Original Article

Visfatin levels in patients with severe pneumonia

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BACKGROUND: As a cytokine highly expressed in internal organs, visfatin could be used as a biomarker of systemic inflammation response for chronic obstructive pulmonary diseases, but few studies have reported the use of visfatin in severe pneumonia. The present study was undertaken to determine the plasma levels of visfatin in patients with severe pneumonia.

METHODS: A total of 70 patients, including 40 patients with severe pneumonia (group A) and 30 patients with non severe pneumonia (group B) who had been admitted to the ICU from June 2009 to June 2010, were enrolled in this prospective study. And another 30 healthy physical examinees served as healthy controls (group C). Patients were excluded if they suffered from severe diseases of the heart, brain and kidney, cancers, autoimmune diseases, or received special treatment in the latest month. The plasma levels of visfatin, IL-6, IL-8 and TNF- α were measured by ELISA, while the level of CRP was determined by immunoturbidimetry, and the routine blood test was performed. Blood gas analysis and Acute Physiology and Chronic Health Evaluation II (APACHE II) were performed in patients with pneumonia. Comparisons between the groups were conducted by Student's t test, ANOVA or nonparametric test. Correlation analysis was carried out by Pearson's correlation test or Spearman's rank-order correlation test.

RESULTS: The plasma level of visfatin in group A was significantly higher than that in groups B and C ($P < 0.001$), and the level of visfatin in group B was significantly higher than that in group C ($P < 0.001$). The plasma level of visfatin was positively correlated with CRP, TNF- α , APACHE II and PMN% in patients with severe pneumonia ($r_{ho} = 0.653$, $r = 0.554$, $r = 0.558$, $r = 0.484$, respectively, $P < 0.05$ for all), while it was negatively correlated with PaO₂ and PaO₂/FiO₂ ($r_{ho} = -0.422$, $r = -0.543$, respectively, $P < 0.05$ for all).

CONCLUSION: Visfatin may be involved in the systematic inflammation response in patients with severe pneumonia as a pro-inflammatory cytokine, and it is valuable in assessing the severity of pneumonia..

KEY WORDS: Severe pneumonia; Visfatin; Interleukin-6; Interleukin-8; Tumor necrosis factor- α ; C-reactive protein; Acute Physiology and Chronic Health Evaluation II (APACHE II); Granulocyte percent (PMN%)

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INTRODUCTION

Severe pneumonia is a common critical disease with a high mortality in clinical practice.^[1] During severe pneumonia, a variety of cytokines and inflammatory mediators such as IL-6, IL-12 and TNF- α are released due to severe infection, which may cause systemic

inflammatory response syndrome (SIRS) and even multiple organ dysfunctions syndrome (MODS).^[2,3] As a cytokine highly expressed in internal organs, visfatin has attracted attention from many researchers in respect of function in inflammatory response.^[4] Liu et al^[5] reported that visfatin could be used as a biomarker of systemic

inflammation response for chronic obstructive pulmonary diseases, but at present, few reports have focused on the use of visfatin in patients with severe pneumonia. In this study, we aimed to explore the changes and role of visfatin in pneumonia.

METHODS

Patients

A total of 70 hospitalized patients with pneumonia, who had been admitted to the ICU in Shanghai Fifth People's Hospital from June 2009 to June 2010, were enrolled in this study. All the patients met the criteria of *Guidelines for the Diagnosis and Treatment of Community-Acquired Pneumonia*^[6] issued by the Society of Respiratory Diseases of the Chinese Medical Association (CMA) in 2006 and *Guidelines for Treatment of Pneumonia*^[7] prepared by ATS/IDSA in 2005. The 70 patients were divided into group A (patients with severe pneumonia) and group B (patients without severe pneumonia).

The patients of group A ($n=40$) met the *Criteria of Diagnosis for Severe Pneumonia* set by AST in 2001.^[8] Among them, 26 were males and 14 females; their age ranged from 25 to 80 (53.16 ± 9.68) years, and the mean average BMI was 24.06 ± 3.73 kg/m². Eleven patients had chronic obstructive pulmonary disease, 9 had coronary heart disease, 5 had type 2 diabetes, and 5 didn't have acute cerebrovascular disease.

In group B ($n=30$), 20 patients were male and 10 female, aged from 27 to 81 (49.83 ± 10.06) years and their average BMI was 23.86 ± 4.67 kg/m². Eight patients had chronic obstructive pulmonary disease, 7 had coronary heart disease, 2 had type 2 diabetes, and 4 didn't have acute cerebrovascular disease.

Thirty patients in group C, selected from healthy physical examinees in the hospital, served as healthy controls. Among them, 19 were male and 11 were female, with age ranging from 26 to 81 (51.16 ± 11.17) years and their average BMI was 25.31 ± 4.90 kg/m².

Exclusion criteria

The exclusion criteria included: 1) patients without any anti-bacterial treatment or those presenting improved clinical symptoms or obvious absorption of pulmonary infiltration; 2) patients who were complicated with severe diseases of the heart, brain, and kidney, or blood vessel and tumor; 3) patients who had autoimmune diseases; 4) patients who had infections in other parts of the body; 5) patients who received special treatment in the latest

month (radiotherapy, chemotherapy, surgery, biotherapy and immunosuppressive therapy, etc); and 6) pregnant patients.

The study was approved by the Ethics Committee of Shanghai Fifth People's Hospital affiliated to Fudan University, and informed consent forms were signed by all subjects.

Laboratory tests

Approximately 4 mL fasting venous blood was collected from patients in groups A and B on the 2nd morning after hospitalization, and from healthy physical examinees in group C on the morning of physical-examination. The plasma levels of visfatin, IL-6, IL-8 and TNF- α were measured using the double antibody ABC-ELISA method. The visfatin kit was purchased from Invitrogen Co., Ltd, USA, and the kits for IL-6, IL-8 and TNF- α were bought from American R&D Company. The plasma CRP was tested by the immune turbidimetry method.

Blood gas analysis and APACHE II score were performed in patients with pneumonia in groups A and B.

Statistical analysis

The data on normal distribution were expressed as mean $\pm$ SD. Student's t test was applied for the comparison between two groups. Oneway ANOVA was used for comparison among multi-groups if the variance was homogeneous, while the nonparametric test (Kruskal-Wallis H) was applied when the variance was heterogeneous, and meanwhile comparison between two groups was performed. The data with non-normal distribution were described with the median (interquartile range). Nonparametric test was used for comparison between the groups (the Mann-Whitney U test for two groups, while the Kruskal-Wallis test for multi-groups), and comparison between two groups was performed. Correlation analysis was conducted with Pearson's correlation coefficient test or Spearman's rank-order correlation test. Data analysis was made with software SPSS 15.0, and $P<0.05$ was considered statistically significant.

RESULTS

General data

There were no significant differences in age, sex and BMI between the three groups ($P>0.05$).

pH, PaO₂, and PaO₂/FiO₂ in group A were significantly lower than those in group B (z values were -9.26 , -6.99

Table 1. General data of patients in the three groups (mean±SD)

Variables	Group A (n=40)	Group B (n=30)	Group C (n=30)	P
pH value	7.27±0.04*	7.37±0.05	-	0.000
PaO ₂ (mmHg)	53.40 (41.93-60.06)*	78.19±7.67	-	0.000
PaO ₂ /FiO ₂ (mmHg)	164.09±52.23*	369.14(351.14-420.88)	-	0.000
WBC(*10 ⁹ /L)	14.27±6.08* [#]	8.40(5.35-12.67) ^Δ	5.22±2.03	0.000
PMN%	0.79±0.10* [#]	0.60±0.08 ^Δ	0.54±0.09	0.000
APACHEII	22.89±3.90*	9.57(7.50-15.10)	-	0.000

Comparison between group A and group B, * $P<0.001$; comparison between group A and group C, [#] $P<0.001$; comparison between group B and group C, ^Δ $P<0.001$.

Table 2. Plasma levels of visfatin, IL-6, IL-8, TNF- α and CRP in the three groups

Variables	Group A (n=40)	Group B (n=30)	Group C (n=30)	χ^2 value	P
Visfatin (ng/mL)	10.39±3.12* [#]	2.56±1.20 ^Δ	1.60±0.26	74.75	0.000
IL-6 (pg/mL)	140.78±49.71* [#]	37.55±11.88 ^Δ	9.64±3.12	83.28	0.000
IL-8 (pg/mL)	78.48±20.22* [#]	21.01±6.98 ^Δ	6.54±1.61	86.73	0.000
TNF- α (pg/mL)	71.76±30.68* [#]	31.31(25.46-39.59) ^Δ	11.40±3.14	75.08	0.000
CRP (mg/L)	71.68(42.72-93.36)* [#]	10.69(7.95-14.52) ^Δ	3.78±1.56	76.37	0.000

Comparison between group A and group B, * $P<0.001$; comparison between group A and group C, [#] $P<0.001$; comparison between group B and group C, ^Δ $P<0.001$.

and -7.12 respectively, $P<0.001$ for all). There were significant differences in WBC and PMN% between the three groups ($\chi^2=42.10$, $P<0.001$; $F=76.92$, $P<0.001$); WBC and PMN% were significantly higher in group A than in group B or in group C ($P<0.001$ for all); and WBC and PMN% were significantly higher in group B than in group C ($P<0.001$).

The APACHE II score in group A was significantly higher than that in group B ($z=-6.97$, $P<0.001$) (Table 1).

Comparison of plasma levels of visfatin, IL-6, IL-8, TNF- α and CRP

There were significant differences in the plasma levels of visfatin, IL-6, IL-8, TNF- α and CRP between the three groups ($P<0.001$ for all). These values were significantly higher in group A than in groups B and C ($P<0.001$ for all), and were higher in group B than in group C ($P<0.001$ for all) (Table 2).

Correlation analysis

In group A, the plasma level of visfatin was positively correlated with CRP, TNF- α , APACHEII and PMN% ($r_{ho}=0.653$, $r=0.554$, $r=0.558$, $r=0.484$, respectively, $P<0.05$ for all), while it was negatively correlated with PaO₂ and PaO₂/FiO₂ ($r_{ho}=-0.422$, $r=-0.543$, $P<0.05$ for all).

DISCUSSION

As a kind of adipocytokine, visfatin can promote pre-B cell to be mature and play a key role in some

inflammatory reactions such as inhibiting neutrophil apoptosis and inducing human pulmonary arterial endothelial cells to secrete IL-8.^[9,10] It has been reported that the level of visfatin was significantly increased in the serum and bronchoalveolar lavage fluids of mouse model with acute lung injury,^[11] while the peptide products of some visfatin genes increased the risk of acute respiratory distress syndrome and mortality of critical patients in ICU. Another study^[12] demonstrated that the plasma level of visfatin in patients with acute pneumonia and septicemia was significantly increased. In this study, the plasma level of visfatin in patients with pneumonia was increased, and the level was significantly higher in patients with severe pneumonia than in those without severe pneumonia. We also compared visfatin level between the COPD patients in group A and group B, and found that visfatin level in group A was significantly higher than that in group B (12.76±2.51 ng/mL vs. 4.08±0.77 ng/mL, $P<0.01$). This finding indicated that visfatin played a critical role in the process of systemic inflammatory response for patients with pneumonia, particularly with severe pneumonia.

Severe pneumonia is often complicated with infection and anoxia, which may activate a variety of inflammatory cells to release inflammatory mediators, and thus result in SIRS. Polymorphonuclear neutrophil (PMN), as an important indicator of inflammation, plays a key role in the development and progress of inflammation. If the apoptosis of PMN is delayed, "respiratory burst" will occur, which leads to a vicious cycle and aggravate the SIRS. Visfatin can obviously

inhibit the neutrophil apoptosis,^[10] and the mechanism is related to the decreased activities of cysteine protease caspases-8 and caspases-3. In addition, it was found that visfatin could extend the survival time of neutrophils, activate neutrophils, increase the expression of intercellular adhesion molecule (ICAM-1) and other costimulatory molecules released by neutrophils, and enhance the adhesion of PMN with vascular endothelium.^[11] The results of our study indicated that both visfatin and neutrophils increased in patients with severe pneumonia, and they were positively correlated. This suggested that visfatin may be involved in inflammation response by enhancing the function of neutrophil, and can be considered as a new pro-inflammatory cytokine.

The major pathophysiological basis of systemic inflammatory response is the uncontrolled release of various inflammatory mediators,^[13] including the pro-inflammatory mediators of TNF- α , IL-6, IL-8 and so on. TNF- α is found to be elevated at the early stage of SIRS, and increased at a higher level under MODS. It is indicated in studies^[14,15] that inflammatory cytokines could regulate the production of visfatin, for example TNF- α could induce the expression of visfatin at the mRNA level, IL-6 and IL-8 could increase the genetic expression of visfatin, while visfatin could also induce the secretion of the above inflammatory cytokines in a dose-dependent way. But there are different points of view. Kralisch et al^[16] found that IL-6 could also inhibit the expression and synthesis of visfatin. Ognjanovic et al^[15] discovered that exogenous IL-8 didn't affect the expression of visfatin. In this study, the level of visfatin in patients with severe pneumonia was positively correlated with TNF- α , but was not significantly correlated with IL-6 and IL-8. This might be due to the relatively small sample size of this study, therefore, the relationship between visfatin and IL-6, IL-8 remains to be further studied.

Ventilation/perfusion imbalance, which leads to hypoxia, is commonly found in patients with severe pneumonia. The gene of visfatin can be activated by the hypoxia-inducible factor-1 under hypoxia, thus increasing its expression.^[17] PaO₂ and PaO₂/FiO₂ can be used to predict the severity of hypoxia for patients with severe pneumonia. The APACHE II score is currently the most commonly used and the most authoritative evaluation indicator for adult patients admitted to ICU. CRP is a sensitive indicator reflecting inflammation response, and obviously increased at the state of bacterial infection, which is consistent with the progress and

severity of diseases. In this study, we found the plasma level of visfatin was positively correlated with APACHE II score and CRP (this is in accordance with the study result of Fasshauer^[18]), and negatively correlated with PaO₂ and PaO₂/FiO₂. This indicated that the plasma level of visfatin could assess the severity of the disease.

Visfatin is not only expressed in adipose tissues, also expressed in many inflammatory cells. When patients with severe pneumonia are infected by pathogenic microorganisms, a large number of inflammatory stimulators appear in plasma, which may activate various inflammatory cells, such as PMN, alveolar macrophages and dendritic cells. In the study, we didn't find that the visfatin level in patients with severe pneumonia was significantly correlated with BMI, which was in accordance with the result of Liu's study.^[5]

The development and progress of severe pneumonia is related to the uncontrolled inflammatory response under infection with pathogens. Our study showed that the increase of plasma visfatin in patients with severe pneumonia was related to neutrophils, TNF- α and hypoxia. Visfatin, as a new pro-inflammatory cytokine, plays an important role in the course of severe pneumonia, and can reflect the severity of pneumonia.

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Ethical approval: The study was approved by the Ethics Committee of Shanghai Fifth People's Hospital Affiliated to Fudan University and informed consent forms were signed by all subjects.

Conflicts of interest: No benefits in any form have been received or will be received from a commercial party related directly or indirectly to the subject of this article.

Contributors: Xie J proposed the study and wrote the first draft. All authors contributed to the design and interpretation of the study and to further drafts.

REFERENCES

- 1 American Thoracic Society and Infectious Diseases Society of America, Guidelines for the management of adults with hospital-acquired, Ventilator-associated for the healthcare-associated pneumonia. *Am J Respir Crit Care Med* 2005;171: 388-416.
- 2 Lee WJ, Ou HC, Hsu WC, Chou MM, Tseng JJ, Hsu SL, et al. Ellagic acid inhibits oxidized LDL-mediated LOX-1 expression, ROS generation, and inflammation in human endothelial cells. *J Vasc Surg* 2010; 52: 1290-1300.
- 3 Zhang MZ, Wang LB, Chen C, Yang Y, Zhang LE. Role of dendritic cells in the pathogenesis of severe pneumonia in children. *Zhonghua Er Ke Za Zhi* 2005; 43: 410-413.

- 4 Samal B, Sun Y, Stearns G, Xie C, Suggs S, McNiece I. Cloning and characterization of the cDNA encoding a novel human pre-B-cell colony-enhancing factor. *Mol Cell Biol* 1994; 14: 1431-1437.
- 5 Liu X, Ji Y, Chen J, Li S, Luo F. Circulating visfatin in chronic obstructive pulmonary disease. *Nutrition* 2009; 25: 373-378.
- 6 The branch of respiratory disease of China medical association (CMA). Guidelines for the diagnosis and treatment of community-acquired pneumonia. *Zhonghua Jie He He Hu Xi Za Zhi* 2006; 29: 649-650.
- 7 American Thoracic Society, Infectious Diseases Society of America. Guidelines for the management of adults with hospital-acquired, ventilator-associated, and healthcare-associated pneumonia. *Am J Respir Crit Care Med* 2005; 171: 388-416.
- 8 American Thoracic Society. Guidelines for the management of adults with community acquired pneumonia. *Am J Crit Care Med* 2001; 163: 1730-1754.
- 9 Jia SH, Li Y, Parodo J, Kapus A, Fan L, Rotstein OD, et al. Pre-B cell colony-enhancing factor inhibits neutrophil apoptosis in experimental inflammation and clinical sepsis. *Clin Invest* 2004; 113: 1318-1327.
- 10 Ye SQ, Zhang LQ, Adyshev D, Usatyuk PV, Garcia AN, Lavoie TL, et al. Pre-B-cell-colony-enhancing factor is critically involved in thrombin-induced lung endothelial cell barrier dysregulation. *Microvasc Res* 2005; 70: 142-151.
- 11 Bajwa EK, Yu CL, Gong MN, Thompson BT, Christiani DC. Pre-B-cell-colony-enhancing factor gene polymorphisms and risk of acute respiratory distress syndrome. *Crit Care Med* 2007; 35: 1290-1295.
- 12 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: 4105-4113.
- 13 Meduri GU, Yates CR. Systemic inflammation-associated glucocorticoid resistance and outcome of ARDS. *Ann N Y Acad Sci* 2004; 1024: 24-53.
- 14 Ognjanovic S, Bao S, Yamamoto SY, Garibay-Tupas J, Samal B, Bryant-Greenwood GD. Genomic organization of the gene coding for human pre-B-cell colony-enhancing factor and expression in human fetal membranes. *Mol Endocrinol* 2001; 26: 107-117.
- 15 Ognjanovic S, Ku TL, Bryant-Greenwood GD. Pre-B-cell colony-enhancing factor is a secreted cytokine-like protein from the human amniotic epithelium. *Am J Obstet Gynecol* 2005; 193: 273-282.
- 16 Kralisch S, Klein J, Lossner U, Bluher M, Paschke R, Stumvoll M, et al. Interleukin-6 is a negative regulator of visfatin gene expression in 3T3-L1 adipocytes. *Am J Physiol Endocrinol Metab* 2005; 289: E586-E590.
- 17 Moschen AR, Kaser A, Enrich B, Mosheimer B, Theurl M, Niederegger H, et al. Visfatin, an adipocytokine with proinflammatory and immunomodulating properties. *Immunol* 2007; 178: 1748-1758.
- 18 Fasshauer M, Waldeyer T, Seeger J, Schrey S, Ebert T, Kratzsch J, et al. Serum levels of the adipokine visfatin are increased in pre-eclampsia. *Clin Endocrinol (Oxf)* 2008; 69: 69-73.

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