ORIGINAL RESEARCH

The Impact of *LEP* rs7799039 Polymorphism and Obesity on the Severity of Coronavirus Disease-19

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Background and Aims: SARS-CoV-2 infection has been recorded in 230 countries to date. Obesity has a negative impact on one's quality of life and is one of the main causes of mortality globally. Obesity affects the immune system, making the host more susceptible to infectious infections. Also, obesity commonly provokes the severity of respiratory diseases so the correlation of *LEP* rs7799039 Polymorphism in corpulent patients with COVID-19 infection was clearly investigated in the current study.

Methods: A total of 232 patients were recruited, 116 patients were obese with COVID-19 infection, and 116 patients were non obese COVID-19. Fasting blood glucose test (FBG), hemoglobin A1C (HbA1C), complete blood count (CBC), international normalized ratio (INR), urea, alanine transaminase (ALT), aspartate aminotransferase (AST), D dimer and C-reactive protein (CRP) were estimated. C.T. scan was performed for each patient, and C.T. severity score was calculated. Genotyping for the *leptin* rs7799039 SNPs was performed by TaqMan® (Applied Biosystems Step One ™ Real-time PCR).

Results: Regarding *LEP* polymorphism, all individuals of non-obese groups significantly had the homozygous allele GG (100%), whereas only 56% of obese groups had GG alleles (P = 0.001). The severity scores significantly (P = 0.001) varied regarding *LEP* polymorphism regarding Rs7799039, where the largest proportion of those with Grade IV had the homozygous allele AA (57.1%).

Conclusion: There was a correlation between the *leptin* gene allelic discrimination and COVID-19 CT brutality in obese patients. The A allele was considered a risk factor for severity in COVID-19 patients while the G allele contributes to decreasing that risk.

Keywords: COVID-19, leptin, gene polymorphism, immunity, obesity

Introduction

A new coronavirus disease (COVID-19) was discovered in Wuhan, Hubei Province, China, in December 2019.[1,2] The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)-instigated coronavirus disease 2019 (COVID-19) has diffused, resulting in a considerable number of cases and deaths.[3]

SARS-CoV-2 instances have been recorded in 230 countries to date.[1] A vast number of diverse hazard factors are concomitant with disease harshness, as respiratory dysfunctions, heart disease, hypertension, older age, diabetes,

hypertension, and most recently obesity.[4] Obesity commonly provokes the severity of respiratory diseases.[5] A rise in studies of clinical reports designate that obesity is a hazard factor for COVID-19 severity.[6]

According to a 100 million health study (2019), the prevalence of obesity in Egypt has climbed in adults to almost 40%, up from 36% in the 2017 STEP SMART survey.[7] Obese people with COVID-19 have a higher risk of severe disease, hospitalization, and mortality, according to several studies.[1,8]

Leptin (LEP) is a peptide that is mostly released in white adipose tissue and operates by interacting to the leptin receptor (LEPR). It was identified in 1994.[9] LEP's key actions are appetite suppression, increased energy expenditure, and lipid metabolism management, all of which help to control obesity as well as its potential as a blockbuster weight loss treatment sparked interest.[10]

Other neuroprotective properties of LEP include increased endothelial cell oxidative stress, stimulation of vascular smooth muscle cell development, induction of arterial vascular wall damage, stimulation of reactive oxygen species generation, and stimulation of the renin-angiotensin–aldosterone system.[11]

Changes in adipokines (such as decreased adiponectin and increased leptin), insulin resistance, higher blood glucose, and chronic low-grade inflammation have all been linked to obesity.[12] Signaling of Leptin is precarious in the retort of T lymphocytes to inflammatory effectors by upregulating cellular glycolysis and boosting the construction of effector cytokines like IFN- and TNF- during infection.[11,12]

These metabolic factors control immune cell metabolism, which impacts how well SARS-CoV-2 viruses work.[13] Additionally, a higher Body Mass Index (BMI) is linked to a higher frequency of the anti-inflammatory CD4 T cell subsets Th2 and T regulatory cells. Increased anti-inflammatory cells may impede the ability to control viral spread by inhibiting the inflammatory responses required to control viral spread. Following infection, Regulatory T cells (Tregs) predominantly resolve immune cell-mediated inflammation.[14]

The concentration of pro-inflammatory cells, such as macrophages, dendritic cells, cytotoxic T cells, and Th1 cells, in the adipose tissue of obese people causes an auxiliary discrepancy in immune cell subsets, causative to the expansion of insulin resistance and chronic inflammation.[15]

Enlarged serum inflammatory cytokines, such as C-reactive protein (CRP), IL-6, type I, and type III interferons, were seen in these pro-inflammatory immune cells and hypertrophic adipocytes.[16] Changes in systemic immune cell populations, as well as their accrual in adipose tissue, have been identified as important mediators of COVID-19 severity in obese people.[17–25]

Surveys on the influenza A virus suggest that persons with obesity may have enhanced viral detaching of SARS CoV 2. Obese people were found to be more infectious than thin patients. Several causes have contributed because of their impaired immune response, symptomatic obese people shed the influenza A virus 42% longer than slender patients.[18] Furthermore, BMI has a favorable relationship with viral consignment in exhaled breath. This indicates that obese patients exhale a higher viral load, therefore increasing the probability to infect others.[19] As SARS-CoV-2 has a similarity to influenza virus strain, it seems likely that these findings can be also applied to obese patients infected with SARS-CoV-2.[20–31] So, the aim of this current study was to evaluate if obesity can be considered an important risk factor for disease severity or not and also to compare between allelic discrimination of the *LEP* rs7799039 Polymorphism in obese COVID-19 patients against non-obese COVID-19 patients.

Methods

Two hundred and thirty-two confirmed COVID-19 patients by PCR were divided into two groups, group I: 116 obese COVID-19, and group II included 116 non obese COVID-19 patients. The study was ethically approved from the institutional ethical committee of the National Hepatology & Tropical Medicine Research Institute, Cairo, Egypt. The study complies with the declaration of Helsinki. Informed consent was acquired from all the studied patients.

Assessment of the Patients
Anthropometric Measurements

The BMI was computed by multiplying the body weight (kg) by the height^2 (m^2). The clinicopathological data of the patients were recorded, including age and gender. Several laboratory tests were carried out including complete blood count, liver function tests, Hb A1C, FBS, PPBS, D-dimer, ferritin and C reactive protein (CRP).

Blood Sample

10 mL venous blood samples were collected from each of the patients for several investigations. For biochemical and enzyme-linked immunosorbent assay (ELISA) analysis (serum leptin) in sterile coagulant-free tubes 3 mL of venous blood was collected from each volunteer while 5 mL of blood was put in Ethylenediaminetetraacetic acid (EDTA) tubes for DNA extraction and gene polymorphism for *leptin* gene polymorphism and last two mL were collected in EDTA tube for complete blood count (CBC).

Biochemical Analysis

Wet chemistry Bachman machine was used to analyze liver enzymes such as Serum Glutamate Oxalate Transaminase (SGOT) IU/L and Serum Glutamate Pyruvate Transaminase (SGPT) IU/L.; total, direct bilirubin, albumin (g/dl), Fasting Blood Glucose (FBG) mg/dl, Lipid profile included triglycerides mg/dl, High-density lipoprotein-cholesterol (HDL-c), cholesterol mg/dl, low-density lipoprotein mg/dl –cholesterol (LDL-c) mg/dl. Complete blood count (CBC) was done for each participant by Beckman coulter while Thermostat Pt Analyzer was used to determine the concentration of INR for each participant. To determine serum leptin levels, after coagulation by centrifugation, serum was instantly collected and stored at −80° until used by Elisa (Sandwich) DRG® Leptin ELISA (EIA-2395), Inc., USA.

Genotyping Testing

In accordance with the manufacturer's recommendations, the genotyping and DNA were retrieved using (QIAGEN GmbH, Hilden, Germany) QIA amp® DNA Blood Mini Kit (Schur, Bjerke et al 2001).

The Nano Drop® (ND-1000) Spectrophotometer was used to determine the concentration of extracted DNA (Nano Drop Technologies Inc., Washington, USA).

The 260/280 nm absorbance ratio of isolated DNA was 1.7–1.9.

Genotyping for the *Leptin* rs7799039 SNPs Polymorphism

On chromosome 7; 128128730 on GRCh38, catalogue number: 4351379. The allelic discrimination was done by a real-time polymerase chain reaction using TaqMan® allelic discrimination test software and an Applied Biosystems Step One TM Real-Time PCR system Thermal Cycling Block (Singapore).

Statistical Analysis

The data was loaded into the computer and assessed with IBM SPSS software version 24.0. The normalized mean values were also used, the significance of the acquired results was determined at a 5% level of significance. ANOVA test: for normally quantitative variables, to compare amid more than two investigated groups, Spearman coefficient; to correlate between two abnormally quantitative variables, Chi-square test; for categorical variables, to differentiate different groups, Student's t-tests; for normally quantitative variables, to differentiate amid II studied groups, ANOVA test; for normally quantitative variables, to compare among more than II investigated groups, Spearman coefficient used.

Results

There was no significant difference in the two groups regarding age and gender (P = 0.12, 0.17, respectively). Table 1 shows a significant difference in mean of BMI values among obese and non-obese groups (P = 0.001).

The laboratory tests performed for the two groups are shown in Table 2. The two groups under study significantly varied regarding the estimated mean values of ferritin (P = 0.001), D-dimer (P = 0.001), CRP (P = 0.001), erythrocyte sedimentation rate (ESR) one hour (P = 0.001), and ESR at two hours (P = 0.001). Obese patients significantly tended to have higher mean levels of the previously estimated factors virtual to the non-obese group. The blood glucose variables, including HbA1c, FBS, and PPBS displayed significant variations among 2 groups (P = 0.001 for each estimated variable). Obese patients significantly displayed higher levels of HbA1c, FBS, and PPBS compared to non-obese patients. Also, the two groups showed significant variations regarding GPT, GOT, LDH, and INR, where Obese patients

Table 1 Comparison Between 2 Studied Groups Regarding Demographic Data

	Group I Obese Group "n=116"		Group II Non Obese Group "n=116"		P value
	No	%	No	%	
Gender Male Female	 72 44	 62.0 38.0	 64 52	 55.17 44.83	0.175 N.S.
Age Range Mean ±SD	 22–66 47.87±12.40		 20–67 45.80±9.82		0.127
BMI Range Mean ±SD	 20–45 32.60±5.64		 20–45 29.56±5.39		0.001*

Note: *Significant difference.
Abbreviations: N.S., Not significant.

Table 2 Comparison Between 2 Studied Groups Regarding Laboratory Findings

Variables	Obese Covid 19 (116)	Non Obese Covid 19 (116)	P value
Ferritin Range Mean ±SD	 30–880 221.32±193.55	 35–201 108.34±36.44	0.001*
D Dimer Range Mean ±SD	 0.5–3.1 1.24±0.52	 0.1–1 0.31±0.21	0.001*
CRP Range Mean ±SD	 1.02–90 16.49±14.99	 0.2–18 5.11±3.16	0.001*
ESR 1 hr Range Mean ±SD	 4–56 16.97±12.92	 3–40 12.12±6.59	0.001*
2 hr Range Mean ±SD	 9–134 39.79±29.83	 7–67 26.28±11.93	0.001*
Hb A1C Range Mean ±SD	 2.9–8.2 5.80±1.39	 1.2–4.5 3.22±0.87	0.001*
FBS Range Mean ±SD	 85–142 101.46±12.53	 67–129 94.16±11.75	0.001*
PPBS Range Mean ±SD	 100–179 121.67±13.92	 86–140 115.10±13.67	0.001*

(*Continued*)

Table 2 (Continued).

Variables	Obese Covid 19 (116)	Non Obese Covid 19 (116)	P value
GPT Range Mean ±SD	20–54 30.47±6.19	10–40 23.78±6.66	0.001*
GOT Range Mean ±SD	18–58 32.84±8.63	17–36 23.70±4.82	0.001*
LDH Range Mean ±SD	120–270 191.27±25.37	100–270 183.66±31.66	0.045*
INR Range Mean ±SD	0.91–2.18 1.23±0.21	0.9–1.4 1.03±0.09	0.001*

Note: *Significant at level 0.05.

had significantly higher mean levels of GPT (P = 0.001), GOT (P = 0.001), LDH (P = 0.045), and INR (P = 0.001) compared to non-obese patients.

The two groups showed significant differences in the mean level of leptin (P = 0.0001), where the mean level of leptin was significantly higher among obese patients in comparison to non-obese patients, Table 3.

The detection of *LEP* polymorphism at rs7799039 showed that all non-obese patients (100%) had the homozygous alleles GG, whereas 65 (56%) of obese groups had the same alleles, followed by 36 (31%) having GA alleles, and 15 (12.9%) having AA alleles; a significant difference was distinguished between the two groups regarding *LEP* polymorphism distribution (P = 0.001), Figure 1.

Regarding *LEP* gene polymorphism, it was found that grad IV of severity which was considered the highest was significantly associated with AA homozygous allele, whereas other less severity grades were more common among those carrying GG alleles (P = 0.001), Table 4. Also, the higher grade of severity was significantly associated with morbid obesity, whereas patients with normal weight significantly with low grade of severity, and those with overweight and obese patients significantly had lower grades of severity compared to those with morbid obesity (P = 0.001), Table 4.

Discussion

Obesity has been linked to the consequences of viral infections in the past, both with the influenza virus and with prior coronaviruses that caused widespread illnesses (SARS, Middle East Respiratory Syndrome (MERS)). Because SARS-CoV-2 (determining COVID-19), SARS-CoV (80%), and MERS-CoV (50%) have greater genetic similarities, this connection is crucial when assessing data.[26–39] Obesity has been linked to an increased risk of COVID-19 severe course in several ways. Obesity has been linked to underlying conditions such type 2 diabetes, hypertension, thrombogenic risk, and lung disease. All these problems make it harder to deal with COVID-19.[27–33]

Table 3 Comparison Between the Studied Groups Regarding Serum Leptin ELISA Test

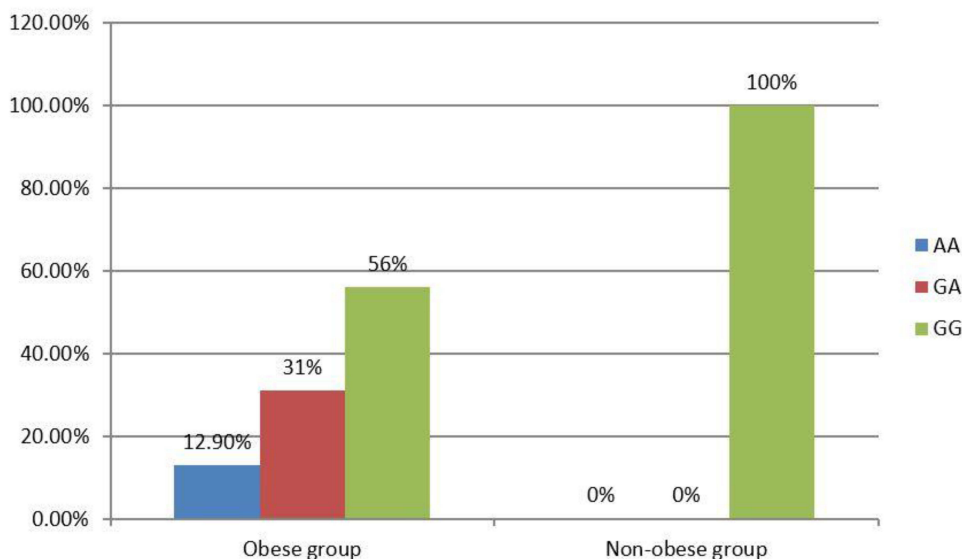| Variables	Group I	Group II	P value
Leptin Range Mean±SD	0.50–37.00 17.43±9.42	0.50–38.00 7.02±9.56	0.000

Figure 1 Comparison between the two studied groups regarding rs7799039.

Obesity is amongst the most prominent factors that increases the mortality risk of SARS-CoV-2 patients tenfold.[28] The current study investigations indicated that obese patients with COVID-19 significantly showed worse laboratory tests compared to non-obese patients with COVID-19 such as D-dimmer, ferritin, HbA1c, liver enzymes, LDH, and INR which is in agreement with previous studies. The current analysis approves that obesity is allied with an increased hazard of hospitalization in patients with COVID-19. This could be concluded through significant association between higher grade of COVID-19 severity that requires hospitalization which means increased cost effectiveness. The increase in the severity score was correlated to the increase with the BMI of COVID-19 patients. These results agreed with Lighter et al,[6] who reported that obesity is allied with an improved threat of hospitalization in patients with COVID-19. Fascinatingly, the reminder of obesity with hospitalization was superior in younger.[6]

The link between obesity and the hazard of hospitalisation is supported by reports of an increase in mortality and ICU admission in obese hospitalized patients with COVID-19.[29–36] The higher severity of COVID-19 in obese people could be due to a variety of factors. Excess weight has been linked to poorer COVID-19 outcomes in a few illnesses (eg, diabetes, cardiovascular diseases (CVDs), and respiratory disease).[30,31] Even after correcting for those factors, the link between obesity and hospitalisation remains statistically significant, implying alternative mechanisms.

Table 4 The Comparison Between Different CT Severity Score Regarding *Leptin* Gene rs7799039 and BMI Category

	CT Severity Score								P value
	Grade I		Grade II		Grade III		Grade IV		
	No	%	No	%	No	%	No	%	
rs7799039									0.001*
AA	4	8.0	0	0.0	3	17.6	8	57.1	
GA	18	36.0	10	28.6	6	35.3	2	14.3	
GG	28	56.0	25	71.4	8	47.1	4	28.6	
BMI category									0.001*
Normal weight	9	18.0	0	0.0	0	0.0	0	0.0	
Overweight	10	20.0	10	28.6	0	0.0	0	0.0	
Obese	16	32.0	15	42.9	3	17.6	1	7.1	
Morbid obese	15	30.0	10	28.6	14	82.4	13	92.9	

Note: *Significant at level 0.05.

Obstructive sleep apnea was not one of the pathophysiological mechanisms that could attribute to the obesity-related risk of severe COVID-19 infection in the current investigation. The current research does not allow for the differentiation of possible pathogenic pathways. It was established that a higher BMI is a negative prognostic factor in COVID-19 patients, and obese patients, as obesity is a major risk factor for hospitalization in COVID-19 patients.[31]

There was no result has been published previously related to association between *LEP* gene polymorphism at rs7799039 and the severity grades of COVID-19.; therefore, we present the first data on this subject. The only limitation of the study may be limited number of patients. So, studies on larger number of patients are recommended to further validate these findings. A significant connotation was perceived between the highest grade of boundary of COVID-19 (grade IV) and the homozygous allele AA. On the other hand, the lower grades from I to III were more probe to be experienced by carriers of GG allele. Hence, we can conclude that the homozygous allele AA can be predictor for higher severity of COVID-19 and therefore poor prognosis. By combining this finding with the previous finding regarding the interconnection among BMI and cruelty of COVID-19, we can conclude that COVID-19 patients who are carriers of AA allele and per morbid obesity are more prone to experience severe COVID-19 infection and experience poorer prognosis. Therefore, we recommend establishing analysis for *LEP* gene polymorphism at rs7799039 among patients with BMI categories including overweight, obese, and morbid obese to provide them the necessary care and to avoid further complications.

Conclusion

Obesity is interrelated to severe COVID-19 and poor prognosis and assist as a prognosticator of deprived outcomes amid COVID-19 patients. Furthermore, the homozygous allele AA of *LEP* polymorphism at rs7799039 is another predictor of higher severity of COVID-19 and poor prognosis. The combination between AA allele and obesity can be additive predictors for superfluous severe COVID-19 and poorer outcomes.

Consent to Participate

Informed consensus was acquired from all studied subject.

Disclosure

The authors report no conflicts of interest in this work.

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