Original Article

The Potential Effect Of Leptin And Il-6 Levels On Covid-19 Severity In Obese Patients

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Abstract

Background: The obesity is one of the high-risk factors in COVID-19 in severe illness and mortality as the pandemic progresses. The pleotropic Leptin is a polypeptide hormone and secreted by adipose tissues. It acts as a proinflammatory cytokine. Leptin is associated with severe conditions known to cause the risk of COVID-19 severity. Acute changes in calorie intake affect Leptin levels, which in turn are related to the amount of adipose tissue. Interleukin 6 (IL-6) also is a pleiotropic cytokine (184 amino acid) and initiates different inflammatory responses in tissues.

The aim of study: The aim of this study is to evaluate the effect of Leptin and IL-6 levels on the COVID-19 severity of an early diagnosis in obese patients infected with COVID-19.

Materials and Methods: This case-control study included 60 obese subjects, divided to 30 obese without any chronic disease and not infected with COVID-19, and 30 individuals were infected with COVID-19, with the age ranged between (25-60) years. COVID-19 patients divided into three categories, (13 mild/moderate), (10 severe) and (7 dead). And thirty (20 male and 10 female) apparently healthy subjects as control group. Their age and sex were comparable to the patients. The enzyme-linked immunosorbent assay (ELISA) kits used to measure serum Leptin and IL-6 levels.

Results: Leptin concentration and IL-6 concentration were significantly higher in obese COVID-19 patients than the obese without COVID-19 and normal weight control group (11.80±1.38, 344.23±70.13 vs 8.64±1.21, 790.34±194.29 vs 4.09±2.15, 138.89±46.36) respectively. However, especially in dead and severe cases increased levels of IL-6 and Leptin than the mild/moderate cases of obese patients infected with COVID-19. Serum leptin level has a positive significant correlation with levels of IL-6, D-dimer, ferritin and NLR in obese COVID-19 patients group.

Conclusions: High circulating IL-6 and Leptin in the obese patients might involve dysregulation of proinflammatory cytokines in obesity and may be used as a useful prognostic tool associated with COVID-19 to predict the SARS-COV-2 severity.

Keywords: Leptin, interleukin 6, SARS-CoV-2 (COVID-19)

INTRODUCTION

This virus has the potential to cause serious infections, including the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) (Anderberg et al., 2021). Patients who have COVID-19 show the most common symptoms of viral. Patients with COVID-19 also have a higher risk of developing severe complications, which include death (D. Tang et al., 2020). There is a large amount of variation in both the development of the disease and the consequences. The majority of patients present with very minor symptoms; however, some patients worsen fast and develop acute respiratory distress syndrome (ARDS) and multiple-organ failure, which results in an extremely high risk of mortality in a short time (Goh et al., 2020).

Adipose tissue is the major source of Leptin in the body. It sends information to the brain about the energy storage in the body, namely the fat reserves that are there. There is a one-to-one correlation between the amount of adipose tissue in a person's body, as measured by their body mass index (BMI), and the amount of Leptin and insulin that is circulating in the blood (Koester-Weber et al., 2014). Leptin is a hormone that when released into the body, causes a feeling of fullness and speeds up the metabolism. The effects that it has on the CNS are what are known as its features. The fact that Leptin is also a protein that promotes inflammation and plays a significant role in the immunity is not very well understood (Abella et al., 2017). Leptin is a pleiotropic hormone as well as a proinflammatory cytokine, meaning that it may influence a wide variety of endocrine activities, including both innate and adaptive immunological responses (La Cava, 2017). It

is possible that Leptin serves as a connection between obesity and metabolism, as well as an enhanced inflammatory environment, cytokine production, and an improperly regulated innate immune response (Victoria & Camelia, 2021).

IL-6 is a cytokine (pleotropic cytokine) generated as a defense mechanism against tissue damage and infections (Tanaka et al., 2014). The synthesis of IL-6 is linked to a diverse range of cell types, including macrophages, mesangial cells, B cells, vascular endothelial cells, fibroblasts, and T cells (Mauer et al., 2015). IL-6 is involved in the control of the immune system as one of the many biological tasks that it mediates.

A protein known as IL-6 plays a crucial role in the maturation and activation of adaptive immune system and innate immune system. Instead of dendritic cells, the innate immune system's Monocytes become macrophages as a result of IL-6's ability to promote differentiation (Pasare & Medzhitov, 2003).

IL-6 also plays a role as a myokine and cytokine, which means that it is created and secreted by muscle tissue in response to the effects of exercise (Raschke & Eckel, 2013), In skeletal muscle, IL-6 mediates anti-inflammatory and metabolic processes by acting in a manner similar to that of an endocrine hormone.

It does this by behaving in a paracrine and autocrine fashion, respectively (Pedersen, 2013). The generation of pro-inflammatory cytokines is suppressed by this IL-6 cytokine, which simultaneously induces the expression of anti-inflammatory cytokines. This combination of effects results in the activation of a response that is anti-inflammatory, IL-6 also induces expression of other anti-inflammatory factors, such as IL-10 (Eckardt et al., 2014). Increased the levels of fatty acid oxidation in skeletal muscle is one of the metabolic effects of IL-6 in human (Catoire & Kersten, 2015), IL-6 also plays a key role in hypertrophic skeletal muscle growth (Serrano et al., 2008).

MATERIALS AND METHODS

A case control study design consisted of a total 90 subjects, 60 Obese patients without any chronic diseas divided into two groups , 30 obese individuals (20 males and 10 females) without COVID-19 and 30 obese individuals (20 males and 10 females) infected with COVID-19 and collected within 8–11 days after the onset of symptoms of COVID-19 which diagnosed by real time reverse transcription-PCR (RT-PCR) and (CXR) chest X-ray or CT scan, with age ranged (25-60) years compared with 30 (10 females and 20 males) normal weight as a control group which are comparable with other groups in sex and age.

Patients were collected from Al-Sader Medical City and Al-Amal Hospital for communicable disease center in Najaf-Iraq within the period from October 2021 to April 2022 after receiving ethical approval from the Iraqi ministry of health and environment ethics committee, and all participants gave informed consent before the study started.

COVID-19 patients were recruited as admission and the diseases severity was assessed using Murray scores (Murray et al., 1988) a patient was considered to have severe COVID-19, if he or she met any of the following:
1. Repertory distraction (≥ 30 /min)
2. Resting oxygen saturated ≤ 90% or
3. Arterial oxygen (PaO2) / fractions of inspired oxygen ≤ 300 mmHg. Or
4. Repertory failure requiring mechanicals ventilation and requires intensive care unit (ICU), and critical patient and then dead will be considered as dead cases.

All participants provided informed permission prior to the start of the trial. The patients were registered and given a file to record their information, including name, age, sex, weight, height, sings, and symptoms. As a control group, 30 subjects with normal weight and apparently healthy were selected. Their age and sex were matching with other groups. Exclusion criteria include volunteers with any chronic diseases, including diabetes, cardiovascular disease, and long-term oral corticosteroid medication, cancer and renal and liver disease patients, as well as smokers, pregnant, anemic subjects, those with systemic immunological disease, and those with thyroid gland disease were all excluded. five milliliters of venous blood were collected from each patient and control group and then divided the samples to two parts: 2 mL from blood were put in EDTA tubes for complete blood count (CBC) analysis, and the remaining blood was added in gel tube and left at room temperature at 10 - 15 minutes for coagulation before being centrifuged (3000 x g) at 10 minutes to separate the serum. Eppendorf tubes were used to divided the serum samples and store at (-20 °C) until biochemical analysis could be performed.

Complete blood count (CBC) was measured by using auto hematology analyzer (linear, Spain). Serum ferritin, and D-dimer levels were measured by fluorescence immunoassay (FIA) (ichromaTM). The concentration of (leptin), (IL-6) assays were determined in serum samples by Enzyme linked immune sorbent assay (ELISA) (Melsin, Chain). Also measured body mass index (BMI) by special equations BMI (Kg/m2) = weight (Kg) / height (m^2).

Statistical Analysis

Using the Statistical Analysis Software for Social Sciences, we evaluated the observed data supplied in the form of mean ± SD (SPSS). Statistically significant differences between the healthy and sick groups were analyzed using the

independent t-test. In this analysis, we used the Pearson correlation coefficient to draw attention to the existence of a statistical connection. For the purpose of illustrating the weight of evidence in favor of significant differences between variables, the thresholds of significance of 5% and 1% ($P \leq 0.05$ and $P \leq 0.01$) were achieved.

RESULTS AND DISCUSSION

This research involved 60 obese patients, 30 of whom were infected with COVID-19, 30 obese persons without COVID-19 (20 men and 10 females), and 30 healthy subjects. The mean age was (49.54 ±10.17) years old. As shown in the Table 1, there were no significant differences in age and sex or gender between the groups (two sick groups and healthy volunteers).

The study was indicating increased levels of Ferritin and D-dimer as well as Neutrophils and Neutrophils/lymphocytes ratio (NLR) when comparing obese patients infected COVID-19 with obese and healthy normal weight groups, especially in critical and severe cases in the obese patients with COVID-19 comparing with mild/moderate cases. The study also, found a decrease in (MLR) in the obese patients with COVID-19 comparing with obese without COVID-19 as shown in Table 1, and normal weight as healthy control groups.

In this investigation, COVID-19 patients were included 13 mild/Moderate, 10 severe, and 7 death cases as shown in Table 2. Dead patients had a much higher Monocyte count, ferritin, D-dimer, NLR, and Neutrophil count than the rest of the cases. Table 3 indicates the correlations between the measured biochemical parameters with Leptin and IL-6 levels among COVID-19 patients. There was a positive link between Leptin and IL-6. This is the first research that, to the best of our knowledge, shows a significant association between Leptin and IL-6 in Iraqi COVID-19 patients.

Table 1. Demographic and General Characteristics in patients and control groups

Parameters	Groups			*P* value
	Obese with COVID-19 Mean±SD	Obese without COVID-19 Mean±SD	Healthy group Mean±SD	
Total number	30	30	30	----
Male/Female	20/10	20/10	20/10	----
Age (Years)	49.54±10.17	48.03±7.20	48.13±7.93	A:0.682 B:0.625 C:0.913
BMI (kg/m²)	33.44±4.76	34.09±3.27	22.52±2.03	A:0.001 B:0.001 C:0.001
SBP (mmHg)	144.49±10.10	147.13±10.02	120.35±5.74	A:0.05 B:0.05 C:0.05
DBP (mmHg)	8.13±1.06	8.20±1.11	7.91±0.63	A:0.891 B:0.617 C:0.701
SpO_2 %	90.64±3.71	96.13±3.34	98.50±0.69	A:0.05 B:0.05 C:0.05
Neutrophil x 10^3 / ml	12.94±4.90 7.32	4.48±0.76 4.55	4.07±0.88 4.14	A < 0.01 B: 0.763 C < 0.01
Lymphocyte x 10^3 / ml	0.91±0.69 0.64	2.52±0.62 2.48	2.40±0.51 2.38	A <0.001 B : 0.626 C :0.001
Monocyte x 10^3 / ml	0.56±0.50 0.42	0.48±0.17 0.47	0.43±0.082 0.44	A :0.683 B :0.803 C :0.729
NLR	14.22±0.62 12.66	1.77±0.56 1.79	1.69±0.53 1.54	A< 0.001 B:0.753 C:0.001

MLR	0.62±0.61	0.21±0.05	0.181±0.32	A :0.01 B :0.05 C :0.001
D-dimer (ng/ml)	1178.17±1120.10	75.34±20.82	60.12±22.15	A:0.0001 B :0.05 C:0.0001
Ferritin (ng/ml)	897.73±12.3	89.24±37.83	72.17±40.28	A:0.0001 B :0.03 C:0.0001
TG (mg/dl)	310.93±75.10	233.19±70.39	109.17±66.97	A: 0.01 B: 0.002 C: 0.01
TC (mg/dl)	170.06±15.62	210.86±32.58	125.26±38.96	A: 0.01 B: 0.47 C: 0.01
HDL.C(mg/dl)	29.10±7.17	30.08±11.36	46.54±11.40	A: 0.052 B: 0.01 C: 0.04
VLDL.C (mg/dl)	54.31±10.23	34.98±15.20	20.59±14.62	A: 0.03 B: 0.01 C: 0.01
LDL.C (mg/dl)	83.08±18.13	120.86±33.24	113.21±10.43	A: 0.001 B: 0.0001 C: 0.05
Leptin (ng/mL)	11.80±1.38	8.64±1.21	4.09±2.15	A: 0.001 B: 0.001 C: 0.024
IL-6 (Pg/mL)	344.23±70.13	790.34±194.29	138.89±46.36	A: 0.0001 B: 0.0001 C: 0.0001

sData represented as Mean ± SD. SD: standard deviation, BMI: body mass index, SBP: Systolic blood presser, DBP diastolic blood pressure, SpO_2: Oxygen saturation.
A= p value (Obese with COVID+Obese), B=p value (Obese with COVID + Healthy), C= p value (Obese+ Healthy), NLR: Neutrophil lymphocyte ratio, MLR Monocyte lymphocyte ratio

Table 2. Demographic and General Characteristics in COVID-19 patient's categories

Parameters	Groups			*P* value
	Dead Mean±SD	Severe Mean±SD	Mild/Moderate Mean±SD	
%	24%	33.33%	43.33%	----
Age (Year)	56.03±3.10	52.13±4.24	40.37±10.16	A:0.178 B:0.05 C:0.05
BMI (kg/m²)	37.12±2.08	33.11±3.90	30.07±2.34	A:0.182 B:0.06 C:0.175
SBP (mmHg)	142.1±3.18	144.6±6.39	146.76±8.01	A:0.186 B:0.200 C:0.187
DBP (mmHg	70.03±10.36	70.11±9.78	75.12±10.18	A:0.662 B:0.05 C:0.05
SpO_2	85.70±6.10	88.35±4.38	94.88±2.80	A:0.06 B:0.04 C:0.04
Neutrophil $x10^3$ / ml	13.87±5.01	12.24±3.45	10.83±2.36	A:0.633 B :0.04 C:0.04
Lymphocyte $x10^3$ / ml	0.82±0.43	0.90±0.56	0.93±0.58	A:0.762 B:0.624 C:0.785
Monocyte $x10^3$ / ml	0.43±0.31	0.60±.47	0.58±0.53	A:0.03 B :0.05 C:0.677
NLR	16.91±11.65	13.6±6.16	11.645±4.068	A:0.01 B :0.01 C:0.05

MLR	0.52±0.72	0.66±0.723	0.623±0.913	A:0.239 B :0.651 C:0.925
D-dimer (ng/ml)	1182.10±1046	1011±976.43	602.04±204.90	A:0.03 B:0.0001 C:0.0001
Ferritin (ng/ml)	1217.4±409.33	1082±310.21	461.50±52.62	A:0.01 B:0.0001 C:0.001
TG (mg/dl)	307.17±73.28	279.98±60.10	350.21±44.35	A:0.041 B:0.026 C:0.013
TC (mg/dl)	160.13±34.75	173.32±22.46	177.03±26.62	A:0.031 B:0.045 C:0.042
HDL.C (mg/dL)	25.10±7.38	29.25±11.30	32.67±9.84	A:0.06 B:0.03 C:0.054
VLDL.C (mg/dL)	60.28±30.07	58.41±16.31	47.92±13.66	A:0.703 B:0.01 C:0.004
LDL.C (mg/dL)	70.37±22.88	88.10±25.17	90.89±24.07	A:0.04 B:0.01 C:0.583
Leptin (ng/mL)	13.73±2.08	11.11±2.12	8.71±2.18	A: 0.048 B: 0.041 C: 0.05
IL-6 (Pg/mL)	1002.17±228.13	961.42±158.36	389.28±101.38	A:0.001 B:0.0001 C: 0.001

Data represented as Mean ± SD. SD: standard deviation, BMI: body mass index, SBP: Systolic blood presser, DBP diastolic blood pressure, SpO_2: Oxygen saturation.
A= p value (Dead+ severe), B=p value (Dead+ moderate) C= p value (severe +moderate), LR: Neutrophil lymphocyte ratio, MLR Monocyte lymphocyte ratio.

Table 3. Correlation of serum Leptin and IL-6 with studied parameters in patients infected with COVID-19

Parameters	Leptin ng/mL		IL-6 Pg/mL	
	r	*p* value	r	*p* value
Age (year)	0.189	0.054	0.162	0.070
BMI kg/m^2	0.493	0.0001	0.436	0.001
Lymphocyte x 10^3 / ml	-0.302	0.01	-0.536	0.0001
Neutrophil. x 10^3 / ml	0.278	0.043	0.581	0.0001
Monocyte x 10^3 / ml	-0.162	0.06	0.306	0.001
NLR	0.241	0.038	0.562	0.0001
MLR	0.187	0.070	-0.347	0.01
D-dimer ng/ml	0.446	0.0004	0.566	0.0001
Ferritin ng/ml	0.395	0.001	0.493	0.0001
IL-6 Pg/ml	0.389	0.002	-	-
Leptin ng/mL	-	-	0.389	0.002

BMI: Body mass index, r: Pearson correlation coefficient, NLR: Neutrophil / lymphocyte ratio, MLR: Monocyte lymphocyte ratio.

It is not quite clear why some individuals with COVID-19 have severe conditions and symptoms, while others have moderate or even asymptomatic. Adipose tissues serve as critical role in controlling the metabolism and the activation of immune cells. When immune cells including lymphocytes, macrophages, and Neutrophils invade adipose tissue, it is a sign of inflammation (Maurya et al., 2021). Leptin promotes T-cell proliferation by increasing the release of inflammatory soluble cytokines. Also, Leptin plays a crucial role in cachexia conditions associated with pathogen-induced inflammatory disorders, collagen vascular disease, and even malignancy. However, persistent inflammation by autoimmune or viral disorders or defective Leptin response to CNS (Leptin resistance)produce weight control resistance, which causes obesity and anorexia (Maurya et al., 2021).

A previous study found that the production of Leptin is regulated by inflammation (Behnes et al., 2012), nevertheless Leptin function in patients with COVID-19 has not been explained. In COVID-19, infection may promote Leptin synthsis, as shown by the more clear increased levels of Leptin in obese and overweight patients. The obese individuals have more adipocytes and so produce more Leptin upon infection. The activation of Monocytes by Leptin during viral infections by STAT3/NF-κB signaling, trigger severe cytokine storms in obese and overweight COVID-19 patients, which form a + feedback loop. Therefore, obese patients are prone to release high cytokines as well as Leptin. Blocking inflammation cytokines like IL-6 seems to reduce the cytokine release syndrome produced by COVID-19 (Liu et al., 2020).

The principle and major function of Leptin is to control energy and regulate appetite (Cava & Matarese, 2004). Leptin is believed to be a proinflammatory cytokine and shows a structural resemblance to IL-6. It reverses the phenotypic of T helper cells by reducing regulatory T-cell (Treg) activity and promoting Th17 polarization (Abella et al., 2017).

Leptin affects macrophage and T-cell activity via the stimulation of the JAK-STAT pathway, resulting in immunological dysregulation and the generation of proinflammatory cytokines (Rebello et al., 2020), It interferes with the (LepR) Leptin receptor, hence contributing to obesity's inflammatory response (Pérez-Pérez et al., 2017). In addition, several additional fatty tissue products, such as TNF-alpha, , IL-6, CCL2, IL-1, and plasminogen activator inhibitor type-1, which play a significant role in immunological dysregulation in obese patients, have been identified (Tilg & Moschen, 2006). Consequently, adipocyte connections with immune system may contribute to the severity of the illness in COVID-19 patients with obesity (Maurya et al., 2021).

Secretion of Leptin in obese individuals is chronically much higher than lean persons. The higher level of (pro-inflammatory) leptin and lower (anti-inflammatory) adiponectin ratio could be contributing to the obesity complication because of prejudiced immune responses (Ouchi et al., 2011). Leptin can tempt and attract the proliferation and stimulation of diverse immune cells, including monocytes, natural killer (NK) cells, and T cells by inducing the release of inflammatory cytokines, such as IL−1, TNF−α, and IL−6 (Acedo et al., 2013; Paz-Filho et al., 2012).

Adipocytes participate in a cross-talk with different immune cells and could be involved in the activation and proliferation, resulting in the production of inflammatory cytokines (Huh et al., 2014). Adipocytes add nearly 35% of the IL-6 concentration to the circulation, along with macrophages that are recruited into the AT by leptin (Sonnenberg et al., 2004). IL-6 is the foremost and common cytokine in acute phase inflammation, an innate immune response by infection. IL-6 also plays important role in improving acquired immunity against pathogens, including the expressions of cytokine and chemokine, stimulation of antibody, macrophage regulation, and dendritic cell differentiation (C.-H. Tang et al., 2007).

T lymphocytes and B lymphocytes can produce IL-6 (Jones & Jenkins, 2018). IL-6 has an important function in infectious disorders such as influenza (Longhi et al., 2008). Loss of IL-6 also causes influenza virus to stay in the lungs, resulting in severe lung damage and mortality (Dienz et al., 2012).

Interleukin 6 (IL-6) levels were elevated in individuals with SARS-CoV-1 who had acute lung lesions. Particularly IL-6 can promote the hyper-innate inflammatory response in response to SARS-CoV-1 respiratory tract invasion (Wang et al., 2004). Interestingly, SARS-CoV-1 was able to produce more IL-6 in human epithelial cells than influenza A virus (Okabayashi et al., 2006). More information reveals that patients with severe respiratory failure and SARS-CoV-2 have immunological disorders or macrophage-activation syndrome, which are both characterized by pro-inflammatory cytokines. Interleukin-6 (IL-6) is responsible for the immunological dysregulation in particular (IL-1beta) (Netea et al., 2020). Two essential characteristics of this immunological dysregulation include: overproduction of pro-inflammatory cytokines by Monocytes and dysregulation of lymphocytes with CD4 lymphopenia (Netea et al., 2020).

Interleukin 6 (IL-6) levels rise in the blood during pro-inflammatory critical care illnesses including sepsis. Increased blood IL-6 levels in individuals with severe COVID-19 have rekindled attention in the cytokine as a potential treatment target. Although IL-6's pro-inflammatory properties are well-known, the cytokine actually serves many important anti-inflammatory and physiological functions. A comprehensive understanding of the intricate processes behind IL-6 signaling is crucial for correct interpretation of IL-6 levels in the body, use of IL-6 as a biomarker in critical care, and development of effective anti-IL-6 treatments (McElvaney et al., 2021).

CONCLUSION:

The current results found elevated serum levels of Leptin and IL-6 in the obese patients with COVID-19 and observed the interplays between Leptin and IL-6 might be to the response that regulate the immune cells and suggested that the obese subjects and metabolic consequences of obesity compromise host antiviral defenses leading to critical outcomes from infection. Leptin and IL-6 levels may be used as a good marker for COVID-19 severity in obese patient.

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Declaration of intersts

The authors declare no conflict of interests

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REFERENCES

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