; p<0.05); the National Early Warning Score 2 (NEWS 2)(r=0.495; p<0.01); quick sequential organ failure assessment (qSOFA)(r=0.538; p<0.001); increased need of oxygen support (r=0.447; p<0.01) and requirement of mechanical ventilation (r=0.378; p<0.05). Tg/HDL c ratio have a negative correlation with partial oxygen saturation/fraction of inspired

oxygen (SaO2/FiO2) ratio (r=-0.332;p<0.05). Linear regression analysis showed that Tg/HDL c ratio can predict increases in inflammatory factors like LDH (p<0.01); ferritin (p<0.01) and D-dimer (p<0.001). Logistic regression model indicated that ≥ 7.45 Tg/HDL c ratio predicts requirement of invasive mechanical ventilation (OR 11.815, CI 1.832-76.186, p<0.01).

Conclusions: The Tg/HDLc ratio can be used as an early biochemical marker of COVID-19 severe prognosis with requirement of invasive mechanical ventilation.

Keywords: Tg/HDLc ratio, COVID-19, HDL c, mechanical ventilation

Introduction

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), also known as coronavirus disease 2019 (COVID-19) is caused by a new positive-sense, single-stranded RNA virus which belongs to the β-coronaviruses lineage [1].

Around 80% of the COVID-19 infected patients are asymptomatic or develop very mild symptoms; however, in the remaining 20% a rapid disease progression leads to severe complications as hypoxemia (dyspnoea, central cyanosis and oxygen saturation lower than 92%), with the risk to develop respiratory distress syndrome; shock and multiple organ failure, increasing the mortality rate to more than 50% [2].

Severe COVID-19 has been linked to a proinflammatory cytokine storm defined as a massive release of tumour necrosis factor alpha (TNFα); interleukins (IL) IL-1β, IL-2, IL-6, IL-8; granulocyte colony-stimulating factor (G-CSF); along with reactive oxygen species and chemokines such as C-C motif chemokine ligands (CCL2, CCL3, CCL5) and interferon gamma-induced protein 10 (IP-10) [2,3].

Little is known about the immunological response against SARS-CoV2; nevertheless, clinical trials with patients infected with other coronaviruses, SARS-CoV and MERS-CoV, shown that increases in the serum concentration of proinflammatory cytokines is linked with lung inflammation and extensive lung injury [4]. The cytokine storm in other coronavirus infections is the result of an increase in neutrophil number; monocyte and macrophage hyperactivation, and lymphocyte Th1/ Th17 activation in the adaptive immune system, intensifying the inflammatory response [3].

Trained immunity is a memory-like feature of cells from the innate immune system like monocytes which develop epigenetic changes when Toll-like receptors (TLR) are activated by diverse antigens, promoting increased release of proinflammatory cytokines in a second exposure to an antigen [5]. The enhanced response depends on the ligand concentration and the type of receptor activated during the first antigen exposure [5]. Remarkably, several

endogenous ligands like saturated fatty acids, oxidized low-density lipoprotein and advanced glycation end products (AGEs) act as damage-associated molecular patterns (DAMPs) and are recognized by Toll-like receptors (TLR2 and TLR4) [6], activating inflammatory responses.

Patients with metabolic syndrome and insulin resistance commonly have higher concentration of these endogenous ligands; therefore, trained immunity could be involved in the increased synthesis of proinflammatory cytokines by the immune system of patients when infected with SARS-CoV-2 [7].

Compared with mild or asymptomatic COVID-19 patients, individuals with severe complications have higher prevalence of comorbidities such as hypertension, cardiovascular disease and type 2 diabetes mellitus [8]. These comorbidities share the common metabolic alterations of insulin resistance and dyslipidaemia; the later has been linked to severe COVID-19 by Choi et al. [9] and Hariyanto et al. [10].

The triglyceride to High Density Lipoprotein-cholesterol (Tg/HDL c) ratio has been associated with reduced insulin sensitivity [11] and adverse cardiovascular events [12] and has even been considered as a better marker of cardiovascular risk than serum concentrations of high- or low-density lipoproteins cholesterol (HDL c or LDL c) alone [12].

Given the lack of studies about a possible association between COVID-19 severity with different components of the lipid profile and particularly with the Tg/HDL c ratio; which represents an easy and affordable estimation of insulin resistance and cardiovascular risk; we here associate this ratio at the time of COVID-19 diagnosis with biochemical values of severity and the development of complications, identifying it as an early marker of negative prognosis.

Materials and Methods

Ethical approval

The research ethics review board at Secretaría de salud de la Ciudad de México (SEDESA) approved the protocol as it represents minimum risk and is in accordance with the WMA Declaration of Helsinki. All participants or their relatives provided written informed consent.

Study design and population

This is an observational cohort study using clinical data of 43 adult patients, 24 (55.8%) male and 19 (44.1%) female admitted from May to October 2020 in our institution with COVID-19 infection, confirmed by quantitative reverse transcriptase-polymerase chain reaction (qRT-PCR) from nasopharyngeal swab specimens in addition to characteristic symptomatology and computerized thoracic tomography imaging.

Patients who had previous dyslipidaemia treatment, uncontrolled type 2 diabetes mellitus or chronic kidney or liver disease were not included.

All patients received a standard clinical laboratory sampling at the baseline (COVID-19 diagnosis). Biochemical parameters were performed on DxC 700 AU chemistry analyser. All laboratory assays had completed the standardization and certification program. Epidemiologic and demographic features as well as the biochemical lipid profile including triglycerides (Tg), total HDL c and LDL c levels were archived. Tg/HDL c ratio was calculated. Other analyses include: hemogram, renal function tests (C cystatin, blood urea nitrogen, creatinine) and severity biochemical parameters (D-dimer, C reactive protein [CRP], erythrocyte sedimentation rate [ESR], ferritin and lactate dehydrogenase [LDH]). Partial oxygen saturation/fraction of inspired oxygen (SaO2/FiO2) ratio was evaluated, and the early warning scores National Early Warning Score 2 (NEWS 2); quick sequential organ failure assessment (qSOFA) and Comorbidity-Age-Lymphocyte Count-Lactate dehydrogenase score (CALL score). For the correlation assessment of the lipid profile with levels of respiratory support these were classified as 1. No complementary oxygen supply,

2. Conventional nasal cannula, 3. Conventional mask, 4. High-flow mask, 5. High-flow nasal cannula, 6. Non-invasive ventilation and 7. Invasive mechanical ventilation. Subsequent clinical assessment was done within 15 days of hospitalization or outpatient management. The incidence of acute kidney injury defined according the KDIGO criteria, requirement of invasive mechanical ventilation, vasopressor support, days of hospitalization and mortality were also assessed.

The criteria for severe COVID-19 were a respiratory rate > 30/min; O2 saturation < 93% and/or SaO2/FiO2 <160 (severe ARDS Acute Respiratory Distress Syndrome by Kigali criteria) and for critical care COVID-19 were requirement of invasive mechanical ventilation, shock and/or multiple organ failure. In this study both, outpatient management and hospitalized patients had antipyretic drugs and 5 mg/12 h of the JAK1/2 inhibitor ruxolitinib in patients with no-requirement of mechanical ventilation and 10 mg/1 2h in patients with mechanical ventilation. All hospitalized patients also had treatment with 6 mg/24 h of dexamethasone for 10 days and low-molecular weight heparin. Patients with critical care COVID-19 were treated at the intensive care unit of our institution.

Statistical analysis

Descriptive tabulation was done using data about age, sex, weight, comorbidities, biochemistry, acute kidney injury, requirement of invasive mechanical ventilation, vasopressor support, days of hospitalization and mortality. The Shapiro-Wilks test was employed to determine normality of data distribution. Statistical analysis of the categorical data was performed using the Chi-square and Fisher exact test. Student´s t-test was used to compare continuous values between two groups in which case data were normally distributed and non-parametric Mann-Witney U test used when data were not normally distributed. Kruskal-Wallis test was used to compare continuous values between three groups in which case data were not normally distributed and followed by a Games-Howell post hoc test. Correlation analysis to study biochemical variables and COVID-19 severity

was done using Spearman test. The simple linear regression analysis and multivariate logistic regression analysis of lipid profile associated with severity of COVID-19 infection. The optimal cut-off values were calculated in accordance with the receiver operating characteristic (ROC) curves and Youden´s index. Data were analysed using IBM SPSS version 20. Statistical test differences were considered significant when *p*-values were < 0.05.

Results

Outpatient management were done in 14 individuals while 29 developed severe COVID-19 and were hospitalized (18 male and 11 females). Average age was 57 (± 13.9) years, not different between outpatient and hospitalized treatment (p= 0.508). Demographic features are shown in **Table 1**.

The most common comorbidities were type 2 diabetes mellitus and hypertension with higher prevalence (2.6 and 1.8 times higher respectively) in hospitalized than outpatient management, **Table 1**. Other comorbidities were chronic obstructive pulmonary disease (COPD) in 4 patients, ischemic cardiomyopathy in 2 patients and vitiligo in 1 patient.

There were statistically significant changes (p<0.001) with a 3.6 times higher NEWS 2 and 11-fold increased qSOFA score as well as decreased SaO2/FiO2 ratio in hospitalized versus outpatient management **Table 1**.

Hospitalized patients also showed higher values of leukocytes (2.1-fold), neutrophil/lymphocyte ratio (2.2-fold), CRP (5.3-fold), LDH (1.76-fold), ESR (1.6-fold), Ferritin (2.2-fold), D-dimer (3.5-fold) and fibrinogen (1.3-fold). All these differences were statistically significant (p<0.01) **Table 1**.

Table 1. Clinical characteristics of patients with COVID-19

	Global (n=43)	Outpatient management (n=14)	Hospitalized (n=29)	
Variable				***p***
Gender (%)				
Male**	24 (55.8)	6 (25)	18 (75)	0.235
Female**	19 (44.1)	8 (42.1)	11 (57.8)	¨
Age (years)*	57.19 (± 13.9)	59.8 (± 17.0)	55.8 (± 12.2)	0.508
Comorbidities (%)				
Diabetes**	25 (58.1)	7 (28)	18 (72)	0.616
Hypertension**	17 (39.5)	6 (35.2)	11 (64.7)	0.616
NEWS 2*	8 (0-16)	2 (0-8)	8 (4-16)	**<0.001**
q SOFA*	1 (0-3)	0 (0-2)	1 (0-3)	**<0.001**
SaO2/FiO2*	265 (81-452)	438 (419-452)	180 (81-438)	**<0.001**
CALL Score*	8 (4-13)	9 (4-12)	8 (4-13)	0.905
Leukocytes (x10e3/µL)*	8.3 (2.7-21.6)	4.7 (2.7-9.7)	10.7 (4.5-21.6)	**<0.001**
Lymphocytes (x10e3/µL)*	1.2 (0.5-7.5)	1.2 (0.5-2.6)	1.3 (0.8-7.5)	0.372
NLR*	3.9 (0.9-17.1)	2.3 (1.5-9.0)	4.5 (0.9-17.1)	**0.003**
CRP (mg/dL)*	9.9 (0.2-39.4)	3.3 (0.2-10.5)	17.6 (0.4-39.4)	**<0.001**
LDH (IU/L)*	326 (149-1053)	219 (149-413)	398 (159-1053)	**0.003**
ESR (mm 3/hr)*	45 (18-60)	27 (18-50)	45 (27-60)	**<0.001**
Ferritin (µg/L)*	601 (41-3629)	361 (41-700)	658 (204-3629)	**0.002**
D-dimer (ng/mL)*	644 (99-4790)	99 (99-2760)	882 (99-4790)	**0.001**
Fibrinogen (mg/ dL)*	508 (250-713)	408.5 (250-533)	584 (294-713)	**0.026**

Data were expressed as mean ± standard deviation or median (range).

The value of *p* was derived from *U Mann Whitney test; **Chi-squared test. Statistical significance *p* values are shown in bold ($p < 0.05$).

Abbreviations: CALL Score, Comorbidity-Age-Lymphocyte count-Lactate dehydrogenase score; ESR, Erythrocyte sedimentation rate; HbA1c %, Glycated hemoglobin; LDH, Lactate dehydrogenase; NLR, Neutrophil-lymphocyte ratio; NEWS 2, National Early Warning Score 2; qSOFA, Quick SOFA score; CRP, Reactive C protein; SaO2/FiO2, Saturation/Fraction of inspired oxygen; SD, Standard deviation.

Regarding the lipid profile, they showed 25 % lower HDL c blood levels ($p<0.01$) and increased Tg/HDL c (2.7-fold) ratio than those of outpatient management ($p<0.01$) **Table 2**.

Table 2. Lipid profile of patients with COVID-19

	Global (n=43)	Outpatient management (n=14)	Hospitalized (n=29)	
Variable		**Median (range)**		***p***
Triglyceride (mg/dL)	147 (72-513)	138 (83-231)	164 (72-513)	0.159
Total Cholesterol (mg/dL)	142.5 (70-283)	147 (70-192)	130 (71-283)	0.554
HDL c (mg/dL)	29.5 (9.6-63.5)	40.5 (20.1-51.6)	25.6 (9.6-63.5)	**0.009**
LDL c (mg/dL)	76.4 (25-166)	68 (28.1-120)	78 (25-166)	0.822
Tg/HDL c	5 (1.3-26.4)	3.3 (2.7-7.2)	6.4 (1.3-26.4)	**0.005**

The value of *p* was derived from U Mann Whitney test. Statistical significance *p* values are shown in bold ($p < 0.05$).

Abbreviations: HDL c, High-density lipoprotein colesterol; LDL c, Low-density lipoprotein colesterol; SD, Standard deviation; Tg /HDL c, Triglyceride to HDL Cholesterol Ratio.

These differences were also observed between the critical care COVID-19 patients and the ones with mild symptoms, Games-Howell post-hoc test indicate significant lower HDL c ($p<0.05$) and higher Tg/HDL c ratio ($p<0.05$) levels **Table 3**.

Table 3. Differences in the lipid profile of patients with COVID-19 by degree of severity

	Global (n=43)	Mild-Moderate (n=10)	Severe (n=17)	Critical (n=16)	
Variable			**Median (range)**		***p***
Triglyceride (mg/dL)	140 (72-513)	142.5 (89-231)	132.5 (83-288)	167 (72-513)	0.421
Total Cholesterol (mg/dL)	142.5 (70-283)	152 (70-192)	128 (80-283)	136 (71-198)	0.732
HDL c (mg/ dL)	29.5 (10.4-63.5)	39.9 (20.1-51.6)	27.6 (19.3-63.5)	22.15 (10.4-56.5)[&]	**0.018**
LDL c (mg/ dL)	76.4 (25-166)	71.3 (28.1-120)	78 (38.6-166)	79.45 (25-125)	0.903
Tg/HDL c	5 (1.3-26.4)	3.45 (2.5-7.2)	5 (1.8-8.2)	7.5 (1.3-26.4)[&]	**0.034**

The value of p was derived from Kruskal-Wallis test. Statistical significance *p* values are shown in bold ($p < 0.05$).

[&] Games-Howell post hoc test ($p<0.05$).

Abbreviations: HDL c, High-density lipoprotein colesterol; LDL c, Low-density lipoprotein colesterol; SaO2/FiO2, Saturation/Fraction of inspired oxygen, Partial Pressure of Arterial Oxygen to Fraction of Inspired Oxygen Ratio; SD, Standard deviation; Tg/HDL-C, Triglyceride to HDL Cholesterol Ratio.

On average, hospitalization time was 15.2 ± 7.9 days. 14 patients (48.2%) had acute kidney injury, 10 (34.4%) vasopressor support and 16 (55.1%) required invasive mechanical ventilation. In the outpatient management, only 4 (28.5%) suffered acute kidney injury. 10 (34.4%) of the hospitalized patients died within the 15 days of the study.

Correlation between lipid profile, Tg/HDL c ratio and severity criteria

A high Tg level at the time of COVID-19 diagnosis was correlated with high LDH level and NEWS 2 **Table 4**. HDL c levels were correlated with SaO2/FiO2 ratio and negatively correlated with LDH, ferritin, NEWS 2, qSOFA, requirement of mechanical ventilation and days of hospitalization **Table 4**. Hence, the Tg/HDL c ratio were also correlated with LDH, NEWS 2, qSOFA as well as requirement of mechanical ventilation and negatively correlated with SaO2/FiO2 ratio **Table 4**. Total cholesterol and LDL c levels showed no correlation with the evaluated parameters **Table 4.**

Table 4. Bivariate correlations between the variables studied and the lipid profile of COVID-19 patients.

Variable	Triglycerides		Total cholesterol		LDL c		HDL c		Tg/HDL c	
	p	*r*	*p*	*r*	*p*	*r*	*p*	*r*	*p*	*r*
CRP	0.745	0.052	0.882	0.024	0.606	0.085	0.146	-0.237	0.193	0.213
LDH	**0.050**	**0.312**	0.323	0.160	0.066	0.306	**0.049**	**-0.326**	**0.030**	**0.356**
ESR	0.707	0.070	0.876	-0.029	0.696	-0.076	0.758	-0.059	0.201	0.240
Ferritin	0.540	0.110	0.400	-0.151	0.817	-0.043	**0.009**	**-0.456**	0.113	0.286
D-dimer	0.513	0.109	0.997	-0.001	0.684	0.071	0.093	-0.288	0.202	0.221
Fibrinogen	0.633	-0.089	0.998	0.001	0.941	0.014	0.907	0.022	0.473	0.136
Leukocytes	0.158	0.224	0.780	0.045	0.922	-0.016	0.380	-0.170	0.171	0.227
Lymphocytes	0.749	-0.052	0.096	-0.263	0.270	-0.183	0.088	-0.281	0.273	0.183
NLR	0.295	0.168	0.347	0.151	0.820	0.038	0.813	0.040	0.714	0.061
SaO2/FiO2	0.201	-0.206	0.623	0.080	0.663	0.074	**0.011**	**0.414**	**0.045**	**-0.332**
NEWS 2	**0.043**	**0.314**	0.952	-0.010	0.991	0.002	**0.002**	**-0.480**	**0.001**	**0.495**
q SOFA	0.070	0.283	0.516	-0.103	0.609	-0.085	**<0.001**	**-0.558**	**<0.001**	**0.538**
CALL score	0.621	0.081	0.385	0.141	0.092	0.281	0.649	0.077	0.896	-0.022
Acute kidney failure	0.213	0.196	0.228	0.190	0.062	0.301	0.865	0.028	0.355	0.152
Vasopressor support	0.254	0.180	0.717	-0.058	0.270	-0.181	0.209	-0.206	0.324	0.162
Invasive mechanical ventilation	0.194	0.204	0.869	-0.026	0.830	0.036	**0.006**	**-0.432**	**0.018**	**0.378**
Days of hospitalization	0.542	0.099	0.419	-0.132	0.683	-0.070	**0.031**	**-0.355**	0.064	0.308
Death	0.476	0.113	0.908	0.018	0.295	0.172	0.119	-0.254	0.167	0.226

The value of *p* was derived from Spearman's Rank Correlation Coefficient. Statistical significance *p* values are shown in bold ($p < 0.05$).

Abbreviations: CALL Score, Comorbidity-Age-Lymphocyte count-Lactate dehydrogenase score; ESR, Erythrocyte sedimentation rate; HDL c, High-density lipoprotein colesterol; LDH, Lactic dehydrogenase; LDL c, Low-density lipoprotein colesterol; NLR, Neutrophil-lymphocyte ratio; NEWS 2, National Early Warning Score 2; qSOFA, Quick SOFA score; CRP, Reactive C protein; SaO2/FiO2, Saturation/Fraction of inspired oxygen; Tg/HDL c, Triglyceride to HDL Cholesterol Ratio.

We found a negative correlation between HDL c level and requirement of higher oxygen supply (r=-0.475; p=0.002) and a positive correlation with the Tg/HDL c ratio (r=0.447; p=0.004).

Simple lineal regression analyses were used to investigate if any of the lipid profile components and/or the Tg/HDL c ratio are predictive of the biochemical variables of severity. LDH blood levels were predicted by Tg concentration (F=11.000; R^2=0.225; Coefficient B=0.90; SE 0.272; p=0.002) and Tg/HDL c ratio (F=12.200; R^2=0.259; Coefficient B=10.6; SE 3.030; p=0.001). Ferritin blood levels were predicted by Tg concentration (F=5.356; R^2=0.147; Coefficient B=2.162; SE 0.934; p=0.027), HDLc level (F=6.583; R^2=0.180; Coefficient B=-20.237; SE 7.887; p=0.016) and Tg/HDL c ratio (F=7.929; R^2=0.209; Coefficient B=28.1; SE 9.980; p=0.009). D-dimer blood levels were predicted by Tg concentration (F=10.985; R^2=0.234; Coefficient B=6.2; SE 1.876; p=0.002) and Tg/HDL c ratio (F=16.237; R^2=0.330; Coefficient B=80.9; SE 20.093; p=<0.001). No association was found for CRP, ESR or fibrinogen.

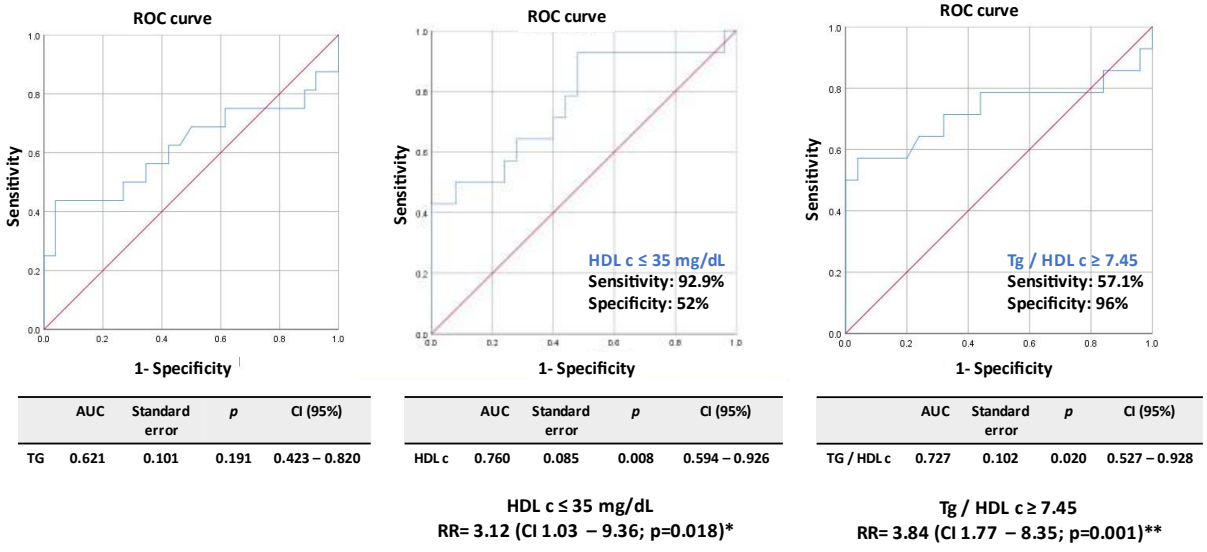
Univariate logistic regression analysis also showed that predictors of invasive mechanical ventilation requirement were Tg concentration (R^2 Nagelkerke=0.173; OR 1.008, CI 1.000 – 1.016; p=0.040), HDL c concentration (R^2 Nagelkerke=0.232; OR 0.919, CI 0.855 – 0.987; p=0.021) and Tg/HDL c ratio (R^2 Nagelkerke=0.394; OR 1.292, CI 1.025 – 1.629; p=0.03). None of the lipid profile components nor the Tg/HDL c ratio were predictors of acute kidney injury, vasopressor support or death.

The prediction for invasive mechanical ventilation requirement was also determined by ROC curve analysis calculating the AUC. For HDL c levels (AUC 0.760) and for Tg/HDL c ratio (AUC 0.727) predicted requirement of invasive mechanical ventilation **Figure 1**.

Determination of cut-off points based on the maximum value of the Youden Index revealed that ≤35 mg/dL of HDL c blood concentration showed a relative risk (RR) YO LE QUITARÍA TAMBIÉN ESTO: of 3.12 (CI 1.03 – 9.36; p=0.018) for the requirement of invasive

mechanical ventilation with 92.9% sensitivity and 52% specificity **Figure 1** and RR of 8.64 (CI 1.23 – 60.60; *p*=0.03) to have a SaO2/FiO2 index <160 with 100% sensitivity, 51.9% specificity **Figure 2**. For the Tg/HDL c ratio a value of ≥ 7.45 had a RR of 3.84 (CI 1.77 – 8.35; *p*=0.001) for requirement of invasive mechanical ventilation with 57.1% sensitivity and 96% specificity **Figure 1** and RR of 3.69 (CI 1.48 – 9.15; *p*=0.009) to have a SaO2/FiO2 index <160 with 58.3 % sensitivity and 92.6 % specificity **Figure 2**.

Figure 1. Analysis of the ROC curve of the triglyceride level, HDL-C and the TG / HDL-C ratio for the requirement of invasive mechanical ventilation in COVID-19 patients



	AUC	Standard error	*p*	CI (95%)
TG	0.621	0.101	0.191	0.423 – 0.820

	AUC	Standard error	*p*	CI (95%)
HDL c	0.760	0.085	0.008	0.594 – 0.926

HDL c ≤ 35 mg/dL
RR= 3.12 (CI 1.03 – 9.36; p=0.018)*

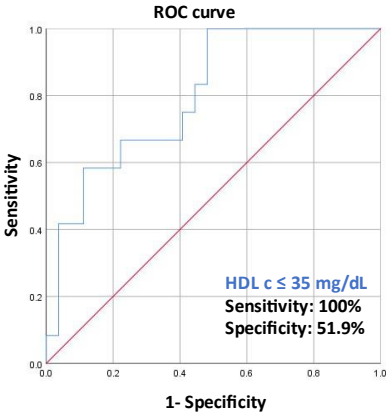
	AUC	Standard error	*p*	CI (95%)
TG / HDL c	0.727	0.102	0.020	0.527 – 0.928

Tg / HDL c ≥ 7.45
RR= 3.84 (CI 1.77 – 8.35; p=0.001)**

The value of p was derived from *Chi-squared test, **Fisher's exact test. Statistical significance *p* <0.05.

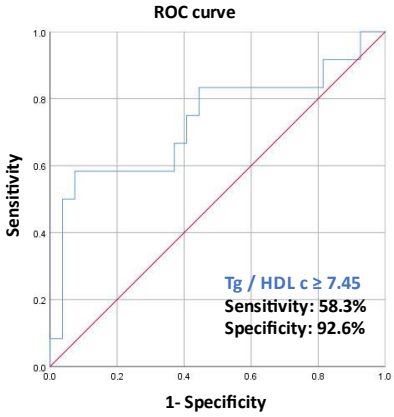
Abbreviations: AUC, Area Under the Curve; CI, Confidence interval; HDL c, High-density lipoprotein colesterol; NEWS 2, National Early Warning Score 2; ROC, Receiver-Operating Characteristic; Tg/HDL c, Triglyceride to HDL Cholesterol Ratio.

Figure 2. Analysis of the ROC curve of the HDL c level and the TG / HDL c ratio with the SaO2/FiO2 index <160 in COVID-19 patients.



	AUC	Standard error	*p*	CI (95%)
HDL c	0.779	0.073	0.003	0.656 – 0.943

HDL c ≤ 35 mg/dL
RR= 8.64 (CI 1.23 – 60.60; p=0.003)*



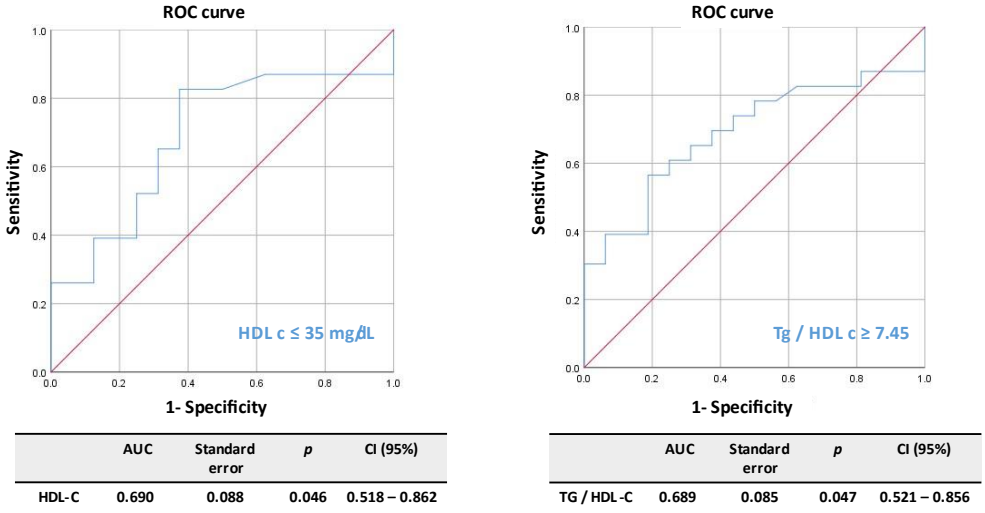
	AUC	Standard error	*p*	CI (95%)
Tg / HDL c	0.731	0.098	0.022	0.539 – 0.924

Tg / HDL c ≥ 7.45
RR= 3.69 (CI 1.48 – 9.15; p=0.009)**

The value of p was derived from *Chi-squared test, **Fisher's exact test. Statistical significance *p* <0.05. Abbreviations: AUC, Area Under the Curve; CI, Confidence interval; HDL c, High-density lipoprotein colesterol; ROC, Receiver-Operating Characteristic; Sa/Fi, Saturation/Fraction of inspired oxygen; Tg/HDL c, Triglyceride to HDL Cholesterol Ratio.

HDL c blood concentration ≤35 mg/dL and Tg/HDL c ratio ≥ 7.45 also predicted a NEWS 2 ≥ 2 (AUC 0.69 and 0.689 respectively) **Figure 3**.

Figure 3. Analysis of the ROC curve of the HDL c level and the Tg / HDL c ratio with NEWS 2 in COVID-19 patients.



	AUC	Standard error	*p*	CI (95%)
HDL-C	0.690	0.088	0.046	0.518 – 0.862

	AUC	Standard error	*p*	CI (95%)
TG / HDL-C	0.689	0.085	0.047	0.521 – 0.856

Statistical significance *p* <0.05. Abbreviations: AUC, Area Under the Curve; CI, Confidence interval; HDL c, High-density lipoprotein colesterol; NEWS 2, National Early Warning Score 2; ROC, Receiver-Operating Characteristic; Tg/HDL c, Triglyceride to HDL Cholesterol Ratio.

Binomial multivariate logistic regression revealed that ≤35 mg/dL HDL c blood concentration (OR 13.716, CI 1.485 – 126.654; *p*=0.021) and ≥ 7.45 Tg/HDL c ratio (OR 11.815, CI 1.832 – 76.186; *p*=0.009) are predictors adjusted by age and gender for the requirement of invasive mechanical ventilation **Table 5**.

Table 5. **HDL c level and Tg / HDL c ratio as risk factors adjusted to age and gender for the requirement of invasive mechanical ventilation in COVID-19 patients.**

	Variable	Sig Chi2	R^2 Nagelkerke	Coefficient B	Standard error	OR Multivariate (CI 95%)	*p*
Step 1	**HDL c ≤ 35 mg/ dL**			2.619	1.134	**13.716** (1.485 – 126.654)	**0.021**
	Tg/HDL c ≥ 7.45			2.469	0.951	**11.815** (1.832 – 76.186)	**0.009**
	Age	**<0.001**	**0.542**	-0.064	0.035	0.938 (0.876 – 1.005)	0.069
	Gender			-0.246	0.873	0.782 (0.141 – 4.325)	0.778
	Constant			1.858	2.220	6.409	0.403
Step 2	**HDL c ≤ 35 mg/ dL**			2.633	1.129	**13.916** (1.523 – 127.171)	**0.020**
	Tg/HDL c ≥ 7.45	**<0.001**	**0.541**	2.463	0.952	**11.742** (1.817 – 75.854)	**0.010**
	Age			-0.064	0.035	0.938 (0.876 – 1.006)	0.071
	Constant			1.694	2.162	5.440	0.433

The value of p was derived from Multivariate logistic regression analysis. Method of successive steps backward (conditional). Statistical significance *p* values are shown in bold ($p < 0.05$).

Abbreviations: HDL c, High-density lipoprotein colesterol; LDL c, Low-density lipoprotein colesterol; OR, odds ratio; SD, Standard deviation; Tg/HDL c, Triglyceride to HDL Cholesterol Ratio.

Discussion

Key factors linked to the development of cardiovascular disease, hypertension and type 2 diabetes mellitus are obesity, dyslipidemia and insulin resistance [13].

Mexico has a high prevalence of metabolic syndrome and related chronic diseases. According to the most recent national health and nutrition survey (*Encuesta Nacional de Salud y Nutrición, [ENSANUT] de Medio Camino 2016)* [14], Mexican adults have a 71.2% prevalence of overweight and obesity, 25.5% have hypertension and 13.7% have type 2 diabetes mellitus [14,15]. In *ENSANUT 2012*, was reported dyslipidaemia in 50- to 59-year-olds with a 36.8% prevalence of ≥ 200 mg/dl total cholesterol; 58.7% of < 40 mg/dl HDL c; 60.1% of ≥ 150 mg/dl Tg and 61.7% of ≥ 100 mg/dl LDL c. A common phenotype in this age group was that of high Tg with low HDL c blood levels [16].

In Mexico, by February 26nd 2021, there was 2,069,370 confirmed cases and 183,692 associated COVID-19 deaths becoming the country with the mortality rate (8.9%) with 145.57 deaths per 100,000 habitants [17].

This high mortality rate in COVID-19 patients may be related with the elevated risk to develop severe complications in individuals with cardiovascular disease, hypertension and type 2 diabetes mellitus [8]. As dyslipidaemia is a common feature in these metabolic disorders and has been linked with severe COVID-19 [9,10]; our results confirm that dyslipidaemia and particularly a high Tg/HDL c ratio at the time of COVID-19 diagnosis can be used as a prognosis factor for disease severity and requirement for invasive mechanical ventilation.

Although we found no differences in gender, age or comorbidities between hospitalized versus outpatient management, a higher percentage of men 75%, required hospitalization compared with 57.8% of women; a point already observed for COVID-19 complications and viremia [8,18,19]. Additionally, 72% of patients with type 2 diabetes mellitus and 64.7% with hypertension were hospitalized. These are commonly reported comorbidities in hospitalized COVID-19 patients [8,18,19].

Our analysis revealed that a low plasmatic level of HDL c and elevated Tg/HDL c ratio at the time of COVID-19 diagnosis is linked with requirement of hospitalization and illness severity as critical care patients showed the lowest HDL c concentration and highest Tg/HDL c compared with severe and mild/asymptomatic COVID-19 patients.

Elevated Tg and low HDL c were constantly associated with clinical and biochemical data of COVID-19 severity like increased LDH levels which is an inflammatory marker previously proposed as predictor of poor prognosis in COVID-19 patients [20,21] and with NEWS 2 an early warning score published by the Royal College of Physicians [22] with a good performance in the prediction of severity and mortality in COVID-19 patients [22–24]. A low HDL c concentration was additionally linked with high ferritin levels, a protein associated

with inflammatory processes and previously reported increased in severe COVID-19 patients [25], related with SARS-CoV-2 viremia [18] and proposed as predictor of mortality for the disease [26,27]. In addition, low HDL c levels were associated with high qSOFA, another early warning score associated with COVID-19 mortality [27]. Remarkably, we found negative correlations between plasmatic HDL c and length of hospitalization along with requirement of invasive mechanical ventilation and a positive correlation with SaO2/FiO2 index, all variables related to the severity of COVID-19.

Our results are in line with those reported previously [28–30] of a higher prevalence of severe COVID-19 cases in patients with low HDL c levels. There is also observed an association between lower concentrations of apolipoprotein ApoA1, one of its major structural components, which is inversely correlated with inflammatory states, disease severity and mortality [30,31].

HDL c has immunomodulatory effects via its binding to pathogen-associated molecules like bacterial-lipopeptides (lipopolysaccharide, lipoteichoic acid) [32] and diacylated peptides like Pam2CSK4, FSL-1 which neutralize the infectious activity by blocking TLR2 and TLR4 responses [33] as well as by inhibiting the production of inflammatory cytokines by macrophages [34].

Besides, elevated serum HDL c can also promote proliferation of IgA in early stages of bacterial infection [35], but it is not known if a similar mechanism could be prevented COVID-19 viremia and derived complications. Nevertheless, as HDL c particles transport paraoxonase 1 which has antiviral properties; this could induce virus inactivation [36] and ApoA1 has shown protective effects in several lung disease conditions, including viral pneumonia [37] although the molecular mechanism involved is not yet elucidated.

Given the observed associations of clinical and biochemical markers of severity with high Tg and low HDL c, our analysis of Tg/HDL c ratio correlations reveal positive associations

with LDH, NEWS 2, qSOFA, length of hospitalization, requirement of invasive mechanical ventilation and a positive correlation with SaO2/FiO2 index.

To our knowledge this is the first study assessing the correlation between Tg /HDL c ratio and COVID-19 complications.

Tg/HDL c ratio has been considered as a better marker of cardiovascular risk than serum concentrations of HDL c and LDL c alone [12] and given its good correlation with the hyperinsulinemic-euglycemic clamp, an accurate estimation of insulin sensitivity; Tg/HDL c ratio can also reflect glucose-metabolic alterations [38].

Hyperinsulinemia of patients with insulin resistance and diabetes could also contribute to increased SARS-CoV-2 viremia [18] as insulin increases membrane expression of angiotensin-converting enzyme 2 (ACE 2) in pneumocyte [39] which function as receptor of the SARS-CoV-2 spike protein causing the cell infection.

In our study, higher Tg/HDL c ratio were observed in hospitalized versus outpatient management and in critical care patients versus mild/asymptomatic individuals. Additionally, high Tg/HDL c ratio were also correlated with increased need for oxygen support.

Greater risk to develop complications and requirement of oxygen assistance are reported in COVID-19 patients with diabetes [18,19]. Hyperinsulinaemia and hyperglycaemia increase inflammation and risk of thrombosis by increasing coagulation [40]. Hyperinsulinemia increases plasminogen activator type 1 levels, promoting thrombi by inhibition of fibrinolysis while hyperglycaemia increase blood coagulation and production of proinflammatory cytokines TNF-alpha and IL-6 [41].

Pulmonary thrombi may contribute to oxygen desaturation and respiratory distress in COVID-19 cases [40] and this could be promoted by insulin resistance which raise glucose and insulin circulating levels.

However, more studies are needed to associate hyperinsulinemia with inflammatory and thrombotic processes in COVID-19 and its direct link with Tg/HDL c ratio in severe patients

could indicate hypoxemia and deficient pulmonary function in COVID-19 severe and critical patients.

Conclusion

Our results emphasize the use of parameters of the lipid profile such as HDL c level as a marker of risk for severe COVID-19 outcomes. Moreover, our findings disclose that the Tg/HDL c ratio is a novel biochemical marker of severe prognosis and requirement of invasive mechanical ventilation in COVID-19 patients.

Limitations

One of the limitations of the present study is the lack of information about the lipid profile of the patients before infection. There are reports about effects of SARS-CoV-2 on metabolic features and one observation is decreased levels of total cholesterol, LDL c and HDL c, that fell continuously until the 9^{th} day of infection and then return to normal levels [36,42]. Therefore, it is not possible to exclude a viral effect on the low HDL c levels found in severe COVID-19 patients at the time of diagnosis. Another limitation is the reduced sample size; however, the strength is the surveillance of the patients during the 15 days following COVID-19 diagnosis, with a very similar treatment scheme for an accurate variable association in a homogeneous population.

Statement of authorship

AAE: Conceptualization, data collection, statistical analysis, data interpretation and article writing. MPE: Conceptualization, data interpretation and article writing. MRF: Conceptualization. BMDR: Conceptualization. GLJA: Data collection. MPMA: Data collection. LNJJ: statistical analysis. AAV: data interpretation and article writing. All authors read and approved the final manuscript.

Funding source

Secretaría de salud de la Ciudad de México (SEDESA).

Conflict of interest

The authors declare no competing interests.

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