

# Serum levels of visfatin, sirtuin-1, and interleukin-6 in stable and acute exacerbation of chronic obstructive pulmonary disease

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**Background:** Visfatin is an adipokine that increased under inflammatory conditions. Moreover, sirtuin-1 possesses regulatory effects on inflammatory factors. In this study, we aimed to evaluate the serum level of visfatin in patients with stable and acute exacerbation of chronic obstructive pulmonary disease (AE-COPD). **Materials and Methods:** In a case-control study, thirty patients with stable COPD (S-COPD), thirty patients with AE-COPD, and thirty control subjects were enrolled. Pulmonary function tests and blood sampling were performed on all participants. Serum visfatin, sirtuin-1, and interleukin (IL)-6 levels were measured using the sandwich ELISA method and assessed their association with study parameters. **Results:** The findings of the current study revealed that serum levels of visfatin in AE-COPD patients were higher than those of healthy controls and S-COPD (for healthy control; standardized mean difference [SMD] = 2.63, 95% confidence interval [CI] = 1.31–2.83,  $P < 0.001$ , and for S-COPD; SMD = 1.53, 95% CI = 0.21–2.85,  $P < 0.05$ ). On the other hand, the serum levels of sirtuin-1 were higher in healthy controls compared to the S-COPD and AE-COPD patients (for S-COPD; SMD = 1.56, 95% CI = 0.018–3.11,  $P < 0.05$ , for AE-COPD; SMD = 1.50, 95% CI = 0.048–3.04,  $P < 0.05$ ). **Conclusion:** Elevated visfatin and IL-6 levels demonstrated their pro-inflammatory effects in patients with COPD, especially in AE-COPD patients. In addition, the negative association found between serum visfatin and sirtuin-1 levels suggested the pathophysiologic and therapeutic roles of these factors in COPD patients.

**Key words:** Chronic obstructive pulmonary disease, interleukin-6, sirtuin-1, visfatin

**How to cite this article:** Ghobadi H, Mokhtari S, Aslani MR. Serum levels of visfatin, sirtuin-1, and interleukin-6 in stable and acute exacerbation of chronic obstructive pulmonary disease. *J Res Med Sci* 2021;26:17.

## INTRODUCTION

The acute exacerbation of chronic obstructive pulmonary disease (AE-COPD) is characterized by the symptoms such as increased systemic inflammation, worsening of pulmonary function tests (PFTs) findings, increased sputum production, worsening of dyspnea and cough, negative impact on survival, and reduced health-associated quality of life.<sup>[1]</sup> Similar to other diseases that are not characterized by a specific etiology, various factors are involved in the pathogenesis of COPD, such as abnormal immune responses,

environmental and hormonal factors, and variable levels of genes expression.<sup>[2,3]</sup>

It has been shown that various factors play an important role in the pathogenesis of systemic inflammation in patients with COPD, including tissue hypoxia, smoking, skeletal muscle dysfunction, and lung hyperinflation.<sup>[4,5]</sup> Systemic inflammatory markers and cytokines such as tumor necrosis factor alpha (TNF- $\alpha$ ), interleukin-8 (IL-8), IL-6, and C-reactive protein (CRP) were well demonstrated to be upregulated in the airways of patients with COPD in the exacerbation phase.<sup>[2,6]</sup> Recently, it was demonstrated that, in chronic lung diseases such as asthma and COPD,

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10.4103/jrms.JRMS\_626\_19

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**Submitted:** 16-Sep-2019 **Revised:** 18-Feb-2020 **Accepted:** 16-Sep-2020 **Published:** 27-Feb-2021

adipose tissue plays a key role in inducing and promoting systemic inflammation.<sup>[7,8]</sup>

As protein mediators involved in regulating energy metabolism and inflammatory responses, adipocytokines are originally secreted from the adipose tissues.<sup>[7]</sup> Visfatin is an adipokine previously known as nicotinamide phosphoribosyltransferase and the pre-B cell colony-enhancing factor.<sup>[9]</sup> It is a pro-inflammatory cytokine involved in inflammatory and innate immune responses.<sup>[10]</sup> Pro-inflammatory cytokines such as TNF- $\alpha$ , IL-6, and IL-1  $\beta$  were shown to induce visfatin expression in granulocytes, monocytes, macrophages, and adipocytes.<sup>[9]</sup> It was demonstrated that, in patients with stable COPD (S-COPD), the serum visfatin level is significantly increased in comparison to healthy individuals.<sup>[11]</sup>

Evidence also suggests accelerated aging of the lung tissue in patients with COPD.<sup>[12]</sup> Since inflammatory processes are one of the most basic mechanisms involved in the progression of COPD, nuclear factor-kappa B (NF- $\kappa$ B), as a redox-sensitive transcription factor, was shown to induce the genes expression of pro-inflammatory factors such as TNF- $\alpha$ , IL-8, and IL-6.<sup>[13]</sup> Numerous studies reported that, by inhibiting NF- $\kappa$ B, sirtuin-1 exerts regulatory effects on the level of pro-inflammatory factors.<sup>[13]</sup> In animal and human studies, sirtuin-1 levels in the lung tissue were significantly reduced in patients with COPD compared to the healthy individuals.<sup>[13]</sup> Considerable amount of evidence showed that visfatin plays a role in regulating sirtuin-1 expression by catalyzing the rate-limiting step in the nicotinamide adenine dinucleotide (NAD) salvage pathway. Furthermore, it was shown that sirtuin-1 is a NAD deacetylase that plays a role in reducing inflammation.<sup>[14]</sup> Since NAD is used as a substrate for sirtuin-1, it can stimulate sirtuin-1 activity in cells.<sup>[15]</sup> Interestingly, visfatin independent of the NAD production pathway was shown to be able to activate the JAK2/STAT3 pathway to induce IL-6 secretion.<sup>[16]</sup>

Little is known about the association between visfatin and sirtuin-1 in various diseases. Findings in this regards are controversial as some studies reported a positive association, and some a negative relationship, while others reported the lack of communication. Therefore, in the present study, we aimed to measure the serum concentrations of visfatin, sirtuin-1, and IL-6 in patients with COPD during the exacerbation and stable phases as well as in healthy controls and examined their relationship with pulmonary function and health status using the COPD assessment test (CAT) score.

## MATERIALS AND METHODS

### Participants

All of the participants signed written consent forms, and the study design was approved by the Ethics Committee of

Ardabil University of Medical Sciences, Ardabil, Iran (No. IR.ARUMS.REC.1396.146). In the current case-control study, patients with S-COPD and AE-COPD were selected from a respiratory clinic and those admitted to the emergency department of Ardabil Imam Khomeini Educational and Clinical Hospital, Ardabil, Iran [Figure 1]. The control group consisted of participants with normal spirometry who had no respiratory symptoms, and they were selected from the same hospital who visited in other outpatient clinics. All participations were male and matched for age. The diagnosis of patients with S-COPD and AE-COPD, as well as cases inclusion and exclusion criteria, was fully addressed previously.<sup>[17,18]</sup> For all participants, PFTs were performed based on ATS guidelines using a spirometer (Chest Inc., 801, Tokyo, Japan). The biochemical and PFT was performed for patients with AE-COPD 1 day after admission to the hospital, while for controls and S-COPD patients, it was performed on the same day. The Persian version of Modified Medical Research Council (mMRC) respiratory questionnaire, CAT questionnaire, and GOLD criteria were validated as addressed previously.<sup>[19]</sup>

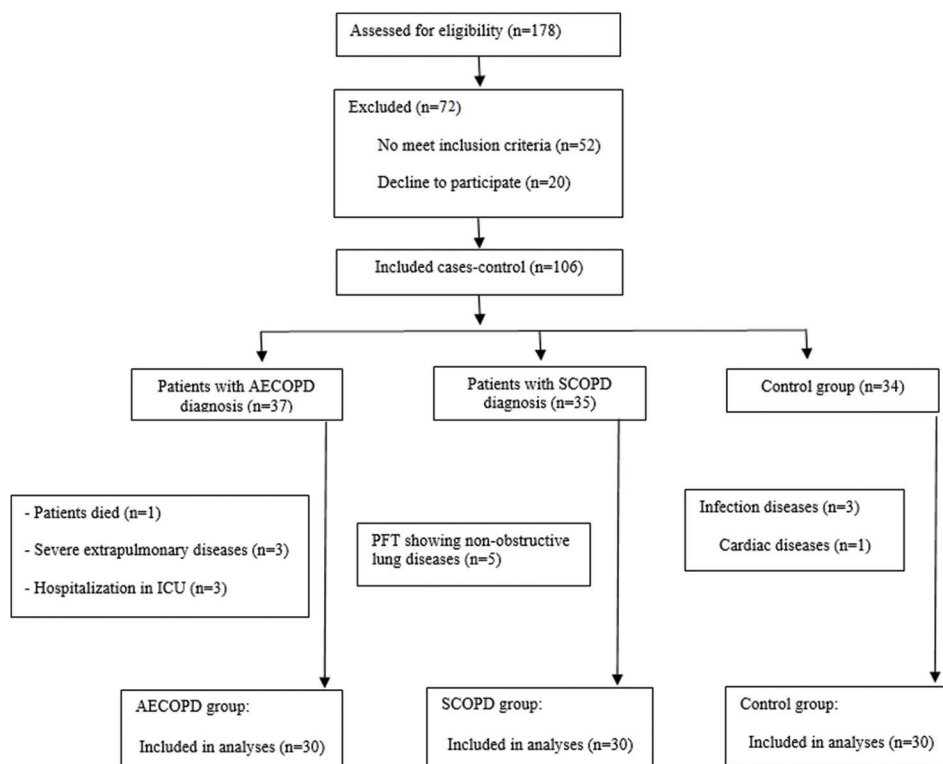
### Biochemical measurements

About 3–5 ml of blood sample was taken from all participants to measure the serum levels of IL-6 and visfatin. Analysis of serum visfatin and IL-6 levels was performed by commercial kits (Crystal Day, China) using the ELISA method. We used an ng/ml to report the results.

### Statistical analysis

The sample size was calculated based on the formula for average comparison with  $\alpha = 0.05$  and  $\beta = 0.1$ ,  $\mu_1 = 2.07$ ,  $S_1 = 0.18$ ,  $\mu_2 = 1.88$ , and  $S_2 = 0.15$  based on the previous study on the serum levels of visfatin:  $n = ([Z_1 - \alpha/2 + Z_1 - \beta]^2 [S_1^2 + S_2^2]) / (\mu_1 - \mu_2)^2$ . Based on the sample size calculation, 22 participants were required in each group. Considering the probability of filling of the samples, finally, 30 participants were recruited in each group (a total of 90 subjects).

Data normality was assessed by Q-Q plot and Kolmogorov-Smirnov. The mean  $\pm$  standard deviation or median and 25<sup>th</sup>–75<sup>th</sup> percentiles were provided to report the results. To compare groups, Kruskal-Wallis test (followed by the Mann-Whitney U-test for *post hoc*) or ANOVA (followed by Tukey-Kramer *post hoc*) were used for nonparametric and parametric data, respectively. Correlation coefficients were evaluated using Spearman rank order test or Pearson's correlation. General linear modeling function analysis was done to adjust for age, body mass index (BMI), and smoking status. Linear regression analysis was also defined based on visfatin as an independent variable and dependent variables including forced expiratory volume in 1 s (FEV1), smoking history (pack/year), SpO<sub>2</sub>, and IL-6.



**Figure 1:** Flowchart of patients included in the study. COPD = Chronic obstructive pulmonary disease; S-COPD = stable COPD patients; AE-COPD = acute exacerbation of COPD patients; PFT = pulmonary function test; ICU = intensive care unit

The multivariate covariance analysis was used to explore the correlation between the serum levels of visfatin and IL-6 to control the effect of age, BMI, and smoking status. Moreover, a  $P < 0.05$  was considered statistically significant. SPSS (version 22; SPSS Inc., Chicago, IL, USA) was used for the statistical analyses.

## RESULTS

The mean age of the control group was  $56.27 \pm 8.12$  years and that of the COPD group was  $59.28 \pm 8.10$  years ( $P = 0.238$ ) [Table 1].

The serum levels of visfatin were significantly lower in control and the S-COPD groups than the AE-COPD group [ $P < 0.001$  and  $P < 0.05$ , respectively; Table 1]. Moreover, the results showed that serum visfatin level was lower in controls in compared with S-COPD patients [ $P < 0.05$ , Table 1]. The serum levels of sirtuin-1 in AE-COPD and S-COPD groups were significantly lower than that of the control group [ $P < 0.05$  for both, Table 1].

In addition, IL-6 results identified a higher level of IL-6 in the AE-COPD group compared to the S-COPD and control groups [ $P < 0.001$  for both, Table 1]. It should be noted that serum level of IL-6 in the control individual was lower than the S-COPD patients [ $P < 0.05$ , Table 1].

## Severity of chronic obstructive pulmonary disease in study groups

Based on the results of GOLD grade in patients with COPD, it was found that there was a statistically significant difference in relation to visfatin ( $P < 0.001$ ), IL-6 ( $P < 0.001$ ), sirtuin-1 ( $P < 0.017$ ), smoking history (pack/year) ( $P < 0.01$ ), FEV1 ( $P < 0.001$ ), SpO<sub>2</sub> ( $P < 0.01$ ), FEV1/forced vital capacity (FVC) ( $P < 0.01$ ), CAT score ( $P < 0.001$ ), and mMRC ( $P < 0.001$ ) [Table 2].

It was found that serum levels of visfatin in Stages I–II decreased compared to Stages III–IV in patients with S-COPD and AE-COPD ( $P < 0.05$  and  $P < 0.001$ , respectively). Interestingly, serum levels of visfatin were lower in the S-COPD group in Stages III–IV compared with AE-COPD [ $P < 0.05$ , Table 2]. Concerning the serum levels of sirtuin-1 based on GOLD criteria, significantly lower levels were found at Stages III–IV compared to Stages I–II in the S-COPD and AE-COPD groups [ $P < 0.05$ , Table 2].

The serum IL-6 level was significantly lower in the AE-COPD and S-COPD patients at Stage I–II compared to Stages III–IV ( $P < 0.05$  for both). In addition, the results showed that serum IL-6 levels in the AE-COPD patients were higher compared to the S-COPD patients at Stages I–II and III–IV [ $P < 0.001$  for both, Table 2]. The results also revealed that in both AE-COPD and S-COPD groups, CAT score

**Table 1: Baseline characteristics of patients with chronic obstructive pulmonary disease and control subjects**

Parameters	Control group (n=30)	COPD group		P
		S-COPD (n=30)	AE-COPD (n=30)	
Mean age (years)	56.27±8.12	58.83±9.47	59.73±6.59	0.238
BMI (kg/m <sup>2</sup> )	26.90±3.91	26.06±5.30	24.35±4.67	0.104
Pulmonary function test				
FEV1 (percentage of predicted)	89.83±8.38	53.13±23.12***	33.95±13.48***,+++	<0.001
FVC (percentage of predicted)	84.63±8.07	68.40±22.57**	50.09±20.68***,++	<0.001
FEV1/FVC	86.76±4.54	58.86±9.99***	55.81±12.43***	<0.001
Visfatin (ng/mL)	3 (2-3)	3 (2-4)*	4.5 (3-6)***,+	<0.001
Adjusted visfatin	2.54±0.75	3.80±0.63	5.14±1.45	<0.001
IL-6 (ng/mL)	56 (44-60)	57.5 (54-63)*	90.5 (73-108)***,+++	<0.001
Adjusted IL-6	49.88±7.92	71.09±6.67	92.74±15.71	<0.001
Sirtuin-1 (ng/mL)	4 (4-7)	3.50 (3-4)*	3 (3-5)*	<0.05
Adjusted sirtuin-1	5.33±0.34	4.67±0.31	3.81±0.57	<0.001

Data are presented as mean±SD or median (25<sup>th</sup>-75<sup>th</sup> percentiles). Kruskal-Wallis and Mann-Whitney tests were used to compare visfatin, sirtuin-1, and IL-6 levels. ANOVA and Tukey's test were used to compare other variables. Adjusting was performed for age, BMI, and smoking history in the study groups. COPD=Chronic obstructive pulmonary disease; BMI=Body mass index; AE-COPD=Acute exacerbation of COPD; S-COPD=Stable COPD; FEV1=Forced expiratory volume in 1 s; FVC=Forced volume capacity; SD=Standard deviation; IL-6=Interleukin 6. To indicate statistically significant differences between the control group and other groups: \**P*<0.05, \*\**P*<0.01, and \*\*\**P*<0.001. To indicate statistically significant differences between stable COPD and the acute exacerbation of COPD: +*P*<0.05, ++*P*<0.01, and +++*P*<0.001

was statistically lower at Stages I–II compared to Stages III–IV (*P*<0.001). However, no statistically significant difference was observed between the S-COPD and AE-COPD subjects for CAT score based on GOLD stages [Table 2].

Furthermore, mMRC results based on GOLD criteria specified that there was a significant difference between the S-COPD with AE-COPD subjects at Stages III–IV and I–II (*P*<0.05 to *P*<0.01, respectively). However, mMRC values were only higher in the S-COPD group at Stages III–IV compared to Stages I–II, based on GOLD criteria [*P*<0.001, Table 2].

### Association of serum levels of visfatin, interleukin-6, and sirtuin-1 with pulmonary function parameters

The results showed that serum levels of visfatin were associated with FVC<sub>% predicted</sub>, FEV1<sub>% predicted</sub> [Figure 2a], dyspnea (according to the mMRC questionnaire), GOLD stages, SpO<sub>2</sub>, smoking history (pack/year) [Figure 2c], and CAT score [Table 3]. In addition, serum sirtuin-1 levels were significantly associated with FEV1<sub>% predicted</sub> [Figure 2b], FVC<sub>% predicted</sub>, smoking history (pack/year) [Figure 2d], and GOLD stages. Moreover, the results indicated significant association between serum IL-6 level and SpO<sub>2</sub>, smoking history (pack/year), and FEV1<sub>% predicted</sub> [Figure 2g]. Furthermore, results identified that significant correlation between serum levels of IL-6 and visfatin [Figure 2e] as well as IL-6 and sirtuin-1 [Figure 2f].

Multiple regression was run to predict visfatin and sirtuin-1 from SpO<sub>2</sub>, FEV1, IL-6, and smoking history (pack/year). These variables could significantly predict only visfatin, *F* (5, 54) = 23.67, *P*<0.001, *R*<sup>2</sup> = 0.687. The results showed that the most significant predictor of visfatin was IL-6 (*P*<0.001) [Table 4].

Regarding the correlation between the two variables of visfatin and sirtuin-1 serum levels and to control the effect of age, BMI, and smoking status, multivariate covariance analysis was used. The results showed that none of the variables, including group, age, BMI, and smoking status, were not significantly correlated with the serum levels of visfatin and sirtuin-1.

## DISCUSSION

In the current study, the serum levels of visfatin and IL-6 were found to be significantly elevated with increasing disease severity based on GOLD stages in patients with stable and AE-COPD; in this context, serum visfatin and IL-6 levels were significantly lower in patients at Stages I–II of COPD compared to those at other stages according to the GOLD criteria. On the other hand, serum sirtuin-1 level was decreased in the AE-COPD and S-COPD groups compared to the control groups. Based on the GOLD criteria, serum sirtuin-1 level in the S-COPD and AE-COPD groups at Stages I–II was significantly higher than that of Stages III–IV. There was a negative association between the serum levels of visfatin and SpO<sub>2</sub> and FEV1. However, there was a positive correlation between the serum levels of visfatin and IL-6, CAT score, mMRC, and the severity of COPD based on GOLD criteria. In addition, there was a positive correlation between sirtuin-1 and FEV1, and there was a significantly negative correlation between sirtuin-1 serum levels and smoking history, GOLD grades, and IL-6 serum levels.

It was demonstrated that adipose-tissue-derived adipokines are responsible for regulating energy metabolism and chronic low-grade inflammation present in inflammatory diseases such as COPD and asthma.<sup>[9,20]</sup> Although there is controversial evidence about the serum levels of visfatin

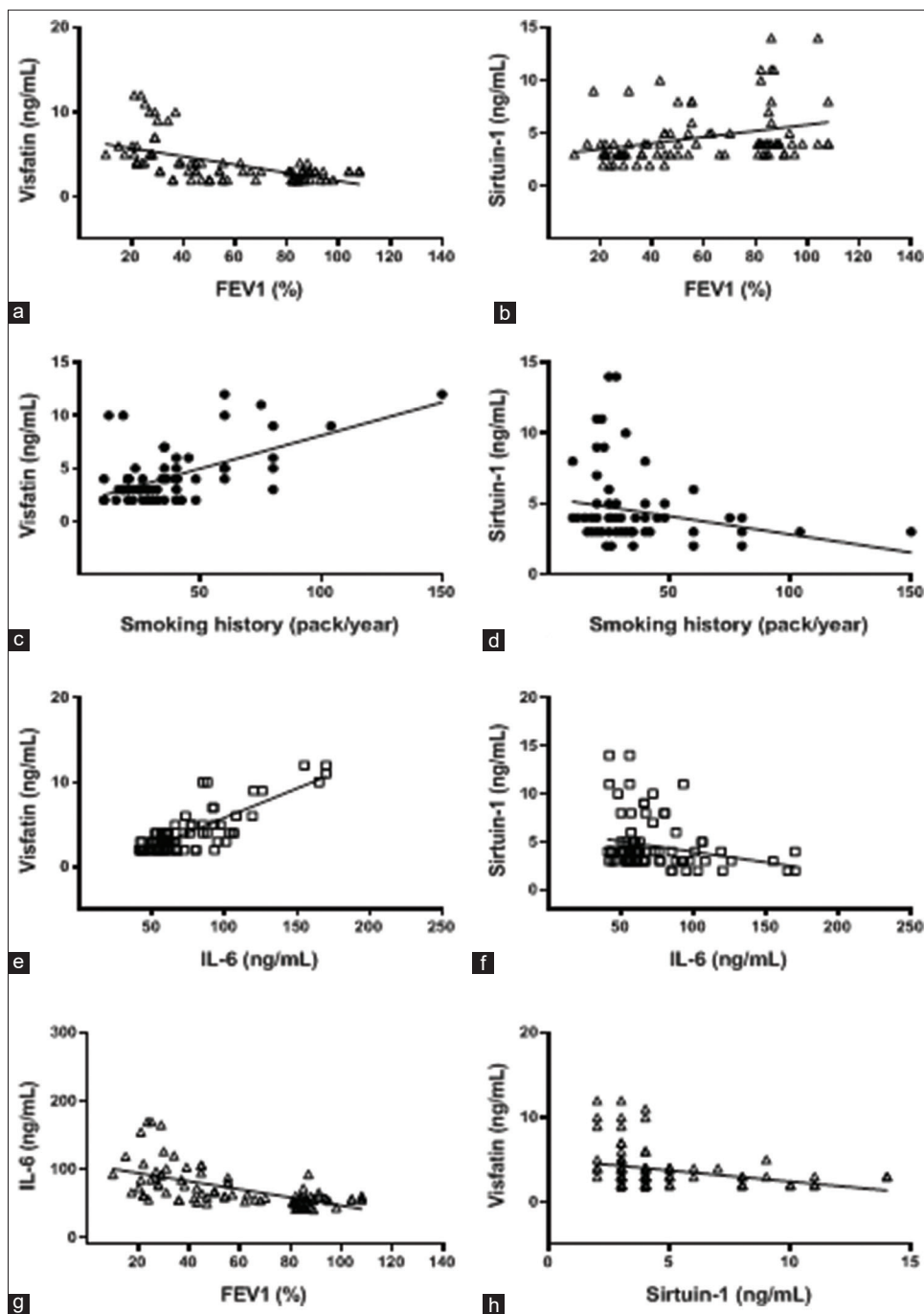
**Table 2: Global initiative for obstructive lung disease groups and baseline characteristics of the study population**

Variables	GOLD I-II		GOLD III-IV	
	S-COPD	AE-COPD	S-COPD	AE-COPD
Number	14	7	16	23
Age (years)	58.36±10.12	62.14±11.48	59.25±9.18	59.00±4.35
BMI (kg/m <sup>2</sup> )	25.52±4.05	25.08±4.15	26.54±6.28	24.13±4.89
Smoking (pack per year)	21 (18-28)	35 (15-40)	34.50 (27-40)	45 (29.50-77.50)
<i>P</i> 1		NS		<i>P</i> <0.05
<i>P</i> 2			NS	NS
FEV1 (% predicted)	74.42±13.29	52.84±2.69	34.50±9.34	28.20±9.46
<i>P</i> 1		<i>P</i> <0.001		<i>P</i> <0.05
<i>P</i> 2			<i>P</i> <0.001	<i>P</i> <0.001
FVC (% predicted)	87.35±15.34	71.28±6.30	51.81±12.34	43.64±19.15
<i>P</i> 1		<i>P</i> <0.05		NS
<i>P</i> 2			<i>P</i> <0.001	<i>P</i> <0.001
FEV1/FVC (%)	66.14±3.00	59.24±6.86	52.50±9.63	54.77±13.63
<i>P</i> 1		<i>P</i> <0.01		NS
<i>P</i> 2			<i>P</i> <0.001	NS
SpO <sub>2</sub> (%)	96 (92-96)	89 (87.50-89.50)	92 (89-95)	86 (82-88)
<i>P</i> 1		<i>P</i> <0.01		<i>P</i> <0.001
<i>P</i> 2			<i>P</i> <0.05	<i>P</i> <0.05
CAT score	11 (8-16)	13 (13-15)	27 (19.50-31)	26 (22-29.5)
<i>P</i> 1		NS		NS
<i>P</i> 2			<i>P</i> <0.001	<i>P</i> <0.001
mMRC	1 (1-2)	2 (2-3)	2 (2-3)	3 (2.5-4)
<i>P</i> 1		<i>P</i> <0.01		<i>P</i> <0.05
<i>P</i> 2			<i>P</i> <0.001	NS
IL-6 (ng/mL)	56 (53-58)	67 (67-80)	61 (56-85)	101 (77-119.50)
<i>P</i> 1		<i>P</i> <0.001		<i>P</i> <0.001
<i>P</i> 2			<i>P</i> <0.05	<i>P</i> <0.05
Visfatin (ng/mL)	3 (2-3)	2 (2-2.5)	4 (2.50-5.50)	5 (4-7.50)
<i>P</i> 1		NS		<i>P</i> <0.05
<i>P</i> 2			<i>P</i> <0.05	<i>P</i> <0.001
Sirtuin-1 (ng/mL)	4 (3-5)	6 (3.50-8)	3 (3-4)	3 (2.50-4)
<i>P</i> 1		NS		NS
<i>P</i> 2			<i>P</i> <0.05	<i>P</i> <0.05

Data are shown as mean±SD or median (25<sup>th</sup>-75<sup>th</sup> percentiles). ANOVA and Tukey's test were used to compare age, BMI, FEV1, FVC, and FEV1/FVC. Kruskal-Wallis and Mann-Whitney tests were used to compare other variables. GOLD=Global initiative for chronic obstructive lung disease; S-COPD=Stable chronic obstructive pulmonary disease; AE-COPD=Acute exacerbation of chronic obstructive pulmonary disease; BMI=Body mass index; FEV1=Forced expiratory volume in 1 s; FVC=Forced volume capacity; SpO<sub>2</sub>=O<sub>2</sub> saturation; SD=Standard deviation; IL-6=Interleukin-6; CAT=COPD assessment test; mMRC=Modified medical research council; NS=Non-significant. *P*1=Statistical differences between S-COPD and AE-COPD; *P*2=Statistical differences between GOLD I-II and GOLD III-IV in groups

in patients with insulin resistance and obesity, elevated serum levels were reported in asthma and COPD.<sup>[11]</sup> We found that serum visfatin level was significantly elevated in patients with stable and AE-COPD compared to healthy controls, which was markedly so in patients with AE-COPD. The results of the current study also showed a significant association between IL-6 and visfatin, consistent with the results of a previous study.<sup>[9]</sup> In the study done by Leivo-Korpela *et al.*, a statistically significant correlation was found between plasma visfatin levels and IL-6, TNF- $\alpha$ , and IL-8.<sup>[9]</sup> Based on these observations and the results of our study, one can state that adipokines such as visfatin may have a role in systemic inflammation in patients with COPD. Interestingly, serum levels of IL-6 and visfatin were higher in the exacerbation phase of COPD compared to the S-COPD.

Macrophages are the main source of pro-inflammatory cytokines TNF- $\alpha$  and IL-6, and activation of macrophages induces the secretion of adipokines.<sup>[21,22]</sup> Perhaps, the relationship observed between visfatin and IL-6 in the present study is due to the activation of macrophages in patients with COPD, which requires further studies. Moreover, inflammation cytokines such as IL-1  $\beta$ , IL-6, TNF- $\alpha$ , and lipopolysaccharide can induce the expression of visfatin.<sup>[9,11]</sup> On the other hand, visfatin inhibits neutrophil apoptosis and may lead to inflammation in patients with COPD.<sup>[9]</sup> According to the results of our study, in the exacerbation of COPD, the elevation of visfatin levels at Stages III-IV of the disease as well as the strong association between the serum levels of IL-6 and visfatin, at least in part, indicate the key role of visfatin in the persistence and



**Figure 2:** Spearman rank order (or Pearson's correlation analysis) of (a) FEV1 and visfatin serum levels (correlation coefficient =  $-0.620$ ,  $P = 0.000$ ), (b) FEV1 and sirtuin-1 serum levels (correlation coefficient =  $0.405$ ,  $P = 0.000$ ), (c) smoking history and visfatin serum levels (correlation coefficient =  $0.451$ ,  $P = 0.000$ ), (d) smoking history and sirtuin-1 serum levels (correlation coefficient =  $-0.344$ ,  $P = 0.001$ ), (e) serum levels of visfatin and IL-6 (correlation coefficient =  $0.635$ ,  $P = 0.000$ ), (f) serum levels of sirtuin-1 and IL-6 (correlation coefficient =  $-0.254$ ,  $P = 0.016$ ), (g): FEV1 and IL-6 serum levels (correlation coefficient =  $-0.626$ ,  $P = 0.000$ ), (h) sirtuin-1 and visfatin serum levels (correlation coefficient =  $-0.337$ ,  $P = 0.001$ ). FEV1 = forced expiratory volume in 1 s, IL-6 = interleukin-6

development of inflammation in COPD. Indeed, increased serum levels of visfatin may be due to systemic or local inflammation in patients with COPD. Although visfatin is essentially produced by adipose tissues, macrophages, and dendritic cells,<sup>[23]</sup> some evidences demonstrated that serum visfatin levels increase in patients with lung injury.<sup>[24]</sup> Increased serum levels of visfatin were also identified in other chronic diseases, including chronic kidney disease,

inflammatory bowel disease, and rheumatoid arthritis.<sup>[25,26]</sup> Nevertheless, the role of visfatin in chronic diseases remains unknown.

A significantly negative association between  $SpO_2$  and visfatin levels that was found in the present study may be another explanation for the increased levels of visfatin. Evidence suggests that increased levels of visfatin are

**Table 3: Spearman correlation analysis of study parameters with visfatin and sirtuin-1**

	Visfatin		Sirtuin-1	
	r	P	r	P
Age	0.205	0.052	-0.052	0.626
Smoking history (/year)	0.451	0.001	-0.344	<0.01
FEV1 (percentage predicted)	-0.620	0.000	0.405	<0.001
FVC (percentage predicted)	-0.624	0.000	0.403	<0.001
FEV1/FVC	-0.420	0.000	0.242	<0.05
GOLD stage	0.612	0.000	-0.382	<0.01
SpO <sub>2</sub>	-0.539	0.000	0.168	0.211
CAT score	0.696	0.000	-0.387	<0.01
mMRC	0.515	0.000	-0.182	0.164
IL-6 (ng/mL)	0.635	0.000	-0.254	<0.05

COPD=Chronic obstructive pulmonary disease; FEV1=Forced expiratory volume in 1 s; FVC=Forced volume capacity; IL-6=Interleukin 6; SpO<sub>2</sub>=O<sub>2</sub> saturation; GOLD=The global initiative for chronic obstructive lung disease; mMRC=Modified medical research council; CAT: COPD assessment test

**Table 4: Associations between visfatin and study parameters**

	Visfatin		
	B	95% CI for B	P
FEV1	-0.120	-0.040-0.010	0.230
Cigarette history (pack/year)	0.057	-0.015-0.027	0.570
SpO <sub>2</sub>	-0.034	-0.082-0.056	0.717
IL-6	0.667	0.040-0.078	<0.001
Sirtuin-1	-0.124	-0.381-0.054	0.138

B represents the unstandardized coefficient. CI=Confidence intervals; FEV1=Forced expiratory volume in 1 s; IL-6=Interleukin-6; SpO<sub>2</sub>=O<sub>2</sub> saturation

occurred by hypoxia-inducible factor 1.<sup>[27]</sup> In patients with COPD, hypoxia developed as a result of airway obstruction and increased expression of hypoxia-inducible factor 1 as a result of hypoxia may lead to increased visfatin levels; nonetheless, further studies are required to clarify this pathway.

We also found that serum levels of sirtuin-1 significantly decreased in patients with COPD compared to the healthy controls, which was consistent with a previous study.<sup>[28]</sup> Reduction of serum sirtuin-1 level was significantly associated with an increase in severity of COPD based on GOLD criteria. Interestingly, this reduction in sirtuin-1 level was associated with the severity of airflow limitation (based on FEV1 values) as well as increased serum IL-6 levels. In a study by Nakamaru *et al.*, mRNA and protein expression as well as the activity of sirtuin-1 was significantly lower in patients with COPD compared to healthy controls, and this decrease was associated with the disease severity.<sup>[28]</sup> They also showed that reduced levels of sirtuin-1 were associated with increased levels of IL-8 and matrix metalloproteinase-9.<sup>[28]</sup> Based on previous research, various types of cellular processes are involved in the pathogenesis of COPD, including inflammation, oxidative stress, autophagy, aging/senescence, proliferation, apoptosis, and autoimmunity.<sup>[29]</sup> Furthermore, sirtuin-1 regulates

numerous processes, including cellular senescence/aging and inflammation.<sup>[30,31]</sup>

Importantly, for the first time, we found the correlation between decreased levels of sirtuin-1 and increased levels of visfatin. Although the exact mechanism of this relationship is not clear, it can be inferred that increased levels of visfatin in patients with COPD, especially under exacerbation conditions, may be resulted from the changes in sirtuin-1 levels. In a study done in ovalbumin-sensitized rats, a significantly positive relationship between visfatin and NF-κB expression levels in lung tissue, was found.<sup>[10]</sup> Furthermore, increased tracheal responsiveness to methacholine in ovalbumin-sensitized rats was associated with increased protein and gene expression levels of visfatin.<sup>[20]</sup> Since sirtuin-1 was shown to be able to mediate inflammatory pathways by reducing the activity of NF-κB, it is concluded that decreased sirtuin-1 activity and increased visfatin levels are associated with the exacerbation of inflammatory conditions in patients with COPD. The interesting finding of the current study is that the effect size for visfatin (1.07) and sirtuin-1 (0.72) is an indicator of the efficacy of the results that can at least partially, explain the generalizability of the results in COPD patients.

In this study, we also found a significantly positive correlation between serum visfatin levels and CAT score and mMRC dyspnea score, but no significant correlation between sirtuin-1 and CAT and mMRC scores was observed. Physical activity is markedly decreased in patients with high stages of COPD,<sup>[32]</sup> which is likely to explain the relationship between increased levels of inflammatory factors and decreased physical activity.<sup>[33]</sup> Considering visfatin pro-inflammatory role reported by various studies, this can be somewhat consistent with the reduction in the physical activity of patients with COPD, especially under acute exacerbation conditions. Possibly, existence of a markedly association between visfatin and IL-6 serum levels and GOLD stages may reflect the effects of systemic inflammation on the quality of life in these patients.

Our study had some limitations. First, we did not include women in this study and did not determine the effect of sex on serum visfatin and sirtuin-1 levels and their association with disease severity. Second, we did not measure the levels of inflammatory markers other than IL-6. Therefore, we are not able to determine the association between the serum levels of visfatin and other inflammatory markers and the risk of COPD. Finally, the sample size of our study was moderate, and future studies must be conducted in larger samples.

It is recommended that future studies evaluate the role of sex differences, other cytokines, and the interaction between

sirtuin-1 and visfatin, in a larger sample size, over longer period.

## CONCLUSION

In the present study, we found that serum visfatin and IL-6 levels increase with increasing severity of airflow limitation in patients with COPD, especially in the acute exacerbation phase. On the other hand, the serum levels of sirtuin-1 were significantly decreased in patients with COPD compared to the healthy individuals. We also found a negative association between serum sirtuin-1 and visfatin and IL-6 levels in these patients. Indeed, the results of the current study suggested that in COPD patients, especially in acute exacerbation phase, various factors including changes in sirtuin-1 and visfatin levels, exacerbate the disease. Therefore, further studies are needed to evaluate the interactions of various factors in COPD.

## Acknowledgments

This is a report on a database from the study entitled "Evaluation of Serum Levels of Visfatin and Sirtuin-1 in Patients with COPD" registered by the Research Committee of Ardabil University of Medical Sciences (Ethics number: IR.ARUMS.REC.1396.146).

## Financial support and sponsorship

Nil.

## Conflicts of interest

There are no conflicts of interest.

## REFERENCES

- Viniol C, Vogelmeier CF. Exacerbations of COPD. *Eur Respir Rev* 2018;27:170103.
- Thomsen M, Ingebrigtsen TS, Marott JL, Dahl M, Lange P, Vestbo J, *et al.* Inflammatory biomarkers and exacerbations in chronic obstructive pulmonary disease. *JAMA* 2013;309:2353-61.
- Ghobadi H, Aslani MR, Hosseini A, Farzaneh E. The correlation of serum brain natriuretic peptide and interleukin-6 with quality of life using the chronic obstructive pulmonary disease assessment test. *Med Princ Pract* 2017;26:509-15.
- Agustí A. Systemic effects of chronic obstructive pulmonary disease: What we know and what we don't know (but should). *Proc Am Thorac Soc* 2007;4:522-5.
- Salimian J, Mirzaei H, Moridikia A, Harchegani AB, Sahebkar A, Salehi H. Chronic obstructive pulmonary disease: MicroRNAs and exosomes as new diagnostic and therapeutic biomarkers. *J Res Med Sci* 2018;23:27.
- Sinden NJ, Stockley RA. Systemic inflammation and comorbidity in COPD: A result of 'overspill' of inflammatory mediators from the lungs? Review of the evidence. *Thorax* 2010;65:930-6.
- Ouchi N, Parker JL, Lugus JJ, Walsh K. Adipokines in inflammation and metabolic disease. *Nat Rev Immunol* 2011;11:85-97.
- Aslani MR, Keyhanmanesh R, Khamaneh AM, Ebrahimi Saadatlou MA, Mesgari Abbasi M, Alipour MR. Lung altered expression of IL-1 $\beta$  mRNA and its signaling pathway molecules in obese-asthmatic male wistar rats. *Iran J Allergy Asthma Immunol* 2016;15:183-97.
- Leivo-Korpela S, Lehtimäki L, Hämäläinen M, Vuolteenaho K, Kööbi L, Järvenpää R, *et al.* Adipokines NUCB2/nesfatin-1 and visfatin as novel inflammatory factors in chronic obstructive pulmonary disease. *Mediators Inflamm* 2014;2014:1-6.
- Aslani MR, Keyhanmanesh R, Alipour MR. Increased visfatin expression is associated with nuclear factor- $\kappa$ B in obese ovalbumin-sensitized male wistar rat tracheae. *Med Princ Pract* 2017;26:351-8.
- Liu X, Ji Y, Chen J, Li S, Luo F. Circulating visfatin in chronic obstructive pulmonary disease. *Nutrition* 2009;25:373-8.
- Yanagisawa S, Papaioannou AI, Papaportfyriou A, Baker JR, Vuppusetty C, Loukides S, *et al.* Decreased serum sirtuin-1 in COPD. *Chest* 2017;152:343-52.
- Chun P. Role of sirtuins in chronic obstructive pulmonary disease. *Arch Pharm Res* 2015;38:1-10.
- Gok O, Karaali Z, Ergen A, Ekmekci SS, Abaci N. Serum sirtuin 1 protein as a potential biomarker for type 2 diabetes: Increased expression of sirtuin 1 and the correlation with microRNAs. *J Res Med Sci* 2019;24:56.
- Zhang T, Kraus WL. SIRT1-dependent regulation of chromatin and transcription: Linking NAD(+) metabolism and signaling to the control of cellular functions. *Biochim Biophys Acta* 2010;1804:1666-75.
- Marotta LL, Almendro V, Marusyk A, Shipitsin M, Schemme J, Walker SR, *et al.* The JAK2/STAT3 signaling pathway is required for growth of CD44+CD24<sup>-</sup> stem cell-like breast cancer cells in human tumors. *J Clin Invest* 2011;121:2723-35.
- Aslani MR, Ghazaei Z, Ghobadi H. Correlation of serum fatty acid binding protein-4 and interleukin-6 with airflow limitation and quality of life in stable and acute exacerbation of COPD Turk J Med Sci 2020;50:337-45.
- Ghobadi H, Hosseini N, Aslani MR. Correlations between serum decoy receptor 3 and airflow limitation and quality of life in male patients with stable stage and acute exacerbation of COPD. *Lung* 2020;198:1-9.
- Amani M, Ghadimi N, Aslani MR, Ghobadi H. Correlation of serum vascular adhesion protein-1 with airflow limitation and quality of life in stable chronic obstructive pulmonary disease. *Respir Med* 2017;132:149-53.
- Keyhanmanesh R, Alipour MR, Ebrahimi H, Aslani MR. Effects of diet-induced obesity on tracheal responsiveness to methacholine, tracheal visfatin level, and lung histological changes in ovalbumin-sensitized female wistar rats. *Inflammation* 2018;41:846-58.
- Barnes PJ. Cellular and molecular mechanisms of chronic obstructive pulmonary disease. *Clin Chest Med* 2014;35:71-86.
- Francisco V, Pino J, Gonzalez-Gay MA, Mera A, Lago F, Gómez R, *et al.* Adipokines and inflammation: Is it a question of weight? *Br J Pharmacol* 2018;175:1569-79.
- Moschen AR, Kaser A, Enrich B, Mosheimer B, Theurl M, Niederegger H, *et al.* Visfatin, an adipocytokine with proinflammatory and immunomodulating properties. *J Immunol* 2007;178:1748-58.
- Ye SQ, Simon BA, Maloney JP, Zambelli-Weiner A, Gao L, Grant A, *et al.* Pre-B-cell colony-enhancing factor as a potential novel biomarker in acute lung injury. *Am J Respir Crit Care Med* 2005;171:361-70.
- Axelsson J, Witasap A, Carrero JJ, Qureshi AR, Suliman ME, Heimbürger O, *et al.* Circulating levels of visfatin/pre-B-cell colony-enhancing factor 1 in relation to genotype, GFR, body composition, and survival in patients with CKD. *Am J Kidney Dis* 2007;49:237-44.



26. Otero M, Lago R, Gomez R, Lago F, Dieguez C, Gomez-Reino JJ, *et al.* Changes in plasma levels of fat-derived hormones adiponectin, leptin, resistin and visfatin in patients with rheumatoid arthritis. *Ann Rheum Dis* 2006;65:1198-201.
27. Bae SK, Kim SR, Kim JG, Kim JY, Koo TH, Jang HO, *et al.* Hypoxic induction of human visfatin gene is directly mediated by hypoxia-inducible factor-1. *FEBS Lett* 2006;580:4105-13.
28. Nakamaru Y, Vuppusetty C, Wada H, Milne JC, Ito M, Rossios C, *et al.* A protein deacetylase SIRT1 is a negative regulator of metalloproteinase-9. *FASEB J* 2009;23:2810-9.
29. Yao H, Rahman I. Current concepts on oxidative/carbonyl stress, inflammation and epigenetics in pathogenesis of chronic obstructive pulmonary disease. *Toxicol Appl Pharmacol* 2011;254:72-85.
30. Rahman I, Kinnula VL, Gorbunova V, Yao H. SIRT1 as a therapeutic target in inflammaging of the pulmonary disease. *Prev Med* 2012;54 Suppl: S20-8.
31. Aslani MR, Matin S, Nemati A, Mesgari-Abbasi M, Ghorbani S, Ghobadi H. Effects of conjugated linoleic acid supplementation on serum levels of interleukin-6 and sirtuin 1 in COPD patients. *Avicenna J Phytomed* 2020;10:305-15.
32. Watz H, Waschki B, Meyer T, Magnussen H. Physical activity in patients with COPD. *Eur Respir J* 2009;33:262-72.
33. Watz H, Waschki B, Kirsten A, Müller KC, Kretschmar G, Meyer T, *et al.* The metabolic syndrome in patients with chronic bronchitis and COPD: Frequency and associated consequences for systemic inflammation and physical inactivity. *Chest* 2009;136:1039-46.