INTRODUCTION

Tuberculosis (TB) is a major infectious disease caused by various strains of mycobacteria, including Mycobacterium tuberculosis (MTB), and pulmonary tuberculosis (PTB) is the most common form of this disease, mainly affecting lung tissue. Currently, PTB poses a massive health challenge owing to its high prevalence and mortality rates, and remains a major public health concern worldwide, especially in...
low-income countries. Therefore, PTB control requires a combination of rapid and reliable diagnosis, appropriate treatment, and timely monitoring. Previous studies had shown that obese hosts, with impaired immune responses, present increased susceptibility to infection with a variety of pathogens, including community-acquired tuberculosis, MTB, and Helicobacter pylori.

The adipose tissue is considered a key organ with important immune functions and can reportedly secrete numerous adipokines, such as leptin, resistin, vaspin, and adiponectin. These adipokines are known to play key roles in regulating energy balance, immune functions, and inflammatory processes, as well as stabilizing endocrine functions. Furthermore, some adipokines were reportedly involved in the development of TB. Ehtesham et al have suggested that the resistin level was significantly higher in TB patients than normal controls, and resistin could serve as an early biomarker for TB diagnosis.

Visfatin is a 52 kDa adipokine, secreted by the adipose tissue and other tissues, including the liver, bone marrow, and skeletal muscles. Visfatin was initially known as a pre-B-cell colony-enhancing factor (PBEF) owing to its ability to enhance differentiation of B-cell precursors synergistically with interleukin (IL)-7 and was also termed nicotinamide phosphoribosyltransferase (NAMPT) owing to its enzymatic activity. As an adipokine with immunomodulatory and pro-inflammatory roles, visfatin could stimulate chemotaxis of activated mononuclear cells and induce monocytes to secrete multiple pro-inflammatory cytokines, including IL-1, IL-6, IL-8, and tumor necrosis factor (TNF)-α. Lipocalin-2, known as neutrophil gelatinase-associated lipocalin (NGAL), is an innate immune protein and was originally considered the main component of neutrophil secondary granules. During the early stages of respiratory mycobacterium infection, lipocalin-2 is reportedly secreted by alveolar macrophages and epithelial cells into the alveolar space. Additionally, it has been suggested that lipocalin-2 binds to bacterial siderophores to isolate the iron required for bacterial growth and was considered to play a protective role in mycobacterial infections.

Therefore, we speculated that these two adipokines (visfatin and lipocalin-2) may be associated with the occurrence of PTB; however, relevant investigations are limited. In peripheral blood mononuclear cells (PBMCs), the abnormal expression of adipokines has been observed in multiple diseases and is expected to be developed as an indicator of disease diagnosis. Therefore, this is an important attempt to explore the expression levels of adipokines in PBMCs from PTB patients. To further investigate the roles of visfatin and lipocalin-2 in the pathogenesis of PTB, we detected the mRNA expression levels of these adipokines in PBMCs from PTB patients and determined their diagnostic role in PTB patients.

2 MATERIALS AND METHODS

2.1 PTB patients and healthy controls

This case-control study was composed of 150 unrelated Han Chinese individuals, including 79 PTB patients and 71 healthy controls. The average age of the PTB patients, including 26 females and 53 males, was 45.16 ± 17.48; the healthy controls were composed of 19 females and 52 males, with a mean age of 44.62 ± 10.00. All PTB patients were consecutively enrolled from the Department of Tuberculosis at Anhui Chest Hospital (Anhui Provincial TB Institute) and defined based on these following criteria: suspicious clinical symptoms, chest radiography, sputum and/or bronchoalveolar lavage fluid MTB culture, microscopy for acid-fast bacilli (AFB), and effect of anti-TB treatment. This study excluded PTB patients with cancer, hepatitis, immunocompromised conditions, and HIV infection. Individuals with normal chest radiographic results and no clinical history of TB were recruited as healthy controls. Basic information concerning study subjects was collected, and several clinical and laboratory parameters of PTB patients were retrieved from their medical records, including chest radiography, sputum smear, complications, erythrocyte sedimentation rate (ESR), total bilirubin (TBIL), alanine aminotransferase (ALT), and aspartate aminotransferase (AST). Peripheral blood samples and relevant information were collected after obtaining informed consent from each subject. This research was approved by the medical ethics committee of Anhui Chest Hospital (K2020-005).

2.2 Quantitative real-time reverse transcription-polymerase chain reaction (qRT-PCR)

In total, 5 mL anticoagulated peripheral blood was extracted from each PTB patient and normal control, and PBMCs were isolated from peripheral blood using Ficoll-Hypaque density gradient centrifugation. Then, total RNA in PBMCs was extracted by TRizol reagent, and the RNA concentration was measured using the NanoDrop 2000 spectrophotometer. Finally, total RNA was reverse-transcribed to cDNA with a PrimeScript™ RT reagent Kit.

Visfatin and lipocalin-2 mRNA expression levels in PBMCs were measured using qRT-PCR with SYBR Green (SYBR Premix Ex Taq II, Takara Bio Inc), and the housekeeping gene β-actin was used as an internal control to calculate the relative expression levels of these adipokines in the same sample. The relative expression levels of visfatin and lipocalin-2 were estimated using $2^{-\Delta\Delta C_t}$ normalized to β-actin.

2.3 Statistical analysis

All statistical analyses were performed using SPSS 23.0. The adipokine mRNA expression levels are presented as median values and interquartile ranges. Differences in adipokine mRNA expressions between PTB patients and healthy controls were analyzed using the nonparametric Mann-Whitney U test. Spearman’s rank correlation coefficient test was used to analyze the correlation between adipokine mRNA expression levels and multiple laboratory indexes investigated in PTB patients. The diagnostic value of adipokine mRNA levels in PTB patients was calculated using receiver operating...
characteristic (ROC) analysis. A $P$ value < .05 was considered statistically significant.

3 | RESULTS

3.1 | Visfatin and lipocalin-2 mRNA levels in PBMCs from PTB patients and controls

In PBMCs from PTB patients and healthy controls, visfatin and lipocalin-2 mRNA expression levels were detected by qRT-PCR. As indicated in Figure 1, the visfatin mRNA expression level in PBMCs from PTB patients was significantly higher than that in healthy controls ($P < .001$). However, no significant difference was observed in the lipocalin-2 mRNA expression level between PTB patients and healthy controls ($P = .933$) (Figure 2). Furthermore, we analyzed the correlation between visfatin and lipocalin-2 mRNA levels in PTB patients and observed no significant result ($P = .475$).

3.2 | Relationship between visfatin and lipocalin-2 mRNA levels and clinical and laboratory data in PTB patients

In the PTB group, 53 (67.09%) and 17 (21.52%) patients were diagnosed as treatment-naive PTB and drug-resistant PTB patients, respectively. Additionally, clinical manifestations in PTB patients included sputum smear-positive (39.24%), pulmonary cavity (34.18%), unilateral tuberculosis foci (26.58%), diabetes (20.25%), extrapulmonary tuberculosis (13.92%), and liver injury (11.39%). To further explore the association between visfatin and lipocalin-2 mRNA levels and disease characteristics, and the severity of PTB patients, we compared the visfatin and lipocalin-2 mRNA levels between PTB patients, with and without the above clinical manifestations. However, no statistical significance was observed (Table 1).

Furthermore, the correlation between visfatin and lipocalin-2 mRNA levels with several laboratory indicators in PTB patients was analyzed, and the results are presented in Table 2. Our results demonstrated that the lipocalin-2 mRNA expression positively correlated with ESR in PTB patients ($P = .010$). However, no significant correlation was observed between visfatin and lipocalin-2 mRNA levels and TBIL, ALT, and AST levels in PTB patients.

3.3 | Diagnostic value of the visfatin mRNA level in PTB

We conducted a ROC curve analysis to calculate the diagnostic significance of the visfatin mRNA expression in PBMCs from PTB patients. As shown in Figure 3, the curve indicated that the visfatin mRNA level could be a potential diagnostic marker for PTB, presenting an area under the curve (AUC) of 0.667 (95% CI: 0.580-0.755). Moreover, the optimal cutoff value of the visfatin mRNA level for PTB diagnosis was 0.645, and the corresponding sensitivity and specificity were 79.7% and 49.3%, respectively.

4 | DISCUSSION

Considering the important roles of adipokines in the innate immune response, several studies had focused on the pathogenicity and expression level of adipokines in patients with TB. In this study, we explored the mRNA expression levels of visfatin and lipocalin-2 in PBMCs from PTB patients and observed increased visfatin mRNA levels in PTB patients. However, no significant difference was observed in the lipocalin-2 mRNA levels between PTB patients and healthy controls.
Currently, a large number of studies have reported that visfatin is involved in pro-inflammatory, immunomodulating processes, attenuating angiogenesis, and endothelial cell repair mechanisms. The role of visfatin in the occurrence and pathogenesis of several respiratory diseases has gradually attracted the attention of several researchers. Toru et al. have observed that the serum visfatin level was significantly increased in asthma patients when compared with healthy controls and could be a potential biomarker for diagnosing and treating patients with asthma. Therefore, it remains necessary to further explore the possible association between visfatin and the pathogenesis of PTB. A previous study has implied that compared with latent TB patients, PTB patients present significantly lower visfatin levels. Moreover, PTB patients with diabetes have revealed a significantly lower visfatin level when compared with latent TB patients with diabetes. Our study was the first to confirm that the visfatin mRNA level in PBMCs was significantly elevated in PTB patients when compared with healthy controls. This further supports the hypothesis that visfatin levels could be correlated with PTB development.

The early and clear diagnosis of PTB is beneficial for reducing the incidence, mortality, and transmission rates; however, despite years of development, PTB diagnosis remains challenging. Based on our findings, we further examined the diagnostic significance of visfatin mRNA levels for PTB by plotting ROC curves. We observed that the AUC was 0.667 (95% CI: 0.580-0.755), and the sensitivity approached 79.7%, when the diagnosis cutoff was 0.645. These results demonstrated that the visfatin mRNA level could be used to differentiate PTB patients from healthy individuals. However, it should be noted that the AUC was not sufficiently

### TABLE 1 The association between visfatin, lipocalin-2 expression level and some clinical features in PTB patients

| Group                              | +/− | Number | Visfatin level | P value | lipocalin-2 level | P value |
|------------------------------------|-----|--------|----------------|---------|-------------------|---------|
| Treatment-naive patients           |     |        |                |         |                   |         |
| +                                  | +   | 53     | 1.039 (0.713, 2.110) | .162    | 0.430 (0.277, 1.158) | .260    |
| −                                  | −   | 26     | 1.000 (0.646, 1.523) | .667    | 0.667 (0.386, 1.181) | .260    |
| Drug-resistant patients            |     |        |                |         |                   |         |
| +                                  | +   | 17     | 1.331 (0.696, 1.789) | .811    | 0.704 (0.386, 1.272) | .166    |
| −                                  | −   | 62     | 1.016 (0.662, 1.988) | .429    | 0.289 (1.075)      |         |
| Liver injury                       |     |        |                |         |                   |         |
| +                                  | +   | 9      | 1.154 (0.727, 2.578) | .487    | 0.408 (0.253, 0.739) | .267    |
| −                                  | −   | 70     | 1.033 (0.662, 1.973) | .534    | 0.317 (1.033)      |         |
| Diabetes                           |     |        |                |         |                   |         |
| +                                  | +   | 16     | 1.318 (0.669, 1.828) | .836    | 0.634 (0.383, 1.137) | .247    |
| −                                  | −   | 63     | 1.027 (0.695, 1.977) | .477    | 0.276 (1.161)      |         |
| Sputum smear-positive               |     |        |                |         |                   |         |
| +                                  | +   | 31     | 0.943 (0.657, 1.533) | .135    | 0.624 (0.360, 1.248) | .059    |
| −                                  | −   | 42     | 1.358 (0.720, 2.026) | .411    | 0.260 (0.710)      |         |
| Unilateral tuberculosis foci       |     |        |                |         |                   |         |
| +                                  | +   | 21     | 1.011 (0.605, 2.013) | .673    | 0.427 (0.268, 1.158) | .450    |
| −                                  | −   | 58     | 1.188 (0.696, 1.902) | .546    | 0.328 (1.188)      |         |
| Pulmonary cavity                   |     |        |                |         |                   |         |
| +                                  | +   | 27     | 1.022 (0.721, 1.657) | .385    | 0.506 (0.360, 0.825) | .992    |
| −                                  | −   | 52     | 1.130 (0.652, 2.062) | .508    | 0.281 (1.246)      |         |
| Extrapulmonary tuberculosis        |     |        |                |         |                   |         |
| +                                  | +   | 11     | 0.943 (0.605, 1.491) | .428    | 0.506 (0.356, 1.295) | .590    |
| −                                  | −   | 68     | 1.110 (0.697, 1.976) | .508    | 0.304 (1.159)      |         |

Note: +/−: with/without; median (interquartile range).

### TABLE 2 The correlation between visfatin, lipocalin-2 mRNA levels and ESR, TBIL, ALT, and AST of PTB patients

| Clinical parameters | Visfatin level $r_s$ | P value | lipocalin-2 level $r_s$ | P value |
|---------------------|----------------------|---------|------------------------|---------|
| ESR                 | −0.199               | .101    | 0.309                  | .010    |
| TBIL                | −0.015               | .898    | −0.065                 | .569    |
| ALT                 | −0.042               | .711    | −0.018                 | .873    |
| AST                 | −0.094               | .409    | 0.101                  | .378    |

Note: $r_s$, Spearman’s rank correlation coefficient.

FIGURE 3 Diagnostic accuracy of visfatin mRNA level in PTB
high, and the specificity was only 49.3%. This could be attributed to the small sample size in this study. Conversely, the low specificity of visfatin could lead to a few healthy individuals being wrongly diagnosed with PTB, resulting in the misuse of medical resources and negative effects on people's health. Based on these results, we concluded that to improve the sensitivity and specificity of PTB diagnosis, a combination of visfatin and other diagnostic methods, including the T-SPOT.TB test and interferon-γ release assay, were necessary in the future.

Previous animal experiments have reported that lipocalin-2 could play an important role in regulating T-cell accumulation and inflammation by restraining inflammatory chemokines and stimulating the recruitment of neutrophils in mycobacterial pulmonary infection. Several investigators have revealed that lipocalin-2 could bind to soluble siderophores of mycobacteria and inhibit the intracellular replication of MTB in cultured macrophage cell lines, as well as the growth of MTB in vitro. Therefore, some studies had explored the role of lipocalin-2, representing an important part of the innate immune response, in patients with PTB. Coussens et al. have analyzed ethnic variations in inflammatory profiles in PTB patients and observed no significant difference in the lipocalin-2 expression among different ethnic groups. Choi et al. have suggested that the serum lipocalin-2 level was lower in PTB patients than healthy controls. However, the expression of lipocalin-2 mRNA in PBMCs from PTB patients has not been evaluated. Our study was the first to demonstrate that lipocalin-2 mRNA levels did not differ between PTB patients and healthy controls. Our findings suggested that the lipocalin-2 mRNA level positively correlated with ESR in PTB patients, implying that this adipokine might possess a direct effect on the laboratory index, and could be used to reflect disease status. Moreover, the precise role of lipocalin-2 in the development of PTB needs to be explored.

In conclusion, our results demonstrated that the visfatin mRNA level was significantly higher, and the lipocalin-2 mRNA level positively correlated with ESR in PTB patients in a Chinese population. Moreover, visfatin could be considered as an auxiliary biomarker for PTB diagnosis. However, several limitations of this study should be acknowledged. First, as a case-control study, we were unable to evaluate the association between adipokine mRNA expression and multiple clinical and laboratory data, as well as the treatment employed in PTB patients within a specific period. Second, our study failed to analyze the potential influence of environmental factors, ethnic backgrounds, and other factors on adipokine mRNA expressions owing to a lack of relevant data. Finally, the sample size of the present study might be insufficient, affecting the power of this study. Hence, further large, prospective, functional studies are crucial to further assess the potential roles of these adipokines in PTB.

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CONFLICT OF INTEREST
The authors declare that they have no conflict of interest.

AUTHOR CONTRIBUTIONS
T-PZ designed the study. H-ML and QH conducted the experiment. S-SC and G-YZ performed the statistical analyses. S-JS and HW participated in sample collection. H-ML drafted the article. YL and T-PZ contributed to the article revision. All the authors approved the final submitted version.

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