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Original Article

Chronic thromboembolic pulmonary hypertension is not associated with iron overload

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ABSTRACT

Background: Chronic thromboembolic pulmonary hypertension (CTEPH) is characterized as the incomplete resolution of emboli after pulmonary embolism (PE) and the subsequent fibrotic organization and remodeling of pulmonary vascular bed. It has been reported that abnormal fibrin probably contributes to the incomplete resolution of emboli. And there is evidence that free iron could convert fibrinogen into fibrin which is remarkably resistant to lysis. Thus, we hypothesized that persistent iron overload might participate in the development of CTEPH.

Materials and methods: A case-control study was conducted. Forty-five CTEPH patients were enrolled as cases, and 36 age and sex frequency-matched chronic PE patients without pulmonary hypertension were selected as controls. Levels of free iron, soluble transferrin receptor (sTfR), ferritin, sTfR/ferritin ratio, hepcidin-25, tumor necrosis factor-alpha (TNF-α), interleukin (IL)-6, and malondialdehyde (MDA) were compared between the two groups. Logistic regression analysis was carried out to estimate odds ratios.

Results: There was no difference of the levels of free iron, hepcidin-25, sTfR, ferritin, sTfR/ferritin ratio, TNF-α, and MDA between CTEPH patients and the controls. Levels of sTfR and ferritin in both groups were within the normal limits. Levels of IL-6 in CTEPH patients were significantly higher than that in the controls. A negative correlation was observed between hepcidin-25 and sTfR (Spearman's $r=-0.438$, $P<.001$), and a positive correlation was observed between hepcidin-25 and ferritin (Spearman's $r=0.503$, $P<.001$). In the univariate logistic regression model, there was no association observed between CTEPH and free iron, hepcidin-25, sTfR, ferritin, sTfR/ferritin ratio, TNF-α, IL-6, and MDA.

Conclusions: CTEPH has no association with iron overload. The iron status evaluated by sTfR and ferritin is within the normal limits in this CTEPH population.

1. Introduction

Chronic thromboembolic pulmonary hypertension (CTEPH) is an uncommon sequel of pulmonary embolism (PE), and a serious clinical situation associated with considerable morbidity and mortality. Presence of unresolved thromboemboli undergoing fibrotic organization is considered the characterization of CTEPH [1]. Why the emboli in some patients evade normal thrombolytic process and become fibrotic organization in CTEPH remains unclear. Based on recent insights, abnormal fibrin probably contributes to the incomplete resolution of emboli after acute PE and the progression to CTEPH [2,3]. Furthermore, studies demonstrated that an excessive accumulation of free (nonprotein-bound) iron in blood could convert fibrinogen into fibrin which is remarkably resistant to lysis [4,5]. And red blood cells (RBCs) were prone to be trapped within iron-induced abnormal fibrin mesh. The enhanced fibrin-RBC interaction which did not exist in blood from healthy subjects would be observed when iron ion was added [6].

These robust evidence draw a hypothesis that the persistent "free iron" or iron overload might play an important role in the formation of unresolved thromboemboli in some patients during the convalescent stage of acute PE, which eventually leads to CTEPH; while iron overload would have not existed in the patients who recuperated from acute PE without pulmonary hypertension (PH). The aim of the study was to explore whether abnormal iron metabolism after acute PE was associated with CTEPH. We conducted assessment of plasma-free iron level, soluble transferrin receptor (sTfR), ferritin, hepcidin-25, and hematologic indices in the present study. sTfR is a more accurate parameter for evaluating iron status in patients compared with serum ferritin and transferrin saturation [7]. And the ratio of sTfR/ferritin is a useful indicator of iron status according to previous studies [8].

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Hepcidin-25, a peptide hormone produced by the liver, constitutes the master regulator of iron homeostasis [9]. In addition, we investigated the role of associated factors, including inflammation and oxidative stress, to explore whether inflammation and/or oxidative stress play a role in development of CTEPH.

2. Materials and methods

2.1. Study design

We used a case-control study to analyze the association between iron metabolic parameters and CTEPH. All study subjects were among patients admitted to the Center for Pulmonary Vascular Diseases of Fuwai Hospital from April 2013 to March 2014. Consecutive patients with confirmed CTEPH diagnosis were enrolled as cases. Subjects with chronic PE—defined as patients recuperated from first episode of acute PE (undergoing at least 6 months of therapeutic anticoagulation and improvement in ventilation–perfusion lung scanning and computed tomography) and without PH—were considered as controls. Before participation, all of the subjects signed written information consent according to the guidelines of the Human Ethics Committee of Fuwai Hospital.

The exclusion criteria were any of the following: (a) age less than 18 years or more than 75 years; (b) anemia; (c) active inflammation condition; (d) liver or kidney dysfunction; (e) left heart failure; and (f) patients who did not sign the written informed consent.

2.2. Diagnosis of CTEPH

CTEPH diagnosis was based on symptoms and signs, ventilation–perfusion lung scanning, computed tomography, right heart catheterization (RHC), and pulmonary angiography, according to the criterion from the scientific statement of the American Heart Association [10]. The diagnosis of CTEPH was confirmed by an independent expert panel composed of four cardiologists in the Center for Pulmonary Vascular Diseases of Fuwai Hospital in Beijing, China.

2.3. Diagnosis of chronic PE

Patients with acute PE were treated according to the management protocol from the scientific statement of the American Heart Association [10]. After 6-month follow-up, chronic PE was defined as (a) significantly improved symptoms; (b) improvements in computed tomography and ventilation–perfusion lung scanning; (c) PH unlikely based on echocardiographic variables: tricuspid regurgitation velocity≤2.8 m/s, pulmonary arterial systolic pressure≤36 mmHg, without additional echocardiographic variables suggestive of PH; and (d) patients with tricuspid regurgitation velocity>2.8 m/s, pulmonary arterial systolic pressure>36 mmHg, or presence of additional echocardiographic variables suggestive of PH undergone RHC to exclude PH. Patients who manifested any signs or symptoms of CTEPH followed the algorithm mentioned above to confirm or exclude the diagnosis of CTEPH. The provoked PE patients were defined as those with trigger factors including active cancer, prolonged immobilization, chronic heart or respiratory failure, recent trauma, surgical intervention, pregnancy, and the use of oral contraceptives or hormone replacement therapy. All other patients were classified as unprovoked PE.

2.4. Laboratory measurements

Blood samples of CTEPH patients were collected when the diagnosis had been confirmed. Blood samples of chronic PE patients were collected at 6-month follow-up. They were obtained by antecubital venipuncture into ethylenediamine tetra acetic acid-coated or sodium citrate-treated polypropylene tubes after overnight fasting. Hematologic indices were determined using the automated hematology analyzer XE-2100 (Sysmex, Co., Kobe, Japan). The plasma supernatants were frozen at −80°C immediately after centrifugation. The free iron levels were measured by the method spectrophoto-metrically with batho-phenanthroline bisulphonate [11]. Levels of tumor necrosis factor-alpha (TNF-α) (Abcam, Cambridge, UK), interleukin (IL)-6 (R&D Systems, Inc., Minneapolis, MN, USA), sTfR (R&D Systems, Inc., Minneapolis, MN, USA), ferritin (Abcam, Cambridge, UK), and malondialdehyde (MDA) (Cloud-Clone Corp, Houston, USA) were determined by enzyme-linked immunoadsorbent assay. Intra- and interassay coefficients of variation were 4.8% and 7.0%, respectively, for TNF-α; 4.2% and 6.4% for IL-6; 4.3% and 6.4% for sTfR; 5.4% and 6.1% for ferritin; 10% and 12% for MDA. The normal range for sTfR is 8.7 nmol/L to 28.1 nmol/L. The normal ranges of ferritin are 6 ng/ml–180 ng/ml for premenopausal females, 8 ng/ml–350 ng/ml for postmenopausal females, and 20 ng/ml–400 ng/ml for males. Hepcidin-25 measurements were made using enzyme immunoassay kit (Bachem Group, Torrance, CA, USA).

2.5. Statistical analysis

Data are presented as medians with interquartile ranges for nonnormally distributed variables and means±standard deviation (S.D.) for normally distributed continuous variables. Skewed variables were log-transformed. Categorical variables were displayed as numbers and percentages. Comparisons between two groups of subjects were made using the Student's *t* tests or the Mann–Whitney *U* test for continuous variables and chi-square tests for categorical variables. Spearman's correlation coefficient was used to test correlation between free iron and hepcidin-25, sTfR, ferritin, sTfR/ferritin ratio, TNF-α, IL-6, and MDA, respectively, and the correlation between hepcidin-25 and the factors described previously. We then used logistic regression models to explore the possible association between CTEPH and all the testing factors mentioned previously. Differences were considered significant when $P<.05$. All statistical analyses were performed using the Statistical Package for Social Science (SPSS) 16.0 for Windows (SPSS Inc., Chicago, IL, USA).

3. Results

3.1. Population characteristics

Forty-five CTEPH patients and 36 age and sex-frequency-matched chronic PE patients were enrolled in present study. Among CTEPH patients, 19 (42%) had a documented previous PE. Clinical characteristics of the 81 patients are displayed in Table 1. The ages of cases and controls were 45–64 years and 52–62 years, respectively. Body mass index (BMI) of CTEPH patients was lower than chronic PE patients. There was no difference of prevalence of hypertension, diabetes, coronary artery disease, and connective tissue disease between two groups. There was no difference of thrombolysis treatment between the two groups. There were 18 (40%) CTEPH patients that were considered to have provoked factors compared with 22 (61%) chronic PE patients in the study population ($P=.088$).

3.2. Iron metabolism, inflammation, and oxidative stress

There was no difference of free iron levels between the cases and the controls. There was no difference of the levels of hepcidin-25, sTfR, TNF-α, and MDA between the two groups. Levels of sTfR and ferritin in both groups were within the normal limits. IL-6 levels in CTEPH patients were significantly higher than that in the controls. Values of hemoglobin and red blood cell distribution width (RDW) were significantly higher in CTEPH patients than that in the controls, while there was no difference of mean corpuscular volume (MCV) between the two groups (Table 2).

A negative correlation was observed between hepcidin-25 and sTfR (Spearman's $r=-0.438$, $P<.001$), and a positive correlation was observed between hepcidin-25 and ferritin (Spearman's $r=0.503$, $P<.001$). There was no correlation between free iron and the other tested factors, for