Detection and significance of TNF-α and hs-CRP in the pleural effusion of patients with diabetes and pulmonary tuberculosis

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Abstract
It is postulated that high-sensitivity C-reactive protein (hs-CRP) and tumor necrosis factor α (TNF-α) are diagnostic utilities for pleural effusion. This study was designed to explore the detection and significance of TNF-α and hs-CRP in the pleural effusion of patients with diabetes and pulmonary tuberculosis. A total of 60 patients with diabetes and pulmonary tuberculosis pleural effusion were selected as the study group, while 60 patients with pulmonary tuberculosis pleural effusion were considered as the control group. The expression of TNF-α and hs-CRP in the two groups was determined from pleural effusion by enzyme-linked immunosorbent assay (ELISA). The expression levels of TNF-α and hs-CRP in pleural effusion of the study group were significantly (P < 0.05) higher than the control group, and the sensitivity and specificity of the combined detection were significantly (P < 0.05) higher than those of the separate detection. The expression of TNF-α and hs-CRP in the pleural effusion of patients with diabetes and pulmonary tuberculosis increased remarkably, which plays an important role in the diagnosis and treatment helping with differential diagnosis and evaluation of severity and prognosis by related detection of changes of these indexes, especially the combined detections.

Keywords
diabetes complicated with pulmonary tuberculosis, high-sensitivity C-reactive protein (hs-CRP), tumor necrosis factor-α (TNF-α)

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Introduction
The causes of pleural effusion are very complicated. At present, the main detection of pleural effusion is mainly limited to identify exudate and leakage. High-sensitivity C-reactive protein (hs-CRP), a protein synthesized by the liver, plays an important role in regulating inflammatory response and exists widely in serum or plasma, interrelated with other cytokines, such as tumor necrosis factor α (TNF-α) and interleukin (IL)-6. Its expression increases significantly when the inflammatory reaction occurs. A large number of hs-CRP can activate the complement system and promote further release of inflammatory mediators and oxyradicals to damage the vascular endothelial cells, which promote and aggravate the disease.1 It is also found that hs-CRP is involved in the formation of thrombosis and atherosclerosis, and is an important inflammatory factor in the development of atherosclerosis.2,3 TNF-α

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is a cytokine produced mainly by activated monocytes and macrophages, with strong inflammatory effect and other biological functions, such as promoting the expression of other cytokines and the accumulation of inflammatory cells, antitumor, and antivirus with the help of interferon, regulating immunity, inducing cells to produce oxyradicals which increase the lipid peroxidation and damage the cell intima, entering cells to bind and release lysosomal enzyme which causes cell autolysis, while there is no obvious effect on normal tissues. Proinflammatory factors are involved in both cell apoptosis and cell regeneration, and participate in the growth and development of organisms with other cytokines, maintaining the stability of the internal environment and participating in inflammation, immunity, and other reactions. In addition, they can expand renal microvessels, enhance the permeability of vascular walls, stimulate glomerular endothelial cells to produce various inflammatory factors, and damage blood vessels and surrounding tissues. They can also stimulate the generation of oxyradicals and prostaglandins in mesangial cells, which can damage basement membrane, promote proteinuria, and participate in the occurrence and development of diabetic nephropathy (DN). The other common causes of pleural effusion include inflammation, tuberculosis, tumor, and connective tissue disease, and the treatment plan and the prognosis vary accordingly. Therefore, it is very important to determine the nature of the pleural effusion. At present, the common diagnosis of pleural effusion is meaningless for a certain disease sometimes, such as routine detection, biochemical detection, or pathological detection. The incidence of diabetes has increased year by year, and the same to pulmonary tuberculosis. It has been found that there are inflammatory reactions involved in diabetes and tuberculosis, which can cause a rise in some inflammatory factors, such as the high expression of TNF-α and hs-CRP in diabetic patients and the high expression of hs-CRP in patients with pulmonary tuberculosis, which can help evaluate the severity, development, and prognosis of these diseases.

The main aim of this study was to explore the detection and significance of TNF-α and hs-CRP in the pleural effusion for clinical application by selecting 60 patients with diabetes and pulmonary tuberculosis (as the study group). It was hypothesized whether hs-CRP and TNF-α are significantly detectable in pleural effusion.

Methodology

Research subjects

A total of 120 patients with diabetes and pulmonary tuberculosis pleural effusion from January 2015 to June 2017 in our hospital were selected as the research subjects. They were divided into two groups: 60 patients were selected as the study group consisting of 38 males and 22 females, aged from 18 to 76 years, with disease course from 5 to 10 years, 10 cases complicated with hypertension and nine cases complicated with coronary heart disease; 60 patients with pulmonary tuberculosis pleural effusion in the same period were selected as the control group including 40 males and 20 females, aged from 19 to 75 years, with disease course from 5 to 11 years, 11 cases complicated with hypertension and 10 cases complicated with coronary heart disease.

Inclusion criteria

Patients enrolled were all diagnosed as type II diabetes with secondary pulmonary tuberculosis according to diabetes diagnostic criteria established by the World Health Organization (WHO) in 1999 and the classification of the Chinese Medical Association for tuberculosis. All patients participated in the study voluntarily and signed informed consent at the beginning.

Exclusion criteria

Patients with rheumatic immune diseases, serious visceral organ diseases, or other malignant diseases were excluded.

Methods

Pleural effusion was extracted from all patients in two groups in equal volume. After centrifugation for 15 min (3000 r/min), the supernatant was kept in the fridge at −20°C and determined within 7 days. All procedures were conducted by the same technician to minimize errors. The enzyme-linked immunosorbent assay (ELISA) detection of TNF-α was performed as follows: adding 50 µL sample in the inspection vessel, then adding 100 µL primary antibody, shaking the vessel for 1 min gently
to mix completely, placing for 30 min at room temperature and avoiding light, cleaning the vessel with 250 µL washing liquor three times, and drying the vessel the third time as possible with water-absorbent paper if necessary; then adding 150 µL secondary antibody, shaking the vessel for 1 min gently to mix completely, covering the vessel, placing for 30 min at room temperature and avoiding light, cleaning the vessel with 250 µL washing liquor three times, and drying the vessel the third time as possible; finally adding 100 µL substrate (TMB), placing for 15 min at room temperature and avoiding light, and adding 100 µL terminator to read at 450 nm. The expression of hs-CRP was determined by immunoturbidimetry.3,4

Statistical methods
All statistical analyses were performed with SPSS version 17.0. The measurement data were expressed as (X ± s) and compared with t-test, and the enumeration data were expressed as n (%) and compared with χ² test, respectively. A P-value of 5% or lower was considered to be statistically significant.

Results

Comparison of pleural effusion TNF-α and hs-CRP between the two groups
The levels of pleural effusion TNF-α and hs-CRP of the study group were significantly higher than those of the control group (P < 0.05) as shown in Table 1.

Comparison of pleural effusion TNF-α and hs-CRP in patients with and without DN in the study group
In the study group, the levels of pleural effusion TNF-α and hs-CRP in patients complicated with DN were significantly higher than those in patients without DN (P < 0.05) as shown in Figure 1.

Comparison of the effect between separate detection and combined detection
The sensitivity and specificity of the combined detection were significantly higher than those of the separate detection (P < 0.05) as shown in Table 2.

Table 1. Comparison of pleural effusion TNF-α and hs-CRP between the two groups (X ± s, mg/L).

| Groups        | n  | TNF-α  | hs-CRP   |
|---------------|----|--------|----------|
| Study group   | 60 | 235.18g | 12.348g  |
| Control group | 60 | 128.39g | 6.0339g  |
| t             | 10.9524 | 8.4219 |
| P             | 0.0367 | 0.0428 |

TNF-α: tumor necrosis factor α; hs-CRP: high-sensitivity C-reactive protein.

Discussion
At present, studies have found that chronic inflammation plays an important role in the occurrence and development of diabetes. At the same time, it can activate the monocyte macrophage system and vascular endothelial system, promote the release of cytokines and inflammatory mediators, and participate in the development of diabetes. And the hyperglycemia of diabetes itself can also promote the secretion of IL-6 that promotes the secretion of hs-CRP in the liver. The above inflammatory factors can cause the death of a large number of pancreatic beta cells, accelerate insulin resistance, and promote the progress of diabetes. Besides, glycation products of diabetes act on the vascular wall, increase the thickness of blood vessels, decrease elasticity, form atherosclerosis, induce oxidative stress, damage vascular endothelial cells, and further aggravate vascular lesions. Therefore, due to the inflammatory status in diabetic patients, it can cause the excessive expression of TNF-α, hs-CRP, and other cytokines, which act as important mediators of inflammation to aggravate the inflammatory reaction and promote the development of diabetes in turn. Therefore, TNF-α and hs-CRP are closely related to the development of diabetes both in reflecting the inflammation status and in monitoring the development of the disease.9,10 As a specific inflammatory disease, tuberculosis itself can stimulate the immune and inflammatory reaction of the body, and increase inflammatory factors such as TNF-α and hs-CRP. Meanwhile, due to undisciplined remedy, more and more drug-resistant tuberculosis increased the difficulty of clinical treatment. And as for diabetic patients, it is very easy to infect with Mycobacterium tuberculosis for poor resistance. The combination of two diseases will undoubtedly increase the difficulty of treatment, and the two diseases interact with each other,
that is, tuberculosis can cause blood glucose disorders, resulting in blood sugar out of control; in return, diabetes leads to the failure of controlling \textit{M. tuberculosis} and promotes further development of the disease.\textsuperscript{11}

Studies about the changes of inflammatory factors in diabetes or tuberculosis have been conducted separately before, while the combined study rarely reported. The results of the study on changes of inflammatory factors in the pleural effusion of patients with diabetes and tuberculosis showed that the levels of pleural effusion TNF-\(\alpha\) and hs-CRP of the study group were significantly higher than those in the control group and within the study group and that the levels of pleural effusion TNF-\(\alpha\) and hs-CRP in patients complicated with DN were significantly higher than those in patients without DN. It indicates that diabetes and pulmonary tuberculosis can promote the release of inflammatory factors such as TNF-\(\alpha\) and hs-CRP, especially when combined with DN. The combined detection of TNF-\(\alpha\) and hs-CRP can effectively improve the sensitivity of the diagnosis and help determine the severity and prognosis of the disease.\textsuperscript{12,13} That is, TNF-\(\alpha\) and hs-CRP in pleural effusion of patients with diabetes mellitus complicated with pulmonary tuberculosis increased significantly, and the sensitivity and specificity of combined detection were 86.67\% and 85.00\%, respectively. If combined with DN, the expression of inflammatory factors will further increase, suggesting that there is a close relationship between them. And the detection of related inflammatory factors can help diagnose and treat diseases effectively.

Some reports concerning the levels of pleural fluid TNF-\(\alpha\) and CRP are documented in the previous literature such as pleural effusion CRP (not hs-CRP) was measured in some studies. We evaluated the level of hs-CRP and found significant results. For instance, Porcel et al. evaluated CRP and several markers such as myeloperoxidase, IL-8, and matrixmetalloproteinase-2, but not hs-CRP, in pleural fluid. They focused on pneumonia and tried to distinguish between complicated and uncomplicated parapneumonia, while in this study we explored TNF-\(\alpha\) and hs-CRP in the pleural effusion of patients with diabetes and pulmonary tuberculosis.\textsuperscript{14}

In conclusion, the expression of TNF-\(\alpha\) and hs-CRP in the pleural effusion of patients with diabetes and pulmonary tuberculosis increased remarkably, which plays an important role in the diagnosis and treatment helping with differential diagnosis and evaluation of severity and prognosis by related

**Figure 1.** Comparison of pleural effusion TNF-\(\alpha\) and hs-CRP in patients with and without diabetic nephropathy. 
\(t = 12.5325\) (TNF-\(\alpha\)), \(t = 6.1757\) (hs-CRP); \(P = 0.0479\) (TNF-\(\alpha\)), \(P = 0.0386\) (hs-CRP).

**Table 2.** Comparison of the effect between separate detection and combined detection (n (%)).

| Indexes       | Sensitivity | Specificity |
|---------------|-------------|-------------|
| Separate detection |             |             |
| TNF-\(\alpha\) | 34 (56.67)  | 36 (60.00)  |
| hs-CRP        | 33 (55.00)  | 37 (61.67)  |
| Combined detection | 52 (86.67)  | 51 (85.00)  |
| \(P\)         | 0.0359      | 0.0347      |

TNF-\(\alpha\): tumor necrosis factor \(\alpha\); hs-CRP: high-sensitivity C-reactive protein.
detection of changes of these indexes, especially the combined detections.

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