ORIGINAL ARTICLE

HUMAN-BETA-DEFENSIN-1: PROGNOSTIC MARKER OF TUBERCULOSIS SEVERITY AND TREATMENT EFFECTIVENESS IN PULMONARY TUBERCULOSIS

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Olha O. Pohorielova, Olga S. Shevchenko
KHARKIV NATIONAL MEDICAL UNIVERSITY, KHARKIV, UKRAINE

ABSTRACT

The aim: Was to investigate human-beta-defensin-1 level in blood serum depending on tuberculosis severity and treatment effectiveness.

Materials and methods: 100 patients with pulmonary tuberculosis and 20 healthy persons were included to the study. HBD-1 level was measured by ELISA in all the healthy persons and in all the patients at the treatment onset and at the end of initial phase of treatment. Additionally, the patients were examined with chest X-ray, sputum microscopy and culture, blood test and blood biochemistry.

Results: HBD-1 level was higher in patients with tuberculosis (21.5 ± 2.9 μmol/L) compared with healthy individuals (8.9 ± 2.5 μmol/L). A positive correlation of middle strength was found between the size of lung lesion and the level of HBD-1 and between the level of HBD-1 and the massiveness of bacterial excretion. We found weakly negative correlations between the level of HBD-1 at the beginning of treatment and parameters of life quality rated on sf-36 scale. Patients with initially high level of HBD-1 had preservation of bacterial excretion, as well as signs of inflammatory activity. In patients with an effective intensive phase of treatment, the initial level of HBD-1.

Conclusions: The larger pulmonary tuberculosis lesion, as well as the more pronounced clinical manifestations lead to the higher level of HBD-1. The possibility of using human-beta-defensin-1 as a prognostic marker of treatment effectiveness is confirmed by the fact that human-beta-defensin-1 level prevails at the beginning of treatment in patients with subsequently non-effective intensive phase of treatment.

KEY WORDS: Human-Beta-Defensin-1, tuberculosis, life quality, diagnosis

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INTRODUCTION

According to WHO, about 10 million people fall ill with tuberculosis annually, while 1.2 million die. This makes tuberculosis one of the 10 most common causes of death in the world. Moreover, about 400 thousand of new TB multi-drug resistant cases are found annually [1].

There are various ways to prevent the development of resistance and increase treatment effectiveness. They are increasing patient's adherence to treatment, strengthening compliance with the therapy regimen, predicting possible treatment failure in the early stages, followed by intensification of the therapy regimen. Thus, study of prognostic markers of the tuberculosis course becomes current issue. Human-Beta-Defensin-1 (HBD-1) can be one of these markers.

β-defensins are small endogenous cationic peptides with an additional N-terminal α-helix, the length of which varies from 33 to 47 amino acid residues (3-5 kDa). They have wide spectrum of microbicidal activity against bacteria, some fungi and viruses [2]. The positive charge of β-defensins allows them to easily interact with negatively charged components on the surface of bacterial cells and integrate into the lipid bilayer, damaging the membrane, causing leakage of ions and metabolites and malfunctioning of membrane-bound protein complexes [3]. In a study by Nurjadi et al. a trend to the development of chronic bacterial infections with the inability to eliminate pathogens in patients with β-defensin deficiency associated with cystic fibrosis was demonstrated [4]. In addition to bactericidal action, β-defensins also have immunomodulating activity, selective inflammatory and anti-inflammatory properties, and additional reparative activity [5].

β-defensins are produced in most organs and systems of the body, including the respiratory system, neutrophils, natural killers, certain types of T-helper cells, monocytes, dendritic cells, and platelets [6]. The largest amount of HBD-1 is produced by epithelial cells, mainly in the respiratory tract [7]. Its expression is stimulated primarily by TNF-α, IL-1, as well as by the direct action of bacterial agents, which makes it potentially sensitive marker of the lesion severity [8]. In addition, the production of HBD-1 stimulates the production of other types of β-defensins (BD-2, -3, -4), continuing the cascade of immune response. In fact, HBD-1 is the only β-defensin that has a basic level of production and is also produced directly under the influence of exogenous factors (pathogens) without endogenous intermediaries. It is noteworthy that HBD-1 is a multifunctional modulator, exhibiting not only innate antimicrobial activity (non-specific bactericidal action associated with inhibition of bacterial cell wall synthesis,

prevention of biofilm formation, as well as direct damage of the cell wall), which provides innate immunity, but also mediates the acquired immune response [9]. Thus, HBD-1 are chemoattractants for CD4+ T-helpers and immature dendritic cells (by binding to the chemokine receptor CCR6), as well as macrophages [3].

These features make it promising to study HBD-1 as a marker of tuberculosis severity and a possible predictor of the treatment effectiveness, as well as a possible additional agent in the pathogenetic treatment of tuberculosis.

THE AIM

The aim of the study was to investigate human-beta-defensin-1 level in blood serum depending on tuberculosis severity and treatment effectiveness.

MATERIALS AND METHODS

100 patients with pulmonary tuberculosis (75 men and 25 women; mean age – 41.8±1.2 years) and 20 healthy persons (13 men and 7 women; mean age – 38.1±1.4 years) were included to the study. HBD-1 level was measured by ELISA (HBD-1 Elisa Kit Genway, USA) in all the healthy persons and all the patients at the treatment onset. They were also interviewed by sf-36 questionnaire to determine life quality. Additionally, the patients were examined according to current TB guidelines, which included chest X-ray (at the treatment onset and in the end of initial treatment phase), sputum microscopy and culture (at the treatment onset and in the end of initial treatment phase for patients with drug-susceptible tuberculosis or monthly for patients with drug-resistant tuberculosis), blood test and blood biochemistry monthly. Criteria for tuberculosis severity were: size of lesion (from one lobe to both lungs), presence of destruction and the massiveness of bacterial excretion detected by the microscopy (from 0 to 3). Treatment was provided according to current guidelines: 4 drugs (Isoniazid, Rifampicin, Pyrazinamide, Ethambutol) for drug-susceptible tuberculosis and individual treatment regimens for drug-resistant tuberculosis. In 2 months of treatment, the patients who have not been excluded from the study due to death or treatment interruptions, were examined again. Later the patients were divided into 2 groups depending on initial phase treatment results. Treatment was considered effective after sputum conversion and good clinical and X-ray dynamics (compaction of infiltrates and reduction of cavities). Thus, we had 2 groups: Group 1 (n=77) – patients with effective initial phase of treatment; Group 2 (n=23) – patients with ineffective initial phase of treatment. As non-parametric distribution was found in groups, statistical data processing was carried out using Statistica 8.0 with Mann-Whitney test for comparing two independent groups and Spearman correlation coefficient. Data were considered reliable at $p<0.05$.

RESULTS

When comparing the study group and the control group, a predominance of HBD-1 level was revealed in patients with tuberculosis (21.5 ± 2.9 μmol/L, median – 7.6 μmol/L) compared with healthy individuals (8.9 ± 2.5 μmol/L, median – 1.9 μmol/L), $p<0.05$, Fig. 1.

Significant differences were found between the quality of life in the group of healthy control and patients with tuberculosis in parameters of physical functioning (PF) (group patients – 55.2 ± 3.0, control group – 95.0 ± 0.8), role-physical functioning (RP) (group of patients – 27.8 ± 4.0, control group – 100.0), pain intensity (BP) (group of patients – 88.0 ± 2.9, control group – 100.0), general health (GH) (group of patients – 19.0 ± 1.8, control group – 91.5 ± 1.2) , vitality (VT) (group of patients – 21.7 ± 2.6, control group – 68.8 ± 1.0), social functioning (SF) (group of patients – 49.0 ± 3.3, control group – 100.0), role-emotional functioning (RE) (group of patients – 32.3 ± 3.9, control group – 100.0) and general mental health (MH) (group of

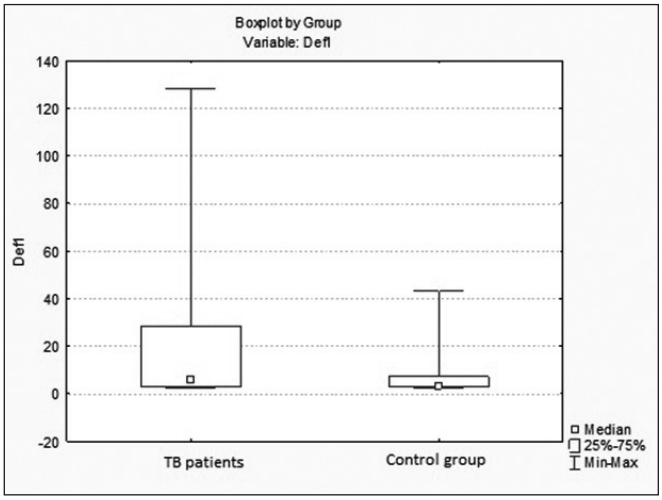

Fig. 1. Comparison of HBD-1 level in tuberculosis patients and healthy persons

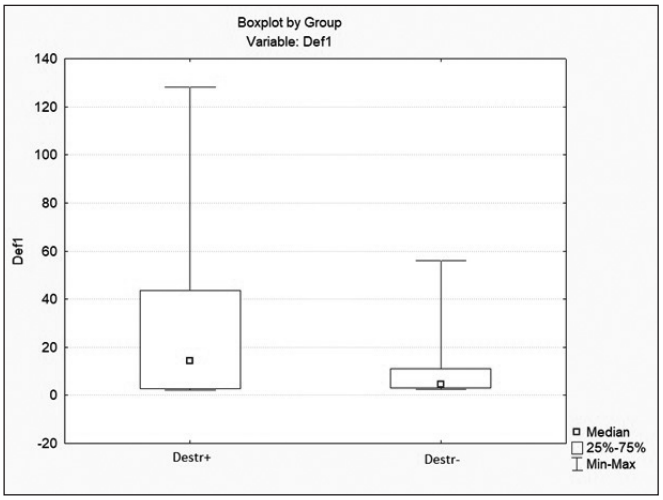

Fig. 2. Comparison of HBD-1 in patients with pulmonary tissue destruction (Destr+) and in patients without pulmonary tissue destruction (Destr-)

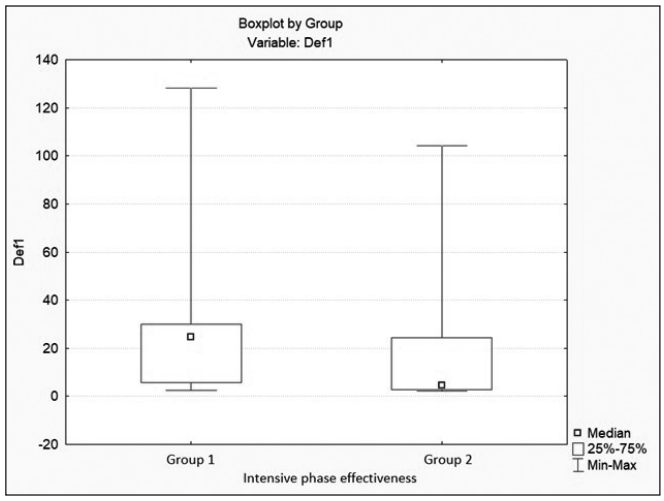

Fig. 3. Comparison of initial HBD-1 level in patients with effective (Group 1) and ineffective (Group 2) initial phase of treatment

patients – 41.8 ± 2.0, control group – 68.0 ± 0.6) (p <0.01). Comparison of the level of HBD-1 in patients with the presence and absence of lung tissue destruction showed a significant difference (p<0.05) with the predominance of HBD-1 in the patients with destruction (27.1 ± 4.7 μmol/L, median – 17.4 μmol/L) over the level of HBD-1 in patients with no lung destruction (10.9 ± 2.7 μmol/L, median – 5.5 μmol/L), Fig. 2.

When searching for correlations between the HBD-1 level and the severity of tuberculosis at the beginning of treatment, a positive correlation of middle strength was found between the size of lung lesion and the level of HBD-1 – r = + 0.53, p <0.05, and also between the level of HBD-1 and the massiveness of bacterial excretion detected by microscopy – r = + 0.55, p <0.05, and by culture on solid nutrient Lowenstein-Jensen medium – r = + 0.51, p <0.05. The dependence of the HBD-1 level on the size of the lesion and the massiveness of bacterial excretion is presented in Table I.

Patients underwent an assessment of the severity of the clinical manifestations of tuberculosis, where 1 point was assigned for each of the symptoms (cough, shortness of breath, weight loss, pain, weakness), and a weakly positive correlation was found between the HBD-1 level and the severity of local and general symptoms – r = +0.44, p<0.05, including medium strength correlation with body temperature – r = + 0.50, p<0.05. A weakly positive correlation was also found between the level of HBD-1 and ESR – r = +0.49, p<0.05. We found weakly negative correlations between the level of HBD-1 at the beginning of treatment and parameters of physical functioning (r = -0.48), role functioning (r = -0.46), general health (r = -0.42) and role-emotional functioning (r = -0.47), rated on sf-36 scale, p<0.05.

Table I. Dependence of HBD-1 level on lesion size and massiveness of bacterial excretion

Parameter of tuberculosis severity	HBD-1 level (μmol/L)
Size of lesion	
1 lobe	8.2±2.2
1 lung	18.5±6.1
2 lungs	28.4±5.2
Massiveness of bacterial excretion	
0	11.5±3.5
1+	26.8±5.9
2+	29.7±5.1
3+	36.7±19.4

An assessment of the patients' state after 2 months of treatment showed that patients with initially high level of HBD-1 had preservation of bacterial excretion, which was expressed in a positive correlation of medium strength (r = + 0.55, p <0.05), as well as signs of inflammatory activity, which was expressed in a positive correlation of middle strength with ESR (r = + 0.50, p <0.05).

Assessment of the initial level of HBD-1 as a prognostic marker of the effectiveness of the intensive phase of treatment showed that in patients with an effective intensive phase of treatment (Group 1), the initial level of HBD-1 was significantly (p <0.05) lower (18.9 ± 3.4 μmol/L, median – 8.2 μmol/L) than in the group with ineffective initial phase pf treatment (Group 2) (34.9 ± 8.6 μmol/L, median – 22.9 μmol/L), Fig. 3.

Table II. Comparison of quality of life in patients with effective and ineffective initial phase of anti-tuberculosis therapy at the second month of treatment

Parameter	Group 1 – Effective initial phase	Group 2 – Ineffective initial phase
Physical Functioning (PF)*	75.6±2.3	54.3±4.8
Role-Physical Functioning (RP)*	60.0±4.9	10.7±5.1
Bodily Pain (BP)	95.8±1.9	76.9±9.6
General Health (GH)*	38.3±2.5	16.4±3.4
Vitality (VT)*	34±2.5	19.3±3.2
Social Functioning (SF)*	63.5±2.2	45.7±5.1
Role-Emotional Functioning (RE)*	72.7±5.0	14.3±6.7
Mental Health (MH)*	53.9±1.7	42.3±2.9

*significant differences between groups, p<0.05

At the same time, the quality of life parameters evaluated on the sf-36 scale in patients in Groups 1 and 2 did not differ at the beginning of treatment ($p > 0.05$), however, after 2 months of anti-tuberculosis therapy, significant differences were found for all parameters, except bodily pain, which is shown in Table II.

A study of the level of HBD-1 after 2 months of treatment revealed its middle positive correlation with the level of bacterial excretion ($r = +0.63$), ESR ($r = +0.66$), alkaline phosphatase ($r = +0.62$), as well as role functioning ($r = + 0.61$), general health ($r = + 0.56$) and role-emotional functioning ($r = + 0.61$), $p < 0.05$.

DISCUSSION

In the context of the spread of drug-resistant *M. tuberculosis*, the study of the innate protective factors of the host organism and their interaction with each other and with the pathogen becomes more and more relevant for expanding the possibilities of diagnosing and predicting the course of the disease, as well as enhancing therapeutic possibilities. One of the components of natural defense is the production of antimicrobial peptides such as β-defensins by respiratory tract epithelial cells that can be produced constitutively, that is, have a basic level of production like HBD-1, or are induced by cytokines and bacterial components (β-defensins-2 , -3, -4) such as polysaccharides, by transcription factors [7]. The study of β-defensins in this aspect is the most promising, since a number of studies have shown that they exhibit significant activity precisely in chronic infections, which can also include tuberculosis [10].

HBD-1 is an atypical chemokine capable of activating chemokine receptors (for example, CCR6, CCL20) and mediate the chemotactic response for immature dendritic cells and memory T cells [11]. The activity of HBD-1 against nonspecific pathogens, in particular *S. aureus* and *P. aeruginosa*, as well as organisms capable of collective defense methods, such as the formation of biofilms, and provoking chronic infectious inflammation [13], has been studied in detail [12], however, data on its antibacterial activity against specific pathogens, namely *M. tuberculosis*, are insufficient. Like other antimicrobial cationic peptides, HBD-1 has several bactericidal mechanisms associated with cell wall damage: destruction of the lipid bilayer of the membrane, attachment to negative phospholipid groups on the surface of the membrane, capsular polysaccharides with subsequent formation of pores in the membrane and leakage cytosolic contents [14]. In a study by Moser et al., it was demonstrated in an experimental mouse model that HBD-1 deficiency delays elimination of pathogens and enhances colonization of pathogens in host tissue [15]. The study of HBD-1 is also interesting because it is the only one of the family of β-defensins that has a basic level of production and is induced directly by bacterial agents, which in turn stimulates the production of other β-defensins (-2, -3, -4), which suggests that the change in the level of HBD-1 will be the earliest and will directly respond to changes in the bacterial load and the severity of tuberculosis [16].

Elevation of BD-1 level in patients with pulmonary tuberculosis compared with the control group, as well as in patients with more severe tuberculosis lesions compared with patients with less severe lesions, confirms the theory of the possibility of using HBD-1 as a marker of the tuberculosis severity. The severity of the tuberculosis in this case can be estimated by the size of pulmonary lesion (from one lobe to the entire pulmonary field), -as well as by the presence or absence of lung tissue destruction. Similar results were observed for other pathogens, such as *E. coli*, *C. albicans*, many gram-positive and gram-negative bacteria and other pathogens, as described in the literature [5-7].

The correlations between the initial level of HBD-1 and the massive bacterial excretion during treatment, as well as the revealed significant difference in the concentration of HBD-1 in patients with effective and ineffective treatment at the end of the intensive phase, allow us to consider HBD-1 as a possible prognostic marker for tuberculosis treatment effectiveness, which can be used to strengthen the chemotherapy regimen in the initial stages in order to achieve greater treatment effectiveness. Similar dynamics of HBD-1 was described by Kumar et al. in patients with tuberculosis and diabetes mellitus comorbidity [17]. Considering the fact that the clinical and radiological evaluation of the effectiveness of anti-TB treatment requires the patient to have fibrosis and healing of lung tissue destruction, an interesting direction in further research may be the identification of the connections between HBD-1 and other inflammatory mediators, as well as destruction and fibrosis factors, in particular with matrix metalloproteinases, which has already been partially described by Wilson et al. [18].

Particulate attention should be payed to a more detailed further study of the relationships of HBD-1 and liver enzymes. In a study by Tsuruta et al. The role of alkaline phosphatase in the functioning of leukocytes and their production of cationic peptides, including HBD-1, was shown [19]. These data were also confirmed in our study as a positive correlation between the level of alkaline phosphatase and HBD-1. Considering also the relationship between the state of protein metabolism and the production of HBD-1 [20], as well as its chemotactic [21] and bactericidal [22-23] functions, further studies of this question may turn out to be promising.

CONCLUSIONS

As a result of the study, it was found that the human-beta-defensin-1 level was significantly higher in tuberculosis patients than in the control group. In addition, it was found that the larger pulmonary tuberculosis lesion, as well as the more pronounced manifestations of local and general symptoms of tuberculosis lead to the higher level of human-beta-defensin-1. A significantly higher level of human-beta-defensin-1 was detected in patients with lung tissue destruction compared with patients without destruction. In other words, the more severe tuberculous lesion leads to the higher the level of human-beta-defen-

sin-1, which allows it to be used as one of the indicators of the severity of the tuberculous process. The possibility of using human-beta-defensin-1 as a prognostic marker of treatment effectiveness is confirmed by the fact that human-beta-defensin-1 level prevails at the beginning of treatment in patients in whom the intensive phase of anti-tuberculosis treatment subsequently was ineffective. These features of human-beta-defensin-1 make it possible to use it as a diagnostic parameter for the severity of the tuberculosis and as a prognostic marker for the effectiveness of anti-tuberculosis therapy, which allows predicting the possible ineffectiveness of anti-tuberculosis therapy at the beginning of treatment and, if necessary, strengthening the chemotherapy regimen.

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ORCID and contributionship:

Olha O. Pohorielova: 0000-0003-4819-9373 [A,B,C,D]

Olga S. Shevchenko: 0000-0002-5476-3981 [E,F]

CORRESPONDING AUTHOR

Olha O. Pohorielova

Kharkiv National Medical University

4 Nauky av., 61062 Kharkiv, Ukraine

tel: +380962802713

e-mail: evildevilolga@gmail.com

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