UDC 615.45:618.13-08
DOI – https://doi.org/10.14300/mnnc.2021.16035
ISSN – 2073-8137

ESTIMATION OF BLOOD ANTIMICROBIAL PEPTIDE LEVELS IN WOMEN OF REPRODUCTIVE AGE WITH PELVIC INFLAMMATORY DISEASE BEFORE AND AFTER ANTIBIOTIC THERAPY

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ОЦЕНКА УРОВНЯ АНТИМИКРОБНЫХ ПЕПТИДОВ В КРОВИ У ЖЕНЩИН РЕПРОДУКТИВНОГО ВОЗРАСТА С ВОСПАЛИТЕЛЬНЫМИ ЗАБОЛЕВАНИЯМИ ОРГАНОВ МАЛОГО ТАЗА ДО И ПОСЛЕ АНТИБАКТЕРИАЛЬНОЙ ТЕРАПИИ

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Blood α-defensin-1 (DEFa1) and β-defensin-1 (DEFb1) levels in 56 women of reproductive age with pelvic inflammatory disease were evaluated before and after antibiotic therapy. DEFa1 levels were decreased by 20 % after tetracycline monohydrate therapy (n=22), by 15.6 % after levofloxacin therapy (n=18), and by 25 % after josamycin therapy (n=16) compared with before therapy. DEFb1 levels were increased by 17 % after tetracycline monohydrate therapy, by 45 % after josamycin therapy, and by 80 % after levofloxacin therapy compared with before therapy. Our study shows that blood antimicrobial peptide levels in women with pelvic inflammatory disease are different and depend on the antibacterial drug used.

Keywords: antimicrobial peptide, α-defensin-1, β-defensin-1, pelvic inflammatory disease, antibiotic therapy, levofloxacin, josamycin, tetracycline monohydrate

Были оценены уровни α-дефензина-1 (DEFa1) и β-дефензина-1 (DEFb1) в сыворотке крови у 56 женщин репродуктивного возраста с воспалительными заболеваниями органов малого таза (ВЗОМТ) до антибактериальной терапии и после неё. Концентрация DEFa1 после терапии тетрациклином моногидратом (n=22) уменьшилась на 20 %, левофлоксацином (n=18) – на 15,6 %, джозамицином (n=16) – на 25 %. В свою очередь, содержание DEFb1 после лечения тетрациклином моногидратом увеличилось на 17 %, джозамицином – на 45 %, левофлоксацином – на 80 %.

Ключевые слова: антимикробные пептиды, α-дефензин-1, β-дефензин-1, воспалительные заболевания органов малого таза, антибактериальная терапия, левофлоксацин, джозамицин, тетрациклина моногидрат.

For citation: Baturin V. A., Boshyan R. O. ESTIMATION OF BLOOD ANTIMICROBIAL PEPTIDE LEVELS IN WOMEN OF REPRODUCTIVE AGE WITH PELVIC INFLAMMATORY DISEASE BEFORE AND AFTER ANTIBIOTIC THERAPY. *Medical News of North Caucasus.* 2021;16(2):159-161. DOI – https://doi.org/10.14300/mnnc.2021.16035

Для цитирования: Батурин В. А., Бошян Р. О. ОЦЕНКА УРОВНЯ АНТИМИКРОБНЫХ ПЕПТИДОВ В КРОВИ У ЖЕНЩИН РЕПРОДУКТИВНОГО ВОЗРАСТА С ВОСПАЛИТЕЛЬНЫМИ ЗАБОЛЕВАНИЯМИ ОРГАНОВ МАЛОГО ТАЗА ДО И ПОСЛЕ АНТИБАКТЕРИАЛЬНОЙ ТЕРАПИИ. *Медицинский вестник Северного Кавказа*. 2021;16(2):159-161.
DOI – https://doi.org/10.14300/mnnc.2021.16035

AMP – antimicrobial peptide
AMR – antimicrobial resistance
DEFa1 – α-defensin-1
DEFb1 – β-defensin-1
PID – pelvic inflammatory disease
r – Pearson's correlation coefficient

Antimicrobial resistance (AMR) is a growing threat to public health and economic growth [1]. Immunological reactivity disorder is one of the causes of antimicrobial resistance. AMR contributes to a change in the microbiocenosis of the urogenital tract and antimicrobial peptide (AMP) levels [2, 3].

AMPs are the primary effectors of the innate immune system. They perform the function of natural antibiotics [4, 5]. Defensins are a family of AMPs, and they have broad-spectrum antimicrobial activity against gram-positive and gram-negative bacteria, viruses, fungi, and protozoa. Defensins participate in cell-mediated

immunity and are chemoattractants for immature dendritic cells [6–8].

Pathogenic bacteria are affected by the action of endogenous AMPs. AMPs are considered as possible therapeutic agents against microorganisms [9, 10]. AMPs have the following properties that make them superior to existing antibacterial drugs: 1) AMPs are active at micromolar concentrations; 2) the resistance of microorganisms is limited to AMPs, and microbes do not significantly change the target or the content of the cell wall; 3) AMPs are synergistic with conventional antibiotics, and thus antibiotics can be designed to enhance their effect [11–13].

This study aimed to determine blood α-defensin-1 (DEFa1) and β-defensin-1 (DEFb1) levels in women of reproductive age with pelvic inflammatory disease (PID) before and after antibiotic therapy.

Material and Methods. Fifty-six patients of reproductive age from 18 to 45 years old with PID were examined. Smears for vaginal and cervical flora, and bacterial cultures from the urethra, cervical canal, and posterior vaginal fornix were obtained (the classical bacteriological method with sensitivity of selected microorganisms to antibacterial drugs was used). Real-time polymerase chain reaction was performed for flora and smears of atypical cells by the Pap test (Papanicolaou smear). Vaginal biocenosis was assessed by Amsel and Nugent criteria. DEFb1 and DEFa1 levels were quantified in the blood serum of the women using an enzyme-linked immunosorbent assay kit (Cloud-Clone Corp., USA) according to the manufacturer's instructions. Venous blood sampling was performed in 56 women before antibiotic therapy and 1.5–2 months after antibiotic therapy. Doses of drugs and the duration of treatment were individually determined. The results of bacteriological culture of flora from the urogenital tract and sensitivity to antibiotics were taken into account. The choice of drugs was made in accordance with the clinical recommendations of the Russian Society of Obstetricians and Gynecologists for the diagnosis and treatment of diseases identified by pathological discharge from the genital tract of a woman. Tetracycline monohydrate 100 mg was administered two times a day orally for 10–14 days, josamycin 500 mg three times a day for 7–10 days, and levofloxacin 250 mg one or two times a day for 5–7 days.

The clinical effectiveness of the therapy was evaluated on a scale comprising the following criteria: 1) quantitative characteristic of vaginal excretion (moderate or plentiful); 2) the nature of the discharge (normal or pathological); 3) smell (periodic or constant); 4) itching (mild, moderate, or severe); 5) burning in the vagina (weak, moderate, or severe); 6) dysuria (frequent urination, burning, or pain when urinating); 7) dyspareunia; 8) the ratio of leukocytes to epithelial cells in smears of the flora (low, moderate, or severe degree); 9) the prevailing pathogen in the microflora of the urogenital tract (conditionally pathogenic or pathogenic); 10) human papilloma virus and its viral load (low, medium, or pronounced clinical significance); and 11) cytological characteristic (results of smears of atypical cells or atypical cells according to Papanicolaou). Scores were evaluated before and after antibiotic therapy. A score of 0–2 points indicated that the infectious process was absent, 2–6 points indicated weak severity of infection, 7–18 points indicated moderate severity, and 19–30 points indicated severe infection [14].

Informed consent was obtained from each patient. Exclusion criteria were gonorrheal cervicitis or herpetic cervicitis. The local ethics committee approved all stages of the study.

Data Processing. The data were statistically analyzed by the unpaired Student's *t*-test, the Mann–Whitney test, and Pearson's correlation coefficient. The selective distribution of quantitative traits was described by the number (n) of patients in the study, median values, and lower (25 %) and upper (75 %) quartiles (Q1 and Q3). Statistical significance was considered as $p < 0.05$.

Results and Discussion. In women with PID, we found aerobic vaginitis (n=18, 32 %), *E. faecalis* (n=15, 26 %), *C. trachomatis* (n=5, 9 %), *E. coli* (n=4, 7 %), *K. aerogenes* (n=3, 5 %), vaginal candidiasis as part of microbial associations (n=3, 5 %), chronic cervicitis (n=3, 5 %), *Ureaplasma* (n=2, 5 %), *S. aureus* (n=1, 2 %), *S. agalactiae* (n=1, 2 %), and *Mycoplasma genitalium* (n=1, 2 %).

Patients were divided into the following three groups: group I comprised patients (n=22, 39 %) who received tetracyclines (tetracycline monohydrate), group II comprised patients (n=18, 32 %) who received fluoroquinolones (levofloxacin), and group III comprised patients (n=16, 29 %) who received macrolides (josamycin). AMP levels in women were quantified before and after antibiotic therapy. DEFa1 levels were decreased by 20 % in group I, 15.6 % in group II, and 25 % in group III after antibiotic therapy compared with before antibiotic therapy (Table 1). DEFb1 levels were increased by 17 % in group I, 80 % in group II, and 45 % in group III after antibiotic therapy compared with before antibiotic therapy (Table 2).

Table 1

Blood DEFa1 levels in women (n=56) with PID before and after antibiotic therapy

AMP (ng/ml)	Group I (tetracycline monohydrate), n=22	Group II (levofloxacin), n=18	Group III (josamycin), n=16	p1;3	p2;3	p1;2
Before therapy	42.2 [35.7–49.2]	41.6 [36.3–48.1]	39.3 [32.1–46.5]	0.036	0.127	0.06
After therapy	33.7 [25.8–42.3]	35.1 [28.3–42.3]	29.6 [22.3–36.6]	0.094	0.1	0.05

Note. Values are median and interquartile range [Q1–Q3] (25th and 75th percentiles).
p values were obtained by the Mann–Whitney test.

Table 2

Blood DEFb1 levels in women (n=56) with PID before and after antibiotic therapy

AMP (ng/ml)	Group I (tetracycline monohydrate), n=22	Group II (levofloxacin), n=18	Group III (josamycin), n=16	p1;3	p2;3	p1;2
Before therapy	21.8 [13.5–28.7]	27.1 [20.1–34.2]	24.9 [18.8–33.2]	0.39	0.339	0.27
After therapy	25.6 [17.3–33.2]	48.8 [39.8–56.7]	36.1 [29.1–43.3]	0.48	0.26	0.18

Note. Values are median and interquartile range (25th and 75th percentiles).
p values were obtained by the Mann–Whitney test.

Investigation of the dependence of AMP levels on microorganisms of the urogenital tract before and after antibiotic therapy showed the following. Women with chlamydial cervicitis (n=5) received josamycin. DEFa1 levels were decreased by one third (30.6 [26.5–34.3]) and DEFb1 levels were increased by 156.7 % (30.6 [26.5–34.3]) after therapy compared with before therapy.

In cases of aerobic vaginitis (n=18), DEFa1 levels were decreased by 35 % (32.8 [28.6–39.4]) in group I (n=3), 22 % (32.6 [28.7–37.3]) in group II (n=12), and 3.3 % (34.1 [29.3–38.2]) in group III (n=3) after therapy compared with before therapy. DEFb1 levels were increased by 27 % (37.5 [33.1–41.2]) in group I, 92 % (58 [50.9–65.3]) in group II, and 30 % (38.1 [34.5–43.1]) in group III after therapy compared with before therapy.

In patients with *E. faecalis* (n=15) in microflora of the urogenital tract, DEFa1 levels were decreased by 27 % (35.1 [30.5–41.3]) in group I (n=10) and 20 % (27.2 [20.2–34.8]) in group III (n=5) after therapy compared with before therapy. DEFb1 levels were increased by 30.2 % (23.7 [17.5–30.3]) in group I and 14 % (23.1 [18.1–28.6]) in group III after therapy compared with before therapy.

The severity of the infectious and inflammatory process before therapy was scored as 17.5±1.1 points in group I, 13.7±0.9 points in group II, and 22.1±1.1 points in group III. After antibiotic therapy, the severity of the infectious and inflammatory process was scored as 1.9±0.2 points in group I, 1.7±0.1 points in group II, and 1.8±0.2 points in group III.

In our earlier study, 120 patients with PID showed the following nosological forms: cervicitis associated with *Enterococcus faecalis* (n=23; 19 %) and *Candida* spp. (n=23; 19 %), bacterial vaginosis (n=11; 9 %), cervicitis associated with *Ureaplasma urealyticum* (n=8; 7 %), *Chlamydia trachomatis* (n=6; 5 %), *Escherichia coli* (n=6; 5 %), *Klebsiella aerogenes* (n=3; 2 %), and *Staphylococcus aureus* (n=2; 2 %) [15]. Aerobic vaginitis was diagnosed when a pathogen was not detected and the ratio of leukocytes to epithelial cells in smears was low [16].

Conclusions. DEFa1 levels are decreased by 20 % and DEFb1 levels are increased by 17 % after tetracycline therapy. DEFa1 levels are decreased by 15.6 % and DEFb1 levels are increased by 80 % after fluoroquinolone therapy. DEFa1 levels are decreased by 25 % and DEFb1 levels are increased by 45 % after macrolide therapy. The clinical efficacy of tetracycline monohydrate therapy is 82 %, levofloxacin is 87 %, and josamycin is 92 %. This study shows that blood AMP levels in women with PID are different and depend on the antibacterial drug used. However, extensive clinical trials with a large number of patients with PID and multidirectional antibacterial drugs need to be conducted in the future.

Disclosures: The authors declare no conflict of interest.

Acknowledgments. We thank Ellen Knapp, PhD, from Edanz (www.edanz.com/ac), for editing a draft of this manuscript.

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