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NEUTROPHIL EXTRACELLULAR TRAPS IN THE BLOOD OF PATIENTS WITH CHRONIC KIDNEY DISEASE OF VARIOUS STAGES

SUMMARY

We studied the possibility of the formation of neutrophil extracellular traps in patients with chronic kidney disease different stages. The object of the study: whole blood of 63 patients with chronic kidney disease distributed in five groups depending on the degree of destruction of renal parenchyma. Formation of net-like structures during NETosis of neutrophil, called neutrophil extracellular traps, having in its composition of DNA, histones, defensins and enzymes (elastase, myeloperoxidase, gelatinase, lactoferrin and lysozyme), was triggered by interaction with lipopolysaccharide substances of exogenous (bacteria) and autologous origin (products of oxidative metabolism), which act through the Fcγ-receptors and activate NADPH-oxidase complex. The results of our study revealed the following picture of the detection of neutrophil extracellular traps: neutrophil extracellular traps were detected in 33 % of patients with chronic kidney disease 1 stage; amount was in the range from 4 to 10 in 100 neutrophils, in the group with 2 stage – in 5 % of patients; amount – 4 in 100 neutrophils, with 3 stage – in 25 % of patients; amount was in the range from 1 to 7 in 100 neutrophils, with 4 stage – in 33 % of patients in an amount from 2 to 12 in 100 neutrophils, in the group with 5 stage – in 35 % of patients in an amount from 2 to 14 in 100 neutrophils. Thus, the highest frequency of detection and the maximum amount of neutrophil extracellular traps was noted in the groups of patients with chronic kidney disease 1, 4 and 5 stages. Some features in morphology of neutrophil extracellular traps different types have been found. Distinctive feature of the first type of neutrophil extracellular traps was hypertrophy of core segments with the concentration of the pellets inside the net-like DNA. Distinctive feature of the second type of neutrophil extracellular traps was rupture of membrane structures of organelles and plasmolemma of neutrophils. The appearance of neutrophil extracellular traps is an unfavorable factor that provokes the activation of procoagulant mechanism of the hemostatic system.

Key words: neutrophil granulocytes, neutrophil extracellular traps, chronic kidney disease.

Introduction. Neutrophil granulocytes, after contact with microorganisms and exogenous cytotoxic agents autogenic origin, throw out into the extracellular space net-like structures, which include DNA, histones, defensins and enzymes such as elastase, myeloperoxidase, gelatinase, lactoferrin and lysozyme. These structures have been named as «neutrophil extracellular traps» (NETs). Formation of NETs – is an alternative neutrophil death, called NETosis. Morphological differences of NETosis compared with apoptosis and necrosis are the decay of the nucleus membrane before disintegration of the cytoplasmic membrane, mixing of nuclear and cytoplasmic material, reorganization of cell cytoskeleton and disappearance of cytoplasmic organelles [2, 4, 7].

NETs formation is studied in various pathological conditions including acute renal lesions. There are studies related to the assessment of the role of extracellular histones and NETs in acute renal lesions [3]. Study of NETs in patients with CKD is not conducted.

The aim of study: identify the possibility of the formation of neutrophil extracellular traps in the blood of patients with chronic kidney disease (CKD) of various stages.

Materials and methods: Object of study is whole blood of 63 patients with CKD. All patients were divided into five groups according to the degree of destruction of renal parenchyma. A group of patients with CKD 1 consisted of 12 persons, with CKD 2 – 5 persons, with CKD 3 – 20 persons, with CKD 4 – 12 persons, with CKD 5 – 14 persons. The control group was represented by 13 healthy donors. Each of the examined gave informed consent to participate in the study.

Detection of neutrophil extracellular traps was performed by the method of Dolgushin et al [1]. Staining of blood smears was performed by the method of May-Grunwald. Chi-square test and Fisher criterion were used for statistical analysis.

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Results and discussion. According to obtained data, NETs were detected in 33 % of patients with CKD 1; amount of NETs was in the range from 4 to10 in 100 neutrophils. In the group with CKD 2 NETs were detected in 5 % of patients; amount – 4 in 100 neutrophils. In the group with CKD 3 NETs were detected in 25 % of patients; amount was in the range from 1 to 7 in 100 neutrophils. In the group with CKD 4 NETs were detected in 33 % of patients in an amount from 2 to 12 in 100 neutrophils. Finally, in the group with CKD 5 NETs were detected in 35 % of patients in an amount from 2 to 14 in 100 neutrophils. In the control group, NETs were not found.

Absence of significant differences in an amount of NETs between the groups of patients with CKD of various stages may be due to insufficient sample size and requires further research with increasing the number of patients in these groups.

Analysis of morphological features of NETs, detected in the blood of patients with CKD different stages, showed the presence of 2 types of NETs (Figure 1 and Figure 2).

Distinctive features of the first type of NETs: the conservation the nuclear apparatus of the cell with hypertrophy of its segments, neutrophil granules are concentrated inside of the formed trap. Distinctive features of the second type of NETs: rupture of membrane structures of neutrophil organelles with formation of retiform DNA.

Conclusion. According to obtained data, NETs were detected in all groups of patients with CKD, but not at all patients in each group, the amount of NETs also was varied. No significant correlation between the amount of NETs and stages of CKD was observed.

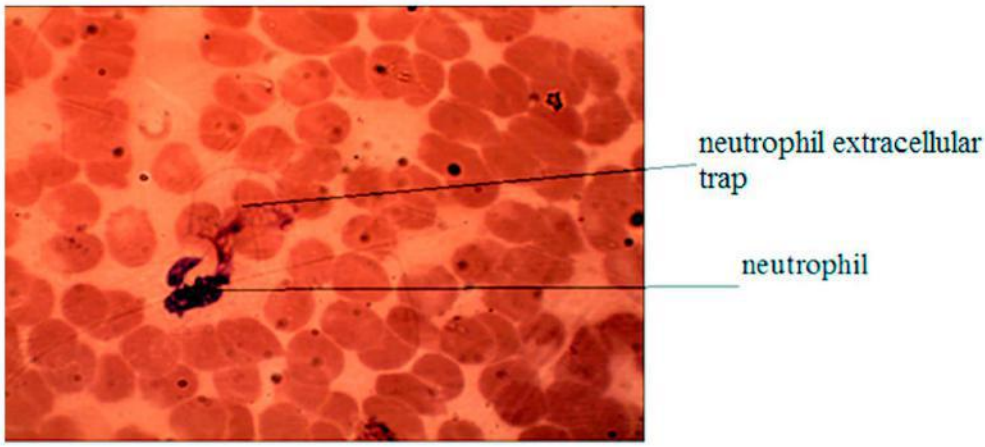

Figure 1 Blood smear of patient with CKD with image of formation the first type of NETs (magnification × 1400, painting with hematoxylin-eosin)

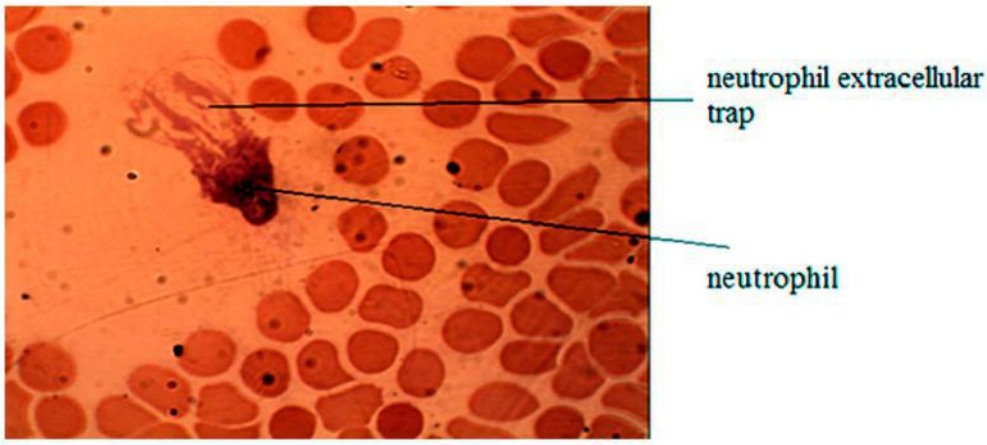

Figure 2 Blood smear of patient with CKD with image of formation the second type of NETs (magnification × 1400, painting with hematoxylin-eosin)

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The appearance of NETs in the blood of patients, in our opinion, is an unfavorable factor. First of all, the formation of NETs in the blood may also render the procoagulant action. As mentioned before, the formation of extracellular net-like structures consisting of DNA and histones takes place during formation of NETs [4, 5]. Extracellular histones can cause endothelial damage also participate in the inflammatory response and provoke the thrombin formation [7, 8], that can undoubtedly aggravate CKD.

Conclusions:

– The highest frequency of detection of NETs was observed in the groups of patients with CKD 1, 4 and 5 stages (from 33 – 35%).

– The largest number of NETs in the blood was also observed in the groups of patients with CKD 1, 4 and 5 stages (from 10 – to 14 pieces).

– NETs with different morphological characteristics can be found in the blood smear of one patient.

LITERATURE

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АННОТАЦИЯ

Изучена возможность образования нейтрофильных внеклеточных ловушек у больных с хронической болезнью почек различной стадии. Объектом исследования служила цельная кровь 63 пациентов с хронической болезнью почек, распределенных в 5 групп в зависимости от степени поражения почечной паренхимы. Образование сетеподобных структур в процессе нетоза нейтрофила, называемых нейтрофильными внеклеточными ловушками, имеющими в своем составе ДНК, гистоны, дефензины и ферменты гранул (эластаза, миелопероксидаза, желатиназа, лактоферрин и лизоцим), было спровоцировано взаимодействием с липополисахаридными веществами экзогенного (микроорганизмы) и аутогенного происхождения (продукты окислительного метаболизма), действующими через Fcγ-рецепторы и активирующими NADPH-оксидазный комплекс. Результаты исследования определили следующую картину обнаружения нейтрофильных внеклеточных ловушек: у больных с хронической болезнью почек 1 стадии – 33 %, количество ловушек варьировало от 4 до 10 на 100 нейтрофилов, 2 стадии – 5 %, количество ловушек составило 4 на 100 нейтрофилов, 3 стадии – 25 %, количество ловушек варьировало от 1 – до 7 на 100 нейтрофилов, 4 стадии – 33 %, количество ловушек варьировало от 2 – до 12 на 100 нейтрофилов, 5 стадии – 35 %, количество ловушек варьировало от 2 – до 14 на 100 нейтрофилов. Таким образом, была отмечена наибольшая частота обнаружения и максимальное количество нейтрофильных внеклеточных ловушек в группах больных с хронической болезнью почек 1, 4 и 5 стадий. Были обнаружены некоторые особенности в морфологии нейтрофильных внеклеточных ловушек разных типов. Отличительной особенностью нейтрофильных ловушек первого типа была гипертрофия сегментов ядра с сосредоточением гранул внутри сетеподобной ДНК. Отличительным признаком нейтрофильных ловушек второго типа был разрыв всех мембранных структур органелл и плазмолеммы нейтрофила. Появление нейтрофильных внеклеточных

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ловушек является неблагоприятных фактором, провоцирующим активацию прокоагулянтного механизма системы гемостаза.

Ключевые слова: нейтрофильные гранулоциты, нейтрофильные внеклеточные ловушки, хроническая болезнь почек.

ТҮЙІН

Әртүрлі сатыдағы созылмалы бүйрек ауруына шалдыққан науқастарда нейтрофильді жасушадан тыс тұтқыштардың пайда болу мүмкіншіліктерін оқыту. Зерттеу нысаны ретінде дәрежесіне қарай 5 топ•а бөлінген бүйрек ұлпасының зақымдануы, созылмалы бүйрек ауруы бар 63 нау•астың тұтас қаны ы•пал етті. NADPH-оксидті, Fcγ-рецепторлары арқылы қолданыстағы экзогенді (микроорганизмдер) және шығу тегі аутогенді қолданыстағы (тотықтырғыш зат алмасу өнімдері) липополисахаридті заттармен өзара әрекеттесуіне акелген, дефензиндер мен гранула ферменттерімен (эластаз, миелопероксидаз, желатиназ, лактоферрин және лизоцим), гистондар, сондай-ақ өзінің құрамында ДНҚ бар, нейтрофил нетроз процесінде желіге ұқсас құрылымның құрылуын нейтрофилді жасушадан тыс қақпан деп атаған. Зерттеу қорытындысымен нейтрофилді жасушадан тыс тұтқышы бар келесідей көрініс анықталды: 1 сатыда созылмалы бүйрек ауруымен ауыратын науқаста р – 33 %, 100 нейтрофильге тұтқыш саны 4-тен 10 дейінді құрайды, 2 сатыда – 5 %, 100 нейтрофильге тұтқыш саны 4 – ті құрайды, 3 сатыда – 25 %, 100 нейтрофильге тұтқыш саны 1-ден 7-ге дейінді құрайды, 4 сатыда – 33 %, 100 нейтрофильге тұтқыш саны 2-ден 12-ге дейінді құрайды, 5 сатыда – 35 %, 100 нейтрофильге тұтқыш саны 2-ден 14-ке дейінді құрайды. Осылайша, созылмалы бүйрек ауруына шалдыққан науқастар тобынан жиілігі 1, 4 және 5 сатыларында нейтрофильді жасушадан тыс тұтқыш сандарының ең көп мөлшері мен жиілігі айтарлықтай атап өтілді. Морфологияда жасушадан тыс тұтқыштардың әр түрлі типтегі кейбір ерекшеліктері анықталды.

Нейтрофильді тұтқыштың бірінші типінің айрықша ерекшелігі ДНҚ ішіндегі жүйе іспеттес гранул топтамасымен ядро сегменттерінің гипертрофиясы болды. Нейтрофильді тұтқыштың екінші типінің айрықша ерекшелігі нейтрофил плазмолеммалары мен органелла құрылымының барлық мембраналық алша•тығы болды. Қан тоқтату жүйесінде прокоагулянт тетігін белсендіру үшін нейтрофильді жасушадан тыс тұтқыштардың пайда болуы қолайсыз фатор болып табылады.

Түйінді сөздер: нейтрофильді гранулоциттер, нейтрофильді жасушадан тыс тұтқыштар, созылмалы бүйрек ауруы.

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