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**Ovcharenko I.A.,**
*PhD-student, Kharkiv National Medical University, Department of Phthisiology and Pulmonology, Kharkiv, Ukraine, IDORCIDorcid.org/0000-0001-6953-9029*
**Shevchenko O.S.,**
*MD, Professor, Kharkiv, Kharkiv National Medical University, Head of the Department of Phthisiology and Pulmonology, Ukraine, IDORCIDorcid.org/0000-0002-5476-3981*
**Todoriko L.D.,**
*MD, Head of the Department of Phthisiology and Pulmonology, MD, Professor, Higher State Educational Establishment „Bukovinian State Medical University“,Chernivtsi, Ukraine, ORCID 0000-0001-9042-0073; Researcher ID: B-9387-2017, pulmonology@bsmu.edu.ua*
**Semianiv I.O.,**
*PhD, assistant of the Department of Phthisiology and Pulmonology, Higher State Educational Establishment „Bukovinian State Medical University“,Chernivtsi, Ukraine, ORCID 0000-0003-0340-0766; Researcher ID: B-9386-2017*
**Vivsyannuk V.V.**
*PhD, assistant of the Department of Internal Medicine, Higher State Educational Establishment „Bukovinian State Medical University“, Chernivtsi, Ukraine, ORCID 0000-0003-0340-0436; Researcher ID: B-9325-2016*

# DYNAMICS OF PULMONARY TISSUE DESTRUCTION MARKERS IN PATIENTS WITH NEW CASES OF TUBERCULOSIS WITH DIFFERENT SUSCEPTIBILITY OF THE PATHOGEN DURING TREATMENT

***Abstract.*** *The study was designed to investigate the difference in the dynamics of levels of pulmonary tissue destruction markers in patients with new tuberculosis cases with different sensitivity of the pathogen on the background of treatment. Materials and methods. 124 patients with pulmonary tuberculosis (TB) were divided into 2 groups: group 1 (n = 84) - patients with MDR-TB; Group 2 (n = 40) - patients with pulmonary tuberculosis susceptible to antimicrobial treatment. The levels of free hydroxyproline (FH), protein-bound hydroxyproline (PBH), matrix metalloproteinase-9 (MMP-9), tissue inhibitor of matrix metalloproteinase-1 (TIMP-1), and aldosterone (A) were investigated at the beginning of treatment and after 2 and 3 months from the start of treatment. Results. Under the influence of 1st line drugs, in the 3rd months of treatment, there was a decrease in the activity of the macrophage system on the background of sputum conversion and lower levels of MMP-9, PBH and A, indicating the inhibition of degradation processes on the background of low fibrotic activity. Under the influence of therapy with second-line drugs for 3 months, the activity of fibrosis formation was higher in Group 1 than in Group 2. It was accompanied by a decrease in the level of PBH and a decrease in the level of FH, indicating inhibition of destructive changes. The slowed conversion of sputum smear in Group 1 on the background of therapy with second-line drugs was accompanied by a deceleration in the decrease of MMP-9 / TIMP-1 ratio. Moreover, the reduction of MMP-9 / TIMP-1 ratio for 2 months of treatment is associated with the growth of TIMP-1, and for 3 months it is associated with further growth of the MMP-9 level, that is, the activity of the destruction processes remains significantly higher. The less pronounced dynamics of decrease in A level and growth of TIMP-1 promotes more active fibrosis formation in Group 2. That is, on the background of treatment with the 1st line drugs, there was a decrease in the activity of the processes of fibrosis, which reduces the amount of residual changes. Conclusions. On the background of treatment with the first-line drugs, patients showed less activity of destruction and less subsequent fibrotic changes, compared with patients treated with second-line drugs.*

***Key words:*** *multi-drug-resistant tuberculosis, aldosterone, protein-bound hydroxyproline, free hydroxyproline.*

**Introduction.** To overcome the epidemic of Multi-drug-resistant tuberculosis (MDR-TB) in the world, World Health Organization (WHO) recommends to achieve 75% level of treatment

effectiveness [1].

Treatment effectiveness is controlled by sputum bacterial conversion and healing of pulmonary destructions at the end of treatment.

It is known that the formation of destruction cavities involves the destruction of the extracellular matrix, which contains collagen fibers that support the pulmonary structure. In the lungs, collagen fibers are destructed by Matrix metalloproteinase (MMP), which can affect all components of the extracellular matrix [2]. One of the products of the collagen fibers destruction in pulmonary tissue is hydroxyproline and its fractions. The process of MMP synthesis is regulated by Tissue inhibitors of metalloproteinase (TIMP), which play an important role in the processes of fibrosis [3]. However, not only the level of MMP is important, but also its ratio to TIMP, which approaches 1 in the absence of pathology [4].

Recent studies indicate the role of A in the processes of fibrosis It is able to activate blood monocytes, induce inflammation, lead to impaired fibrinolysis, strengthen and accumulate collagen. [5] Thus, the destruction of the extracellular matrix is one of the most important pathological events in the formation of residual changes in the pulmonary tissue during tuberculous inflammation [6, 7].

**Aim.** The study was designed to investigate the difference in the dynamics of levels of pulmonary tissue destruction markers in patients with new tuberculosis cases with different sensitivity of the pathogen on the background of treatment.

**Materials and methods.** 124 patients with new cases of pulmonary Tuberculosis (TB) were examined. Patients were divided into Group 1 (n = 84) - MDR-TB, and Group 2 (n = 40) - pulmonary TB with preserved sensitivity of the pathogen to anti-tuberculosis drugs.

The patients were aged from 18 to 55 years, the average age was 35.6 ± 3.1 years; men - 72.5%, women - 27.5% ($p < 0.05$).

In Group 1, all patients had destruction of the pulmonary tissue and bacterial excretion.

Patients with HIV, diabetes, Hepatitis B / C, COPD, cardiovascular disease were excluded from the study.

Clinical, biochemical, microbiological and instrumental studies were performed in all patients at the beginning of treatment, as well as after 2 and 3 months from the start of treatment. The levels of free hydroxyproline (FH), protein-bound hydroxyproline (PBH), MMP-9, TIMP-1, and aldosterone (A) were studied.

Statistical data processing was carried out using non-parametric statistics by STATISTICA application software package. To compare the values on 3 stages of the dynamic study within the combined group, as well as within the Groups 1 and 2, the Friedman nonparametric method was used, followed by pairwise comparison of groups using the Wilcoxon criterion. To compare Groups 1 and 2 at different stages of dynamic treatment, the Mann-Whitney test was used. To present the results, the median, interquartile range (Lower - lower quartile, Upper - upper quartile) and sample size (min - minimum, max - maximum value) were used. To establish functional relationships between the parameters, the Spearman correlation coefficient R was calculated, with statistical significance at $p < 0.05$.

**Results.** The dynamics of the levels of pulmonary tissue destruction factors was studied in patients with TB in the course of treatment with standard chemotherapy regimens.

According to sputum microscopy data, bacterial excretion at the beginning of treatment was observed in 85.7 ± 3.8% of cases in Group I and in 100% of Group 2; after 2 months of treatment, bacterial excretion was observed in 40.5 ± 5.4% of cases in Group 1 and in 17.5 ± 6% of cases in Group 2 ($p < 0.05$); at the 3rd month of treatment, bacterial excretion was observed in 11.9 ± 3.5% of cases in Group 1, while in group II sputum smear conversion was recorded in 100% of cases, which was accompanied by 100% positive x-ray dynamics in the form of partial resorption of infiltrations and reduction of destructions, whereas in Group 1, positive radiological dynamics was observed in 60.7% of cases only.

The difference in the dynamics of indicators of tissue destruction factors, A and collagen metabolism products in patients in Groups 1 and 2 was analyzed.

It was found that within 3 months of treatment, the level of FH in Group 1 was significantly higher than in Group 2 (by 11.3%, 6.7%, 10.2%, respectively, at the beginning of treatment, after

2 and 3 months of treatment (p <0.05 for all cases)).

The level of PBH was higher in Group 2, and it significantly increased in Group 1 by 8.9% during treatment, in contrast to the dynamics in Group 2, where we observed a significant decrease in its level by 53.2% (p <0.05).

The initial level of TIMP-1 was higher in Group I, and it increased within 3 months of treatment in both groups. The intensity of TIMP-1 level increase in Group 2 was significantly higher (by 39.2%) compared with an increase (by 27.4%) in Group 1 (p <0.05)).

The initial level of A in Group 1 was lower by 6.5% (p <0.05). During treatment, there was a decrease in its level in both groups: in Group 1 by 34.3% and in Group 2 by 59.6% (p <0.05). The intensity of the decrease in the level of aldosterone was significantly more pronounced in Group 2 (by 34.5%, (p <0.05)).

The initial levels of MMP-9 and its dynamics during treatment in the groups did not have a significant difference. There was a trend of an increase in the level of MMP-9 in both groups.

To assess the balance of the processes of destruction and repair of tissues, the ratio of MMP-9 / TIMP-1 was applied, which approaches 1 in the absence of pathology. In both study groups, this parameter was increased to 2.7 and 2.9 in groups 1 and 2 respectively, indicating an active inflammatory process with a predominance of destruction. In the course of treatment, a more intensive dynamics of ratio decrease was observed in Group 2 with a significant decrease by 25% in contrast to 16.6% in Group 1 (p <0.05). Also, a higher ratio of MMP-9 / TIMP-1 in patients from Group 1 in the third month of treatment was observed in the absence of sputum conversion in 11.9 ± 3.5% of cases.

As is known, the production of MMP-9 stimulates macrophages activated by the Mycobacterium tuberculosis (MTB). This is confirmed by the correlation links in Group 1 (between MMP-9 and monocytes (r = 0.56, p = 0.003), TIMP-1 and monocytes (r = 0.89, p = 0.00001) and FH and monocytes (r = 0.82, p = 0.00001)), and correlations in Group 2 (between MMP-9 and monocytes (r = 0.65, p = 0.001), TIMP-1 and monocytes (r = 0, 74, p = 0.00005), FH and monocytes (r = 0.92, p = 0.00001) and PBH and monocytes (r = 0.82, p = 0.00001).

An increase in the level of TIMP-1 was accompanied by an increase in the level of MMP-9 in dynamics during treatment. This is indicated by the correlation relationships between them at the 2nd month of treatment in Group 1 (r = 0.79, p = 0.00001) and at the 3rd month of treatment in Group 1 (r = 0.8, p = 0, 00001) and in Group 2 (r = 0.64, p = 0.005).

High levels of FH in Group 1 are associated with higher activity of MMP-9 in this group and are confirmed by the dynamics of these parameters and correlations in Group 1 obtained during the 2nd month of treatment (r = 0.37, p = 0.00005). In Group 2, the increase in the level of FH on the 2nd month of treatment was associated with an increase in the level of MMP-9 (r = 0.8, p = 0.00001).

The decrease in the level of PBH in Group 2 is accompanied by a decrease in the induction of collagen by aldosterone, as indicated by direct correlations that appeared at the 2nd month of treatment (r = 0.33, p = 0.043) and intensified at the 3rd month of treatment (r = 0, 55, p = 0.0001).

**Discussion.** The obtained dynamics of tissue destruction markers indicates a more favorable effect of the 1st line drugs compared with the 2nd line drugs on the reparation processes.

The decrease in the level of FH in both groups on the 3rd month of treatment is associated with the subsequent activation of TIMP-1, but in Group 2 this dynamics is more pronounced, which indicates inhibition of destructive processes.

The decrease in the level of PBH in Group 2 is accompanied by a decrease in the induction of collagen by aldosterone. In Group 1, the slower dynamics of A decrease and the increase in MMP-9 reflect the preservation of high activity of the processes of fibrosis and the formation of fibrotic changes, as indicated by an increase in the level of PBH and a slower X-ray dynamics.

Thus, under the influence of 1st line drugs, on the 3rd month of treatment, there was a decrease in the activity of the macrophage system on the background of sputum conversion and a decrease in the levels of MMP-9, PBH and A, which indicates the suppression of destruction processes on the background of low fibrotic activity.

Under the influence of treatment with second-line drugs, at the 3rd month of treatment,

fibrosing activity was higher in Group 1 than in Group 2, which was accompanied by a decrease in PBH and a decrease in FH level, which indicates inhibition of destructive changes. Slow sputum smear conversion in Group 1 during the treatment with second-line drugs was accompanied by a slower decrease in the ratio MMP-9 / TIMP-1. Moreover, the decrease of the ratio MMP-9 / TIMP-1 in the 2nd month of treatment is associated with an increase in the level of TIMP-1, and in the 3rd month it is associated with a further increase in the level of MMP-9, that is, the activity of the destruction processes remains significantly high. A less pronounced decrease in A level and growth of TIMP-1 promotes active fibrosis formation in Group 2. That is, during treatment with 1st-line drugs, there was less activity of fibrosing processes, which reduces the amount of residual changes.

Obtained data reflect an important role of free hydroxyproline, protein-bound hydroxyproline and aldosterone as well as the role of the matrix-metalloproteinase-9 and tissue inhibitor of matrix metalloproteinase-1 in regulation of fibrotic and reparation processes in pulmonary tissue. It means that these markers can predict the processes of destruction healing in pulmonary tissue.

Comparison of these parameters in patients treated with first- and second-line drugs showed less activity of destruction and less subsequent fibrotic changes in patients treated with first-line drugs, compared with patients treated with second-line drugs.

**Conclusions**

Under the influence of 1st line drugs, on the 3rd month of treatment, there was a decrease in the activity of the macrophage system on the background of sputum conversion and a decrease in the level of MMP-9, PBH and A, which indicates the suppression of destruction processes on the background of low fibrotic activity. Under the influence of treatment with second-line drugs, at the 3rd month of treatment, the activity of fibrosis was higher and was accompanied by an increase in the level of PBH and a decrease in the level of FH. Slow sputum conversion during therapy with second-line drugs was accompanied by a slowdown (8.2%) in a decrease of MMP-9 / TIMP-1 ratio due to the further increase in the level of MMP-9 and a significantly high activity of the destruction processes. That is, against the background of treatment with 1st line drugs, there was less activity of the processes of fibrosis, which reduces the amount of residual changes.

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