U.D.C.: 616.98:578.828BIJI:616.24-002]-078:57.083.3
DOI: https://doi.org/10.52692/1857-0011.2024.2-79.18

DYNAMICS OF FIBROSIS MARKERS ON THE BACKGROUND OF TB/HIV CO-INFECTION TREATMENT

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Summary.
TB/HIV co-infection remains one of the biggest medical problems in Ukraine and the world. In tuberculosis, MTB stimulates immunocompetent cells to produce MMP-9, which destroys collagen fibers. The inhibitor of MMP-9 is TIMP-1, and the product of destruction of collagen fibers is oxyproline. In HIV/TB co-infection, the immune response is altered. Therefore, we performed a study of the levels of MMP-9, TIMP-1 and oxyproline in patients with MDR-TB with HIV co-infection. The comparison group included TB patients without HIV. Our research revealed that the level of MMP-9 in patients with MDR-TB remained stable during the study. In patients with HIV/TB co-infection, there is a violation of the regulation of the balance of MMP-9 and TIMP-1, which affects the processes of fibrosis and causes a severe disease course.

Key words: tuberculosis, HIV infection, matrix metalloproteinase-9, tissue inhibitor of metalloproteinase-1, oxyproline.

Rezumat. **Dinamica markelor de fibroza pe fundalul tratamentului co-infecției TB/HIV.**
Co-infectia TB/HIV ramane una dintre cele mai mari probleme medicale din Ucraina si din lume. În tuberculoză, MBT stimulează celulele imunocompetente să producă MMP-9, care distruge fibrele de colagen. Inhibitorul MMP-9 este TIMP-1, iar produsul distrugerii fibrelor de colagen este hidroxiprolina. În co-infecția TB/HIV, răspunsul imun este modificat. Prin urmare, am efectuat un studiu al nivelurilor de MMP-9, TIMP-1 și hidroxiprolină la pacienții cu MDR-TB asociată cu co-infecție HIV. Grupul de comparație a inclus pacienți cu TB fără HIV. Cercetarea noastră a arătat că nivelul MMP-9 la pacienții cu MDR-TB a rămas stabil în timpul studiului. La pacienții cu co-infecție TB/HIV, există o încălcare a reglementării echilibrului MMP-9 și TIMP-1, care afectează procesele de fibroză și provoacă o evoluție severă a bolii.

Cuvinte cheie: Tuberculoză, infecție HIV, metaloproteinaza-9 matriceală, inhibitor tisulare al metaloproteinazei-1, hidroxiprolină.

Introduction.

Tuberculosis (TB) and infection caused by the human immunodeficiency virus (HIV) currently remain one of the main urgent medical problems in the world and in Ukraine in particular [1]. The incidence of TB increased for the past 2 years, after an encouraging multi-year downward trend [2]. The HIV epidemic is also increasing in recent years. This disappointing epidemiological situation is deepened due to the military conflict in Ukraine, which leads to the displacement of large sections of the population both within the country and outside its borders, to a significant deterioration of their social status, and in some situations, to the impossibility of receiving medical care in time. All this contributes to the deterioration of the epidemic situation in relation to each of these diseases separately, as well as in relation to HIV/TB co-infection.

It is well known that HIV affects the course of the tuberculosis process, and Mycobacterium tuberculosis (MTB), in turn, affects the immune response in HIV patients. Thus, people living with HIV (PLHIV) have a 19 times higher risk of TB disease compared to people without HIV [1, 3]. In addition, the features of the immune response in people with HIV significantly affect the features of TB infection course. This applies to a smaller number of destructive forms of TB, the presence of the pathogen in sputum and the methods by which it can be found, differences in blood tests. First of all, with HIV, the amount of CD4 decreases,

which in turn inhibits an adequate immune response to MTB and creates conditions for the rapid progression of TB [3]. However, even with a normal level of CD4, people with HIV have a several times higher risk of developing TB. [4] Adaptive immunity in TB is triggered 6 weeks after infection and is manifested by the appearance of a positive tuberculin skin test. In the presence of HIV infection, this mechanism is inhibited due to a delayed reaction of CD4 T-lymphocytes in regional lymph nodes. It is known that MTB is able to influence the function of macrophages and change them according to their needs. [5] Monocytes from bone marrow and blood migrate to sites of infection and transform into tissue macrophages and dendritic cells. [6] T-lymphocytes, macrophages, monocytes and other immune cells, as well as MTB stimulate the synthesis of gelatinase B (matrix metalloproteinase-9 (MMP-9)), which destroys collagen, the breakdown product of which is oxyproline.

The purpose of the study was to compare dynamics of oxyproline, matrix metalloproteinase-9 and tissue inhibitor of metalloproteinases-1 in patients with multidrug-resistant tuberculosis without HIV and with HIV/TB co-infection

Materials and methods.

The study included 56 patients with new multidrug-resistant tuberculosis (MDR-TB) with bacterial excretion. The patients were divided into 2 groups: Group 1 (n=16) included patients with TB/HIV co-infection; Group II (n=40) included HIV-negative patients. In patients from group I, HIV was confirmed for the first time in their lives during an examination for TB. Patients from Group I did not receive antiretroviral treatment (ARVT) as according to the Order of the Ministry of Health of Ukraine No 1039, ARVT should start in 2-8 weeks after start of effective anti-tuberculosis treatment. [7] Antituberculosis treatment was provided according to the current orders of Ministry of Health of Ukraine.

The groups were similar by gender and age. In Group 1, the average age was 39 years, in group II - 37 years. The ratio of men to women in both groups was 1:1. All the patients were examined at the beginning of treatment and 2 months after the start of treatment. An examination included disease history, objective examination, routine clinical, biochemical, microbiological and instrumental methods of examination, levels of tissue markers of fibrosis, in particular, total oxyproline (OT), MMP-9, tissue inhibitor of metalloproteinases-1 (TIMP-1).

Blood sampling was performed at the beginning and 2 months after the start of TB treatment. The determination of the levels of total oxyproline was carried out according to the method of P. N. Sharayev (mg/L) according to the principle of the oxidative polycondensation reaction with subsequent quantitative determination of substances using a KFK-2 photoelectrocolorimeter (Ukraine) on the basis of the Department of Laboratory Diagnostics of the State University “Institute of Spine and Joint Pathology named after Prof. M. I. Sitenko National Academy of Sciences of Ukraine”. Levels of MMP-9 and TIMP-1 were investigated by ELISA using standard test systems in the Central Research Laboratory of Kharkiv National Medical University.

Statistical data processing was performed using the Statistica for Windows software package. Spearman correlation coefficient was used to find correlations; Mann-Whitney coefficient was used to compare two independent samples; Wilcoxon coefficient was used to compare two dependent samples; descriptive statistics.

Results.

According to the orders of Ministry of Health of Ukraine regarding the management of TB cases, during the treatment, the effectiveness of the treatment is monitored by clinical, laboratory and X-ray indicators.

At the beginning of treatment, bacterial excretion confirmed by culture was observed in 100% of patients in both groups. However, microscopy was positive in 50% of patients with HIV/TB co-infection and in 90% of patients without TB. After 2 months of treatment in Group 1, the number of patients with bacterial excretion decreased to 25%, i.e. bacterioscopically, the number of patients with bacterial excretion decreased by 2 times, and culturally by 4 times. In Group 2, the number of patients with bacterial excretion also decreased: 42% of positive results were obtained microscopically, and 47% of culture results, which is almost 2 times less than at the beginning of treatment. According to chest X-ray, at the beginning of treatment, 50% of patients from Group 1 and 95% from Group 2 had destruction of pulmonary tissue. According to the TB case management protocol, a chest X-ray control performed 4 months after the treatment onset. Thus, positive dynamics in the form of resorption and compaction was observed in 25% of patients in Group 1 and in 50% of patients in Group 2. The results of the levels of the studied indicators in the dynamics are shown in the Table 1.

As can be seen from the table, at the beginning of treatment, the MMP-9 in Group 1 was 11% lower than in Group 2. During 2 months of treatment, its level increased significantly in Group 1 by 18.4%. (Fig. 1). In Group 2 it almost did not change during the study. At the beginning of treatment, the level of TIMP-1 differed significantly between groups

Table 1.

Median levels of fibrosis markers in groups at baseline and after 2 months of treatment

		MMP-9 (ng/mL)	TIMP-1 (ng/mL)	Total oxyproline (mg/L)
Group	**Blood sample**	**Me**	**Me**	**Me**
I	1	326,5	154,6	3,95
	2	386,4	174	5,2
II	1	361,4	127,8	3,33
	2	363,2	160,2	3,15

and was 17.3% higher in Group 1. (Fig. 2) After 2 months of treatment, the level of TIMP-1 increased significantly in both groups: in Group 1 by 12.5% (Fig. 3), and in Group 2 by 25%. (Fig. 4)

The level of TO in Group 1 at the beginning of treatment was higher and reliably increased by 31.6% in 2 months (Fig. 5). In Group 2, at the beginning of treatment, the level of TO was lower by 18.6% compared to Group 1, and during 2 months of treatment, the substance did not change (it decreased by 5%). After 2 months of treatment, the significant difference in TO levels between groups was 1.7 times.

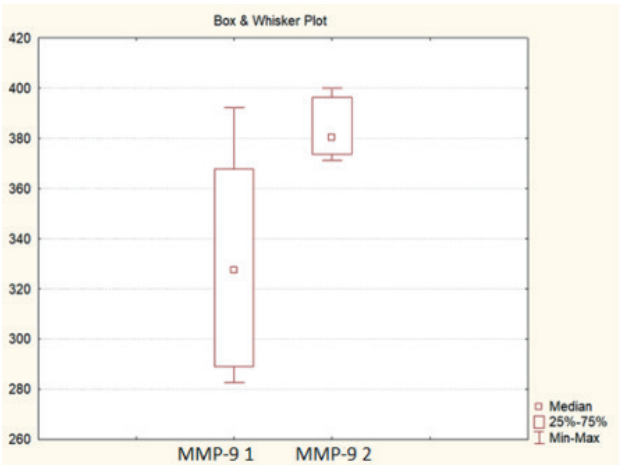

Figure 1. Dynamics of MMP-9 in Group 1.

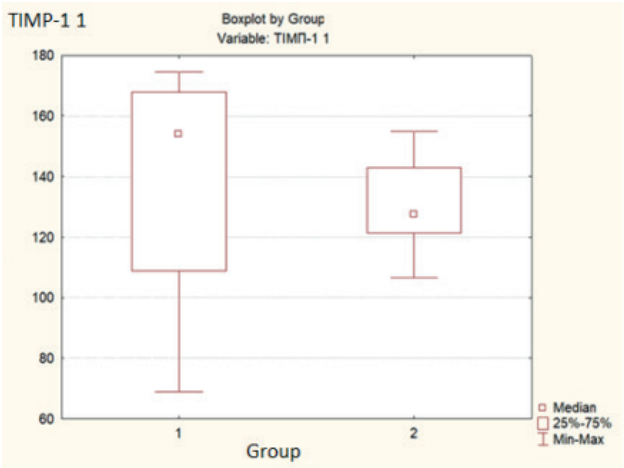

Figure 2. Difference of TIMP-1 between groups at the treatment onset.

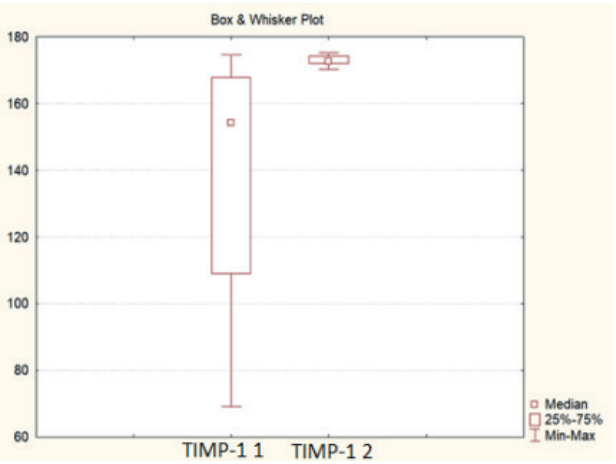

Figure 3 Dynamics of TIMP-1 in Group 1.

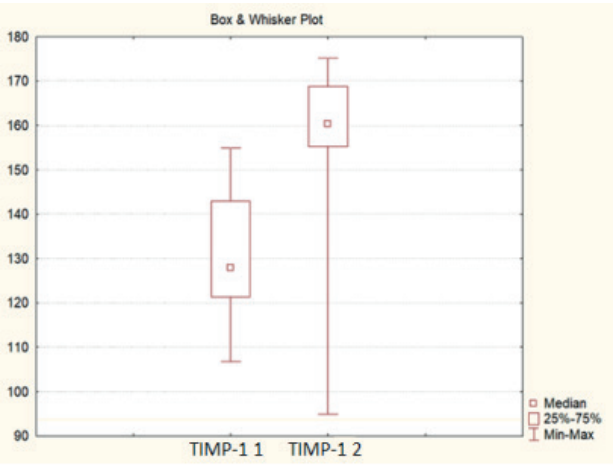

Figure 4. Dynamics of TIMP-1 in Group 2

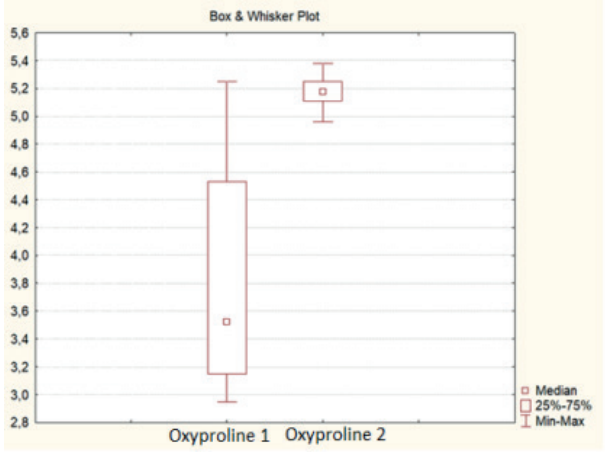

Figure 5. Dynamics of TO in Group 1.

Discussion.

Under physiological conditions, MMP-9 activity is regulated by TIMP-1, which is a specific inhibitor for this MMP. Under normal conditions, lung cells do not express MMP-9. [8, 9] During infectious and inflammatory processes, bronchial epithelial cells, alveolar cells, smooth muscle cells, and fibroblasts begin to synthesize MMP-9. In TB patients, MTB is able to stimulate the expression of MMP-9 in the host's body. The level of MMP-9 can be three times higher than that of healthy individuals. [8, 9] Our research revealed that the level of MMP-9 in Group 2 remained stable throughout the study, which was achieved by increasing the level of TIMP-1. This is confirmed by the direct correlation of mean strength (+0.45, $p < 0.05$) at the beginning of treatment, and the strengthening of this relationship after 2 months of treatment (+0.77, $p < 0.05$).

In pathological conditions, lower levels of oxyproline are maintained due to more adequate regulation of MMP-9 secretion by its inhibitor [10]. The balance between the breakdown and synthesis of collagen depends on these processes. In Group 2, oxyproline levels are lower throughout the study compared to Group 1. This is confirmed by a direct correlation of medium strength (+0.55, $p < 0.05$), which disappears after 2 months. These processes are accompanied by positive dynamics of the disease in Group 2, which is confirmed by laboratory and X-ray data. Thus, bacterial excretion, which was determined microscopically and culturally, decreased twice, and positive X-ray dynamics was obtained during the follow-up examination in 50% of patients of Group 2. At the beginning of treatment in Group 1, a strong inverse correlation between the levels of MMP-9 and TO was found (-0.85, $p < 0.05$). That is, the higher the level of MMP-9 in a particular patient, the lower the level of TO, and vice versa, with a low level of MMP-9, there will be a high level of TO. MMP-9 should be used for the formation of TO. This correlation indicates the atypicality and imbalance of these processes against the background of HIV. In patients from Group 1 compared to Group 2, the level of MMP-9 is unstable and increases during 2 months, and the increase in TIMP-1 by 12.5% in dynamics is not able to inhibit the increased production of MMP-9 (Fig. 3). The result is a higher level of oxyproline during the study and a significant increase of TO by 31.6% at 2 months of the study, which causes a decrease in the ability to rebuild connective tissue. This is confirmed by laboratory and X-ray examination. Thus, positive X-ray dynamics was observed in only 25% of patients, which is 2 times less compared to Group 2. Although the number of bacterial isolates in Group 1 decreased by 2 times after 2 months, the weaker positive X-ray dynamics against the background of 100% bilateral spread of processes is a sign of generalization of the process in most patients from this group. This indicates the preservation of MTB activity and the inability of the cellular immunity to localize the TB process.

Thus, in patients with HIV/TB co-infection, there is a violation of the regulation of the balance of MMP-9 and TIMP-1, which affects the processes of fibrosis and causes a severe course of the disease.

Conclusions.

In MDR-TB patients who did not have HIV infection, the processes of lung tissue reorganization are more typical and the balance of MMP-9, TIMP-1 and oxyproline levels is seen, which promotes fibrosis to limit specific inflammation in the pulmonary tissue. In MDR-TB patients with HIV-TB co-infection, there is an imbalance of fibrosis factors, which entails the impossibility of limiting specific inflammation and leads to the generalization of the tuberculosis process.

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