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DDC-UDC: 616.314.5-018.4-037 DOI:10.19221/202111

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THE EFFECT OF PRESCRIBING A COMPLEX OF ESSENTIAL AMINO ACIDS ON THE LEVEL OF HUMAN-BETA-DEFENSIN-1 IN PATIENTS WITH DRUG-SUSCEPTIBLE AND DRUG-RESISTANT PULMONARY TUBERCULOSIS

Abstract. *With the expansion of M. tuberculosis resistance, more and more attention is being paid to alternative pathogenetic therapies that can stimulate the host's immune response. The purpose of the study was to determine the effect of prescribing a complex of essential amino acids on the level of Human-beta-defensin-1 in patients with drug-susceptible and drug-resistant pulmonary tuberculosis. Materials and methods. 50 patients with drug-susceptible tuberculosis (TB) and 50 patients with drug-resistant TB (multidrug-resistant and extensively drug resistant TB) were included to the study. The patients with were divided into 3 groups: patients in Group 1 did not receive additional treatment, patients in Group 2 received essential amino acids in tablets for 30 days, patients in Group 3 received injectable essential amino acids for 10 days and after that they received essential amino acids in tablets for 20 days. HBD-1 level was measured in blood serum by ELISA at the treatment onset, after 30 and after 60 days in all the patients. Results. Comparison of the HBD-1 level in patients with susceptible TB after 30 days of treatment showed its highest level in Group 3 (21.59±5.33 µmol/L, median – 17.14 µmol/L), lower level in Group 2 (19.41±3.66 µmol/L, median – 20.17 µmol/L) and the lowest level in Group 1 (9.25±1.95 µmol/L, median – 4.42 µmol/L), p<0.05. After 60 days of treatment, patients with drug susceptible TB showed the opposite results with the highest level of HBD-1 in Group 1 (26.18±2.82 µmol/L, median – 29.17 µmol/L), lower level in Group 2 (16.57±3.95 µmol/L, median – 11.11 µmol/L) and the lowest level in Group 3 (6.32±1.44 µmol/L, median – 3.99 µmol/L). Comparison of the HBD-1 level in patients with drug resistant TB after 30 days of treatment also showed its highest levels in Group 2 (21.65±3.27 µmol/L, median – 20.19 µmol / L) and Group 3 (20.98±7.91 µmol/L, median – 11.01 µmol/L), and significantly lower level in Group 1 (10.79±2.91 µmol/L, median – 4.09 µmol/L), p<0.05. After 60 days of drug resistant TB treatment, the highest HBD-1 level was observed in Group 1 (63.24±9.73 µmol/L, median – 58.15 µmol / L), its lower level was in Group 2 (18.99±2.09 µmol/L, median – 20.26 µmol/L) and the lowest level was in Group 3 (13.86±3.63 µmol/L, median – 13.97 µmol/L), p<0, 05. Conclusions. The appointment of the complex of essential amino acids in the pathogenetic therapy of tuberculosis allows to increase the production of Human-beta-defensin-1 in both patients with drug susceptible and drug resistant tuberculosis, which leads to a more balanced immune response and an increase in the effectiveness of anti-tuberculosis therapy.*
Key words: *tuberculosis, amino acids, MDR-TB, treatment*

Introduction. With the expansion of *M. tuberculosis* resistance, more and more attention is being paid to alternative pathogenetic therapies that can stimulate the host's immune response.

One of the important elements of anti-tuberculosis immunity is Human-beta-defensin-1 (HBD-1). HBD-1 is a cationic peptide of the immune system, which has a direct bactericidal effect on MTB due to the destruction of its cell wall. It also has an indirect immune effect as a chemoattractant for immune cells [1].

We have previously investigated the possibility of HBD-1 use as a marker of the severity of tuberculosis (TB) and the effectiveness of anti-

tuberculosis therapy [2]. However, the value of HBD-1 is not limited to diagnostic capabilities alone. The possibilities of HBD-1 using in therapy against various types of viruses [**Ошибка! Источник ссылки не найден.**], bacteria [4] and fungi [5] have been described. The therapeutic effect of HBD-1 in patients with TB has also been suggested [6], but studies in human have not been provided. One of the main problems in the therapeutic use of HBD-1 is the complexity of its preparation and the instability of this cationic peptide with a short half-life. In this regard, a possible way of using HBD-1 in pathogenetic therapy is the stimulation of its production through the use of drugs containing amino acids that are part of HBD-1. A similar study was performed by Rivas-Santiago et al. and it showed the effectiveness of L-isoleucine in stimulating the production of HBD-1 and pathogenetic therapy of TB in mice [7]. In addition, the prescribing of a complex of amino acids may be promising, since HBD-1 contains predominantly lysine, threonine, leucine, valine, and phenylalanine in addition to L-isoleucine [8].

Taking into account the deficiency of amino acids that occurs during long-term course of TB [9], especially in drug-resistant forms, the use of a complex of amino acids in pathogenetic therapy of TB may be a promising method for increasing the effectiveness of anti-tuberculosis therapy.

The purpose of the study was to determine the effect of prescribing a complex of essential amino acids on the level of Human-beta-defensin-1 in patients with drug-susceptible and drug-resistant pulmonary tuberculosis.

Materials and methods. 50 patients with drug-susceptible TB and 50 patients with drug-resistant TB (multidrug-resistant and extensively drug resistant TB) were included to the study. The patients were examined, get standard anti-tuberculosis treatment and underwent treatment effectiveness monitoring according to WHO guidelines and Ukrainian state protocols. Patients with drug-resistant TB were divided into 3 groups: patients in Group 1 (25 patients) did not receive additional treatment, patients in Group 2 (13 patients) received essential amino acids in tablets for 30 days, patients in Group 3 (12 patients) received injectable essential amino acids for 10 days and after that they received essential amino acids in tablets for 20 days. Patients with drug susceptible TB were also divided into 3 groups: patients in Group 1 (25 patients) did not receive additional treatment, patients in Group 2 (12 patients) received essential amino acids in tablets for 30 days, patients in Group 3 (13 patients) received injectable essential amino acids for 10 days and after that they received essential amino acids in tablets for 20 days. Injectable drug contained essential amino acids in following dosage: isoleucine - 4.4 mg, valine - 4.9 mg, leucine – 9.8 mg, lysine hydrochloride - 11.5 mg, methionine – 5.7 mg, threonine – 4.3 mg, tryptophan – 1.44 mg, phenylalanine – 7.0 mg (№UA/5616/01/01; 15.02.2017). Tablets contained essential amino acids in following dosage: isoleucine 50 mg, valine 60 mg, leucine 80 mg, lysine 80 mg, methionine 25 mg, threonine 40 mg, tryptophan 25 mg, phenylalanine 40 mg (№ 05.03.02-04/49900; 18.10.2006). HBD-1 level was measured in blood serum by ELISA at the treatment onset, after 30 and after 60 days in all the patients. Statistical data processing was performed using Statistica 8.0.

Results. Comparison of the HBD-1 level in patients with susceptible TB after 30 days of treatment showed its highest level in Group 3 (21.59±5.33 µmol/L, median – 17.14 µmol/L), lower level in Group 2 (19.41±3.66 µmol/L, median – 20.17 µmol/L) and the lowest level in Group 1 (9.25±1.95 µmol/L, median – 4.42 µmol/L), $p<0.05$.

After 60 days of treatment, patients with drug susceptible TB showed the opposite results with the highest level of HBD-1 in Group 1 (26.18±2.82 µmol/L, median – 29.17 µmol/L), lower level in Group 2 (16.57±3.95 µmol/L, median – 11.11 µmol/L) and the lowest level in Group 3 (6.32±1.44 µmol/L, median – 3.99 µmol/L), $p <0.05$, Fig. 1.

At the same time, there was a significant difference in the effectiveness of the intensive phase of anti-tuberculosis therapy between the groups. In Group 1, 72.0% of patients effectively completed the intensive phase of treatment after 2 months, in Group 2 – 92.3% and in Group 3 - 91.7% ($p <0.05$). The ineffectiveness of the intensive phase in other patients was associated with treatment interruption, treatment failure or death. In addition, some of them required an extension of

the intensive phase of treatment due to the persistence of bacterial excretion and the absence of positive clinical and X-ray dynamics. Such patients prevailed in Group 1, who did not receive amino acids as an additional pathogenetic therapy.

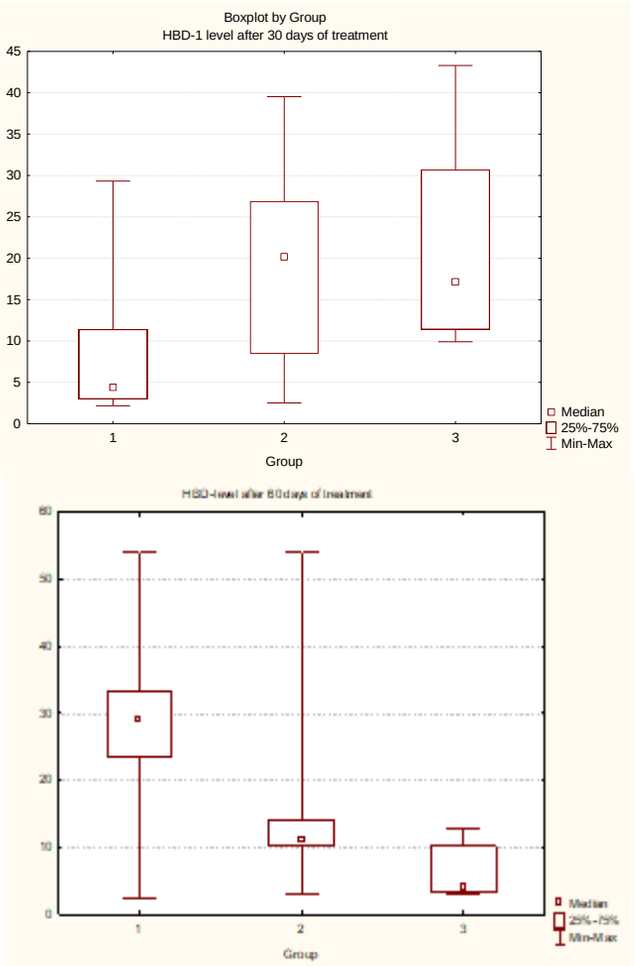

Figure 1. Comparison of HBD-1 level in groups of patients with drug susceptible TB after 30 and 60 days of treatment

Comparison of the HBD-1 level in patients with drug resistant TB after 30 days of treatment also showed its highest levels in Group 2 (21.65±3.27 µmol/L, median – 20.19 µmol / L) and Group 3 (20.98±7.91 µmol/L, median – 11.01 µmol/L), and significantly lower level in Group 1 (10.79±2.91 µmol/L, median – 4.09 µmol/L), p<0.05.

After 60 days of drug resistant TB treatment, the highest HBD-1 level was observed in Group 1 (63.24±9.73 µmol/L, median – 58.15 µmol / L), its lower level was in Group 2 (18.99±2.09 µmol/L, median – 20.26 µmol/L) and the lowest level was in Group 3 (13.86±3.63 µmol/L, median – 13.97 µmol/L), p<0, 05.

In patients with drug resistant TB, significant dynamics of HBD-1 level in the groups was revealed. Thus, in Group 1, there was a decrease in the HBD-1 level from 24.66±6.67 µmol/L (median – 5.76 µmol/L) to 10.79±2.91 µmol/L (median – 4.09 µmol/L) to 30 doses and an increase to 63.24±9.73 µmol/L (median – 58.15 µmol/L) to 60 doses, p<0.05. In Group 2, there was an increase in the level of HBD-1 from 16.99±12.49 µmol/L (median – 4.04 µmol/L) to 21.65±3.27 µmol/L (median – 20.19 µmol/L) to 30 doses and the same level of HBD-1 to 60 doses (18.99±2.09 µmol/L, median – 20.26 µmol/L), p<0.05. In Group 3, there was a tendency to an increase of HBD-1 from 19.95±11.54 µmol/L (median – 3.36 µmol/L) to 20.98±7.91 µmol/L (median – 11.01 µmol/L) to 30 doses and its slight decrease to 13.86±3.63 µmol/L (median – 13.97 µmol/L) to 60 doses. The data are presented in Fig. 2.

At the same time, there was a significant difference in the effectiveness of the intensive phase of anti-tuberculosis therapy between the groups. In Group 1, 64.0% of patients effectively completed the intensive phase of treatment. Treatment effectiveness in Group 2 was 84.6%. In Group 3, all patients effectively completed the intensive phase of treatment (p<0.05). Sputum conversion in patients who did not receive additional pathogenetic therapy with amino acids after 2 months of treatment was recorded in 80.0% of patients, while in patients receiving additional therapy - in 95.8% (p<0.05).

Significant differences in the HBD-1 level when comparing patients with drug susceptible and drug resistant pulmonary TB were found only in Group 1 after receiving 60 doses of anti-tuberculosis drugs with a significantly higher HBD-1 level in patients with drug resistant TB (63.24±9.73 µmol/L, median – 58.15 µmol / L) compared with drug susceptible cases (26.18±2.82 µmol/L, median – 29.17 µmol/L), p<0.05, Fig. 3. At the same time, in patients from Groups 2 and 3, there was no significant difference in the parameters and dynamics of the HBD-1 level in patients with drug susceptible and drug-resistant TB.

Discussion. The dynamics of the HBD-1 level showed similar results in patients with drug susceptible and drug resistant TB, namely, a significant increase by the 30th day of treatment and a decrease by the 60th day of treatment in

patients receiving a complex of essential amino acids. It was more pronounced in patients receiving the injectable form of the drug. In the comparison group, the opposite dynamics was obtained: a decrease in the HBD-1 level by the

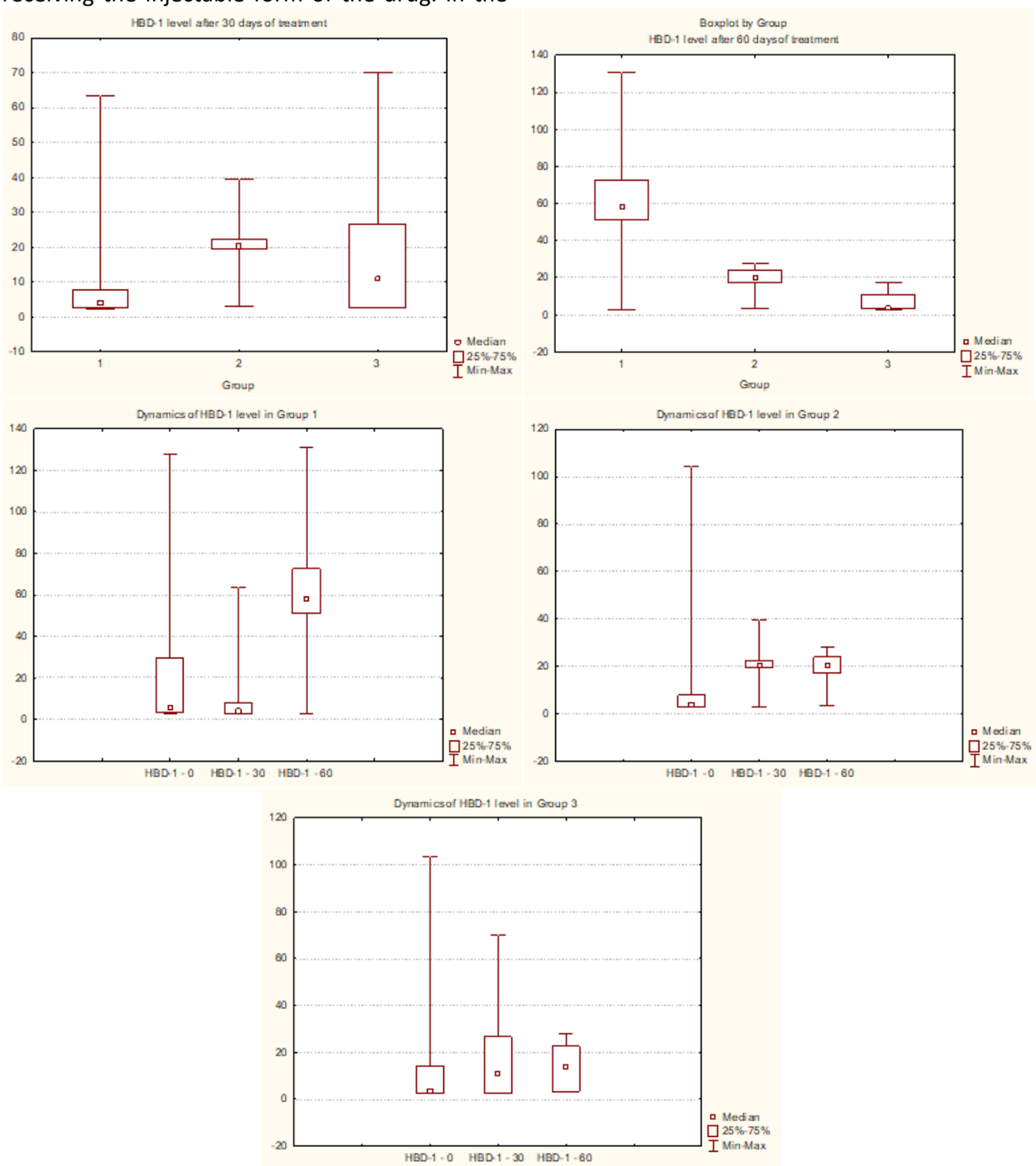

Figure 2. Comparison of HBD-1 level in groups of patients with drug resistant TB

30th day of therapy and its increase by the 60th day treatment. It can be explained by the positive role of the amino acid complex in replenishing the HBD-1 level, which was most pronounced by the 30th day of treatment, i.e. by the end of the course of essential amino acids intake. During this period, HBD-1 had a pronounced bactericidal and immunomodulatory effect, which was subsequently confirmed by a significantly more frequent sputum after 60 days of treatment in patients receiving additional therapy with a complex of amino acids. Subsequently, the produced HBD-1 gradually decreased by the 60th day of therapy. In patients who did not receive additional therapy, the immune response was delayed and an increase in the HBD-1 level was

observed only by the 60th day of treatment. An active bactericidal and immunostimulating effect appeared later, so a greater number of patients had bacterial excretion at the 60th dose of anti-tuberculosis chemotherapy. The positive effect of the appointment of a complex of essential amino

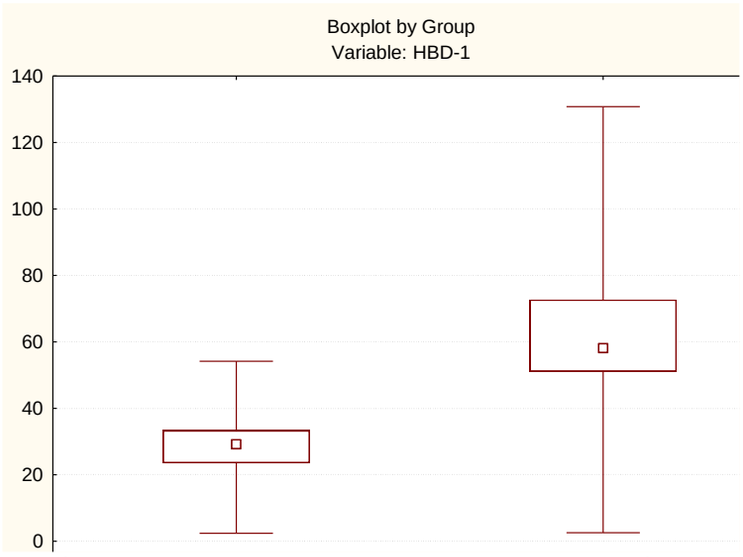

Figure 3. Comparison of HBD-1 level in patients with drug susceptible TB (Drug resistance – 0) and drug resistant (Drug resistance 1) TB.

acids on the anti-tuberculosis immune response is also confirmed by the higher effectiveness of the intensive phase of anti-tuberculosis therapy in the groups of patients who additionally received amino acids in pathogenetic therapy, both in sensitive and in drug resistant TB.

The results are consistent with previous studies by Rivas-Santiago et al. [7] in a murine model, in which a decrease in the β-defensins level of was also obtained after 4 weeks of the course of the disease without additional therapy with L-isoleucine, and vice versa - their increase with the introduction of L-isoleucine.

Against the background of additional pathogenetic therapy with a complex of amino acids at 60 doses, identical levels of HBD-1 were observed both in patients with drug susceptible and drug resistant TB, in contrast to the comparison group, where high HBD-1 level remained at 60 doses in patients with drug resistant TB with dynamics towards its normalization in patients with drug susceptible TB. This fact probably indicates the preservation of an active inflammatory process during treatment with second-line anti-tuberculosis drugs at the stage of 60 doses. Considering that HBD-1 is a marker of the TB treatment effectiveness [8], the appointment of a complex of amino acids in the pathogenetic therapy of TB brings the effectiveness of the treatment of multidrug resistant and extensively drug-resistant TB closer to the effectiveness of the treatment of drug susceptible TB, and also reduces the phase of active inflammation in patients with drug resistant TB by reducing the pool of active MTB. A similar bactericidal effect was demonstrated by Kalita et al. [9] in vitro: it was demonstrated that HBD-1 has a direct damaging effect on the MTB cell wall, and also facilitates the penetration of antimycobacterial drugs into bacterial cells, but such studies on humans have not been performed.

Conclusions. The appointment of the complex of essential amino acids in the pathogenetic therapy of tuberculosis allows to increase the production of Human-beta-defensin-1 in both patients with drug susceptible and drug resistant tuberculosis, which leads to a more balanced immune response and an increase in the effectiveness of anti-tuberculosis therapy.

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