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Determine the effect of the lipid peroxidation end product (4-Hydroxynonenal) in the context of atherogenic among COVID-19 cases

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***Abstract*---**Background: Coronavirus disease 2019 (COVID-19) manifests as multiple clinical and pathological organ dysfunctions. It also disrupts metabolic profile due to the release of pro-inflammatory cytokines causing a systemic inflammation reaction. As the number of COVID-19 complications continues to rise worldwide, the identification of risk factors for the disease is an urgent issue, and it remains controversial whether atherogenic lipid-related traits including lipid peroxidation product 4-Hydroxynonenal and lipid profile panel levels, are risk factors. The aim of this study was to estimate causal effects of lipid-related factors on COVID-19 risk Methods: This case control study includes (90) subjects, were divided into: (30) critical, (30) sever COVID-19 groups, and (30) control group. Samples of COVID-19 patients were collected from Al-Hayat unit at Imam Hussein Medical City/ Iraq. This study was included all patients that confirmed with positive RT-PCR in the hospital, and only smoked patients were excluded….Results: The results of this study found significant increase in HNE levels in sever and critical groups compared to control group. Lipid profile, in critical COVID-19 group, showed very high significant increase in means of TG, VLDL, LDL, and non-HDL when compared with control group, and a massive significant decreases in means of HDL compared with control group. While T-Cholestrol displayed a significant differences in mean when compared with control group. Atherogenic indices, in sever COVID-19, AC and CRI-I, C-index and CRI-II were showen a significant increases. On the other hand, critical group indicated a statistically significant difference among all atherogenic indices. Conclusion : COVID-19 may be the trigger factor in the progression of the

atherosclerotic process up tomaking it clinically manifest. The thrombotic complications can involve in the atherosclerotic plaque due to increasing the levels of atherogenic context.

***Keywords*---**4-hydroxynonenal (HNA), COVID-19, Lipid Profile, Atherogenic indices.

Introduction

The coronavirus pandemic appeared as the worst global health disaster of the century; humankind has experienced the most challenging health emergencies. The novel respiratory disease (SARS-CoV-2) emerged in Wuhan at the end of December 2019. As on 1 July 2022, the WHO reported that there were 564,126,546 confirmed cases worldwide with 6,371,354 confirmed deaths. Therefore, it is important to know the best biomarkers which might be associated with disease severity in order to avoid more death cases. The biomarkers that studied in COVID-19 were varied and they had significant role in the diagnosis, treatment, and prediction of the clinical outcomes of patients. Currently, there are few reports about the relationship between HNE and COVID-19 severity.

1.1. Lipid peroxidation and Atherosclerosis

lipid molecule is oxidized upon lipid radical formation. The initiators of non-enzymatic lipid peroxidation are mainly hydroxyl and hydroperoxyl radicals that initiate oxidative chain reactions. The most sensitive molecules that undergo peroxidation are membrane phospholipids containing polyunsaturated fatty acids (PUFAs), including arachidonic, linoleic, linolenic, eicosatetraenoic, and docosahexaenoic acids. Enzymatic lipid peroxidation is primarily catalyzed by cyclooxygenases (COX)***(Liu et al., 2015),*** lipoxygenases (LOX)***(Murakami, 2015),*** and phospholipase A (PLA)***(Adibhatla & Hatcher, 2006).***

Among the many different aldehydes which can be formed as secondary products during lipid peroxidation, malondialdehyde (MDA), propanal, hexanal, and 4-hydroxynonenal (4-HNE). MDA appears to be the most mutagenic product of lipid peroxidation, whereas 4-HNE is the most toxic***(Girotti, 1998).***

1.2. 4-Hydroxynonenal

HNE is the major type of 4-hydroxyalkenals end product, generated by decomposition of arachidonic acid and PUFAs, through enzymatic or nonenzymatic processes. The affinity of HNE to bind to proteins, modifying their structure and function and acting dynamically in a concentration-dependent manner not only as a cytotoxic product of lipid peroxidation, but also as a signaling, regulatory molecule, makes HNE a valuable bioactive marker for various diseases associated with oxidative stress(Zarkovic et al., 2017) and their respective therapies(Jaganjac et al., 2020), suggesting it could also be a predictive biomarker of SARS-CoV-2 induced oxidative stress. 4-HNE directly contributes to many atherosclerosis-based cardiovascular diseases. 4-HNE is present in vascular smooth muscle cells of the atherosclerotic tissue, and its detection in

both oxidized low-density lipoprotein (Ox-LDL) and fibrotic plaques in atherosclerotic patients has demonstrated its potential involvement in the pathogenesis of atherosclerosis(Leonarduzzi et al., 2005). Accordingly, this is an active area of research , more data are needed to clarify similarities and differences of the 4-Hydroxynonenal (HNA) level in covid-19 (moderate, sever) cases and healthy. Such issues are especially relevant given the potential use 4-Hydroxynonenal (HNA) as a marker of promote iron accumulation which happens in the infected cells.

2. Data Collection

Samples of patients with full clinical history, clinical examination, and relevant laboratory investigations of covid19 were collected .The diagnosis of the Covid 19 clinical conditions was established according to the latest clinical practice guidelines by the WHO.

A detailed questionnaire was prepared to obtain information that helps in selecting people according to the selection criteria for research. The sociodemographic data of the patients were involved age, gender, and having any current chronic diseases (Hypertension, Diabetes, and Autoimmune diseases). Smoking state, body temperature, dry cough, and Oxygen-level saturation were taken from each patient. Categorical variables were described as frequency rates and percentages, and continuous variables were described using Mean ±SD values. The clinical demographic characteristics and laboratory parameters of different stages of COVID-19 severity were summarized in Table (1).

Table (1): Demographic characteristics and medical history of Covid-19 patients and control groups

Characteristics		Severe Covid-19 N=30	Critical Covid-19 N=30	Control Group N=30
Age (Mean ± SD)		55.45 ± 13.86	61.4±17	57.7±9.2
Gender N (%)	Male	13 (43.3)	12 (40)	13 (43.3)
	Female	17 (56.6)	18 (60)	17 (56.6)
DM N (%)		0 (0%)	10 (33.3%)	0 (0%)
HT N (%)		0 (0%)	5 (16.7%)	0 (0%)
SPO2 (Mean ± SD)		96.1 ± 2.9	88.7 ± 7.6	98±1

DM: Diabetes Mellitus, HT: Hypertension, N=Number of subjects, %= Percent of subjects.

3. Methods

The medical data of (60) COVID-19 patients admitted to Al-Hayat unit at Imam

Hussein Medical City/ Kerbala were collected. Levels of HNE was measured using ELISA Technique and lipid profile were measured by UV-VIS Spectrophotometer.

4. Statistical Analysis

The data analysis for this work was generated using the Real Statistics Resource Pack software for Mac (Release 7.2) of the resource pack for Excel 2016. The distribution of the data was checked for normality using the Box plot test. ANOVA test was used to obtain the differences in mean between group. Pearson correlation coefficient test was used to calculate the association coefficient (r) by using R software (Version 4.0.0). $P < 0.05$ was considered statistically significant.

5. Results

5.1. Results of HNE

Mean values of HNE levels for patient groups (sever , critical) COVID-19 and control group were 2.78, 2.62, 2.39 ng/ml, respectively, as *shown* in Figure (1).

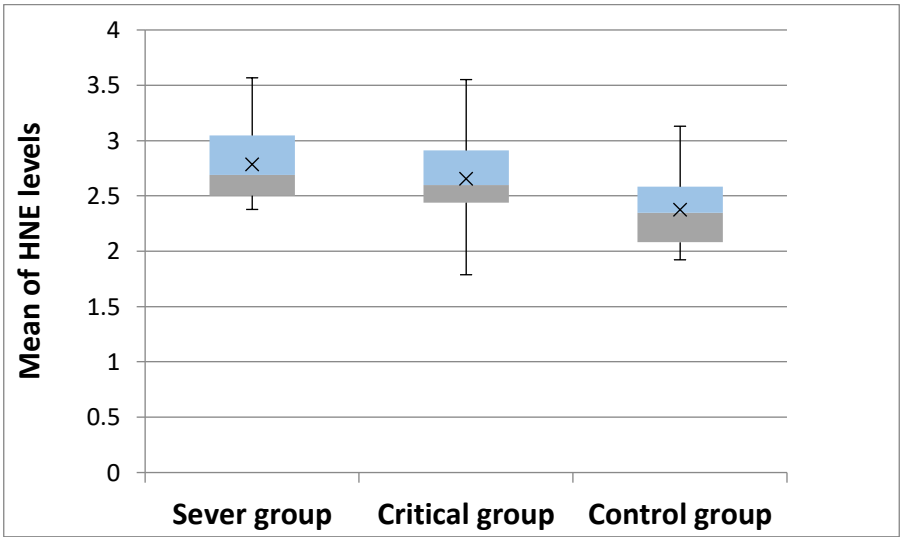


Figure (1): Boxplot explain HNE level in control and COVID-19 groups.

The analysis of HNE levels in this study indicated a significant increase in HNE levels in sever and critical groups compared to control group. This comparison of COVID-19 displayed statistically significant difference, there were a huge significant increase ($P < 0.001$) in mean of sever group verses control group. while there was high significant difference ($P<0.01$) between critical and control groups.

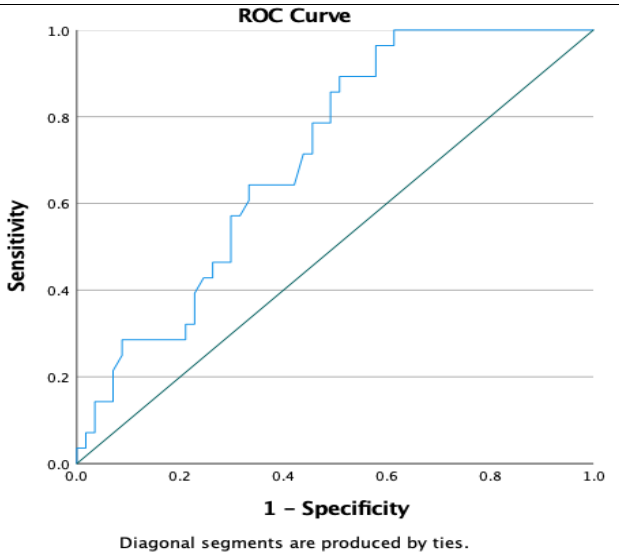
Table (2): Mean ± SD of HNE level in (sever and critical) Covid-19 groups and control group

Parameters	Groups		
	Severe N = 30	Critical N = 30	Control N = 30
HNE ng/ml	2.78±0.32$^{a▲***}$	2.62±0.36$^{a▲*, b\ (N.S)}$	2.39±0.35

a= ANOVA test between severe, critical versus control group; b= ANOVA test between severe and critical group; ***= very high significant (P<0.001); * = significant (P<0.05); (N.S) =insignificant differences.

5.1.1. Receiver operating characteristic curve of HNE

Receiver operator characteristic curve (ROC) analyses of serum HNE showed fair diagnostic performances, as shown in Figure (2). In this figure the cut-off value was = 2.53 ng / ml; at this cut-off value the sensitivity equal to 0.71% and specificity was 0.56% and the area under the curve (AUC) was 0.7.

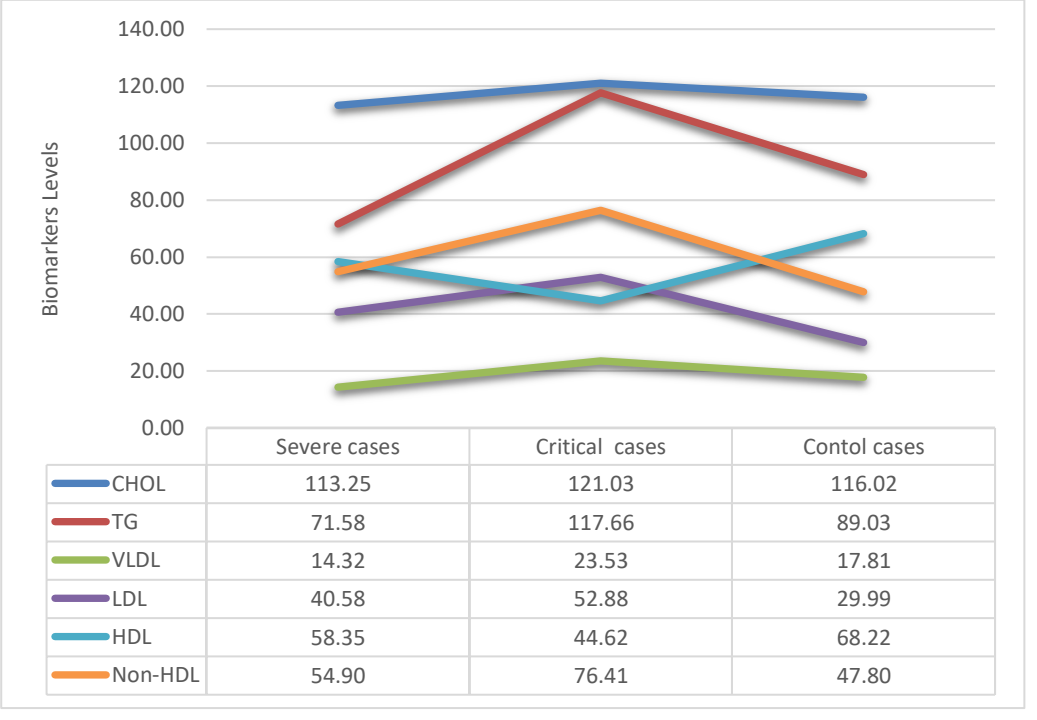


Test Variable	AUC	Sensitivity %	Specificity %	Cut-off points
HNE	0.7	0.71	0.56	2.53

Figure (2): ROC showing sensitivity and specificity of HNE in Critical COVID-19 group

5.2. Result of Lipid Profile as a Risk Indices in Covid-19

Mean values of lipid profile parameters Total cholesterol (CHOL), Triglyceride (TG), very Low-density lipoprotein (VLDL), Low-density lipoprotein (LDL), High-density lipoprotein (HDL), and Non-HDL (T-C - HDL), for patient groups (sever and critical) COVID-19 compared to control group were *shown* in Figure (2).



	Severe cases	Critical cases	Contol cases
CHOL	113.25	121.03	116.02
TG	71.58	117.66	89.03
VLDL	14.32	23.53	17.81
LDL	40.58	52.88	29.99
HDL	58.35	44.62	68.22
Non-HDL	54.90	76.41	47.80

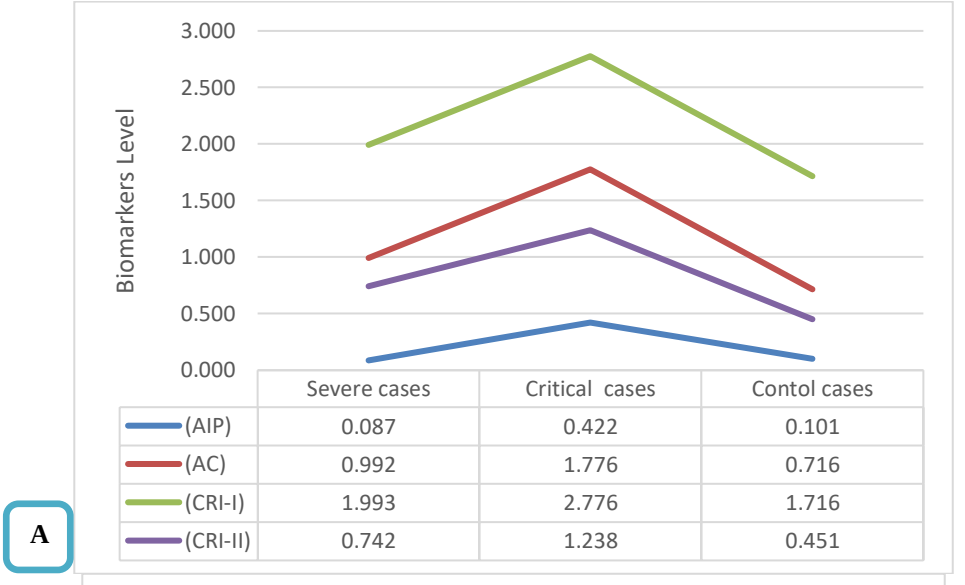
Figure (2): Comparisons of lipid profiles parameters between the Covid-19 and healthy group. TG: Triglyceride, HDL: High-density lipoprotein, LDL: Low density lipoprotein; VLDL: Very Low-Density Lipoprotein-, CHOL: Total cholesterol; non-HDL= (Total cholesterol - HDL).

In this study, critical COVID-19 group showed a massive significant increase ($P<0.001$) in means of TG and VLDL, LDL, and non-HDL when compared with control group, and a significant decreases ($P<0.001$) in means of HDL compared with control group. While CHOL displayed insignificant differences ($P>0.05$) in mean when compared with control group. Sever COVID-19 group showed insignificant differences ($P>0.05$) in CHOL and Non-HDL, and very high significant increases ($P=0.001$) in TG and VLDL when compared with control group. LDL displayed significant increases ($P<0.05$) and HDL displayed very high significant decreases ($P<0.001$) in mean when compared with control group.

5.2. Result Of Atherogenic Indices

Mean values of atherogenic indices [Atherogenic coefficient (AC), Atherogenic index (AIP), Castelli's risk index-I (CRI-I), and Castelli's risk index-II (CRI-II)] of

(sever and critical) COVID-19 compared to control groups was presented in Figure (3).



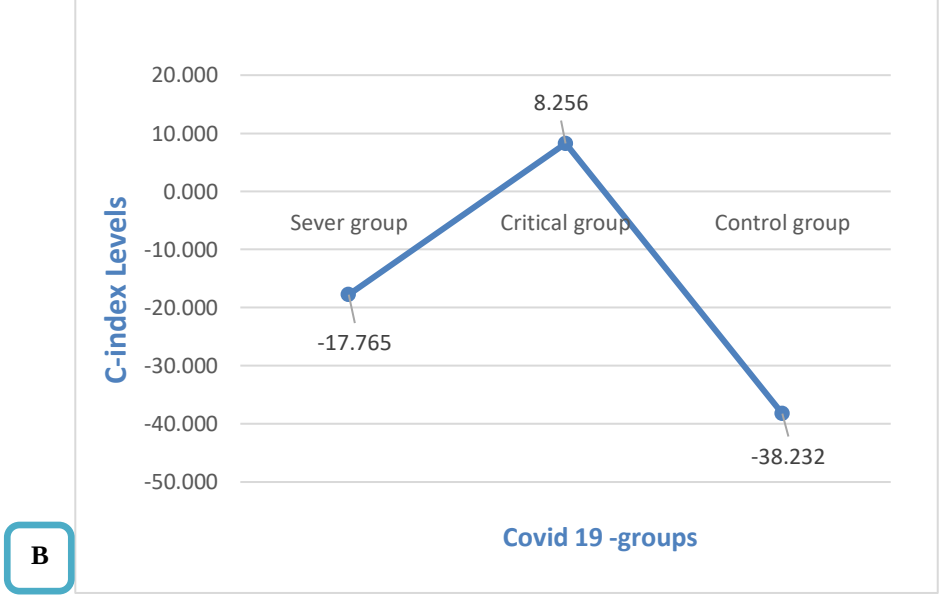


Figure (3): Mean levels of atherogenic indices parameters of the (sever and critical) Covid-19 groups and healthy group. A: levels of AC, AIP, CRI-I, CRI-II. B: levels of C-index. AC = non-HDL/HDL, AIP = log (TG/HDL), CRI-I= TC/HDL ratio, CRI-II = LDL/HD.

ANOVA analysis indicated that in critical group, there was a significant increases ($P<0.001$) of all atherogenic indices AIP, AC, CRI-I, and CRI-II when compared with control group. Sever COVID-19 group displayed insignificant differences ($P>0.05$) in AIP when compared with control group. AC and CRI-I showed significant increases ($P <0.05$) as compared with control group. While C-index and CRI-II displayed a significant increases ($P<0.01$) in mean when compared with control group. In comparison to sever group, critical group displayed very high significant increases ($P<0.001$) in all atherogenic indices AC, AIP, C-index CRI-I, and CRI-II.

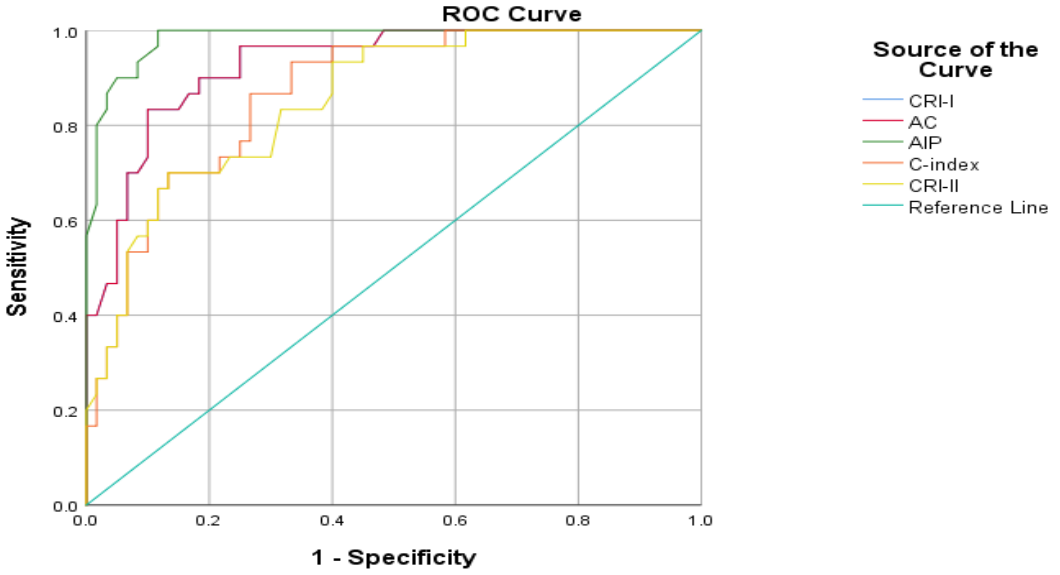
5.4. Results of correlation coefficient between HNE and Atherogenic Indices

Sever and critical COVID-19 groups, showed insignificant correlation between HNE and Atherogenic Indices ($p > 0.05$).

5.3. Receiver operating characteristics of atherogenic indices as a diagnostic markers of COVID-19 severity

Receiver operator characteristic curve was used for the analysis of significant differences of atherogenic indices of COVID-19 patient groups. Determination of the diagnostic performance is based on the area under the curve (AUC) as follows: AUC = 0.9–1.0, excellent; AUC = 0.8–0.9, good; AUC = 0.7–0.8, fair; AUC= 0.6–0.7, poor; and AUC <0.6, not useful**(Anjum *et al.*, 2020), (Emon *et al.*, 2020)**

AIP, AC, C-index, CRI-I and CRI-II showed excellent diagnostic performances according to ROC analysis, as shown in Figure (4).



Test Variable positive actual critical state.	AUC	Sensitivity %	Specificity %	Cut-off points	P vale
AC	.928	.967	0.5	0.8100	0.000
AIP	.983	.967	0.2	0.2550	0.000
C-index	.868	.967	0.63	-33.8250	0.000
CRI-I	.928	.967	0.5	1.8100	0.000
CRI-II	.853	.967	.62	0.4450	0.000

Figure (4): Receiver operating characteristic curve showing sensitivity and specificity of Atherogenic Indices in Critical COVID-19 group.

Discussion

One of the underlying mechanisms of organ damage in COVID-19 could be mitochondrial dysfunctions resulting in excessive production of ROS, triggered by inflammatory response to SARS-CoV-2 infection, hence resembling other types of sepsis characterized by severe inflammation **(Nagar, Piao and Kim, 2018)**. Namely, under such aggressive oxidative stress, polyunsaturated fatty acids in cellular biomembranes become targets for ROS-induced damage, triggering a self-catalyzed, non-enzymatic chain reaction of LPO. Thus damaged cells in the affected tissues together with erythrocytes in blood generate ROS and release of free iron which can consequently amplify LPO through Fenton reactions and promote LPO and ferroptosis**(Cecchini and Cecchini, 2020)**, resulting in membrane dysfunction/destruction and multiorgan failure in COVID-19 patients.

During the lipid peroxidation of polyunsaturated fatty acids, 4-hydroxynonenal (HNE) is one of the most important lipid-derived compounds, which act as "second messenger" of free radicals, generated through the oxidation of 7-6 polyunsaturated fatty acids. It is a highly cytotoxic compound, which alters several cellular functions such as membrane integrity and function, mitochondrial respiration and cell death.**(Vlahakos *et al.*, 2021).**

(Skesters *et al.*, 2022) revealed that the HNE adducts as an indicator of oxidative stress, the oxidative stress markers HNE were many times higher than the accepted upper limit**, w**hile another study conducted by **(Martín-Fernández *et al.*, 2021)** focused on the comparison of oxidative stress molecules' Malondialdehyde and 4-Hydroxynonenal levels across COVID-19 severity, were revealed that only lipid peroxidation was statistically different between mild and intubated/ death COVID-19 patients.

A key enzyme participating in lipid alterations is lipoprotein lipase (LPL) **(Mead, Irvine and Ramji, 2002)**, in the presence of inflammation, several mediators interfere with glucose and lipid metabolism**(Kersten, 2014).** Several cytokines and inflammatory mediators that are overexpressed during COVID-19 may interact directly with LPL or its regulatory proteins such as apo CII **(Ng *et al.*, 2010)**, both cytokine and α-adrenergic stimulation appear to alter the activity of several lipolytic enzymes. Lipoprotein lipase is critical to hepatic lipid metabolism as once fatty acids reach the liver, they can either be oxidized as an immediate energy source or re-esterified to VLDL triglycerides and redistributed throughout the body. Since lipoprotein lipase cleaves fatty acids from triacylglycerols its inhibition results in a decreased ability to catabolize and thus clear circulating triglycerides**(Trinder, Boyd and Brunham, 2019)**,**(Saballs *et al.*, 2016).**

The results of present study were in agreement with the previous studies, which state that the triglyceride levels were found to be higher in SARS-CoV-2 infected patients than in the healthy controls**(Roccaforte *et al.*, 2021)**,**(Masana *et al.*, 2021)**,**(Govindula, 2020).** A cross-sectional analysis of hospitalized patients with COVID-19 showed that low HDL and high TG before infection and upon admission were strong predictors of disease severity**(Masana *et al.*, 2021).** In addition, the TG/HDL-C ratio was markedly increased in the severe patients verses non-severe patients**(Zhang *et al.*, 2020).** High triglyceride levels during hospitalization should be considered high risk markers for COVID-19**(Masana *et al.*, 2021).**

(Bellia *et al.*, 2021) reported significant association between detection of atherogenic dyslipidemia at admission and subsequent adverse outcome of disease in patients hospitalized for COVID-19. More specifically, atherogenic dyslipidemia was strongly related to mortality, resulting in more than threefold increased probability of association with in-hospital death. Triglycerides levels detected at admission were higher in patients with critical COVID-19**.**

Conclusion

The danger of HNE induction in severe COVID-19 patients is quite great, so the verification of this hypothesis seems to be an urgent task as detection of the

agents leading to cause systemic complications through SARS-COV-2 infection which might save many human beings. C-index and CRI-II emerges as a good candidate to be used as an early biomarker for prediction covid compication in cases that need intensive care. Hence, regular check of these markers in COVID-19 patients can improve management of these patients and prevent deterioration of the disease.

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