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Original article

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.

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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 had pneumonia (controls; 44 patients). All patients were counseled by a psychiatrist. Standard tuberculosis diagnostic procedure was carried out. During the first two days, a skin test with tuberculosis recombinant allergen (Diaskintest) was performed. 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 ± σ. 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.

Table 1. Diagnosis of tuberculosis

Methods	*Group 1 (cases), n=42*		*Group 2 (control subjects), n=44*		*p*
	Coverage, % (number of patients)	Positive result, % (number of patients)	Coverage, % (number of patients)	Positive result, % (number of patients)	
Skin test with tuberculosis recombinant allergen (Diaskintest)	100 (42)	14.3 (6)	100 (44)	0	0.01
Ziehl-Neelsen sputum smear microscopy	100 (42)	9.5 (4)	100 (44)	0	0.037
Ziehl-Neelsen microscopy of bronchoalveolar lavage	90.5 (38)	15.5 (6)	95.5 (42)	0	0.008
GeneXpert MTB/RIF assay	85.7 (36)	100 (36)	100 (44)	0	<0.001
BACTEC MGIT test	85.7 (36)	100 (36)	100 (44)	0	<0.001

Table 2. Adverse social factors

Socially significant factors	*Group 1 (cases), n=42*		*Group 2 (control subjects), n=44*		*p*
	n	%	n	%	
Unemployed status	34	81	22	54.2	0.047
Absence of a family	26	61.9	30	68.2	0.7
Alcohol abuse	7	16.7	10	23.8	1.0
Smoking	24	57.1	30	68.2	1.0
Drug use (intravenous)	19	45.2	26	59.1	0.343

Table 3. CD4+ T-cell distribution

CD4+ T-cell count, cells/uL	*Group 1 (cases), n=42*		*Group 2 (control subjects), n=44*		*p*
	n	%	n	%	
<50	0	0	30	68.18	<0.001
51-100	7	16.67	9	20.45	0.652
101-200	18	42.86	2	4.54	<0.001
201-300	11	26.19	2	4.54	0.006
301-400	4	9.52	0	0	0.037
401-500	2	4.76	1	2.27	0.53
>500	0	0	0	0	1.0
Median	175.5 (127; 265)		34.5 (13; 57)		<0.001

Table 4. Patient complaints

Complaints	*Group 1 (cases), n=42*		*Group 2 (control subjects), n=44*		*p*
	n	%	n	%	
Dyspnea	15	35.7	38	90.5	0.122
Weakness	33	78.6	40	95.2	0.387
Cough	28	66.7	37	88.1	0.15
Sputum production	18	42.9	35	83.3	0.438
Fever	36	85.7	41	97.6	1.0

Clinical and social characteristics of the patients were analyzed. We revealed that patients in both groups were affected by negative social factors equally often (*Table* 2).

The duration of the HIV history from the diagnosis of HIV to current hospitalization was determined. Median duration constituted 7.5 (4; 12) yrs. in Group 1. The shortest duration was approximately 1 yr., while the longest was nearly 19 yrs. Median duration in Group 2 was 8.5 (7.5; 11) yrs. However, 9% of patients (n=4) in this group first learned about their HIV status during current hospitalization. The longest duration of the HIV history from the diagnosis to current hospitalization was 14 yrs. No intergroup difference in the duration of HIV anamnesis was detected (p=0.603). Patients received antiretroviral therapy: 16.7% (n=7) in Group 1 and 31.8% (n=14) in Group 2. Median viral load was lower in Group 1: 8,241 (687; 59,786) copies of HIV RNA/mL vs. 446,330 (58,259.5; 1,061,576.0) copies of HIV RNA/mL in Group 2 (p<0.001). Median CD4+ T-cell count was much lower in Group 2 (p<0.0001), as shown in *Table* 3.

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:

$$Y=a+b_1x_1+b_2x_2+\ldots+b_nx_n \quad (1)$$

After logit transformation:

$$P=1/(1+e^{-y}) \quad (2)$$

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:

$$Y=-5.0016 + 0.0000081*X_1-0.1993*X_2-0.3874*X_3 + 1.80963*X_4 + 0.01844*X_5, \quad (3)$$

$$P=1 / (1 + 2.7182^{(5.0016-0.0000081*X1 + 0.1993*X2 + 0.3874*X3 - 1.80963*X4 - 0.01844*X5)}), \quad (4)$$

where: *P* is the probability of tuberculosis; X_1 is hepcidin level; X_2 is iron concentration; X_3 is WBC count; X_4 is RBC count; X_5 is CD4+ T-cell count.

Table 5. Laboratory test results

Parameters	*Group 1 (cases), n=42*	*Group 2 (control subjects), n=44*	*p*
RBC count, x1012 cells	4.2±0,67	3.44±0.64	<0.001
Hemoglobin level, g/L	122.26±17,6	105.73±18.4	<0.001
WBC count, x109 cells	5.5 (4.3; 7,0)	6.8 (4.65; 9.1)	0.039
Platelet count, x109 cells	182 (123; 265)	221.5 (180; 242)	0.093
ESR, mm/hr	31.3±13.4	31.7±13.6	0.895
Hepcidin level, pg/mL	14,670 (12,065; 62,010)	10,442 (7,505; 14,175)	<0.001
Ferritin level, µg/L	248.55 (150.3; 746)	440.15 (175.7; 763)	0.383
Iron concentration, µmol/L	7.25 (5.2; 9,3)	10.9 (7.4; 17.1)	<0.001

Table 6. Anemia severity

Anemia severity	*Group 1 (cases), n=42*		*Group 2 (control subjects), n=44*		*p*
	n	%	n	%	
None	21	50	15	34.1	0.135
Mild	20	47.6	17	38.6	0.4
Moderate	1	2.4	11	25	0.003
Severe	0	0	1	2.3	0.326

Table 7. Predictors of tuberculosis

Variable	*Coefficient*	*Standard error*	*Wald*	*P*
Hepcidin level	0.0000081	0.0000039	4.34	0.03
Iron concentration	-0.1993	0.0828	5.79	0.016
WBC count	-0.3874	0.187	4.29	0.038
RBC count	1.80963	0.7527	5.78	0.016
CD4+ T-cell count	0.01844	0.00513	12.9	<0.001
Constant	-5.0016			

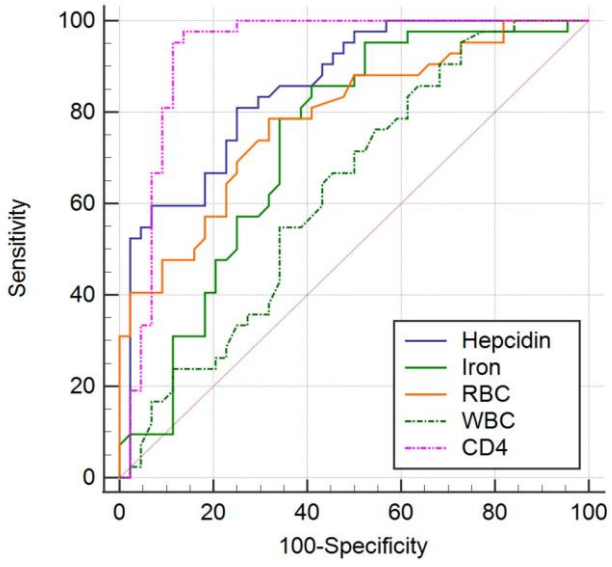

Figure 1. ROC curves of iron concentration, hepcidin level, RBC count, WBC count, CD4+ T-cell count. The sensitivity of the method is 92.86% and the specificity is 90.91%. The percentage of correctly classified cases is 91.86%. The area under the curves is 0.958 in this model.

There is a high probability of tuberculosis if P>0.5, and tuberculosis is unlikely if P≤0.5.

The coefficient of determination, R^2=0.7736, implies high goodness of fit of the regression model. The method of ROC analysis was employed to determine the numerical value of the reliability of the difference between the information content of used methods. For this purpose, the area under the curve (AUC) was calculated; AUC=0.958 in our model. This corresponds to high information content (*Figure* 1).

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 sensu 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.

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