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Human-Beta-Defensin-1, Ferritin, Interleukin-6 and their Relationship with Clinical and Laboratory Parameters of the Severity of the Tuberculosis Process

Establishing relationships between clinical and laboratory parameters, such as general patient's state, parameters of complete blood count and blood biochemistry and markers of the course of tuberculosis in the future can be used to predict the severity of dysfunction of various organs and tissues in patients with tuberculosis and in particular in patients who receive anti-tuberculosis treatment.

Objective — to investigate the relationship between biochemical markers, namely Human-beta-defensin-1, ferritin and interleukin-6, and clinical and laboratory indicators of the severity of the tuberculosis process.

Materials and methods. 100 patients diagnosed with pulmonary tuberculosis were included in the study. After receiving 60 doses of anti-tuberculosis treatment, the patients were retrospectively divided into 2 groups. Group 1 (n = 77) consisted of patients in whom sputum conversion was observed after 60 doses of treatment, determined by sputum microscopy. Group 2 (n = 23) comprised patients in whom bacterial secretion was maintained after 60 doses of treatment, as detected by microscopy. In addition to the routine studies provided for the monitoring of patients with tuberculosis by the current orders of the Ministry of Health of Ukraine, the levels of Human-beta-defensin-1, ferritin and interleukin-6 (IL-6) in the fasting blood were additionally measured by ELISA at the beginning of treatment and after 60 days. Statistical data processing was carried out using the Statistica 8.0 software environment.

Results. A comparison of the investigated parameters between groups at the beginning of treatment showed significantly higher values of Human-beta-defensin-1 (Group 1 — (18.97 ± 2.42) pg/ml, Group 2 — (55.02 ± ± 15.69) pg/ml), ferritin (Group 1 — (94.86 ± 6.02) ng/ml, Group 2 — (141.61 ± 24.66) ng/ml) and IL-6 (Group 1 — (80.33 ± 5.03) pg/ml, Group 2 — (110.13 ± 10.35) pg/ml) in patients with positive sputum microscopy after 60 doses of treatment, $p < 0.05$. All studied markers demonstrated a reliable positive relationship with the massiveness of bacterial excretion, a conditional indicator of the severity of clinical symptoms and signs, ESR and urea level, as well as reliable negative correlations with creatinine level. In addition, patients with a lower body mass index were found to have higher levels of Human-beta-defensin-1 and ferritin. Higher levels of Human-beta-defensin-1 and ferritin are associated with lower hemoglobin levels (Human-beta-defensin-1 was also negatively correlated with erythrocyte count). An increase in the leukocytes level is accompanied by a significant increase in the level of Human-beta-defensin-1 and IL-6. Additionally, a significant negative correlation was found between the level of glucose and ferritin, as well as between the level of bilirubin and ferritin and IL-6.

Conclusions. The determined significantly higher levels of Human-beta-defensin-1, ferritin and interleukin-6 in patients in whom sputum microscopy was positive after 60 doses of treatment allow considering the investigated biochemical parameters as markers of the ineffectiveness of anti-tuberculosis therapy. The identified positive relationships with the severity of clinical symptoms indicate the possibility of using the studied parameters as markers of the severity of the tuberculosis course. The possibility of using Human-beta-defensin-1 and ferritin as markers of anemia was also found. Correlations with parameters of blood biochemistry allow us to talk about increased levels of Human-beta-defensin-1, ferritin and interleukin-6 against the background of kidney damage.

Keywords

Tuberculosis, Human-beta-defensin-1, ferritin, interleukin-6, prognostic markers.

Tuberculosis is a serious chronic infectious disease that leads not only to the destruction of pulmonary tissue, but also to the damage of other organs and systems and violation of their functioning. Later this becomes the cause of invalidation of patients, decrease of life quality, adherence to anti-tuberculosis treatment and treatment effectiveness.

One of the most common disorders in tuberculosis patients is anemia. Release of interleukin-6 (IL-6) in tuberculosis patients' stimulates the synthesis of hepcidin, which is a negative regulator of iron processing and absorption, including due to its binding to ferritin. As a result, the availability of iron for erythropoiesis decreases, which is manifested by an increase in the level of ferritin and a decrease in the level of iron [5].

As for the leukocyte branch of hematopoiesis, patients with tuberculosis may have various disorders: leukopenia, neutropenia, lymphocytopenia, monocytopenia, leukocytosis, neutrophilia, lymphocytosis, monocytosis, and pancytopenia (in patients with miliary tuberculosis) [6] .

Also, tuberculosis is associated with thrombocytosis, which in turn is associated with an increase in the level of IL-6, which is responsible for the state of hypercoagulation. The degree of thrombocytosis correlates with common markers of inflammation, such as C-reactive protein and ESR [13].

Anti-tuberculosis treatment also has a significant impact on the patients state. Metabolism of *Isoniazid* by the liver due to N-acetyltransferase-2 and microsomal cytochrome P4502E1 enzyme, potentiation of hepatotoxicity of other drugs by rifampicin due to the hepatocellular pattern of DILI, inhibition of CYP45058 by pyrazinamide and a number of other adverse reactions of antituberculosis drugs lead to hepatotoxicity and corresponding changes in biochemical blood analysis [8, 12, 15].

Some anti-tuberculosis drugs, such as *Rifampicin*, have a nephrotoxic effect, in particular, due to a type II or III hypersensitivity reaction with antibodies against *Rifampicin* form immune complexes that are deposited in the renal vessels, glomerular endothelium, and interstitium [11]. This leads to the narrowing of blood vessels and tubular ischemia, acute tubular necrosis and acute interstitial nephritis [3].

So, the question arises: is there a relationship between clinical and laboratory parameters, such as general patient's, parameters of complete blood count and blood biochemistry, and markers of the tuberculosis course, in particular, ferritin, Human-beta-defensin-1 and IL-6 which were studied in our work? Establishing such relationships in the future can be used to predict the severity of dysfunction of various organs and tissues in patients with tuberculosis, and in particular in patients receiving anti-tuberculosis treatment.

Objective — to investigate the relationship between biochemical markers, namely Human-beta-defensin-1, ferritin and interleukin-6, and clinical and laboratory parameters of the severity of the tuberculosis process.

Materials and methods

100 patients with pulmonary tuberculosis were included in the study. After receiving 60 doses of anti-tuberculosis treatment, the patients were retrospectively divided into 2 groups: Group 1 (n = 77) comprised patients in whom sputum conversion was observed (determined by sputum microscopy) after 60 doses of treatment, while Group 2 (n = 23) comprised patients without sputum conversion (determined by microscopy) after the same duration of treatment.

In addition to the routine studies provided for the monitoring of tuberculosis patients by the current orders of the Ministry of Health of Ukraine, the levels of Human-beta-defensin-1, ferritin and IL-6 in fasting blood by the ELISA method were determined at the beginning of treatment and after 60 days. For the numerical expression of clinical symptoms and signs, a conditional score was used, in which each of the main symptoms of tuberculosis (cough, shortness of breath, chest pain, weight loss, weakness) was evaluated as 1 point.

Statistical data processing was carried out using the Statistica 8.0 software environment using descriptive statistics (mean, standard error, median) and non-parametric statistics (Spearman's correlation coefficient, Mann—Whitney coefficient); the obtained results were considered statistically significant at $p < 0.05$.

Results

A comparison of the investigated indicators between the groups at the beginning of treatment (Fig. 1) showed a significantly higher level of Human-beta-defensin-1 (Group 1 — (18.97 ± 2.42) pg/ml (median — 6.81 pg/ml), Group 2 — (55.02 ± ± 15.69) pg/ml (median — 57.85 pg/ml)), ferritin (Group 1 — (94.86 ± 6.02) ng/ml (median — 78.41 ng/ml), Group 2 — (141.61 ± 24.66) ng/ml (median — 135.81 ng/ml)) and IL-6 (Group 1 — (80.33 ± 5.03) pg/ml (median — 67.93 pg/ml), Group 2 — (110.13 ± 10.35) pg/ml (median — 105.83 pg/ml)) in patients with positive sputum microscopy after 60 doses of treatment ($p < 0.05$).

A comparison of the studied parameters between the groups after 60 doses (Fig. 2) showed significantly higher levels of Human-beta-defensin-1 (Group 1 — (15.44 ± 2.24) pg/ml (median — 6.76 pg/ml), Group 2 — (71.25 ± 12.53) pg/ml (median — 54.90 pg/ml)), ferritin (Group 1 —

(60.39 ± 6.60) ng/ml (median — 33.15 ng/ml), Group 2 — (135.63 ± 21.61) ng/ml (median — 115.55 ng/ml)) and IL-6 (Group 1 — (38.04 ± 2.88) pg/ml (median — 24.18 pg/ml), Group 2 — (99.87 ± 15.86) pg/ml (median — 105.80 pg/ml)) in patients with positive sputum microscopy after 60 doses of treatment ($p < 0.05$).

When studying correlations between Human-beta-defensin-1, ferritin, IL-6 and clinical and laboratory parameters of the severity of the tuberculosis process, all studied markers demonstrated a reliable positive relationship with the massiveness of bacterial excretion, a conditional indicator of the severity of clinical symptoms, ESR and urea level, as well as reliable negative correlations with creatinine level. In addition, patients with a lower body mass index were found to have higher levels of Human-beta-defensin-1 and ferritin. Higher levels of Human-beta-defensin-1 and ferritin are associated with lower hemoglobin levels (Human-beta-defensin-1 was also

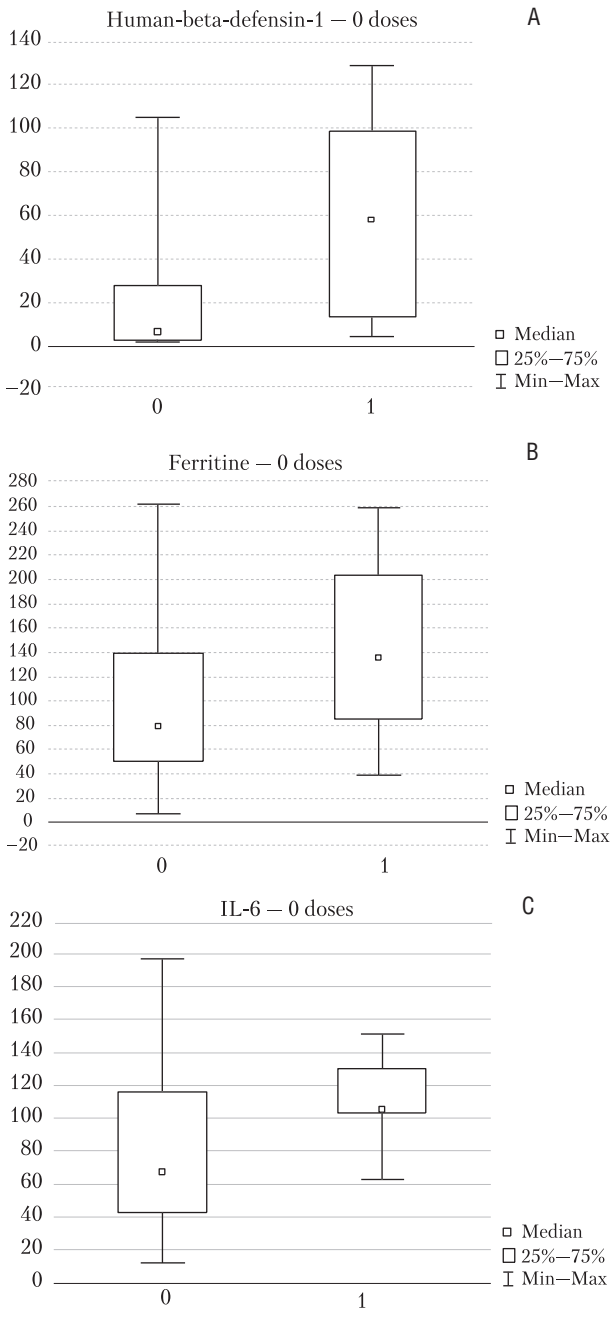

Fig. 1. **Comparison of the levels of Human-beta-defensin-1 (A), ferritin (B) and IL-6 (C) at the beginning of treatment between patients in whom sputum microscopy was negative after 60 doses of treatment (0) and patients in whom sputum microscopy was positive after 60 doses treatment (1)**

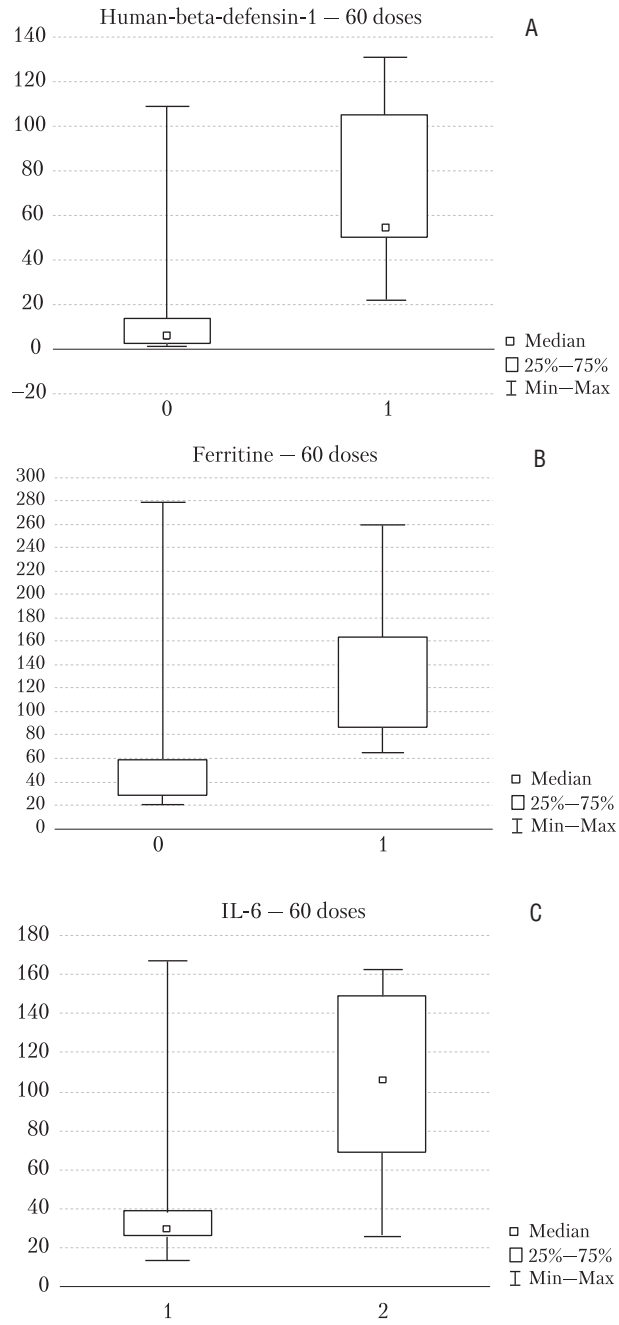

Fig. 2. **Comparison of the levels of Human-beta-defensin-1 (A), ferritin (B) and IL-6 (C) after 60 doses between patients in whom sputum microscopy was negative after 60 doses of treatment (0) and patients in whom sputum microscopy was positive after 60 doses of treatment (1)**

Table. **Correlations between clinical and laboratory parameters of the severity of the tuberculosis process and the investigated biochemical markers**

	Human-beta-defensin-1	Ferritin	IL-6
Body mass index (BMI)	–0.32	–0.26	—
Conventional indicator of severity of clinical symptoms	+0.52	+0.29	+0.26
The massiveness of bacterial excretion, determined by sputum microscopy	+0.48	+0.43	+0.44
The massiveness of bacterial excretion determined by sputum culture on Lowenstein—Jensen medium	+0.33	+0.30	—
Hemoglobin	–0.26	–0.24	—
Erythrocytes	–0.27	—	—
Leukocytes	+0.29	—	+0.35
ESR	+0.53	+0.37	+0.23
Creatinine	–0.36	–0.33	–0.28
Bilirubin	–0.40	–0.22	–0.25

Note. All the given parameters have reliable correlations ($p < 0.05$); «–» the absence of a reliable correlation ($p > 0.05$).

negatively correlated with erythrocytes level). An increase in leukocytes level is accompanied by a significant increase in the levels of Human-beta-defensin-1 and IL-6. In addition, a significant negative correlation was found between the level of glucose and ferritin, as well as between the level of bilirubin and ferritin and IL-6. The obtained data are presented in the Table.

Discussion

In previous works, we considered the possibility of using ferritin, Human-beta-defensin-1, and IL-6 as markers of the tuberculosis treatment effectiveness, but their relationship with clinical and radiological parameters of the severity of the tuberculosis process was not sufficiently studied [1, 16].

In the course of the study, significantly higher levels of Human-beta-defensin-1, ferritin, and IL-6 were found in patients who, after 2 months of anti-tuberculosis treatment, maintained bacterial excretion, which allows us to consider the investigated biochemical indicators as markers of the effectiveness of tuberculosis treatment.

The revealed inverse relationship between BMI, Human-beta-defensin-1 and ferritin is probably a consequence of the fact that with a massive tuberculosis lesion, which leads to exhaustion of the patient, the severity of the immune response increases, which is manifested by an increase in the studied inflammatory markers. Interestingly, no significant relationship between BMI and IL-6 was found. This can be explained by the fact that the production of IL-6 is one of the first to increase when immune cells are in contact with *M. tuberculosis,* and IL-6 remains a key inducer of the immune response regardless of the volume of the tuberculosis lesion and the general patient's state [9].

In all three investigated markers, reliable positive correlations were observed with the severity of clinical symptoms and the massiveness of bacterial excretion, which allows them to be considered as markers of the tuberculosis course severity. At the same time, the highest correlation strength was observed in Human-beta-defensin-1, which makes it the most sensitive of the studied markers.

Ferritin plays an important role in the anti-tuberculosis immune response not only by acting as a pro-inflammatory cytokine [10], but also by reducing the availability of iron to *M. tuberculosis*, as iron is an important cofactor of metabolic enzymes of mycobacteria [1]. In fact, in tuberculosis, there is a transition from available, transferrin-bound iron to accumulated iron, that is, ferritin [14]. However, reducing the availability of iron for the pathogen, ferritin also reduces its availability for the host's body, which is reflected in the negative relationship between the level of ferritin and hemoglobin that we found. In turn, iron deficiency leads to a violation of the production of antimicrobial peptides, including the family of beta-defensins, and reduces their bactericidal activity [11]. The negative correlations we found between the level of Human-beta-defensin-1 and hemoglobin and erythrocytes confirm this fact.

A positive relationship between the levels of Human-beta-defensin-1, IL-6 and leukocytes was expected, since the latter are the main producers of these cytokines [12, 13].

The revealed negative relationship between the levels of Human-beta-defensin-1, ferritin and IL-6 on the one hand and the levels of bilirubin and creatinine on the other indicates a malfunction of the liver and kidneys against the background of active tuberculosis inflammation and taking anti-tuberculosis drugs.

Conclusions

The determined significantly higher levels of Human-beta-defensin-1, ferritin and interleukin-6 in patients in whom sputum microscopy was positive after 60 doses of treatment allow considering the investigated biochemical parameters as markers of the ineffectiveness of anti-tuberculosis therapy. The identified positive relationships with the severity of clinical symptoms indicate the possibility of using the studied parameters as markers of the severity of the tuberculosis course. The possibility of using Human-beta-defensin-1 and ferritin as markers of anemia was also found. Correlations with parameters of blood biochemistry allow us to talk about increased levels of Human-beta-defensin-1, ferritin and interleukin-6 against the background of kidney damage.

No conflict of interest.
Participation of the authors: concept and design of the study — O.S. Shevchenko, L.D. Todoriko; collection of material — I.A. Ovcharenko; processing of material — O.M. Shvets, R.S. Shevchenko; writing the text — O.O. Pohorielova; text editing — S.L. Matvyeyeva, E. Tudor.

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β-дефензин-1, феритин, інтерлейкін-6 та їхній зв'язок із клінічними та лабораторними показниками тяжкості туберкульозного процесу

Установлення зв'язків між клініко-лабораторними параметрами (загальний стан пацієнта, показники клінічного і біохімічного аналізів крові) та маркерами перебігу туберкульозу в перспективі може бути використане для прогнозування тяжкості порушення функціонування різних органів і тканин у хворих на туберкульоз, зокрема в пацієнтів, які отримують протитуберкульозне лікування.

Мета роботи — дослідити зв'язки між біохімічними маркерами (β-дефензин-1, феритин, інтерлейкін-6 (ІЛ-6)) і клінічними та лабораторними показниками тяжкості туберкульозного процесу.

Матеріали та методи. У дослідження було залучено 100 хворих на туберкульоз легень. Після отримання 60 доз протитуберкульозного лікування пацієнтів ретроспективно розподілили на дві групи: 77 пацієнтів, у яких зареєстрували припинення бактеріовиділення (за даними мікроскопії мокротиння), і 23 пацієнти, у яких зберігалося бактеріовиділення (за даними мікроскопії мокротиння). Крім рутинних досліджень, передбачених для моніторингу пацієнтів із туберкульозом наказами МОЗ України щодо ведення хворих на туберкульоз, вимірювали рівень β-дефензину-1, феритину та ІЛ-6 у крові натще методом імуноферментного аналізу на початку лікування та через 60 днів. Статистичну обробку даних проведено за допомогою програмного середовища Statistica 8.0.

Результати та обговорення. Порівняння досліджуваних показників у групах на початку лікування виявило статистично значущо ($p < 0,05$) вищий рівень у групі 2 β-дефензину-1 ((18,97 ± 2,42) та (55,02 ± 15,69) пг/мл), феритину ((94,86 ± 6,02) і (141,61 ± 24,66) нг/мл) та ІЛ-6 ((80,33 ± 5,03) і (110,13 ± 10,35) пг/мл). Усі досліджувані маркери мали статистично значущий прямо пропорційний зв'язок з масивністю бактеріовиділення, умовним показником виразності клінічних симптомів, ШОЕ та рівнем сечовини, а також статистично значущий обернено пропорційний зв'язок із рівнем креатиніну. У пацієнтів з меншим індексом маси тіла зареєстровано вищі показники β-дефензину-1 і феритину. Більша концентрація β-дефензину-1 та феритину пов'язана з нижчим рівнем гемоглобіну (для β-дефензину-1 також установлено обернено пропорційний зв'язок із кількістю еритроцитів). Збільшення кількості лейкоцитів супроводжувалося статистично значущим підвищенням рівня β-дефензину-1 та ІЛ-6. Установлено статистично значущий обернено пропорційний зв'язок між вмістом глюкози та феритину, а також між рівнем білірубіну і феритину та ІЛ-6.

Висновки. Статистично значущо вищі рівні β-дефензину-1, феритину та ІЛ-6 у пацієнтів, у яких через 60 доз лікування зберігалося бактеріовиділення, дають підставу розглядати досліджувані біохімічні показники як маркери неефективності протитуберкульозної терапії. Установлені прямо пропорційні зв'язки з тяжкістю клінічної симптоматики свідчать про можливість застосування досліджуваних параметрів як маркерів тяжкості перебігу туберкульозу. Виявлено можливість використання β-дефензину-1 та феритину як маркерів анемії. Наявність кореляції з показниками біохімічного аналізу крові свідчить про підвищення рівня β-дефензину-1, феритину та ІЛ-6 на тлі ураження нирок.

Ключові слова: туберкульоз, β-дефензин-1, феритин, інтерлейкін-6, прогностичні маркери.

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