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DYNAMICS OF ALDOSTERONE, CONNECTIVE TISSUE REORGANIZATION AND GLUCOSE LEVEL AS MARKERS FOR TUBERCULOSIS TREATMENT EFFECTIVENESS

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Abstract

The objective of the study was to evaluate the dynamics of reorganization of connective tissue, aldosterone and glucose level as markers of favorable course of multidrug-resistant pulmonary tuberculosis.

Materials and methods. The study was performed on 84 patients with firstly diagnosed multidrug-resistant pulmonary tuberculosis. Aldosterone, free and protein-bound hydroxyproline and glucose levels were measured in all patients.

Results. It was found that in group I patients, free hydroxyproline levels are lower than in group II during the first two months of treatment for 60% at treatment onset and for 29.4% after 2 months of treatment. There was an increase in this index during the first 2 months of treatment in both groups. Protein-bound

Résumé

La dynamique du niveau de l'aldostérone, de la réorganisation du tissu conjonctif et du taux de glucose comme marqueurs de l'efficacité du traitement de la tuberculose

Objectif. L'étude visait à évaluer la dynamique de la réorganisation du tissu conjonctif, les taux d'aldostérone et de glucose en tant que marqueurs de l'évolution favorable de la tuberculose pulmonaire multirésistante.

Matériels et méthodes. L'étude a été réalisée sur 84 patients atteints de tuberculose pulmonaire multirésistante diagnostiquée pour la première fois. Les taux d'aldostérone, d'hydroxyproline et de glucose libres et liés aux protéines ont été mesurés chez tous les patients.

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hydroxyproline level was higher in group I by 21.7%. The level of aldosterone at the treatment onset was almost identical in both groups. After 2 months of treatment, its level decreased in both groups. However, after 2 months of treatment in group I, its index was lower than in group II by 12.7%.

Conclusions. Our results suggest that patients with a favorable course of multidrug-resistant tuberculosis have more pronounced decrease in aldosterone level, which may act as a pro-inflammatory agent and free hydroxyproline, which is a marker for the destruction of connective tissue. Inverse correlation between protein-bound hydroxyproline and glucose levels confirm the importance of normal glucose concentration for repair process.

Keywords: multidrug-resistant tuberculosis, glucose level, aldosterone, protein-bound hydroxyproline, free hydroxyproline.

Abbreviations list

FHP – free hydroxyproline
HIV – Human Immunodeficiency Virus
HP – hydroxyproline
IP – intensive phase
MTB – *Mycobacterium tuberculosis*
MDR TB – Multidrug-resistant tuberculosis
PBHP – protein-bound hydroxyproline
TB – tuberculosis
THP – total hydroxyproline

Résultats. Il a été constaté que, chez les patients du groupe I, les taux d'hydroxyproline libre étaient inférieurs à ceux du groupe II pendant les deux premiers mois de traitement pour 60% au début du traitement et pour 29,4% après 2 mois de traitement. Il y avait une augmentation de cet indice au cours des 2 premiers mois de traitement dans les deux groupes. Le taux d'hydroxyproline liée aux protéines était plus élevé dans le groupe I de 21,7%. Le niveau d'aldostérone au traitement était presque identique dans les deux groupes. Après 2 mois de traitement, son niveau a diminué dans les deux groupes. Cependant, après 2 mois de traitement dans le groupe I, son indice était inférieur de 12,7% à celui du groupe II.

Conclusion. Nos résultats suggèrent que les patients présentant une évolution favorable de la tuberculose multirésistante présentent une diminution plus prononcée des taux d'aldostérone, ce qui peut agir en tant qu'agent pro-inflammatoire et en hydroxyproline libre, marqueur de la destruction du tissu conjonctif. La corrélation inversée entre les niveaux d'hydroxyproline et de glucose liés aux protéines confirme l'importance d'une concentration en glucose normale pour le processus de réparation.

Mots-clés: tuberculose multirésistante, taux de glucose, aldostérone, hydroxyproline liée aux protéines, hydroxyproline libre.

Introduction

Tuberculosis is an important cause of death because of infectious diseases[1]. In the last decade, the increase of the number of patients with drug-resistant tuberculosis raised particular concerns. Multidrug-resistant tuberculosis (MDR TB) takes a special place in the structure of resistant forms of tuberculosis, when *M. tuberculosis* (MTB) is resistant to at least two antimicrobial agents of the first line, namely isoniazid and rifampin[2]. Such patients require long-term treatment with the use of second line drugs (aminoglycosides, 4th generation fluoroquinolones and others). However, the effectiveness of treatment in patients with MDR TB remains low[3].

Improving the treatment effectiveness of these patients is one of the important factors in the fight against this pathology, as it helps to reduce the number of individuals in the population who can be sources of further spread of the disease[4].

The criteria for effective treatment of patients with MDR TB include the cessation of bacterial excretion and the healing of pulmonary cavities[5]. However, many patients complete their treatment with large residual changes in the lungs, which can serve as a source of tuberculosis recurrence. Therefore, the study of the processes of destruction and subsequent reorganization of the lung tissue in patients with MDR TB is relevant.

It is known that the formation of cavities is associated with the destruction of the extracellular matrix and collagen fibers, which ensure the integrity of the lung tissue[6]. One of the destruction products of collagen fibers is hydroxyproline (HP) and its fractions. Free hydroxyproline (FHP) is considered a marker of connective tissue destruction, and protein-bound hydroxyproline (PBHP) is considered a repair marker[7,8]. The study of the collagen degradation products levels is relevant, since the destruction of lung tissue with the subsequent formation of fibrotic changes is one of the main pathomorphological events in tuberculosis.

The state of carbohydrate metabolism has a significant effect on the destructive changes progression, since the presence of diabetes mellitus significantly complicates the course of TB and worsens the healing of destructive changes. Thus, monitoring of

the carbohydrate metabolism state in patients with pulmonary MDR TB allows early prediction of the delayed mechanism of connective tissue reorganization.

Recently, close attention has been paid to the study of aldosterone, which can act as a fibrosis factor[9]. It is known that aldosterone acts as a pro-inflammatory agent. However, its role in the processes of fibrosis in tuberculosis patients is not well understood[10,11]. Mainly, the role of aldosterone in the tuberculous process as the main mineralocorticoid hormone was studied[12-14].

The aim of the study (prospective cohort) was to evaluate the dynamics of reorganization of connective tissue, aldosterone and glucose level as markers of favorable course of multidrug-resistant pulmonary tuberculosis.

Materials and methods

The study was performed on 84 patients aged 18-55 years, 50 men (59.5%) and 34 women (40.5%), new cases of pulmonary MDR TB. Patients were treated in Kharkiv Regional TB Dispensary No. 1, Ukraine, from 2014 to 2016, and at the time of the study they completed the main course of chemotherapy with anti-TB drugs, according to the order of the Ministry of Health of Ukraine No. 620 of 14.09.2014. Patients with comorbidities (HIV, cardiovascular disease, chronic obstructive pulmonary disease) were excluded from the study. Informed consent was obtained from all the patients included in the study. All patients had bacterial excretion and lung tissue destruction.

Treatment of patients with pulmonary MDR TB requires at least 8 months of the intensive phase (IP) of chemotherapy with anti-TB drugs. During this time, patients were monitored for treatment efficacy: a monthly sputum test to identify MTB, and an X-ray examination 4 and 8 months after the onset of anti-TB therapy. Depending on the results of the monitoring, the patients were divided into groups: Group I (n = 40) included patients in whom the cavities were closed and the bacterial excretion was stopped after 8 months of treatment – favorable course of TB; Group II (n = 44) included patients with destructions and/or bacterial excretion after 8 months of treatment – unfavorable course of TB.

Plasma glucose levels, total hydroxyproline (THP), FHP, PBHP and aldosterone levels were studied in all patients at the beginning of treatment, and at 2 and 3 months after the treatment onset. THP and its fractions (mg/L) were studied by Sharayev[15]. The aldosterone levels were tested by ELISA using standard Direct ELISA Kit,the EiAsyTM Way ALDOSTERON systems on a Labline-90 analyzer, according to the instructions.

Statistical processing of the obtained results was carried out by analyzing the contingency tables using the StatisticaBasicAcademic 13 for Windows software package (LicenseNumber: 139-956-866). We used the median (Me) interquartile range (Lower – lower quartile, Upper – upper quartile) and sample size (min – minimum, max – maximum value). The difference between groups was determined by non-parametric statistics using the Mann-Whitney criteria. To study the independent variables, we used the non-parametric Kruskal-Wallis criteria. The correlation Spearman coefficient R was calculated to find functional relationships between the parameters, with statistical significance at $p < 0.05$.

The study was performed according to the requirements for researches with the participation of patients: Statute of the Ukrainian Association for Bioethics and the GCP norms (1992), requirements and norms of ICH GLP (2002), typical ethics provisions of the Ministry of Public Health of Ukraine 66 dated February 13, 2006.

Results

The study of the dynamics of collagen degradation product levels showed that patients with MBR TB at the beginning of treatment, 2 and 3 months after the treatment onset had the FHP, PBHP indicators presented in the Table 1.

During all monitoring periods, the patients had higher FHP levels. By the second month of treatment, the level of FHP increased by 7.2%, while the PBHP decreased by 6.2%. After 3 months of treatment, the level of FHP decreased by 10.2% compared with its level at the beginning of treatment and by 18.2% compared with the previous month. The level of PBHP increased by 8.2% after 3 months of treatment compared with its level at the start of treatment and by 13.9% compared with the previous month.

The levels of aldosterone in patients with MDR TB at the beginning of treatment, after 2 and 3 months from the start of treatment, are presented in Table 2.

When comparing groups with favorable and unfavorable tuberculosis, it was found that the level of aldosterone was almost the same at the beginning of treatment. However, after 2 months of treatment, it decreased by 33.4% in Group I and by 25.3% in Group II, and its level in Group I was lower by 12.7% than in Group II ($p<0.05$) (Fig. 1).

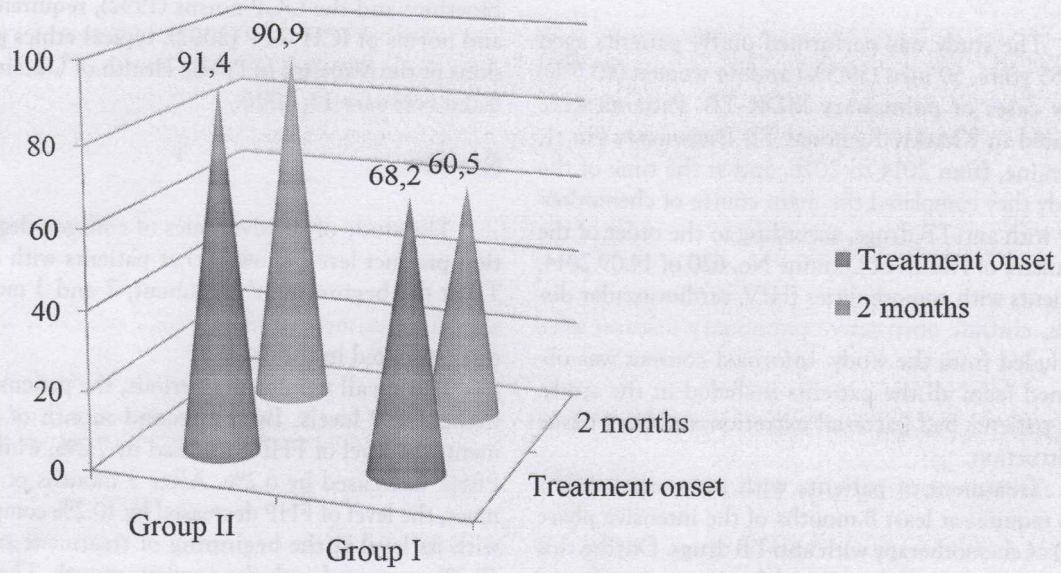
The level of FHP was lower by 60% in patients from Group I at the beginning of treatment, and by

Table 1. Dynamics of parameters of connective tissue reorganization markers in patients with pulmonary MDR TB ($p < 0.05$)

Parameter, units, time	Group (n=84)	mean	median	min	max	lower	upper	Stn.dev
FHP, mg/L, treatment onset	MDR TB	0.99	0.97	0.45	1.95	0.61	1.30	0.439
FHP, mg/L, 2 months	MDR TB	1.12	1.04	0.65	1.95	0.67	1.63	0.438
FHP, mg/L, 3 months	MDR TB	0.88	0.88	0.79	0.97	0.79	0.97	0.096
PBHP, mg/L, treatment onset	MDR TB	2.41	2.25	0.97	5.26	1.37	2.98	1.174
PBHP, mg/L, 2 months	MDR TB	2.08	2.11	0.85	4.21	1.45	2.5	0.854
PBHP, mg/L, 3 months	MDR TB	2.45	2.45	1.85	3.05	1.85	3.05	0.641

Table 2. Dynamics of aldosterone in patients with pulmonary MDR TB ($p < 0.05$)

Parameter, units, time	Group (n=84)	mean	median	min	max	lower	upper	Stn. dev.
Aldosterone, mg/L, treatment onset	MDR TB	93.665	91.11	27.81	262.16	47.64	110.8	55.9
Aldosterone, mg/L, 2 months	MDR TB	65.716	61.41	39.29	101.46	57.99	68.5	16.4
Aldosterone, mg/L, 3 months	MDR TB	59.865	59.87	57.91	61.82	57.91	61.8	2.1



*-significantly when comparing the two groups ($p<0,05$)

Figure 1. The dynamics of aldosterone (pg/mL) in patients with different outcomes of pulmonary MDR TB treatment.

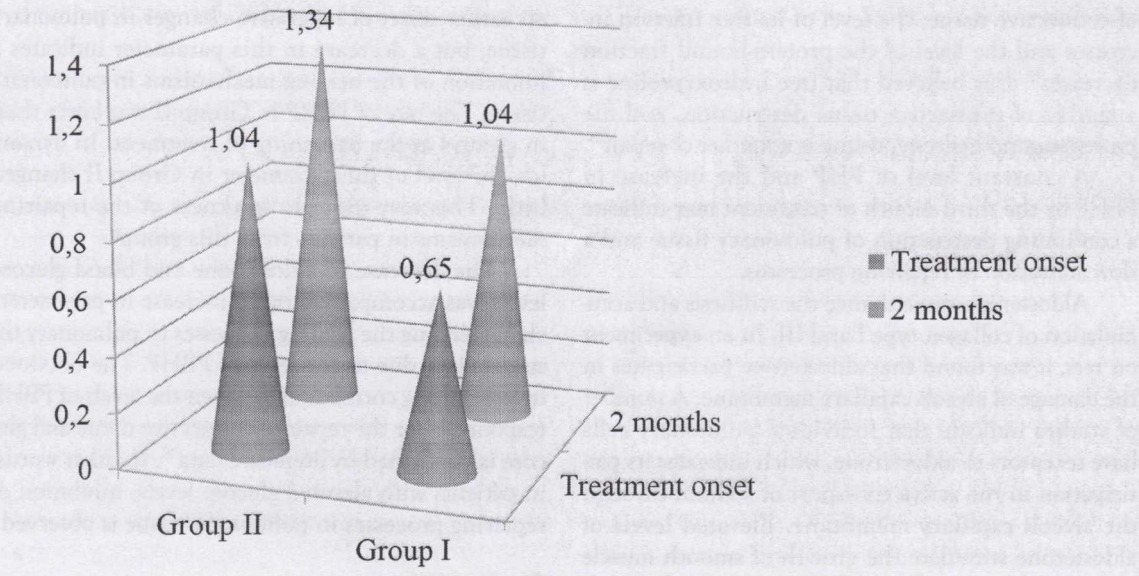
29.4% after 2 months of treatment. The dynamics of its growth in Group I was 59.2%, and in Group II it was 28.9% ($p<0.05$) (Fig. 2).

The level of PBHP in patients from Group II was higher than in Group II by 21.7%, and after 2 months of treatment it was 55.5% lower ($p <0.05$). In two months of treatment, it decreased by 46.2% in Group I, and slightly increased (by 6.9%) in Group II ($p<0.05$) (Fig. 3).

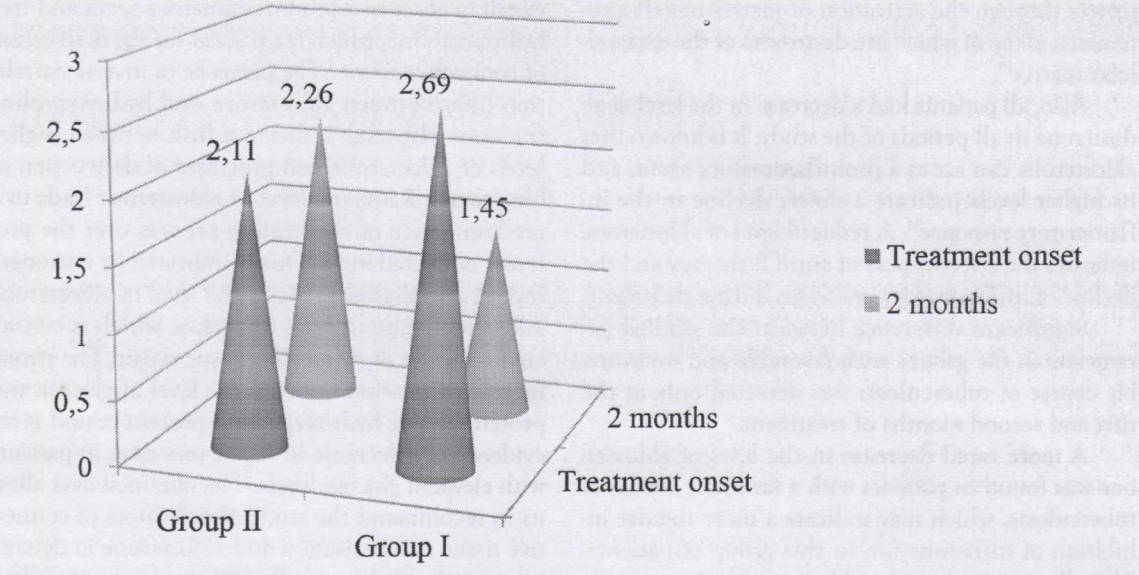
We performed a correlation analysis between collagen degradation products, aldosterone, and blood glucose levels. At the beginning of the treatment, a strong inverse correlation was found between glucose level and PBHP ($r = - 0.86$, $p<0.05$). After 2 months of treatment, a strong inverse correlation was found between aldosterone and PBHP ($r = -0.83$, $p <0.05$) and the inverse correlation of average strength between aldosterone and FHP ($r = -0.56$, $p <0.05$).

Discussion

The amino acid oxyproline is the product of the hydrolysis of collagen and gelatin. It is a heterocyclic amino acid that is part of gelatin (13%), as well as



*-significantly when comparing the two groups ($p<0,05$)
Figure 2. The dynamics of free hydroxyproline (mg/L) in patients with different outcomes of pulmonary MDR TB treatment



*-significantly when comparing the two groups ($p<0,05$)
Figure 3. The dynamics of protein-bound hydroxyproline (mg/L) in patients with different outcomes of pulmonary MDR TB treatment.

collagen. Hydroxyproline is the main marker that allows to describe the processes of collagen catabolism in the body. When collagen and gelatin are destroyed, hydroxyproline is released into the bloodstream both as a free oligopeptide and in polypeptide form, since it cannot be reused for the synthesis of collagen and gelatin. Therefore, a significant part of endogenous hydroxyproline, which is found in biological fluids, is the product of the destruction of various forms of collagen and gelatin. The normal concentration of hydroxyproline (in healthy individuals) in blood serum is 12.68 μmol/L, in urine it is 172.5 mol/L[15]. Hydroxyproline is almost exclusively found in collagen and gelatin, so its concentration in the blood and urine indicates the intensity of the destruction of these structures[16]. In disorders of the metabolism

of connective tissue, the level of its free fraction increases and the level of the protein-bound fraction decreases[17]. It is believed that free hydroxyproline is a marker of connective tissue destruction, and the protein-bound hydroxyproline is a marker of repair[18].

A constant level of FHP and the increase in PBHP by the third month of treatment may indicate a continuing destruction of pulmonary tissue and a slow activation of repairing processes.

Aldosterone can enhance the synthesis and accumulation of collagen type I and III. In an experiment on rats, it was found that aldosterone participates in the damage of alveoli capillary membrane. A number of studies indicate that individual pulmonary cells have receptors to aldosterone, which indicates its participation in the active transport of sodium through the alveoli capillary membrane. Elevated levels of aldosterone stimulate the growth of smooth muscle cells, fibroblasts and contribute to the development of fibrosis in the lungs. The synthesis of collagen in the pulmonary tissue is controlled by the state of the extracellular matrix. There is evidence that aldosterone can increase the degradation of the extracellular matrix through the activation of matrix metalloproteinases, some of which are destroyers of the extracellular matrix[19].

Also, all patients had a decrease in the level of aldosterone in all periods of the study. It is known that aldosterone can act as a proinflammatory agent, and its higher levels indicate a slower decline in the inflammatory response[11]. A reduced level of aldosterone indicates the effectiveness of anti-TB therapy and the decline of inflammatory processes during treatment.

Significant difference between the studied parameters in the groups with favorable and unfavorable course of tuberculosis was detected only in the first and second months of treatment.

A more rapid decrease in the level of aldosterone was found in patients with a favorable course of tuberculosis, which may indicate a more intense inhibition of inflammation in this group of patients. This allows us to consider aldosterone as a prognostic marker of effective treatment.

In both groups, there was an increase in the level of FHP, which indicates the continuing processes of the pulmonary tissue destruction during the first 2 months of treatment. However, the level of this parameter was significantly lower in Group I than in Group II, which may indicate a predominance of destruction processes over repair processes in patients with ineffective treatment of MDR TB.

In the group with a favorable course of tuberculosis, there was a decrease in PBHP, while in the group with an unfavorable course there was a mild increase of its level. Such dynamics may indicate an earlier onset of reparative changes in pulmonary tissue, but a decrease in this parameter indicates a limitation of the healing mechanisms in pulmonary tissue. The rate of PBHP in Group II was lower than in group I at the beginning of treatment. In dynamics, the level of this parameter in Group II changed little. This may indicate weakness of the repairing mechanisms in patients from this group[6].

The decrease in aldosterone and blood glucose levels was accompanied by an increase in parameters characterizing the healing processes in pulmonary tissue, mainly due to the level of PBHP. The obtained inverse strong correlation between the levels of PBHP responsible for the repair of connective tissue and glucose is confirmed by literature data[20]. In other words, in patients with elevated glucose levels, inhibition of repairing processes in pulmonary tissue is observed.

Conclusions

Our results suggest that patients with a favorable course of multidrug-resistant tuberculosis have more pronounced decrease in aldosterone level, which may act as a pro-inflammatory agent and free hydroxyproline, which is a marker for the destruction of connective tissue. The presence of inverse correlation links between aldosterone and hydroxyproline fractions also may indicate a link between higher levels of aldosterone and processes of destruction of lung tissue. Reducing level of aldosterone leads to a predominance of the healing process over the processes of degradation. This is indicated by a stronger inverse correlation between the level of aldosterone and protein-bound hydroxyproline which is considered a marker of connective tissue repair. The strong inverse correlation between the level of glucose and protein-bound hydroxyproline protein-bound gives evidence of a decrease in repair processes in patients with elevated glucose levels. The obtained data allow us to recommend the study of indicators of connective tissue reorganization and aldosterone in dynamics, as early markers of effective treatment of multidrug-resistant pulmonary tuberculosis.

Compliance with Ethics Requirements:

„The authors declare no conflict of interest regarding this article"

„The work was performed according to the requirements for researches with the participation of people: Statute of the Ukrainian Association for Bioethics and the GCP norms (1992), requirements and norms of ICH GLP (2002), ethical standards in the Helsinki Declaration of 1975, as revised in 2008, typical ethics provisions of the Ministry of Public Health of Ukraine 66 dated February

13, 2006. The work was approved by Ethical Commission of Kharkiv National Medical University, Ukraine (Protocol No 2 of 02.02.2015; chairmen – MD, professor Ospanova T.S. (+ 38 050 9988242))."

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