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DEPENDENCE OF HUMAN-BETA-DEFENSIN-1 LEVEL ON DRUG-RESISTANCE SPECTRUM AND TREATMENT REGIMENS IN PATIENTS WITH PULMONARY TUBERCULOSIS

Abstract. *Taking into account the constant expansion of M. tuberculosis drug resistance, the study of the host immune response components, which can be used in diagnosis, prediction of tuberculosis course and pathogenetic treatment, is becoming an increasingly urgent issue. The purpose of the study was to compare Human-beta-defensin-1 level in different types of drug-resistance in patients with pulmonary tuberculosis and its dynamics during use of standard and individualized regimens of anti-tuberculosis treatment. Materials and methods. 100 patients with tuberculosis were included in the study. Human-beta-defensin-1 (HBD-1) level was measured in all the patients at the treatment onset and 2 months later. The patients also underwent chest X-ray, sputum smear microscopy, sputum molecular-genetic study, sputum culture on liquid and solid media, blood count, blood biochemistry. Patients were treated with standard and individualized anti-tuberculosis treatment regimens. Statistical data processing was carried out using Statistica 8.0. Results: After 2 months of anti-tuberculosis treatment HBD-1 level was significantly higher in patients with drug-resistance (42.1±8.2 µmol/L, median – 16,8 µmol/L) than in patients with drug-susceptible tuberculosis (15.9±5.3 µmol/L, median – 38.4 µmol/L), and there was a significant ($p<0.05$) difference between HBD-1 levels in patients with different types of drug-resistance with its increase in wider resistance spectrum: in mono-resistant TB – 12.6±5.8 µmol/L (median – 12.0 µmol/L), in MDR-TB – 38.1±6.9 µmol/L (median – 56.2 µmol/L), in XDR-TB – 71.8±28.5 µmol/L (median – 104.8 µmol/L). The study of the dynamics of HBD-1 in patients with drug-resistant tuberculosis showed its increase by the second month of treatment from 21.3±4.8 µmol/L (median - 3.7 µmol/L) to 42.1±8.2 µmol/L (median - 52.7 µmol/L). Conclusions. The spectrum of drug-resistance does not affect the level of Human-beta-defensin-1 in patients with pulmonary tuberculosis. The initial level depends on the pulmonary lesions size, the presence of bacterial excretion and liver function impairment. Treatment of drug-susceptible tuberculosis leads to a significant decrease in Human-beta-defensin-1 by the second month, in contrast to cases of drug-resistant tuberculosis. The dependence of the effectiveness of treatment of drug-resistant tuberculosis on the initial level of Human-beta-defensin-1 was revealed: the lower the level of Human-beta-defensin-1, the higher the treatment effectiveness, which makes it possible to use this parameter as a prognostic marker of the treatment effectiveness.*

Key words: *tuberculosis, Human-beta-defensin-1, drug-resistance*

Introduction. Taking into account the constant expansion of *M. tuberculosis* drug resistance, the study of the host immune response components, which can be used in diagnosis, prediction of tuberculosis course and pathogenetic treatment, is becoming an increasingly urgent issue.

One of these components are cationic peptides, in particular the β-defensin family. Recent study of Zhu et al. [1] showed high effectiveness of Human-beta-defensin-3 against methicillin-resistant *Staphylococcus aureus* in murine model. The similar study was provided earlier by Maisetta et all. [2] in vitro against multidrug-resistant strains of *aureus*,

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Enterococcus faecium, *Pseudomonas aeruginosa*, *Stenotrophomonas maltophilia* and *Acinetobacter baumannii.*

Despite the fact that β-defensins-2, -3 and 4 are not primary in the immune response, do not have a basic level of production and their production is stimulated by β-defensin-1 [3], detailed study of β-defensin-1 role in immune response was not provided until recently. Only in 2019, a study by Wendler et al. [4] showed the model of β-defensin-1 influence on antibiotic-resistant strains of *E. coli*, *P. aeruginosa*, and *C. albicans*, and suggested the possibility of using β-defensin-1 as a component of pathogenetic therapy supporting the host's immunity.

It should be noted that ongoing studies of the activity of β-defensins included the study of their effect on common species of the families *Candida*, *Staphylococcus*, *Escherichia*, but did not include the effect on *M. tuberculosis*, which is nevertheless a complex epidemiological problem for many countries [5]. A detailed study of the effect of various regimens of anti-tuberculosis therapy with the use of first and second line anti-tuberculosis drugs was not carried out.

Therefore, the dynamics of Human-beta-defensin-1 level described in this work under various resistance spectra of *M. tuberculosis* and anti-tuberculosis treatment regimens is a topical study, and the results obtained can be used in the future to improve the diagnosis and treatment of drug-resistant tuberculosis.

The purpose of the study was to compare Human-beta-defensin-1 level in different types of drug-resistance in patients with pulmonary tuberculosis and its dynamics during use of standard and individualized regimens of anti-tuberculosis treatment.

Materials and methods. 100 patients were included in the study and divided into groups: Group 1 – 52 patients with drug-susceptible tuberculosis (TB), Group 2 – 48 patients with drug-resistant tuberculosis (Group 2.1 – mono-resistant tuberculosis (n=15), Group 2.2. – multidrug-resistant tuberculosis (MDR-TB) (n=21), Group 2.3 – extensively drug-resistant tuberculosis (XDR-TB) (n=12)). Human-beta-defensin-1 (HBD-1) level was measured in all the patients at the treatment onset and 2 months later. The patients were also examined according to the current Order of Ministry of Health of Ukraine which included chest X-ray, sputum smear microscopy, sputum molecular-genetic study, sputum culture on liquid and solid media, blood count, blood biochemistry. Patients were treated with standard and individualized treatment regimens which included during intensive phase: 4 first-line drugs for patients with susceptible tuberculosis; 4 first- and second-line drugs for patients with mono-resistant tuberculosis, 4-5 second-line drugs for patients with MDR-TB and XDR-TB with or without added bedaquiline. Due to treatment interruption or death to the second month of treatment the number of patients changed and was: Group 1 – 43 patients, Group 2.1 – 15 patients, Group 2.2 – 17 patients, Group 2.3 – 10 patients. Statistical data processing was carried out using Statistica 8.0.

Results. Comparison of HBD-1 level in groups with drug-susceptible and drug-resistant tuberculosis at the treatment onset showed no significant difference ($p>0.05$): Group 1 - 20.4±3.6 µmol/L, Group 2 - 21.4±4.8 µmol/L. However, after 2 months of anti-tuberculosis treatment HBD-1 level was significantly higher in Group 2 (42.1±8.2 µmol/L, median – 16,8 µmol/L) than in Group 1 (15.9±5.3 µmol/L, median – 38.4 µmol/L), $p<0.05$ (Fig. 1)

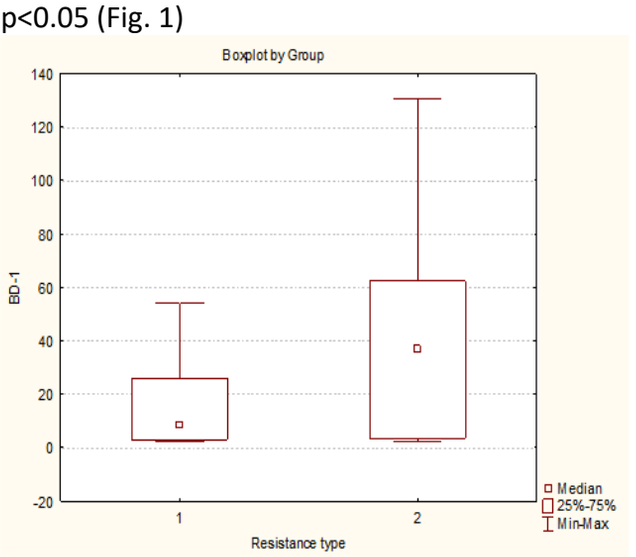

Figure 1. Comparison of Human-beta-defensin-1 level in patients with drug-susceptible (Group 1) and drug-resistant (Group 2) tuberculosis after 2 months of anti-tuberculosis treatment

To study the influence of resistance type on HBD-1 level we compared groups with different types of drug-resistance. There was no significant difference at the treatment onset ($p<0.05$), but

there was a tendency to HBD-1 increase with widening of drug-resistance spectrum. HBD-1 level was: in mono-resistant patients – 9.7±4.7 µmol/L, in MDR-TB patients – 17.3±4.3 µmol/L, in XDR-TB patients – 32.5±14.0 µmol/L. After 2 months of treatment, there was a significant ($p<0.05$) difference between HBD-1 levels in patients with different types of drug-resistance with its increase in wider resistance spectrum: in mono-resistant TB – 12.6±5.8 µmol/L (median – 12.0 µmol/L), in MDR-TB – 38.1±6.9 µmol/L (median – 56.2 µmol/L), in XDR-TB – 71.8±28.5 µmol/L (median – 104.8 µmol/L) (Fig. 2).

Further study of correlations of HBD-1 and other parameters was provided independently in groups. HBD-1 level did not influence treatment effectiveness in patients with drug-susceptible tuberculosis which can be due to small size of group of patients with non-effective treatment. Despite this we noted that higher HBD-1 level correlated with larger pulmonary lesions ($r=+0.44$, $p<0.05$) and presence of pulmonary tissue destruction: patients with pulmonary tissue destruction had HBD-1 level 25.7±5.0 µmol/L (median – 23.6 µmol/L), patients without pulmonary tissue destruction had HBD-1 level 12.9±4.4 µmol/L (median – 7.1 µmol/L), $p<0.05$ (Fig. 3).

Since HBD-1 is a natural cationic immune peptide, its synthesis also depends on the state of the protein-synthetic function of the liver, which was confirmed by positive correlations between the level of HBD-1 and total protein ($r = + 0.66$, $p <0.05$) and also between the level of HBD-1 and thymol test ($r = + 0.45$, $p <0.05$). The functioning of HBD-1 as an inflammatory agent was confirmed by its positive correlation with such a nonspecific marker of inflammation as ESR ($r = + 0.78$, $p <0.05$).

The study of the dynamics of HBD-1 in patients with drug-resistant tuberculosis showed its increase by the second month of treatment from 21.3±4.8 µmol/L (median - 3.7 µmol/L) to 42.1±8.2 µmol/L (median - 52.7 µmol/L), $p <0.05$ (Fig. 4).

Comparison of the HBD-1 level in patients with drug-resistant tuberculosis depending on the effectiveness of the intensive phase of anti-tuberculosis treatment showed that initial HBD-1 was significantly higher in patients who completed the intensive phase of treatment ineffectively (36.9±11.8 µmol/L, median - 30.4 µmol/L) than in those who completed it effectively (16.4±4.9 µmol/L, median - 3.2 µmol/L), $p<0.05$ (Fig. 5).

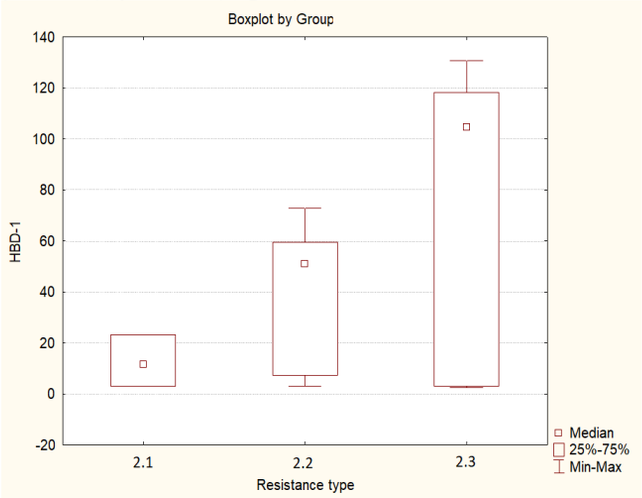

Figure 2. Comparison of Human-beta-defensin-1 level in patients with mono-resistant TB (Group 2.1), MDR-TB (Group 2.2) and XDR-TB (Group 2.3) after 2 months of anti-tuberculosis treatment

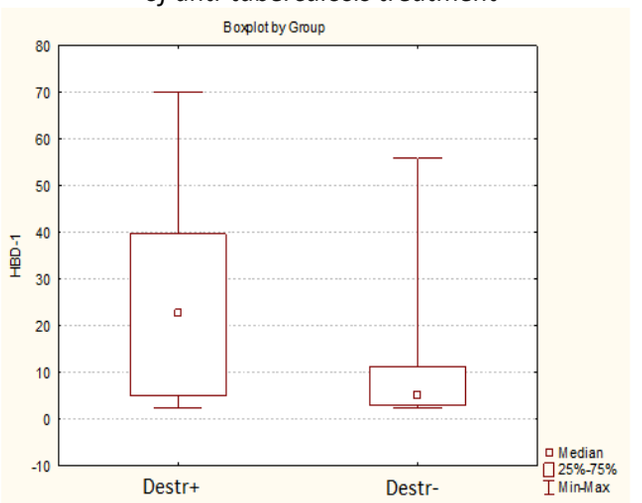

Figure 3. Comparison of Human-beta-defensin-1 level in patients with and without destruction of lung tissue

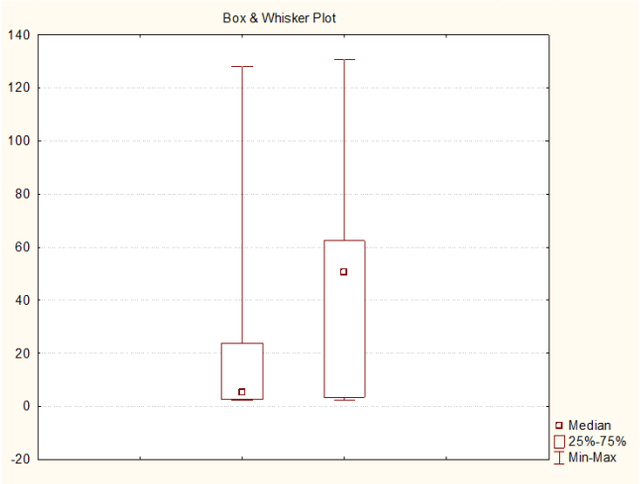

Figure 4. Dynamics of Human-beta-defensin-1 level in patients with drug-resistant tuberculosis

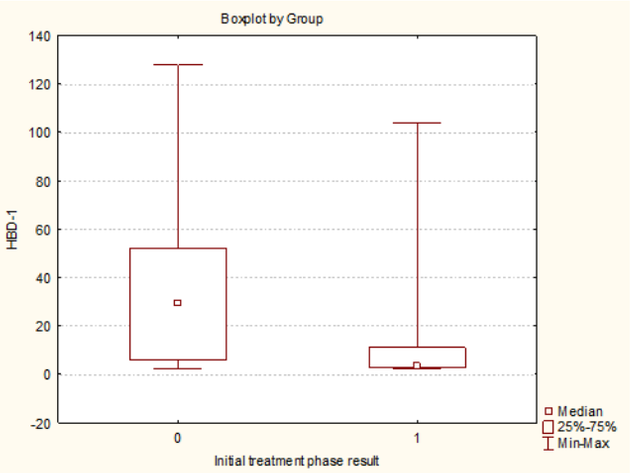

Figure 5. Comparison of Human-beta-defensin-1 level at the treatment onset in patients who completed initial phase of anti-tuberculosis treatment ineffectively (0) and effectively (1)

An increased initial HBD-1 level led to the persistence of a greater bacterial excretion at the 2nd month of treatment (r = +0.57). In addition, we found a correlation between an increased HBD-1 and impaired liver function, namely increased ALT (r = +0.55, p<0.05) and AST (r = +0.54, p <0.05).

It was interesting to compare the effect of different regimens of anti-tuberculosis treatment of MDR-TB and XDR-TB on the HBD-1 level. It was found that in patients who received new anti-tuberculosis treatment regimens with the addition of bedaquiline, the level of HBD-1 at the second month of treatment was significantly lower (19.1±13.4 µmol/L (median - 12.8 µmol/L) than in patients who received individualized 20-month courses of treatment without bedaquiline (45.7±5.3 µmol/L (median - 52.3 µmol/L), p<0.05 (Fig. 6).

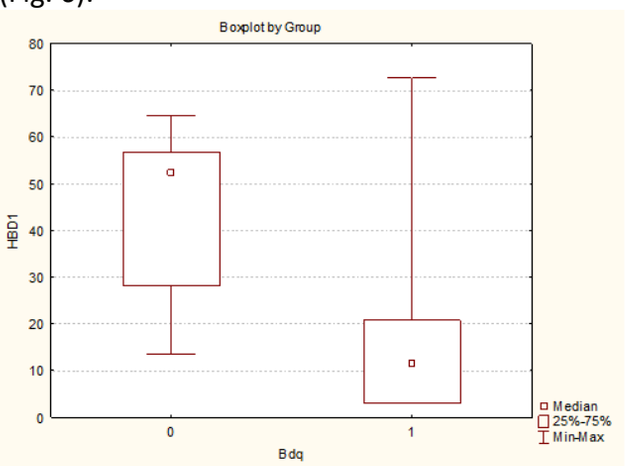

Figure 6. Comparison of Human-beta-defensin-1 level at the 2nd month of treatment in patients with MDR-TB and XDR-TB receiving treatment regimens with (1) and without (0) bedaquiline

At the same time, 100.0% of patients who received treatment regimens with the addition of bedaquiline had sputum conversion by the 2nd month of treatment, while patients treated without bedaquiline had sputum conversion be the 2nd month of treatment only in 73.1% of cases. (p <0.05).

Discussion. The obtained results of the comparison of the dynamics of the HBD-1 levels can be explained by the peculiarities of its production directly in response to the effect of microbial antigens [6]. Thus, at the beginning of treatment, when the antigenic load is high, all patients show increased HBD-1 level. After 2 months of treatment, the bacterial load in drug-susceptible TB is significantly reduced, patients start supportive phase of treatment and the production of HBD-1 also decreases. In drug-resistant tuberculosis, in particular MDR-TB and XDR-TB, a high activity and concentration of *M. tuberculosis* is still preserved at the 2nd month of treatment, which explains the persistence of high HBD-1 level in these patients. This fact is confirmed by the positive correlation of the average strength between the HBD-1 level and the massiveness of bacterial excretion at the 2nd month of treatment. Moreover, many patients with drug-resistant TB have severe intoxication and cachexy, so that the normalization of metabolism, particularly protein exchange, starts to normalize only by the 2nd month of treatment crating favorable conditions for the production of cationic peptides as factors of the immune response. This explains the growth the HBD-1 level in the group of patients with drug-resistant tuberculosis. Thus, in a study by Mao et al. in an animal model, normalization of protein metabolism by the introduction of amino acids and microelements was able to stimulate the production of β-defensin and increase the level of resistance of the host organism to microbial agents [9]. On the other hand, such an increase in HBD-1 levels may also be associated with the hepatotoxic effect of anti-tuberculosis drugs. A recent study by Kaltsa et al. showed a relationship between an increase of HBD-1 level and an increase of liver enzymes in patients with liver cirrhosis [8]. In our study, this hypothesis is confirmed by the positive correlation between the HBD-1 level and the levels of ALT and AST. In a study by Aksoy et al. [7] the same correlation was

observed in patients with Crimean-Congo hemorrhagic fever, but in their study such a correlation was a positive prognostic marker and was observed only in the group of survivors.

The introduction of new treatment regimens for MDR-TB and XDR-TB using bedaquiline makes it possible to accelerate the elimination of *M. tuberculosis*, which is confirmed by a significantly more frequent sputum conversion at the 2nd month of treatment. Therefore, in these patients we see lower level of HBD-1, which indicates a decrease in the severity of inflammatory reactions and a normalization of the immune response.

A promising direction is the study of the possibilities of HBD-1 using as a prognostic marker. In our study, a relationship between an increase of HBD-1 level and an increase of lesion size was found, as well as the formation of destruction of pulmonary tissue, which allows us to consider it as a marker of the severity of tuberculous lesion. In addition, the correlation between HBD-1 level and the massiveness of bacterial excretion at the 2nd month of treatment in patients with drug-resistant tuberculosis, as well as a higher initial level of HBD-1 in patients with an ineffective intensive phase of anti-tuberculosis therapy, allows using it as a prognostic marker of the anti-tuberculosis treatment effectiveness.

Conclusions. The spectrum of drug-resistance does not affect the level of Human-beta-defensin-1 in patients with pulmonary tuberculosis. The initial level depends on the pulmonary lesions size, the presence of bacterial excretion and liver function impairment. Treatment of drug-susceptible tuberculosis leads to a significant decrease in Human-beta-defensin-1 by the second month, in contrast to cases of drug-resistant tuberculosis. The dependence of the effectiveness of treatment of drug-resistant tuberculosis on the initial level of Human-beta-defensin-1 was revealed: the lower the level of Human-beta-defensin-1, the higher the treatment effectiveness, which makes it possible to use this parameter as a prognostic marker of the treatment effectiveness. The use of bedaquiline in treatment regimens for multidrug-resistant and extensively drug-resistant tuberculosis showed a more significant decrease in the level of Human-beta-defensin-1 by the second month of therapy with sputum conversion in all the patients, which confirms the effectiveness of new regimens for multidrug-resistant tuberculosis and extensively drug-resistant tuberculosis treatment.

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