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ORIGINAL ARTICLE

Diagnostic and Prognostic Relevance of Serum Irisin Level in Patients with Pulmonary Embolism

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SUMMARY

Background: **We aimed to determine the diagnostic and prognostic value of serum irisin level in patients with acute pulmonary embolism (PE) admitted to the emergency department.**
Methods: **Ninety patients who underwent computed tomography pulmonary angiography (CTPA) due to suspected PE were included in the study. Demographic data, PE risk factors, and associated diseases, vital signs, Wells score, Revised Geneva score, pulmonary embolism severity index (PESI), and simplified PESI (sPESI) were recorded. Irisin levels were measured by enzyme linked-immunosorbent assay.**
Results: **Serum irisin level in patients with confirmed PE (n = 45) was significantly lower than that in patients (n = 45) without PE (p = 0.001). On receiver operating characteristic curve analysis, use of optimal irisin cutoff level of 8.6 µg/mL for diagnosis of PE was associated with 82.2% sensitivity, 60% specificity, 67.3% positive predictive value (PPV), and 77.1% negative predictive value (NPV) [area under the curve (AUC): 0.744, 95% confidence interval (CI): 0.641 - 0.830, p < 0.001)]. Use of optimal D-dimer cutoff level of 1,720 µg/L was associated with 86.7% sensitivity, 62.2% specificity, 69.6% PPV, and 82.4% NPV (AUC: 0.801, 95% CI: 0.704 - 0.878, p < 0.001). Irisin level showed no significant correlation with Wells score or revised Geneva score; however, irisin level showed a significant negative correlation with PESI and sPESI.**
Conclusions: **Patients with acute PE showed significantly lower serum levels of irisin. The sensitivity, specificity, NPV, and PPV of irisin level for diagnosis of PE were lower than those of D-dimer.**
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INTRODUCTION

Acute pulmonary embolism (PE) is the third most common cause of cardiovascular death after ischemic heart disease and cerebrovascular disease. Rapid diagnosis and treatment of PE is a key imperative owing to the high associated morbidity and mortality [1-3]. However, fast and accurate diagnosis of PE is not always possible owing to considerable variability in the clinical presentation, ranging from lack of any symptoms to sudden death [1].
Irisin is a 112-amino-acid protein (molecular weight: 12

kDa) produced by proteolytic cleavage of fibronectin type III domain-containing protein 5 (FNDC5). Irisin converts white adipose tissue cells to brown adipose tissue cells. The newly formed brown adipose tissue is rich in mitochondrial uncoupling protein 1 (UCP1) [4]. Increased expression of UCP 1 inhibits ATP synthesis and increases heat production, which causes energy consumption in the cell; this ensures thermogenesis and glucose homeostasis. Binding of irisin to its receptors on adipocytes upregulates lipolysis and increases energy expenditure [4,5]. Furthermore, irisin has been shown to alleviate oxidative stress and apoptosis [6].

Studies have found a strong association of irisin level with several diseases such as diabetes, obesity, cardiovascular diseases, fatty liver disease, chronic cartilage disease, psoriasis, osteoporosis, chronic kidney disease, chronic obstructive pulmonary disease, and cancer [5,7-9]. Irisin has been shown to exhibit a protective effect against cardiovascular diseases [6,10]. Studies have indicated a potential anti-atherosclerotic effect of irisin [5, 11,12]. In addition, irisin may play a key role in maintaining endothelial cell function; low irisin level was shown to induce endothelial dysfunction and increase the incidence of atherosclerosis [13].

Several studies have investigated the role of irisin in cardiovascular diseases; however, the relationship between PE and serum irisin level is not well characterized [14,15]. In this study, we aimed to investigate the diagnostic and prognostic relevance of serum irisin level in patients with acute PE admitted to the emergency department.

MATERIALS AND METHODS

The prospective study was initiated after obtaining approval of the local ethics committee. Patients admitted to the emergency department during a one-year period with chief complaint of shortness of breath and/or acute chest pain and who underwent computed tomography pulmonary angiography (CTPA) due to suspected acute PE were included in the study. All patients were evaluated by the emergency physician. The evaluation included calculation of Wells score and the revised Geneva score. CTPA was performed in patients with a high clinical probability of PE as well as in patients with low or intermediate clinical probability of PE but who had a positive D-dimer test. Patients with acute renal failure, acute cerebrovascular disease, diabetic ketoacidosis, hyperglycemic hyperosmolar nonketotic coma, pulmonary edema, and acute coronary syndrome were excluded from the study.

During the study period, a total of 138 patients underwent CTPA. Of these, 28 patients did not qualify according to the inclusion criteria for this study. Out of the remaining 110 patients, acute PE was detected in 45 patients. Forty-five patients with similar clinical and demographic characteristics and in whom PE was ruled out after CTPA were included in the control group. To measure the level of irisin, 5 mL venous blood samples were obtained and placed in tubes containing aprotinin. Samples were centrifuged at 4,000 rpm for 5 minutes. The separated serum was stored at -80°C until the day of the analysis. The irisin levels were measured by enzyme linked-immunosorbent assay (Human Irisin ELISA kit, Cat. No: YHB1765Hu). D-dimer and troponin I levels were analyzed using the AQT90 flex (Radiometer Medical ApS, Denmark); these parameters were determined from the venous blood samples collected in ethylenediaminetetraacetic acid tubes at the time of admission of the patient to the emergency department. The lactate level was determined using heparinized arterial blood sample obtained for arterial blood gas analysis using an ABL800 flex (Radiometer Medical ApS, Denmark).

Sample size was calculated using sample size calculating software G*Power version 3.1.9.2 (Germany). With power of 80%, 0.05 level of statistical significance, and effect size of 0.6, sample size for each group was calculated to be 45. Statistical Package for the Social Sciences (SPSS 22, IBM SPSS Statistics for Windows; IBM Corp, Armonk, NY, USA) was used for statistical analysis. Normally distributed continuous variables are presented as mean ± standard deviation, while non-normally distributed continuous variables are presented as median (interquartile range). Normality of distribution of variables was assessed using the Kolmogorov-Smirnov and Shapiro-Wilk tests. Between-group differences with respect to non-normally distributed variables were assessed using the Kruskal-Wallis test. Mann-Whitney U test was used to determine the relationship between the groups. Spearman's correlation test was used to evaluate the correlation of serum irisin level with the ordinal variable scales. Receiver operating characteristic (ROC) curve analysis was performed to assess the ability of irisin level to predict PE. ROC curve analysis results are presented as sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), area under the curve (AUC), and confidence intervals (CI). p-values < 0.05 were considered indicative of statistical significance.

RESULTS

Data pertaining to the 45 patients with PE (PE group) and the 45 patients without PE (control group) were included in the analysis. The demographic, clinical, and laboratory data of patients in the two groups are summarized in Table 1.

Risk factors for PE are presented in Table 2.

Serum irisin level in the PE group was significantly lower than that in the control group ($p = 0.001$). On ROC curve analysis, use of the optimal serum irisin cutoff level of 8.6 µg/mL for diagnosis of PE was associated with 82.2% sensitivity, 60% specificity, 67.3% PPV, and 77.1% NPV (AUC = 0.744, 95% CI: 0.641 - 0.830, $p < 0.001$). Use of optimal D-dimer cutoff level of 1,720 µg/L for diagnosis of PE was associated with 86.7%

Table 1. Demographic, clinical, and laboratory data of PE and the control group.

Data of patients	PE (n = 45)	Control (n = 45)	p-value
Age (years)	67.4 ± 17.3	66.0 ± 20.5	0.87
Gender (female/male)	23/22	31/14	0.08
BMI (kg/m^2)	24.9 ± 4.5	24.1 ± 4.3	0.32
Time (h) *	7.4 ± 6.3	9.2 ± 7.2	0.22
SBP (mmHg)	125.3 ± 21.7	129.7 ± 25.1	0.54
DBP (mmHg)	75 ± 11.4	80.1 ± 15.5	0.17
Heart rate (bpm)	101 ± 21.9	100 ± 18.1	0.97
Respiratory rate	23.8 ± 4.4	21.5 ± 5.4	0.02
sO2 (%)	89 ± 6.08	91 ± 5.96	0.06
Glasgow coma score	14.8 ± 0.63	15 ± 0.01	0.08
Wells score	4.1 ± 1.6	3.1 ± 1.3	0.003
Revised Geneva score	6.3 ± 3	5.5 ± 2.2	0.23
PESI (mean ± std. dev)	102.9 ± 34.8	95.2 ± 31.1	0.39
sPESI (mean ± std. dev)	1.68 ± 1.04	1.53 ± 0.94	0.39
Irisin (µg/mL) (median) (IQR)	4.7 (3.2 - 7.5)	9.2 (5.1 - 14.1)	0.001
D-dimer (µg/L) (median) (IQR)	4,080 (2,350 - 7,540)	1,590 (913 - 2,620)	0.001
Troponin (ng/mL) (median) (IQR)	0 (0 - 0.15)	0 (0 - 0.1)	0.39
Lactat (mg/dL) (median) (IQR)	1.9 (1.25 - 2.65)	1.6 (1.15 - 2.05)	0.08

*** Time interval from the onset of symptoms until blood sample is taken.**
BMI - body mass index, SBP - systolic blood pressure, DBP - diastolic blood pressure, PESI - pulmonary embolism severity index, sPESI - simplified pulmonary embolism severity Index.

Table 2. Risk factors of the groups.

Risk factors (n)	PE	Control	p-value
Hypertension	13	19	0.18
Diabetes mellitus	5	7	0.53
Ischemic heart disease	9	5	0.24
Congestive heart failure	8	13	0.21
Malignancy	5	7	0.53
Chronic obstructive pulmonary disease	9	7	0.58
Previous deep vein thrombosis	4	-	0.04
Previous pulmonary embolism	1	1	1
Immobilization	10	6	0.27
Long bone-pelvic fracture	3	3	1
Recent surgery	1	2	0.55
Recent trauma	1	-	0.31
Pregnancy/post-partum period	1	1	1
Previous stroke	5	3	0.45
No risk factor	6	7	0.76

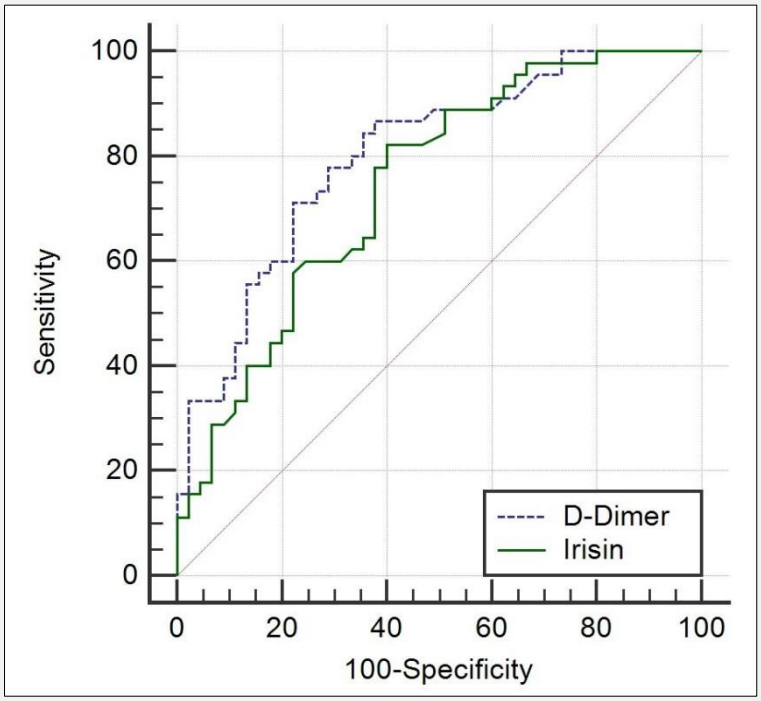

Figure 1. ROC analysis showing specificity and sensitivity of serum irisin and D-dimer levels in the diagnosis of PE.

AUC - area under the curve, CI - confidence intervals, PPV - positive predictive value, NPV - negative predictive value.
AUC = 0.801 (95% CI: 0.704 - 0.878)
Best cutoff point for D-dimer 1,720 µg/L
Sensitivity: 86.7.2%
Specificity: 62.2%
PPV: 69.6%
NPV: 82.4%
AUC = 0.744 (95% CI: 0.641 - 0.830)
Best cutoff point for irisin: 8.6 µg/mL
Sensitivity: 82.2%
Specificity: 60%
PPV: 67.3%
NPV: 77.1%

sensitivity, 62.2% specificity, 69.6% PPV, and 82.4% NPV (AUC = 0.801, 95% CI: 0.704 - 0.878, $p < 0.001$) (Figure 1).

We observed no significant correlation of irisin level with age ($r = -0.152$, $p = 0.15$), body mass index ($r = -1.97$, $p = 0.63$), or the time elapsed since the onset of symptoms ($r = 0.76$, $p = 0.47$).

Irisin level showed a negative correlation with Wells score ($r = -0.11$, $p = 0.27$) and revised Geneva Score ($r = -0.12$, $p = 0.24$); however, the correlation was not statistically significant. There was a significant negative correlation of irisin level with pulmonary embolism severity index (PESI) ($r = -0.248$, $p = 0.019$) and simplified PESI (sPESI) ($r = -0.242$, $p = 0.021$). There was no significant correlation between serum lactate ($r = -0.175$, $p = 0.1$) and troponin ($r = -0.112$, $p = 0.29$) levels of the patients; however, there was a significant negative correlation between D-dimer and irisin levels ($r = -0.358$, $p = 0001$).

DISCUSSION

In our study, serum irisin level in the PE group was significantly lower than that in the control group. Use of irisin cutoff value of 8.6 µg/mL for diagnosis of PE was associated with 82.2% sensitivity, 60% specificity, 67.3% PPV, and 77.1% NPV. Irisin level showed a significant negative correlation with D-dimer level which is used for the diagnosis of PE. There was no significant

correlation of irisin level with Wells score or revised Geneva score which are clinical decision scores. Irisin level showed a significant negative correlation with PESI and sPESI scores which are used to estimate the 30-day mortality risk of patients with PE.

The non-specific signs and symptoms tend to delay the diagnosis and treatment of PE. The imaging methods used for the diagnosis of PE are relatively costly and entail exposure to radiation and contrast media [3]. Current guidelines recommend assessment of the clinical probability of PE using Wells score or Geneva score prior to imaging for a definitive diagnosis of PE. D-dimer values are used for the diagnosis of PE in patients with a low or moderate clinical probability of PE. Owing to the low PPV and high NPV of D-dimer, it is typically used to rule out PE [16]. In a study by Glober et al., PE was detected in 198 of 3,523 patients who were admitted with suspected PE in the emergency department; the sensitivity and specificity of D-dimer for diagnosis of PE was 95.7% and 40.0%, respectively [17]. Sikora-Skrabaka et al. examined 370 hospitalized patients and diagnosed PE in 134 patients; patients with D-dimer level > 2,152 ng/mL showed a significantly increased risk of PE (PPV: 82%) [3]. Nilsson et al. investigated the diagnostic performance of D-dimer level in a study of 90 hemodynamically stable patients with symptoms of PE; the optimal cutoff D-dimer level of 0.5 mg/L was associated with 79% sensitivity, 88% specificity, 81% PPV, and 87% NPV [18]. In the present study, use of cutoff D-dimer level of 1,720 µg/L was associated with 86.7% sensitivity, 62.2% specificity, 69.6% PPV, and 82.4% NPV.

Previous studies have shown that serum irisin level decreases in acute myocardial infarction (MI) [19-22]. Studies have also suggested that low serum irisin levels can be used to predict the severity of coronary disease [21,22]. Deng reported a negative relationship of serum irisin level with the presence and severity of coronary artery disease in patients with angiographically proven disease [21]. Similarly, in the study by Anastasilakis et al., circulating irisin levels in patients with acute MI and coronary artery disease were significantly lower than those in the control group; in addition, the extent of decrease was associated with the degree of coronary stenosis [22]. Decreased serum irisin level in the setting of acute MI is believed to be a protective mechanism against energy loss from myocardial cells; the decreased blood irisin level reduces the energy requirement in the body which has a cardio protective effect [19]. Low level of irisin is believed to protect cells from necrosis to some extent by preventing the consumption of ATP [20]. We believe that the decreased serum irisin level observed in PE patients in our study may represent a protective mechanism against necrosis, similar to that observed in acute MI.

PE can cause ischemia-reperfusion (IR) induced lung injury. Post-ischemic injury tissue reperfusion induces activation of inflammatory response and increased production of reactive oxygen species, leading to disruption of mitochondrial function and impaired integrity of plasma membrane [14]. In addition to energy metabolism, irisin stimulates the mechanism that regulates mitochondrial biogenesis and oxidative metabolism [5]. Irisin was shown to reduce myocardial apoptosis and help maintain mitochondrial function during IR damage, leading to a reduction in infarct size [6,23].

Chen et al. documented a time-dependent increase in serum irisin level in rats subjected to ischemia/reperfusion of extremities; in addition, in mice exposed to lung IR injury, they demonstrated accumulation of irisin in damaged lung tissue alongside a significant decrease in serum irisin level compared to that in the sham control group. Furthermore, intravenous administration of exogenous irisin was found to alleviate IR-induced pulmonary damage in mouse models. The authors suggested that serum concentration of irisin may be a potential marker for human lung diseases and that increasing serum irisin concentrations may be useful for the treatment of lung injuries [14]. Our finding of decreased serum irisin level in PE patients supports this assertion. In a recent study, Sun et al. divided 86 patients with confirmed diagnosis of acute PE into low irisin (< 6.9 µg/mL) and high irisin (> 6.9 µg/mL) groups based on the median plasma irisin level. Patients in the low irisin group had higher levels of N-terminal pro-brain natriuretic peptide, mean pulmonary artery pressure, systolic pulmonary artery pressure, mean right ventricular pressure, and systolic right ventricular pressure; in addition, patients in the low irisin group showed significantly higher clinical deterioration rate. Patients in the low irisin group exhibited less favorable hemodynamic profile, and there was a negative correlation between irisin level and hemodynamic parameters [15]. In our study, we observed a significant negative correlation of irisin level with PESI and sPESI, both of which are used for prognostic assessment and for guiding treatment decision-making.

In our study, patients with acute PE showed a decreased level of serum irisin, which explains the significant negative correlation with PESI and sPESI. However, we concluded that compared to D-dimer level, low irisin level has poor sensitivity, specificity, NPV, and PPV for the diagnosis of PE. This shows the poor diagnostic performance of serum irisin level for diagnosing PE. Further research is required to assess the diagnostic and prognostic relevance of irisin level in the context of acute PE.

Study limitations:

Our study has several limitations. First, the level of irisin was measured at the time of initial admission to the emergency department; subsequent serial measurements of irisin level were not performed. Second, we did not evaluate the irisin level in the lung parenchyma of patients with PE. Larger and in-depth studies are required to provide more definitive evidence of the relationship between PE and serum irisin level.

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Declaration of Interest:
There are no conflicts of interest.

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