Features of iron metabolism in patients with HIV infection and pulmonary dissemination in tuberculosis and pneumonia: An exploratory case-control study

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Abstract: Background — Differential diagnosis of pneumonia vs. tuberculosis in patients with HIV infection and pulmonary dissemination syndrome is an important problem in contemporary clinical medicine. The goal of our study was to determine the features of iron metabolism in patients with HIV infection and pulmonary dissemination in tuberculosis and pneumonia. Methods — The case-control study was conducted on 42 cases (patients with tuberculosis, hereinafter referred to as Group 1) and 44 control subjects (patients with pneumonia, hereinafter referred to as Group 2). All of them had HIV infection and syndrome of pulmonary dissemination. For subjects in both groups, we analyzed clinical and social characteristics, along with laboratory data. We also examined hepcidin content, iron concentration, and ferritin level in blood serum, and performed statistical data processing. Findings — There were significant differences between cases and controls in terms of iron metabolism. Hepcidin and hemoglobin levels were statistically significantly higher (p < 0.001), whereas iron concentration was lower (p = 0.0002) in patients with tuberculosis (cases). Furthermore, there were statistically significant differences between groups in CD4+ T-cell count, viral load, and RBC and WBC counts in complete blood count tests; in duration of complaints prior to hospital admission; and in terms of job stability. Conclusion — The quantitative parameters with the greatest statistical significance of the differences between the groups were iron concentration, hepcidin level, RBC count, WBC count, and CD4+ T-cell count.

Keywords: HIV infection, tuberculosis, pneumonia, iron metabolism, hepcidin.

Introduction

Respiratory diseases are currently most common in the structure of morbidity in the population of many countries. Disseminated lung diseases occupy a large share of those. Such diseases have a similar change in radiography albeit different etiology, pathogenesis, and treatment [1, 2]. HIV infection is among the causes of the increase in the number of patients with disseminations, including disseminated tuberculosis [3-5]. Patients with HIV infection have a higher risk of tuberculosis [6].

When examining such subjects, it is often difficult to diagnose pulmonary dissemination syndrome [7-9]. The most likely diagnoses for HIV infection and pulmonary dissemination are pneumonia caused by Pneumocystis jirovecii and disseminated pulmonary tuberculosis [10, 11]. Verification of these diagnoses presents substantial difficulty because the results of specific diagnostic tests are often negative [12]. Investigating vital roles of microorganisms in recent years, many studies were dedicated to the importance of iron. A review of published sources over the past decade has revealed that some studies have demonstrated changes in iron metabolism in tuberculosis [13-15], while other studies identified the features of the anemic syndrome developing in tuberculosis patients [13, 14, 16]. The clinical significance of hepcidin as a marker of inflammation in community-acquired bacterial pneumonia was determined without specifying the causative agent of pneumonia [17]. A few studies presented the results of examining the concentration of iron and iron-binding proteins in the bronchial fluid of patients with Pneumocystis pneumonia [18] and showed that the level of serum ferritin was associated with its severity [19]. At the same time, in domestic and foreign publications, there are no data on the details of iron metabolism in disseminated pulmonary tuberculosis and pneumonia of various etiologies in patients with HIV infection in a comparative aspect, which suggests the relevance of this study.

An important feature of disseminated tuberculosis pathogenesis is the hematogenous spread of mycobacteria [20]. Iron is among the most important substances in the life of mycobacteria. Also, iron belongs to the essential trace elements for humans [21-24]. Mycobacterium tuberculosis employs several mechanisms for obtaining iron. First, with the help of iron-chelating microbial siderophores; second, from iron-containing enzymes. This method is not typical for most microorganisms [21, 24, 25]. Tuberculosis patients may develop iron metabolism disorders in the form of a combination of anemia of chronic disease and iron deficiency anemia. Iron deficiency in anemia of chronic disease is relative in contrast to iron deficiency anemia [21, 24]. Transferrin and its receptors, ferritin, ferroportin,
ferroxidases and hepcidin are the most important substances in human iron metabolism [16, 23, 26]. Hepcidin synthesis is regulated by both increased content of free iron and the infectious process. Lipopolysaccharide macromolecules of bacterial cells act on macrophages, induce the interleukin-6 production, which stimulates the production of hepcidin [16, 27]. The study by Hella, Cercamondi, Mhimbira, et al. (2018) demonstrated that hepcidin was associated with the severity of tuberculosis and the development of active tuberculosis, but not with HIV-co-infection or helminths. It was also established that viruses of hepatitis B and C had almost no effect on hepcidin homeostasis [14].

The relevance of our study is suggested by the need to optimize the differential diagnosis of pneumonia vs. tuberculosis in patients with HIV infection and pulmonary dissemination, and by insufficient comparative laboratory data on the features of iron metabolism in such patients.

We searched eLIBRARY.RU (Russian electronic scientific library) and PubMed using the following keywords: disseminated tuberculosis, pneumonia, HIV infection, iron metabolism and hepcidin. Our search covered the studies published in 2010-2020, with no language restrictions. Most studies demonstrated the role of hepcidin as a marker of inflammation. Hepcidin is also known to increase in bacterial pneumonia similar to inflammatory process. We identified published studies on iron metabolism in patients with tuberculosis and pneumonia, but without comparative data on the characteristics of iron metabolism in patients with HIV infection and pulmonary dissemination.

The goal of our study was to identify the peculiarities of iron metabolism in patients with HIV infection and pulmonary dissemination in tuberculosis vs. pneumonia.

Material and Methods

Study design and population

This study was an exploratory, single-center case-control research conducted at Samara City Pulmonary Center. The study was approved by the Bioethics Committee at Samara State Medical University. For this type of study, formal consent was not required. Patients were eligible for recruitment if they were over 18 years, HIV positive and had pulmonary dissemination on radiographs. We excluded patients who received iron supplements less than 10 days prior to the study. The gender, age and race of patients did not matter. Subjects in Group 1 (cases; 42 patients) had a confirmed diagnosis of tuberculosis. Subjects in Group 2 (controls; 44 patients). All patients were counseled by a psychiatrist. Standard tuberculosis diagnostic procedure was performed three times. If it was negative, fiberoptic bronchoscopy with biopsy was carried out. During the first two days, a skin test with purified protein derivative (PPD) and a PPD skin test were done. The terms for diagnosing tuberculosis ranged from 3 to 21 days (mean: 14±5.3 days). Ziehl-Neelsen sputum smear microscopy (of the morning sputum portion) was performed three times. If it was negative, fiberoptic bronchoscopy and Ziehl-Neelsen microscopy of bronchoalveolar lavage were also performed. GeneXpert MTB/RIF assay and BACTEC MGIT test were carried out as well. The terms for diagnosing tuberculosis ranged from 3 to 21 days (mean: 14±5.3 days). Ziehl-Neelsen sputum microscopy detected M. tuberculosis in 9.5% of cases (4/42 – i.e., 4 patients of 42). Microscopy of bronchoalveolar lavage fluid detected M. tuberculosis in 15.5% of cases (6/38). GeneXpert MTB/RIF demonstrated 100% diagnostic result (36/36). It was further supported by the BACTEC MGIT test result (Table 1).

Data collection

Characteristics of social status, clinical manifestations, and examination results of both groups were analyzed. The parameters of the complete blood count test were assessed. Additionally, the indicators of iron metabolism were identified in blood serum (hepcidin, ferritin, and iron concentration).

Laboratory studies

The collection of blood serum was conducted no later than three days before admission to the pulmonary department. Serum was collected in Eppendorf tubes and stored at -20 °C until the analytical examination. The samples were defrosted at the temperature of 23-25 °C before testing. Repeated freezing of the samples was not permitted. Samples with hemolysis and lipemia were excluded from the study. We collected a total of 42 serum samples from the patients with tuberculosis and 44 samples from the subjects with pneumonia. The determination of the of iron and ferritin concentrations were determined on Cobas Integra 400+ automatic biochemical analyzer (Roche-Diagnostics, Switzerland), using commercially available reagent kits (Roche-Diagnostics, Switzerland). Hepcidin concentrations were determined via ELISA, using the ELISA Kit for Hepcidin (China) on Lazurit automatic analyzer (Vector-Best, Russia).

Statistical analysis

Statistical data processing was carried out using MedCalc 19.2.6 statistical software. The Shapiro-Wilk test was employed to assess the distributions of parameter values. Normally distributed parameters are presented as arithmetic mean and standard deviation, M ± o. Parameters with distributions other than normal are presented as median and interquartile range, Me (Q1-Q3). The statistical significance of intergroup differences was assessed using the Student’s t-test or Mann-Whitney test, depending on the distribution. Differences between groups were considered statistically significant at p<0.05 (exact p-values are indicated in parentheses). ROC curves were drawn to graphically display the values of investigated parameters, and areas under the curve (ROC AUC) were calculated for quantitative interpretation of the curves. Multivariate logistic regression analysis with subsequent logit transformation was performed to estimate the probability of tuberculosis in patients with HIV infection and dissemination syndrome. We developed a diagnostic model. Regression equations with various combinations of independent variables were compiled. The dependent variable was binary (presence or absence of tuberculosis). A stepwise regression for independent variables (regressors, or predictors, of tuberculosis) was chosen: P<0.05 was considered statistically significant.

Results

Of 42 patients in Group 1, there were 16.7% of women (n=7) and 83.3% of men (n=35). Median age was 39 (35; 44) yrs. The youngest patient in the group was 34 y/o, and the oldest was 55 y/o. Of 44 patients in Group 2, there were 36.3% of women (n=16) and 63.6% of men (n=28). Their median age was 40.4 (36; 43) yrs. The youngest patient in the group was 25 y/o, while the oldest was 65 y/o. There were no age differences (p=0.846) between the groups according to Mann-Whitney test.
Median duration of complaints prior to hospital admission was 4 (3; 4) wks. in Group 1, and 1 (0.75; 3) wk. in Group 2 (p<0.0001). Patients of both groups had complaints of weakness, dyspnea, cough, sputum production, and fever (Table 4).

The results of complete blood count tests in both groups were analyzed. Statistically significant differences were found in RBC count, WBC count and hemoglobin level (Table 5). Anemia severity was classified sensu World Health Organization criteria: 50% (n=21) had anemia in Group 1, and 65.9% (n=29) had anemia in Group 2 (p=0.135) (Table 6).

The parameter values of iron metabolism (iron concentration, ferritin, hepcidin) in the serum of patients were determined. Statistically significant differences were found for hepcidin level and iron concentration. Median hepcidin was 1.4 times higher in Group 1 (p <0.001), and median iron concentration was 1.5 times lower in Group 1 (p<0.001) (Table 5). In addition, there was no relationship between the severity of anemia and hepcidin content in patients with HIV and dissemination syndrome.

We performed multivariate logistic regression analysis with subsequent logit transformation to estimate the probability of tuberculosis and selected an equation, which included five predictors with the highest levels of statistical significance. These were iron concentration, hepcidin level, RBC count, WBC count, CD4+ T-cell count (Table 7).

The logistic regression equation is:

\[ Y = a + b_1 x_1 + b_2 x_2 + \cdots + b_n x_n \]  

(1)

After logit transformation:

\[ P = 1 / (1 + e^{-Y}) \]  

(2)

where: Y is a dependent binary variable, a is a constant (represented by an intercept of the estimate line), b1-n denotes regression coefficients, x1-n denotes predictors (regressors), e is an exponent (e=2.7182=const), P designates the probability.

Hence, the obtained model is as follows:

\[ Y = -5.0016 + 0.0000081 * X_1 - 0.1993 * X_2 - 0.3874 * X_3 + 1.80963 * X_4 + 0.01844 * X_5 \]  

(3)

\[ P = 1 / (1 + 2.7182 ^ {(-5.0016 + 0.0000081 * X_1 - 0.1993 * X_2 - 0.3874 * X_3 + 1.80963 * X_4 + 0.01844 * X_5)}) \]  

(4)

where: P is the probability of tuberculosis; X1 is hepcidin level; X2 is iron concentration; X3 is WBC count; X4 is RBC count; X5 is CD4+ T-cell count.
Table 5. Laboratory test results

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Group 1 (cases), n=42</th>
<th>Group 2 (control subjects), n=44</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>RBC count, x1012 cells</td>
<td>4.2±0.67</td>
<td>3.44±0.64</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Hemoglobin level, g/L</td>
<td>122.26±17.6</td>
<td>105.73±18.4</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>WBC count, x109 cells</td>
<td>5.5 (4.3-7.0)</td>
<td>6.8 (4.65-9.1)</td>
<td>0.039</td>
</tr>
<tr>
<td>Platelet count, x109 cells</td>
<td>182 (123-265)</td>
<td>221.5 (180-242)</td>
<td>0.093</td>
</tr>
<tr>
<td>ESR, mm/hr</td>
<td>31.3±13.4</td>
<td>31.7±13.6</td>
<td>0.895</td>
</tr>
<tr>
<td>Hepcidin level, pg/mL</td>
<td>14,670 (12,065; 62,010)</td>
<td>10,442 (7,505; 14,175)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Ferritin level, μg/L</td>
<td>248.55 (150.3; 746)</td>
<td>440.15 (175.7; 763)</td>
<td>0.383</td>
</tr>
<tr>
<td>Iron concentration, μmol/L</td>
<td>7.25 (5.2; 9.3)</td>
<td>10.9 (7.4; 17.1)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Table 6. Anemia severity

<table>
<thead>
<tr>
<th>Anemia severity</th>
<th>Group 1 (cases), n=42</th>
<th>Group 2 (control subjects), n=44</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>21 (50)</td>
<td>15 (34.1)</td>
<td>0.135</td>
</tr>
<tr>
<td>Mild</td>
<td>20 (47.6)</td>
<td>17 (38.6)</td>
<td>0.4</td>
</tr>
<tr>
<td>Moderate</td>
<td>1 (2.4)</td>
<td>11 (25)</td>
<td>0.003</td>
</tr>
<tr>
<td>Severe</td>
<td>0 (0)</td>
<td>1 (2.3)</td>
<td>0.326</td>
</tr>
</tbody>
</table>

Table 7. Predictors of tuberculosis

<table>
<thead>
<tr>
<th>Variable</th>
<th>Coefficient</th>
<th>Standard error</th>
<th>Wald</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hepcidin level</td>
<td>0.0000081</td>
<td>0.0000039</td>
<td>4.34</td>
<td>0.03</td>
</tr>
<tr>
<td>Iron concentration</td>
<td>-0.1993</td>
<td>0.0828</td>
<td>5.79</td>
<td>0.016</td>
</tr>
<tr>
<td>WBC count</td>
<td>-0.3874</td>
<td>0.187</td>
<td>4.29</td>
<td>0.038</td>
</tr>
<tr>
<td>RBC count</td>
<td>1.80963</td>
<td>0.7527</td>
<td>5.78</td>
<td>0.016</td>
</tr>
<tr>
<td>CD4+ T-cell count</td>
<td>0.01844</td>
<td>0.00513</td>
<td>12.9</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Constant</td>
<td>-5.0016</td>
<td></td>
<td></td>
<td></td>
</tr>
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</table>

Discussion

There are difficulties in the differential diagnosis of pneumonia vs. tuberculosis when examining patients with HIV infection and pulmonary dissemination syndrome. It is crucial to consider an absence of specific symptoms, along with taking into account the results of physical examinations; furthermore, it is imperative to suspect tuberculosis in such patients.

Anemia often complicates tuberculosis. We discovered that there was no relationship between hepcidin as the main regulator of iron metabolism and the severity of anemia in disseminated tuberculosis. Some studies have also examined the relationship between hepcidin and the severity of anemia in HIV and tuberculosis [13, 28], but their findings still remain controversial.

There is evidence of a high hepcidin level in patients with disseminated tuberculosis [13]. It is recognized that an increase in hepcidin content occurs in pneumonia as well [29, 30]. However, we did not come across studies, in which hepcidin was investigated as a potential marker for the differential diagnosis of pneumonia vs. tuberculosis. In our research, we planned to examine hepcidin and other indicators of iron metabolism in a comparative aspect in disseminated tuberculosis vs. pneumonia.

We studied two groups of patients with HIV and dissemination on radiographs (cases: patients with tuberculosis; controls: patients with pneumonia). We established differences in the parameters of iron metabolism. Hepcidin and hemoglobin levels were statistically significantly higher (p<0.001), while iron concentration was lower (p=0.0002), in tuberculosis. Also, there were statistically significant differences between groups in terms of CD4+ T-cell count, viral load, RBC and WBC counts, setting the results of complete blood count tests. Other significant differences included duration of complaints prior to hospital admission, and job stability.

Also, we identified quantitative parameters with the highest statistical significance (iron concentration, hepcidin level, RBC count, WBC count, CD4+ T-cell count) and developed a formula for calculating the probability of tuberculosis. This mathematical model could be used as a supplementary technique for assessing the probability of tuberculosis in patients with HIV infection and pulmonary dissemination in case of negative results, yielded by bacterioscopic and immunological diagnostic tests, with persisting suspicion of tuberculosis, based on clinical evidence.

Study limitations

This study has a number of limitations. First, it is a cross-sectional case-control study. Patients were preassigned to either a group of cases or a group of controls. In future studies, it would be necessary to conduct a cohort study on iron metabolism parameters and observe patients during the whole period from detection of disseminated lung disease to verification of the
diagnosis. Second, this was a single-center study with a small number of observations. It did not take into account climatic and geographical features. Consequently, the obtained formula for calculating the probability of tuberculosis is solely a mathematical model, thereby requiring further studies.

**Conclusion**

It is necessary, first of all, to suspect tuberculosis, when examining a patient with HIV infection and pulmonary dissemination syndrome. Differential diagnosis of tuberculosis in such patients is difficult, because there are no specific complaints, symptoms, examination results or radiological data. An iron-regulating peptide hormone hepcidin is of great practical importance for further study as a potential marker for the diagnosis of tuberculosis.

**Conflict of Interest**

The authors declare no conflicts of interest.

**References**


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