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Leptin and linterlukin-6 relationship and influence of mortality in sepsis

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Abstract

Background Sepsis is a severe and life-threatening disease involving multiple risk factors. Leptin has been suggested to play a role in modulating the inflammatory response in sepsis and improving outcomes; however, there are conflicting results regarding the outcome of sepsis. The present study aims to clarify the expression of leptin in patients with sepsis, and its association with other cytokines.

Method The retrospective study enrolled 165 adults with sepsis from medical intensive care units (ICU)s, and collected leptin, glucose levels, and cytokines such as IL-6, IL-1RA, IL-10, IL-17, TNF-α, IFN-γfor analysis. Leptin levels were divided into three groups based on concentration: Low (≤ 3.78 ng/mL), Medium (3.78 < leptin ≤ 23.2 ng/mL), and High (> 23.2 ng/mL). Survival curve analysis and comparisons among groups were performed. A subgroup analysis by sex (male and female) was also conducted. Finally, a multiple-factor logistic regression model was used to evaluate the interaction between leptin and other factors.

Result The high leptin groups were the oldest (low vs. medium vs. high: 60 vs. 66 vs. 78, $p < 0.0001$) and had the highest body mass index (BMI) (19.8 vs. 23.9 vs. 24.2, $p < 0.0001$), the highest percentages of women (28.6 vs. 34.1 vs. 65.9 $p = 0.001$), and the most comorbidities (1 vs. 1 vs. 2, $p = 0.001$). After controlling IL-6, day 1 leptin had a trend associated with lower mortality in the hospital ($\beta = 0.984$, $p = 0.062$). The highest IL-6 group had a significantly higher mortality rate among three IL-6 level patients ($p = 0.015$), but in the high leptin subgroup analysis, the significant effect of high IL-6 on mortality disappeared. Besides, the subgroup analysis of men, the high leptin group had a trend of better survival than the medium and low leptin groups.

Conclusion High leptin levels may mitigate the adverse prognostic impact of elevated IL-6 on septic mortality. At comparable IL-6 levels, leptin could serve as a predictor of septic outcomes. Leptin might act as a protective factor in men. Future research should explore leptin's role in IL-6-mediated inflammation and its potential protective effect in high IL-6 sepsis cases.

Keywords Leptin, Sepsis, Interleukin 6, Mortality

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Introduction

Sepsis is a severe and heterogeneous disease that makes life life-threatening [1]. Precious studies found many risk factors associated with septic mortality such as comorbidity [2], poor nutrition [3], infection source [4], diagnosis timing [5], abnormal clinical data [6], and immunocompromised [7]. Signaling transduction plays an important role in affecting and regulating the outcome of sepsis [8]. Many cytokines such as TNF-α, IL-1, and IL-6, release and regulate the signaling pathway during the acute stage [3, 8], which influences patients' clinical symptoms, signs, and laboratory presentation [8]. Scientists tried to use therapeutic strategies targeting on inhibition of specific components of inflammatory responses, however, over the past two decades those therapies did not work [8]. Behind the inflammatory cytokine, many components interact with those signaling pathways that may influence the results. Further research is needed to clarify the interactions among these substances and their impact on sepsis outcomes.

Leptin secreted by adipose tissue is a member of the helical cytokine family, which includes IL-6, IL-11, IL-12, and G-CSF [9]. Leptin not only plays an important role in the regulation of body weight and energy expenditure [10] but also influences the inflammatory signaling pathways [11–14]. Many studies suggest that survivors of sepsis with high levels of leptin compared to those with mortality [11, 13–15]. In contrast, Some studies suggest that leptin has no significant impact on sepsis outcomes [11, 16, 17], or provides protective effects only for males, while higher levels in females are associated with increased mortality [18]. A study related to chronic obstructive pulmonary disease (COPD) indicated that high leptin levels in women were associated with high C-reactive protein (CRP) levels [19]. Another study on diabetes risk also indicated that high leptin levels were associated with an increased risk of diabetes in men, but not in women [20]. Previous studies have also indicated that female adipocytes have a higher capacity for leptin secretion compared to males [18, 21]. This might contribute to the difference in baseline leptin levels between men and women. The results of these studies are inconsistent, highlighting the need for further research to clarify the role of leptin in sepsis patients.

In animal studies, leptin injection decreased the mortality of septic mice and decreased the level of IL-6 and TNF-α [12, 22]. Animal studies have shown that in hamsters with bacterial infections, leptin mRNA levels in adipose tissue increase, indicating a regulatory relationship between infection and leptin production [23]. Leptin levels reduce induced malnutrition and immunodeficiency [11]. The leptin receptor, linked to the STAT3 signaling pathway, has been associated with increased susceptibility to Clostridium difficile infectious colitis and bacterial peritonitis. Consequently, leptin appears to play a protective role in maintaining the intestinal mucosal defense against C. difficile infections in both mice and humans [11]. Those studies prove leptin may offer protective potential for mortality and poor outcomes in sepsis patients.

As mentioned above, leptin on sepsis remained inconsistent in human studies. Most research on the interactions between cytokine and inflammatory factors has focused on mice, with a lack of human data. This study aims to investigate the impact of leptin levels on mortality in ICU patients and explore its interactions with other inflammatory factors in greater depth.

Methods

Participants

The current retrospective study enrolled 165 adult patients with sepsis from medical ICUs (34 beds) at Kaohsiung Chang Gung Memorial Hospital between August 2013 and January 2017. Sepsis was defined according to the criteria of the Third International Consensus Definitions for Sepsis and Septic Shock (Sepsis-3) [24]. Clinical parameters, including age, sex, BMI, APACHE II score, Charlson comorbidity index, SOFA score, comorbidities, laboratory data, and day of mortality were obtained from medical records. All participants agreed to undergo blood sampling during the ICU hospitalization period. They or their family members provided written informed consent for the use of residual blood samples. The study design was approved by the Institutional Review Board (ID: 202001696B0C501) of Chang Gung Memorial Hospital.

Measures and design

The levels of leptin and biomarkers, including IL-6, IL-1RA, IL-10, IL-17, TNF-α, IFN-γ in the plasma, and monocyte HLA-DR expression have been mentioned in our previous reports [7, 25]. Whole blood samples were collected from patients by heparinized tube (BD, Franklin Lakes, NJ, USA) when they were admitted to ICU. Using a Ficoll-Paque (Amersham Biosciences, Uppsala, Sweden), whole blood was centrifuged at 400 g × 30 min to separate the plasma and peripheral blood mononuclear cells (PBMCs).

Plasma leptin level analysis was conducted with ELISA kits. IL-10, IL-6, IL-17, TNF-α, and IFN-γ, were quantified using a Human Cytokine/Chemokine Magnetic Bead Panel customized Milliplex MAP kit (#HCYTOMAG-60 K, EMD Millipore, Darmstadt, Germany) [7]. Using the PBMCs, monocyte HLA-DR expression was measured by flow cytometry [25] (Cytomics FC500; Beckman Coulter, Inc., Fullerton, CA, USA).

This study distinguished leptin into three groups according to quartile: patients with day 1 leptin levels

above the top 25% were categorized into the high leptin groups; patients with day 1 leptin levels in the interquartile range were categorized into the medium leptin groups; and patients with day1 leptin groups below the lowest 25 percents were categorized into the low leptin group. The study analyzed the differences among three leptin groups and stratified analysis by gender. The factors that were significantly different among the three groups would input secondary analyses of multiple regression models to clarify the interactions with leptin that contributed to mortality in the hospital. The multiple regression models were built based on the leptin day 1 variable (Model 0). Model 1 was adjusted for the basic characteristics of the cases; therefore, the variables in Model 1 were retained in the subsequent three models. Model 2 adjusted for comorbidities, Model 3 adjusted for cytokines, and Model 4 adjusted for laboratory data.

Statistical analyses

The data was analyzed by SPSS software (version 22.0; IBM Corp., Armonk, NY, USA). The women and men were compared in terms of demographic characteristics, baseline characteristics, and outcomes. The Mann-Whitney U test was used for continuous variables and the chi-square test was used for categorical variables. Subsequently, the demographic characteristics, baseline characteristics, and outcomes were compared across the three different leptin levels. The Kruskal-Wallis test was used for continuous variables.

and the chi-square test was used for categorical variables. The survival curve was constructed according to the 90-day mortality that was determined based on the different day 1 leptin levels. Subgroup analysis stratified participants by gender.

Sequentially, the dependent variable of the logistic regression model was the 90-day mortality rate. The variance Inflation Factor (VIF) was checked for continuing variables. The unadjusted model (Model 1) included intercept leptin, age, sex, BMI, and intercept. Models 2–4 were adjusted for the same variables used in Model 1. Model 2 further included comorbidities which were different among the three leptin groups, Model 3 included cytokines which were different among the three leptin groups, and Model 4 included laboratory data which were different among the three leptin groups. Those models were quadratic by Nagelkerke R^2. The significance level for all tests was set at an alpha level = 0.05.

Results

Baseline characteristics and outcomes of the patients with sepsis

Among 165 patients, 98 of them were men, and 67 of them were women. Compared to men, women exhibited a higher percentage of patients with diabetes mellitus (men vs. women; 41.8% vs. 58.2%, $p = 0.039$), a higher leptin concentration on day 1 (7.1 vs. 16.0, $p < 0.0001$) and day 3 (5.4 vs. 18.4, $p < 0.0001$), and a higher glucose level on day 1 (171 vs. 221, $p = 0.036$) (Table 1). 42, 82, and 41 patients were categorized into three groups based on their leptin levels on day 1 (low: leptin ≤ 3.78, medium: 3.78 < leptin ≤ 23.2, high: leptin > 23.2 (ng/dL)). The patients in the high leptin group were the oldest (low vs. medium vs. high: 60 vs. 66 vs. 78, $p < 0.0001$) and had the highest BMI (19.8 vs. 23.9 vs. 24.2, $p < 0.0001$), the highest percentages of women (28.6 vs. 34.1 vs. 65.9 $p = 0.001$), the most comorbidities (1 vs. 1 vs. 2, $p = 0.001$), including coronary artery disease (16.7 vs. 25.6 vs. 48.8, $p = 0.003$), stroke (21.4 vs. 14.6 vs. 34.1, $p = 0.044$) hypertension (40.5 vs. 54.9 vs. 75.6, $p = 0.005$) and diabetes mellitus (35.7 vs. 42.7 vs. 73.2, $p = 0.001$), the highest glucose level (148 vs. 205 vs. 216, $p = 0.034$) and the lowest CRP level on day 1 (239.0 vs. 174.6 vs. 109.6, $p = 0.009$). They also had the highest leptin concentration on day 1(1.2 vs. 9.9 vs. 44.3, $p < 0.0001$), and day 3 (2.1 vs. 7.4 vs. 32.3 $p < 0.0001$), while the lowest IL-6 (112.2 vs. 45.8 vs. 41.3, $p = 0.008$) and IL-10 (43.9 vs. 16.1 vs. 10.9, $p = 0.013$) levels on day 1.

The relationship between leptin and mortality of men

Table 2 revealed the characteristics of the 98 male patients. 30, 54, and 14 patients were categorized into three groups based on their leptin levels on day 1. In the male subgroup, the high leptin group was associated with the oldest age (low vs. medium vs. high: 59 vs. 67.5 vs. 78, $p = 0.007$), the highest rate of coronary artery disease (13.3 vs. 24.1 vs. 50, $p = 0.031$) and hypertension (46.7 vs. 45.3 vs. 87.5, $p = 0.024$), and the lowest level of IL-10(38.4 vs. 13.6 vs. 7.7, $p = 0.017$). Compared to the low leptin group, both the medium and high leptin groups had higher BMI (19.4 vs. 24.1 vs. 22.3, $p = 0.014$).

Figure 1 revealed the 90-day survival curve of men distinguished by different day 1 leptin levels. The high leptin group had a trend of better survival than the medium and low leptin groups, but the p-value was non-specific ($p = 0.441$).

The relationship between leptin and mortality of women

Table 3 revealed the characteristics of the 67 female patients. 12, 28, and 27 patients were categorized into three groups based on their leptin levels on day 1. The high leptin group was associated with the oldest age ((low vs. medium vs. high: 69.5 vs. 65 vs. 79, $p = 0.006$), the highest BMI (20.4 vs. 23.7 vs. 24.7, $p = 0.028$), the most comorbidities (1 vs. 1 vs. 2, $p = 0.008$), the highest rate of diabetes mellitus (33.3 vs. 50 vs. 77.8, $p = 0.018$), and the lowest CRP level (260.0 vs. 253.1 vs. 109.8, $p = 0.020$). The high leptin group also showed a trend of the highest glucose level (168 vs. 229. vs. 231, $p = 0.081$). Compared to the low leptin group, both medium and high leptin

Table 1 Baseline characteristics and outcomes of the patients with sepsis

	ALL (*n* = 165)	**Male (*n* = 98)**	**Female (*n* = 67)**	***P***	**Leptin ≤ 3.78 (*n* = 42)**	**3.78 < Leptin ≤ 23.2 (*n* = 82)**	**Leptin > 23.2 (*n* = 41)**	***P***
Demographic characteristics, Median (Quartile)								
Age (years)	68 (55–80)	65.5(51.8–79.3)	74 (59–80)	0.067	60.0 (51.8–76.3)	66 (51.8–79.0)	78 (68.5–85)	< 0.0001*
BMI, kg/m^2	22.9(19.6–26.3)	22.3 (19.0-25.7)	23.6 (20.6–28.6)	0.102	19.8 (16.7–24.8)	23.9 (20.2–26.8)	24.2 (22.0-28.8)	< 00001*
Sex (female), n (%)					12 (28.6)	28 (34.1)	27 (65.9)	0.001*
Score, Median (Quartile)								
APACHE II	25.0 (20–33)	24.5 (20–33)	27 (20–32)	0.790	25.5 (20–33)	25.0 (20.8–33)	24.0 (19.5–32.5)	0.746
Charlson comorbidity index	2 (2–2)	2 (1.75–2.25)	2 (2–2)	0.964	2 (1-2.3)	2 (2-2.3)	2 (2–2)	0.276
SOFA	9 (7–12)	9 (7–12)	9 (7–12)	0.653	11 (7–13)	10 (7–12)	8 (7-10.5)	0.128
Comorbidity (number)	2 (1–2)	1 (1–2)	2 (1–2)	0.511	1 (0–2)	1 (1–2)	2 (1–3)	0.001*
Comorbidities, n (%)								
Coronary artery disease	48(29.1)	24(24.5)	24(35.8)	0.116	7 (16.7)	21 (25.6)	20 (48.8)	0.003*
History of stroke	35(21.2)	23(23.5)	12(17.9)	0.391	9 (21.4)	12 (14.6)	14 (34.1)	0.044*
Hypertension	90(56.4)	51(52.0)	42(62.7)	0.176	17 (40.5)	45 (54.9)	31 (75.6)	0.005*
COPD	23(13.9)	16(16.3)	7(10.4)	0.284	4 (9.5)	11 (13.4)	8 (19.5)	0.414
Cancer	31(18.8)	21(21.4)	10(14.9)	0.294	11 (26.2)	15 (18.3)	5 (12.2)	0.261
CKD	37(22.4)	19(19.4)	18(26.9)	0.258	5 (11.9)	21 (25.6)	11 (26.8)	0.165
Liver cirrhosis	7(4.2)	6(6.1)	1(1.5)	0.147	5 (11.9)	7 (8.5)	1 (2.4)	0.265
Diabetes mellitus	80(48.5)	41(41.8)	39(58.2)	0.039*	15 (35.7)	35 (42.7)	30 (73.2)	0.001*
Biomarker, Median (Quartile)								
Leptin day1	9.8 (3.8–23.2)	7.1 (3.2–16.2)	16.0 (5.4–41.8)	< 0.0001*	1.2 (0.7-3.0)	9.9 (6.2–15.6)	44.3 (30.9–72.3)	< 0.0001*
Leptin day3	8.5 (3.0-22.3)	5.4 (2.4–13.9)	18.4 (4.3–38.5)	< 0.0001*	2.1 (1.0-3.8)	7.4 (4.4–15.0)	32.3 (16.2–54.8)	< 0.0001*
IL-6 day1	57.6 (24.5-144.6)	41.9 (24.8–119.0)	62.4 (21.0-164.5)	0.336	112.2 (44.6-269.7)	45.8 (17.2-141.4)	41.3 (15.1–89.6)	0.008*
IL-1RA day1	72.3 (17.9–224.0)	51.7 (112.3-184.3)	74.8 (19.1-268.1)	0.209	120.6 (23.0-298.7)	56.0 (14.2-231.3)	66.5 (17.0-144.9)	0.188
IL-10 day1	22.9(3.9–70.6)	25.2 (5.2–88.3)	20.5 (3.6–65.5)	0.628	43.9 (16.7–98.4)	16.1 (2.7–60.9)	10.9 (3.6–56.3)	0.013*
IL-17 day1	4.6 (2.1–11.3)	5.5(1.9–12.7)	4.5 (2.2–11.2)	0.880	4.6 (2.1–17.2)	5.6 (2.2–13.8)	2.9 (2.0-8.1)	0.111
TNF-α day1	35.2 (19.6–72.1)	36.4 (20.8–67.0)	34.5 (18,8-74.4)	0.994	53.6 (24.0-84.7)	32.9 (18.1–78.9)	32.6 (20.7–52.8)	0.164
IFN-γ day1	5.0 (2.4–23.0)	6.1 (2.3–35.2)	4.5 (2.4–16.0)	0.303	5.3 (2.6–29.6)	5.4 (2.4–19.7)	4.5 (2.2–22.5)	0.495
HLA-DR expression (%) day1	92.0 (80.4–97.6)	91.3 (71.3–97.6)	92.5 (83.3–97.6)	0.362	90.8 (76.7–98.0)	90.6 (81.5–97.3)	94.3 (85.8–97.6)	0.823
Laboratory data, Median (Quartile)								
HbA1c (%)	6.9 (6.0–8.0)	6.8 (6.0–8.0)	7.0 (5.9–8.2)	0.903	7.1 (5.9–7.8)	6.8 (5.9–7.9)	7.3 (6.0-8.3)	0.723
Glucose (mg/dL) day1	187 (124.3–259)	171 (132–245)	221 (159–266)	0.036*	148 (127.8–220.0)	205.0 (143.0-264.0)	216 (164.5-278.8)	0.034*
CRP day1	172.0 (54.2-286.7)	157.7 (55.5-243.6)	208.6 (47.6-315.1)	0.284	239 (140.9-330.9)	174.6 (55.9–279)	109.6 (24.3-237.3)	0.009*
WBC (X1000) day1	15.4 (9.9–21.1)	15.5 (9.8–23.7)	14.6 (10.0-19.2)	0.251	16.2 (9.0-24.2)	14.4 (9.2–20.3)	15.5 (12.6–20.5)	0.396
Lactate day1	22.2 (15.3–39.3)	21.3 (14.9–41.7)	23.6 (17.8–38.6)	0.384	22.9 (17.3–50.0)	22.4 (14.8–40.0)	20.9 (15.9–32.2)	0.634
Admission days, Median (Quartile)								
ICU days	10.8 (7.1–16.8)	11.8 (7.0-16.9)	10.0 (7.1–16.0)	0.333	13.5 (7.6–18.5)	10.0 (7.0-14.9)	10.9 (6.5–18.2)	0.315
LOS	25.5 (15.8–47.9)	26.1 (17.3–55.1)	24.9 (15.3–42.7)	0.365	25.6 (15.4–44.7)	25.2 (16.0-43.1)	26.4 (15.9–63.7)	0.731
Mortality, n (%)								
7-day mortality	15 (9.1)	9 (9.2)	6 (9.0)	0.960	3 (7.1)	8 (9.8)	4 (9.8)	0.879

Table 1 (continued)

	ALL (*n* = 165)	Male (*n* = 98)	Female (*n* = 67)	*P*	Leptin ≤ 3.78 (*n* = 42)	3.78 < Leptin ≤ 23.2 (*n* = 82)	Leptin > 23.2 (*n* = 41)	*P*
28-day mortality	34 (20.6)	20 (20.4)	14 (20.9)	0.939	9 (21.4)	19 (23.2)	6 (14.6)	0.538)
90-day mortality	58 (35.2)	34 (34.7)	24 (35.8)	0.882	14 (33.3)	29 (35.4)	15 (36.6)	0.951

***$p < 0.001$, **$p < 0.01$, *$p < 0.05$, †$p < 0.09$

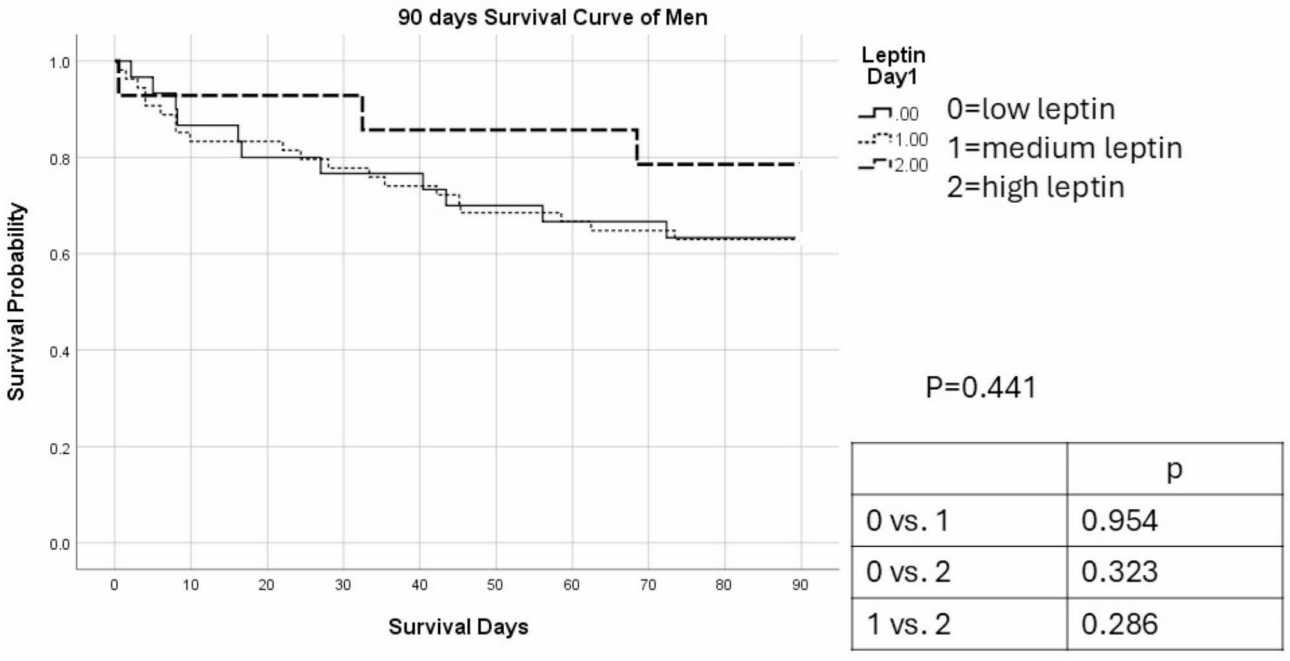
Abbreviations: BMI: Body Mass Index, APACHE II: Acute Physiology and Chronic Health Evaluation II, SOFA: Sequential Organ Failure Assessment

COPD: Chronic Obstructive Pulmonary Disease, CKD: Chronic Kidney Disease, HbA1c: Glycated Hemoglobin, CRP: C-reactive Protein, WBC: White Blood Cell Count (commonly abbreviated as WNC in some contexts), ICU: Intensive Care Unit, LOS: length of stay, IL-6, interleukin 6; IL-1RA, interleukin-1 receptor antagonist; IFN-γ, interferon gamma; IL-10, interleukin 10; IL-17, interleukin 17; TNF, tumor necrosis factor; HLA-DR, human leukocyte antigen-DR

Table 2 Baseline characteristics and outcomes of the male patient with sepsis

	Male (*n* = 98)	Leptin ≤ 3.78 (*n* = 30)	3.78 < Leptin ≤ 23.2 (*n* = 54)	Leptin > 23.2 (*n* = 14)	*P*
Demographic characteristics, Median (Quartile)					
Age (years)	65.5(51.8–79.3)	59 (49-75.3)	67.5 (48-78.3)	78 (73.5–87.3)	0.007**
BMI, kg/m^2	22.3 (19.0-25.7)	19.4 (16.3–24.9)	24.1 (19.5–26.2)	22.3 (21.8–28.3)	0.014*
Score, Median (Quartile)					
APACHE II	24.5 (20–33)	25 (19.5–35)	24 (20.8–33)	25 (19.5–33.5)	0.981
Charlson comorbidity index	2 (1.75–2.25)	2 (1-3.75)	2 (1.75-3.0)	2 (2–2)	0.672
SOFA	9 (7–12)	11 (7–13)	9 (7-11.3)	9 (6.8–10.5)	0.370
Comorbidity (number)	1 (1–2)	1 (0.75-2.0)	1 (1–2)	2 (1.75-3.0)	0.107
Comorbidities, n (%)					
Coronary artery disease	24(24.5)	4(13.3)	13 (24.1)	7 (50)	0.031*
History of stroke	23(23.5)	8(26.7)	9(16.7)	6(42.9)	0.106
Hypertension	51(52.0)	14 (46.7)	25 (45.3)	12 (87.5)	0.024*
COPD	16(16.3)	4 (14.3)	9 (16.7)	3 (21.4)	0.791
Cancer	21(21.4)	9 (30.0)	11 (20.4)	1 (7.1)	0.218
CKD	19(19.4)	4 (13.3)	12 (22.2)	3 (21.4)	0.601
Liver cirrhosis	6(6.1)	2 (6.7)	4 (7.4)	0 (0)	0.582
Diabetes mellitus	41(41.8)	11 (36.7)	21 (38.9)	9 (64.3)	0.181
Biomarker, Median (Quartile)					
Leptin day1	7.1 (3.2–16.2)	1.1 (0.6-3.0)	8.9 (5.8–15.6)	44.0 (26.5–64.9)	< 0.001***
Leptin day3	5.4 (2.4–13.9)	2.1 (1.0-3.1)	7.2 (3.6–12.2)	22.6 (14.9–35.8)	< 0.001***
IL-6 day1	41.9 (24.8–119.0)	113.7 (50.2-339.7)	51.7 (10.1-137.4)	41.5 (15.6–90.7)	0.026*
IL-1RA day1	51.7 (112.3-184.3)	137.8 (27.1-570.8)	47.7 (10.7-295.2)	96.92 (44.1-170.2)	0.150
IL-10 day1	25.2 (5.2–88.3)	38.4 (15.1-138.6)	13.6 (2.7–54.1)	7.7 (3.5–56.3)	0.017*
IL-17 day1	5.5(1.9–12.7)	5.1 (2.1–17.2)	4.6 (2.3–17.8)	3.5 (2.6–5.1)	0.507
TNF-α day1	36.4 (20.8–67.0)	54.3 (25.7–84.7)	30.9 (17.2–76.9)	32.1 (21.8–52.5)	0.180
IFN-γ day1	6.1 (2.3–35.2)	4.5 (2.5–17.2)	5.4 (2.5–20.2)	2.9 (2.2–11.8)	0.417
HLA-DR expression (%) day1	91.3 (71.3–97.6)	25.7 (13.0-32.8)	22.0 (14.8–34.3)	21.8 (14.8–30.6)	0.910
Laboratory data, Median (Quartile)					
HbA1c (%)	6.8 (6.0–8.0)	7.3 (7.0-7.3)	7.3 (6.8–7.3)	7.3 (7.3–8.1)	0.169
Glucose (mg/dL) day1	171 (132–245)	147.5 (124-235.3)	171.5 (128.8-237.8)	181 (152.5-314.5)	0.272
CRP day1	157.7 (55.5-243.6)	227.5 (87.4-332.4)	128.4 (54.1-239.6)	96.7 (26.3-232.9)	0.116
WBC (X1000) day1	15.5 (9.8–23.7)	19.3 (10.3–25.9)	14.4 (9.2–20.3)	16.7 (12.6–24.3)	0.139
Lactate day1	21.3 (14.9–41.7)	25.3 (17.4–57.0)	18.5 (13.7–34.6)	20 (15.0-34.4)	0.203
Admission days, Median (Quartile)					
ICU days	11.8 (7.0-16.9)	12.2 (7.6–18.3)	9.1 (7.0-14.6)	9.0 (6.6–13.1)	0.338
LOS	26.1 (17.3–55.1)	23.4 (15.4–44.2)	25.5 (14.7–42.2)	23.2 (14.7–60.2)	0.984
Mortality, n (%)					
7-day mortality	9 (9.2)	2 (6.7)	6 (11.1)	1 (7.1)	0.764
28-day mortality	20 (20.4)	7 (23.3)	12 (22.0)	1 (7.1)	0.410
90-day mortality	34 (34.7)	11 (36.7)	20 (37.0)	3 (21.4)	0.530

***$p < 0.001$, **$p < 0.01$, *$p < 0.05$, †$p < 0.09$



	p
0 vs. 1	0.954
0 vs. 2	0.323
1 vs. 2	0.286

Fig. 1 90 days survival curve of men distinguished by different day 1 leptin level

groups had higher rates of hypertension (25.0 vs. 71.4 vs. 70.4, $p = 0.012$).

Figure 2 revealed the 90-day survival curve of women distinguished by different day 1 leptin levels. The mortalities were non-specific differences among different leptin levels.

IL-6 prediction power of mortality interacted with leptin level

Table 4 revealed the odds ratio of day 1 leptin associated with mortality in the hospital. The VIF values for BMI, leptin, IL-6, IL-10, glucose, and CRP were 1.160, 1.259, 1.311, 1.321, 1.114, and 1.107, respectively, indicating no significant multicollinearity. The day 1 leptin had a trend associated with lower mortality in the hospital ($\beta = 0.984$, $p = 0.062$) after controlling IL-6 in Model 3. IL-6 also had a trend associated with higher mortality in the hospital ($\beta = 1.003$. $p = 0.053$). Model 3 of cytokine (IL-6 & IL10) Model 3 showed the greatest improvement in goodness-of-fit, increasing Nagelkerke R^2 from 0.035 in Model 1 to 0.085.

Figure 3 showed the high IL-6 group had a significantly higher mortality rate among three IL-6 level patients ($p = 0.015$). In subgroup analysis, the high IL-6 still predicted mortality of patients with low (medium IL-6 vs. high IL-6, $p = 0.016$) and medium (medium IL-6 vs. high IL-6, $p = 0.048$) day 1 leptin (Fig. 4A and B). However, IL-6 association with mortality was absent in the high leptin groups (Fig. 4C).

Discussion

The present study revealed a negative correlation between leptin and IL-6, and after controlling for IL-6, leptin levels show a trend toward predicting mortality. Besides, IL-6 had a significant prediction power for septic mortality; however, in the high leptin group, IL-6 lost its ability to predict mortality. Leptin levels alone could not predict mortality; however, in the survival curve (Fig. 1) for males, the high leptin group shows a trend toward better survival rates. Leptin levels and blood glucose levels in sepsis patients exhibit gender differences, being higher in females.

Leptin and IL-6 interaction in sepsis

In the comparison of three groups divided by leptin levels, the highest leptin group shows the lowest IL-6 levels in both overall analysis and subgroup analysis by gender. This finding is consistent with previous studies that mentioned survivors of sepsis had high levels of leptin and lower levels of IL-6 [13, 15, 22]. According to the Koca et al. study, injecting leptin into septic mice could significantly reduce IL-6 levels [22]. Leptin regulated inflammation by inhibiting the hypothalamic-pituitary-adrenal axis, whereas IL-6 stimulated it [15, 17]. This might explain why the two components are negatively correlated. However, the relationship and potential interaction between IL-6 and leptin in sepsis remain unclear. In our study, after controlling IL-6, leptin had a trend to predict septic mortality. Besides, IL-6 shows significant

Table 3 Baseline characteristics and outcomes of the female patient with sepsis

	Female (*n* = 67)	Leptin ≤ 3.78 (*n* = 12)	3.78 < Leptin ≤ 23.2 (*n* = 28)	Leptin > 23.2 (*n* = 27)	*P*
Demographic characteristics, Median (Quartile)					
Age (years)	74 (59–80)	69.5 (54-77.8)	65 (55.8–79)	79 (66–86)	0.006**
BMI, kg/m^2	23.6 (20.6–28.6)	20.4 (17.7–22.6)	23.7 (21.3–28.7)	24.7 (22.2–29.1)	0.028*
Score, Median (Quartile)					
APACHE II	27 (20–32)	27.5 (21.0-38.3)	28 (20.3–32)	24 (19–32)	0.624
Charlson comorbidity index	2 (2–2)	2 (1–2)	2 (2–2)	2 (2–2)	0.225
SOFA	9 (7–12)	10 (6.3–12.5)	10 (7.3–12)	8 (7–11)	0.279
Comorbidity (number)	2 (1–2)	1 (0–2)	1 (1–2)	2 (1–3)	0.008**
Comorbidities, n (%)					
Coronary artery disease	24(35.8)	3 (25.0)	8 (28.6)	13 (48.1)	0.219
History of stroke	12(17.9)	1 (8.3)	3 (10.7)	8 (29.6)	0.119
Hypertension	42(62.7)	3 (25.0)	20 (71.4)	19 (70.4)	0.012*
COPD	7(10.4)	0 (0)	2 (7.1)	5 (18.5)	0.165
Cancer	10(14.9)	2 (16.7)	4 (14.3)	4 (14.8)	0.981
CKD	18(26.9)	1 (8.3)	9 (32.1)	8 (29.6)	0.273
Liver cirrhosis	1(1.5)	1 (8.3)	0 (0)	0 (0)	0.098
Diabetes mellitus	39(58.2)	4 (33.3)	14 (50)	21 (77.8)	0.018*
Biomarker, Median (Quartile)					
Leptin day1	7.1 (3.2–16.2)	1.5 (1.0–3.0)	11.0 (7.0-15.6)	44.3 (34.0-78.6)	< 0.001***
Leptin day3	5.4 (2.4–13.9)	2.1 (0.9–6.7)	12.6 (5.4–23.6)	39.2 (22.8–56.4)	< 0.001***
IL-6 day1	41.9 (24.8–119.0)	86.8 (28.6-148.3)	40.2 (24.8-151.3)	41.3 (11.3–89.9)	0.431
IL-1RA day1	51.7 (12.3-184.3)	65.7 (8.9-266.4)	78.7 (24.1-188.6)	31.3 (11.2-125.7)	0.443
IL-10 day1	25.2 (5.2–88.3)	52.3 (20.1–88.2)	23.8 (3.3-100.9)	14.1 (3.5–86.3)	0.415
IL-17 day1	5.5(1.9–12.7)	4.0 (2.4–27.3)	9.2 (2.1–16.1)	2.9 (1.3-9.0)	0.250
TNF-α day1	36.4 (20.8–67.0)	40.5 (21.3–86.3)	37.9 (20,8-90.3)	32.6 (18.2–53.9)	0.604
IFN-γ day1	6.1 (2.3–35.2)	21.0 (5.1-173.8)	5.1 (2.2–21.4)	5.0 (2.2–55.9)	0.099
HLA-DR expresssion (%) day1	91.3 (71.3–97.6)	26.7 (16.6–43.2)	21.3 (14.5–30.0)	18.3 (13.9–36.9)	0.621
Laboratory data, Median (Quartile)					
HbA1c (%)	7.0 (5.9–8.2)	6.5(6.1–7.4)	6.9 (6.4–7.7)	6.5 (6.0-7.8)	0.435
Glucose (mg/dL) day1	221 (159–266)	168 (133.8–203)	229 (159-337.5)	231 (177–273)	0.081†
CRP day1	208.6 (47.6-315.1)	260 (221.7-338.6)	253.1 (70.7-348.2)	109.8 (22.6-291.6)	0.020*
WBC (X1000) day1	14.6 (10.0-19.2)	11.1 (3.7–16.7)	15.1 (8.1–20.3)	14.9 (12.5–18.9)	0.230
Lactate day1	23.6 (17.8–38.6)	21.2 (14.6–29.9)	27.2 (17.4–40.6)	20.9 (18.9–31.0)	0.432
Admission days, Median (Quartile)					
ICU days	10.0 (7.1–16.0)	13.8 (7.3–19.6)	11.5 (7.9–15.7)	11.0(6.2–19.1)	0.647
LOS	24.9 (15.3–42.7)	33.4 (16.6–52.3)	24 (17.8–50.5)	27.3 (16.6–65.0)	0.768
Mortality, n (%)					
7-day mortality	6 (9.0)	1 (8.3)	2 (7.1)	3 (11.1)	0.843
28-day mortality	14 (20.9)	2 (16.7)	7 (25.0)	5 (18.5)	0.776
90-day mortality	24 (35.8)	3 (25.0)	9 (32.1)	12 (44.4)	0.438

***$p < 0.001$, **$p < 0.01$, *$p < 0.05$, †$p < 0.09$

predictive power for mortality in sepsis, but in the subgroup analysis of the high leptin group, IL-6 loses its predictive ability. These results suggest that the concentrations of leptin and IL-6 may influence each other in predicting sepsis mortality. Future studies are needed to further investigate whether high leptin levels have the potential to reduce the mortality risk associated with IL-6 and cytokine storms.

Leptin shows a trend toward predicting mortality in male sepsis patients

Leptin levels alone could not predict mortality, but it had a trend in predicting septic mortality in men. According to baseline characteristics of patients with sepsis, the patients with high leptin levels were older, had higher BMI, more comorbidities, female participants, lower IL-6, IL-10, CRP, and higher glucose. Age, comorbidities, and IL-6 were known as bad risk factors for sepsis before, while Mild increases in BMI and blood glucose levels

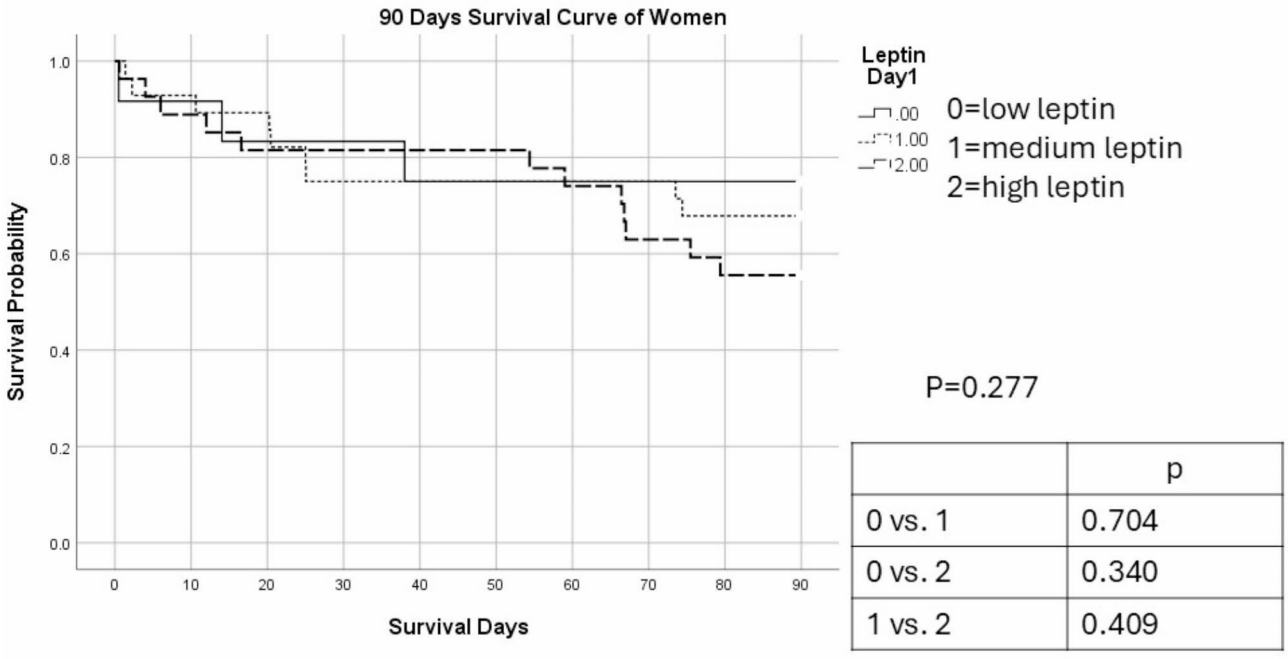


Fig. 2 90 days survival curve of women distinguished by different day 1 leptin level

Table 4 Odds ratio of day1-leptin associated with mortality of hospital

	Model 0	Model1	Model 2	Model 3	Model 4
Leptin D1	0.989	0.990	0.990	0.984†	0.998
Age		0.992	0.992	0.994	0.989
Sex (male)		0.936	0.948	0.967	0.653
BMI		1.010	1.013	1.017	0.968
Coronary artery disease			0.748		
History of stroke			0.886		
Hypertension			1.217		
Diabetes mellitus			1.118		
IL-6 day1				1.0003†	
IL-10 day1				0.9998	
Glucose (mg/dL) day1					1.002
CRP day1					1.001
Intercept	0.686†	0.940	1.021	0.711	1.392
Nagelkerke R^2	0.025	0.032	0.040	0.082	0.035

***$p < 0.001$, **$p < 0.01$, *$p < 0.05$, †$p < 0.09$

are not necessarily harmful to sepsis patients [3, 26, 27]. Jacobsson et al. revealed similar results that high leptin in the acute phase was associated with increased mortality in women while being protective in men [18]. However, in the women subgroup analysis, our study does not find significant differences in mortality. Why leptin has a protective effect in the acute phase of male sepsis but not in females remains unclear. However, it may be related to differences in leptin signaling pathways between males and females [18, 28, 29]. In an animal study, leptin injection on food intake suppression in mice shows that only female mice experience suppressed appetite at 24 h, while male mice remain unaffected [29]. Besides, previous studies have shown that women have higher baseline leptin levels [18, 21], which may contribute to increased leptin resistance [30]. This might be one of the reasons for the different effects of leptin on men and women during the acute phase of sepsis. Although elevated leptin levels appear to have a protective effect in males, previous experiments on leptin treatment in septic mice have shown inconsistent results [12, 17, 22, 31]. Therefore, more research is needed to elucidate its mechanism before leptin can be applied in clinical treatment.

Leptin levels exhibit gender differences

Leptin levels and blood glucose levels in sepsis patients were higher in females. This might be related to the body mass distribution in females, as women had more adipocytes, which could lead to higher leptin levels [29]. Additionally, female adipocytes had a higher capacity for leptin secretion [21]. The higher blood glucose levels in females compared to males were speculated to be related to the higher prevalence of diabetes in the female population.

This study presents a new perspective: under conditions of high leptin, the significance of IL-6 in predicting poor sepsis outcomes disappears, suggesting that leptin might suppress IL-6-related cytokine storms and associated damage. Another study also indicated that leptin can inhibit dendritic cells from producing IL-10, and protect dendritic cells from apoptosis [9]. Many previous studies have suggested that leptin might have a protective effect

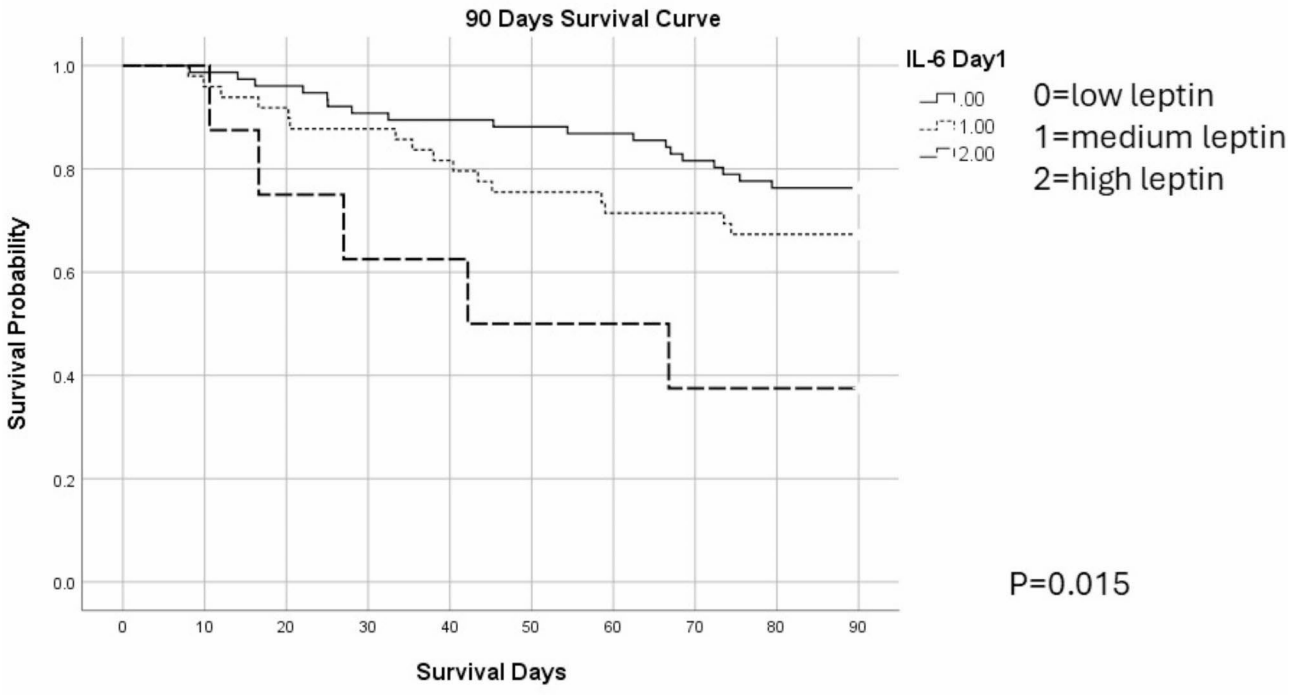


Fig. 3 90 days survival curve distinguished by different day 1 IL-6

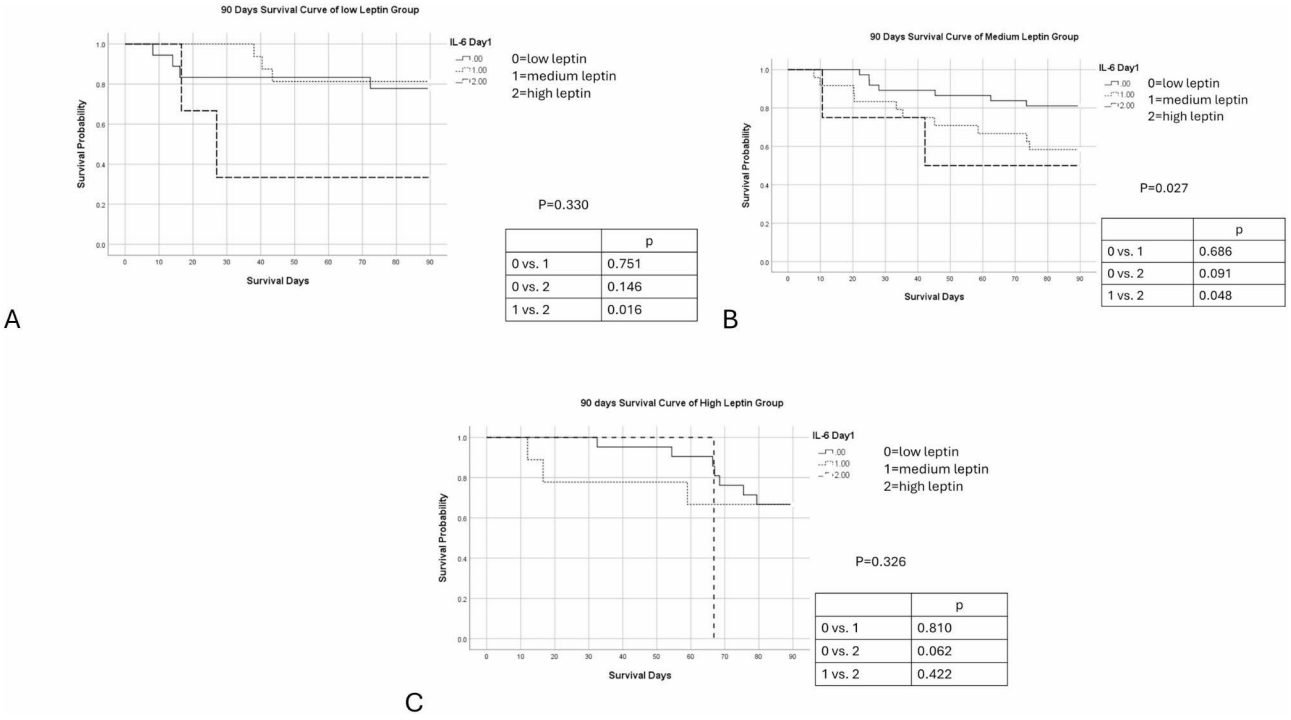


Fig. 4 90 days survival curve of different leptin levels distinguished by different day 1 IL-6 (4a: low leptin, 4b: medium leptin, 4c: high leptin)

on sepsis; however, due to the lack of clear mechanistic evidence, consistent therapeutic experimental results have not been achieved. Our findings highlight the interaction between leptin and IL-6, providing a focused perspective on the role of leptin in anti-inflammatory processes and further clarifying its function in sepsis. Besides, gender differences in leptin signaling pathways were another issue. Future studies are needed to clarify how leptin regulates inflammation differently between male and female sepsis patients.

This study had certain limitations. First, the retrospective design and the limited number of cases with leptin restrict further analysis. If more cases could be obtained, the statistical significance might improve. Second, HbA1C data were not available for every case. For the missing data, we replaced the missing values with predicted values from linear regression [32]. Third, this study lacks baseline leptin levels of patients to determine whether high leptin levels in certain cases were pre-existing or a response to the acute phase. Sepsis is an unpredictable disease, and leptin is not part of routine clinical blood tests. Obtaining baseline leptin data before sepsis occurred in septic patients would be challenging unless conducted through large-scale cohort studies in human trials.

In conclusion, high leptin levels might counteract the adverse prognostic effect of elevated IL-6 on septic mortality. At the same IL-6 level, leptin could predict septic mortality. Leptin might be a protecting factor for men and gender-different signaling pathways related to sepsis need further study. This study's strength was focused on the relationship between different cytokines and leptin and pointed out the interaction between IL-6 and leptin. Future research can clarify the mechanisms by which leptin influences IL-6 regulation of inflammation and further evaluate whether administering leptin in cases of high IL-6 sepsis could provide a protective effect.

Abbreviations

IL-6	Interleukin 6
IL-1RA	Interleukin-1 receptor antagonist
IFN-γ	Interferon gamma
IL-10	Interleukin 10
IL-17	Interleukin 17
TNF-α	Tumor necrosis factor-α
HLA-DR	Human leukocyteantigen-DR

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Author contributions

Conceptualization and methodology: Y.-H.T, K-Y. H, and W.-F.F.; acquisition, analysis, or inter-pretation of data: Y.-H.T. K.-Y.H., and W.-F.F.; drafting the manuscript and revising it critically for important intellectual content: Y.-H.T. and W.-F.F. All authors have read and approved the final manuscript.

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Data availability

The data are not publicly available due to patients' privacy. If the data are required for research purposes, please contact the corresponding author.

Declarations

Ethics approval and consent to participate

The study was conducted according to the guidelines of the Declaration of Helsinki and approved by the Institutional Review Board of Chang Gung Memorial Hospital (ID: 202001696B0C501; approval date: 21 December 2020).

Consent for publication

Informed consent was obtained from all participants involved in the study from whom blood samples were collected.

Competing interests

The authors declare no competing interests.

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References

1. Evans L, Rhodes A, Alhazzani W, Antonelli M, Coopersmith CM, French C, Machado FR, Mcintyre L, Ostermann M, Prescott HC. Surviving sepsis campaign: international guidelines for management of sepsis and septic shock 2021. Crit Care Med. 2021;49(11):e1063–143.
2. Yang Y, Yang KS, Hsann YM, Lim V, Ong BC. The effect of comorbidity and age on hospital mortality and length of stay in patients with sepsis. J Crit Care. 2010;25(3):398–405.
3. Tsai Y-H, Lin C-Y, Chen Y-M, Chang Y-P, Hung K-Y, Chang Y-C, Chen H-C, Huang K-T, Chen Y-C, Wang Y-H. Impact of body mass index on the survival of patients with sepsis with different modified NUTRIC scores. Nutrients. 2021;13(6):1873.
4. van Vught LA, Klouwenberg PMK, Spitoni C, Scicluna BP, Wiewel MA, Horn J, Schultz MJ, Nürnberg P, Bonten MJ, Cremer OL. Incidence, risk factors, and attributable mortality of secondary infections in the intensive care unit after admission for sepsis. JAMA. 2016;315(14):1469–79.
5. López-Mestanza C, Andaluz-Ojeda D, Gómez-López J, Bermejo-Martín J. Clinical factors influencing mortality risk in hospital-acquired sepsis. J Hosp Infect. 2018;98(2):194–201.
6. Fang W-F, Chen Y-M, Wang Y-H, Huang C-H, Hung K-Y, Fang Y-T, Chang Y-C, Lin C-Y, Chang Y-T, Chen H-C. Incorporation of dynamic segmented neutrophil-to-monocyte ratio with leukocyte count for sepsis risk stratification. Sci Rep. 2019;9(1):19756.
7. Fang W-F, Douglas IS, Chen Y-M, Lin C-Y, Kao H-C, Fang Y-T, Huang C-H, Chang Y-T, Huang K-T, Wang Y-H. Development and validation of immune dysfunction score to predict 28-day mortality of sepsis patients. PLoS ONE. 2017;12(10):e0187088.
8. Zhang Y-y. Ning B-t: signaling pathways and intervention therapies in sepsis. Signal Transduct Target Therapy. 2021;6(1):407.
9. Mattioli B, Straface E, Quaranta MG, Giordani L, Viora M. Leptin promotes differentiation and survival of human dendritic cells and licenses them for Th1 priming. J Immunol. 2005;174(11):6820–8.
10. Pérez-Pérez A, Sánchez-Jiménez F, Vilariño-García T, Sánchez-Margalet V. Role of leptin in inflammation and vice versa. Int J Mol Sci. 2020;21(16):5887.
11. Birlutiu V, Boicean LC. Serum leptin level as a diagnostic and prognostic marker in infectious diseases and sepsis: A comprehensive literature review. Medicine 2021, 100(17).
12. Negrin LL, Jahn A, Van Griensven M. Leptin protects against mortality and organ dysfunction in a two-hit trauma/sepsis model and is IL-6-dependent. Shock. 2017;48(1):130–7.
13. Bornstein S, Licinio J, Tauchnitz R, Engelmann L, Negrao A, Gold P, Chrousos G. Plasma leptin levels are increased in survivors of acute sepsis: associated loss of diurnal rhythm in cortisol and leptin secretion. J Clin Endocrinol Metabolism. 1998;83(1):280–3.
14. Maurya R, Bhattacharya P, Dey R, Nakhasi HL. Leptin functions in infectious diseases. Front Immunol. 2018;9:2741.
15. Torpy D, Bornstein S, Chrousos G. Leptin and interleukin-6 in sepsis. Horm Metab Res. 1998;30(12):726–9.
16. Koch A, Weiskirchen R, Zimmermann HW, Sanson E, Trautwein C, Tacke F. Relevance of serum leptin and leptin-receptor concentrations in critically ill patients. Mediators of inflammation 2010, 2010.
17. Basharat S, Parker JA, Murphy KG, Bloom SR, Buckingham JC, John CD. Leptin fails to blunt the lipopolysaccharide-induced activation of the hypothalamic–pituitary–adrenal axis in rats. J Endocrinol. 2014;221(2):237.
18. Jacobsson S, Larsson P, Johansson G, Norberg M, Wadell G, Hallmans G, Winsö O, Söderberg S. Leptin independently predicts development of sepsis and its outcome. J Inflamm. 2017;14(1):1–10.
19. Breyer M-K, Rutten EP, Vernooy JH, Spruit MA, Dentener MA, van der Kallen C, Marleen M, Wouters EF. Gender differences in the adipose secretome system

in chronic obstructive pulmonary disease (COPD): a pivotal role of leptin. Respir Med. 2011;105(7):1046–53.
20. Johnston M, Hu JT, Fardo WW, Greco DJ, Perry S, Montine GJ, Trojanowski TQ, Shaw JM, Wesson Ashford L, Tezapsidis J. Low plasma leptin in cognitively impaired ADNI subjects: gender differences and diagnostic and therapeutic potential. Curr Alzheimer Res. 2014;11(2):165–74.
21. Hellström L, Wahrenberg H, Hruska K, Reynisdottir S, Arner P. Mechanisms behind gender differences in Circulating leptin levels. J Intern Med. 2000;247(4):457–62.
22. Koca C, KAVAKLI HŞ, ALICI Ö. Immunomodulatory role of leptin treatment in experimental sepsis caused by gram negative bacteria. Turk J Med Sci. 2011;41(2):251–8.
23. Grunfeld C, Zhao C, Fuller J, Pollack A, Moser A, Friedman J, Feingold KR. Endotoxin and cytokines induce expression of leptin, the Ob gene product, in hamsters. J Clin Investig. 1996;97(9):2152–7.
24. Singer M, Deutschman CS, Seymour CW, Shankar-Hari M, Annane D, Bauer M, Bellomo R, Bernard GR, Chiche J-D, Coopersmith CM. The third international consensus definitions for sepsis and septic shock (Sepsis-3). JAMA. 2016;315(8):801–10.
25. Hung K-Y, Chen Y-M, Wang C-C, Wang Y-H, Lin C-Y, Chang Y-T, Huang K-T, Lin M-C, Fang W-F. Insufficient nutrition and mortality risk in septic patients admitted to ICU with a focus on immune dysfunction. Nutrients. 2019;11(2):367.
26. Tsai Y-H, Hung K-Y, Fang W-F. Use of peak glucose level and peak glycemic gap in mortality risk stratification in critically ill patients with sepsis and prior diabetes mellitus of different body mass indexes. Nutrients. 2023;15(18):3973.
27. Hung K-Y, Tsai Y-H, Lin C-Y, Chang Y-C, Wang Y-H, Lin M-C, Fang W-F. Application of peak glucose range and diabetes status in mortality risk stratification in critically ill patients with sepsis. Diagnostics. 2021;11(10):1798.
28. Meli R, Pacilio M, Raso GM, Esposito E, Coppola A, Nasti A, Di Carlo C, Nappi C, Di Carlo R. Estrogen and raloxifene modulate leptin and its receptor in hypothalamus and adipose tissue from ovariectomized rats. Endocrinology. 2004;145(7):3115–21.
29. Clegg DJ, Riedy CA, Smith KAB, Benoit SC, Woods SC. Differential sensitivity to central leptin and insulin in male and female rats. Diabetes. 2003;52(3):682–7.
30. Letizia Hribal M, Vanessa Fiorentino T, Sesti G. Role of C reactive protein (CRP) in leptin resistance. Curr Pharm Design. 2014;20(4):609–15.
31. Shapiro NI, Khankin EV, Van Meurs M, Shih S-C, Lu S, Yano M, Castro PR, Maratos-Flier E, Parikh SM, Karumanchi SA. Leptin exacerbates sepsis-mediated morbidity and mortality. J Immunol. 2010;185(1):517–24.
32. Haitovsky Y. Missing data in regression analysis. J Roy Stat Soc: Ser B (Methodol). 1968;30(1):67–82.

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