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MICROBIAL LOAD OF THE URINE IN PATIENTS WITH RECURRENT UROLITHIASIS AND ITS CORRECTION

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Among the causes of stone formation in the urinary system, an important role belongs to the microbiome, which affects the stability of the colloidal system of urine. Understanding the level of microbial tension in the urine allows to use pathogenetic approaches both for prevention and for the relapses of stone formation. The study included 162 patients with recurrent urolithiasis, 12 patients underwent complex anti-relapse therapy after surgical treatment (or spontaneous discharge of concretions after litokinetic therapy). It was determined that along with the known factors, there is a violation of quantitative indicators of urine microbiota. An increase of the microbial load of urine leads to a violation of the structure of uromodulin (Tamm – Horsfall protein), which in turn determines the high frequency of recurrence of urinary lithogenesis. Thus, a comprehensive assessment of the urine microbiota and its impact on the state of uromodulin, lead to an improvement in the quality of urolithiasis metaphylaxis. In the complex of metaphylaxis and prevention of urolithiasis, it is advisable to use antimicrobial agents (uroseptics) not only to reduce the risk of infectious and inflammatory complications, but also to increase the stability of the colloidal properties of urine.

Keywords: urolithiasis; urine microbiota; metaphylaxis; antibacterial therapy.

МИКРОБНАЯ НАГРУЗКА МОЧИ ПРИ РЕЦИДИВИРУЮЩЕМ УРОЛИТИАЗЕ И ЕЕ КОРРЕКЦИЯ

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Основная роль камнеобразования в моче принадлежит микробиому, влияющему на стабильность коллоидной системы мочи. Понимание уровня микробной напряженности в моче позволяет использовать патогенетические подходы как для профилактики, так и для предупреждения рецидивов камнеобразования. В исследование были включены 162 пациента с рецидивирующим уролитиазом, 12 пациентам после хирургического лечения (или спонтанного отхождения конкрементов после литокинетической терапии) проведена комплексная противорецидивная терапия. Определено, что, наряду с известными факторами, имеется нарушение количественных показателей микробиоты мочи. Увеличение микробной нагрузки мочи приводит к нарушению структуры уромодулина (белок Тамма – Хорсфалла), что в свою очередь определяет высокую частоту рецидива мочевого литогенеза. Таким образом, комплексная оценка микробиоты мочи и ее влияния на состояние уромодулина ведут к улучшению качества проводимой метафилактики уролитиаза. В комплексе метафилактики и профилактики уролитиаза целесообразно применять противомикробные средства (уросептики) не только с целью снижения риска развития инфекционно-воспалительных осложнений, но и для повышения стабильности коллоидных свойств мочи.

Ключевые слова: мочекаменная болезнь; микробиота мочи; метафилактика; антибактериальная терапия.

INTRODUCTION

Microorganisms can influence metabolism and the functioning of the urinary and endocrine systems, either directly or through the human metagenome. Therefore, the development of microbiology and the emergence of new data on microorganisms necessitates a clarification of the nature of microorganisms' influence. In recent years, the concept of microbiocenosis has undergone significant changes because of the introduction of new molecular and genetic research methods that have enabled the identification of several bacteria, which cannot be cultivated and have not been studied previously [1, 2].

One of the most widespread diseases is urolithiasis, with an incidence of 5%–10% in the European population and up to 20% in the Eastern Arabic countries [3]. The Russian Federation has observed a persistent tendency toward an increase of 0.5%–5.3% annually in the incidence of urolithiasis. Moreover, the number of patients with urolithiasis in Russia, regardless of sex and age, has increased by more than 1.5 times over the past decade [4]. Besides an increase in the incidence of urolithiasis, there is a high rate of recurrence of lithogenesis, 50%–75% within the period of 5 to 10 years [5, 6]. This situation has intensified the search for newer drug treatments and metaphylaxis of urolithiasis [7–9]. Recurrent kidney lithogenesis is accompanied by hypercoagulation, hypovolemia of various origins, decreased anticoagulant and fibrinolytic activity of blood and urine – all of which need to be considered for metaphylaxis of urolithiasis [10, 11].

The current methods of detecting microbial agents in the urinary tract and studies on the role of metabolic syndrome offer new prospects in clarifying the pathogenesis of urolithiasis [6, 12]. Nevertheless, despite a significant number of studies, the influence of the infectious factor on the mechanisms of urinary calculi formation have been insufficiently studied [13, 14]. Studies aimed at exploring lithogenesis have emphasized the crucial role of the primary stabilizer of the urine colloidal system, namely uromodulin or the Tamm-Horsfall protein (THP), which exhibits protective properties against urinary pathogens [15–17]. However, the influence of the urine microbiome on the THP structure remains unclear.

Recent studies have indicated the need, especially in systemic, recurrent, and bilateral nephrolithiasis, for an in-depth analysis of the role of the urinary microbiota that represents a combination of microorganisms coexisting under normal and pathological conditions in the urinary tract and participating in physiological and pathophysiological reactions [13, 18]. Notably, an imbalance in the microbiota could be one of the critical factors in the pathogenesis of urolithiasis [19], and influencing the microbiota could increase the effectiveness of treatment and metaphylaxis of the disease.

This work aimed to establish the relationship between the qualitative and quantitative indicators of urine microbiota with changes in qualitative deviations in the THP structure, to assess the possibility of their correction to optimize treatment and metaphylaxis in patients with recurrent urolithiasis.

PATIENTS AND METHODS

This study involved 162 patients with recurrent urolithiasis, including 93 (57.4%) men and 69 (42.6%) women. The average age of patients was 46.6 ± 15.7 years, and the disease duration was more than 2 years. In 99 (61.1%) patients, a unilateral process was determined; in 30 (18.5%) patients, calculi were localized in the kidney; 6 (3.7%) patients had a coral calculus; and in 69 (42.6%) patients, calculi were localized in the ureter. In 63 (38.9%) patients, a bilateral process was registered.

As part of the study, 12 patients with recurrent urolithiasis (7 [58.3%] men and 5 [41.7%] women) aged 44.5 ± 1.5 years, with a disease duration of more than 3 years, received a complex of anti-relapse therapy, which combined urinary antiseptics, herbal diuretics, and fibrinolysis activators.

The control group consisted of 35 healthy people.

In addition to standard laboratory studies, the microbiota of urinary calculi and urine was determined using gas chromatography-mass spectrometry (GC-MS) that determined microbial markers of bacteria (including anaerobic), viruses, and fungi in 104 samples. Moreover, the method of dynamic light scattering with programmed cooling was used for 162 samples to determine the size and structure of THP complexes.

RESULTS AND DISCUSSION

The results of GC-MS revealed that in all patients with recurrent urolithiasis, the disease proceeded with an extremely high level of total bacterial load

of urine, which was determined to be in the range of 59,661–65,769 CFU (62,715 ± 3,054 CFU), with the results in the control group ranging as 2,250–2,474 CFU (2,362 ± 112 CFU). Using the method of dynamic light diffusion, we obtained similar results and noted that in patients with recurrent urolithiasis, the molecular THP complexes had a significantly larger size (1518.4 ± 12.3 nm), whereas in the control group, this value was much lower (111.4 ± 4.8 nm) (see Table).

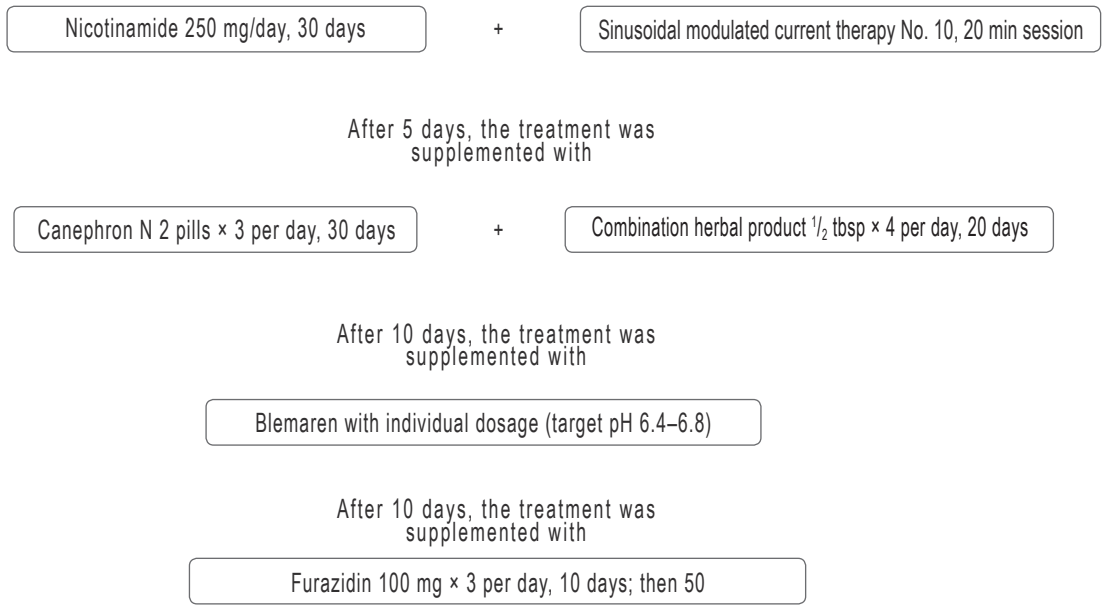
For anti-relapse therapy, patients were treated per the following scheme: nicotinamide 250 mg once a day (for 30 days) plus physiotherapy using sinusoidal modulated currents No. 10 (a 20 min session); after 5 days, Canephron N two pills three times a day (for 30 days) was added to the treatment plus a combination of herbal products half tablespoon four times a day (for 20 days); after another 10 days, Blemaren was added (target pH 6.4–6.8); and after 5 days, Furazidin 100 mg three times a day (10 days) was administered, followed by a decrease in the dosage by 50 mg three times a day for another 10 days (see Figure).

After the treatment course, the GC–MS revealed a statistically significant ($p < 0.001$) decrease in the total bacterial load to a level of 6078–6556 CFU (6317 ± 239 CFU), and the data of the dynamic light diffusion method revealed a significant ($p < 0.001$) decrease in the size of the THP complexes to 177.6 ± 6.4 nm (see Table 1). Therefore, the findings indicate a direct relationship between the total bacterial load level and THP complex size.

Characteristics of urine obtained by gas chromatography – mass spectrometry and dynamic light scattering before and after a course of complex anti-relapse therapy

Характеристики мочи, полученные при помощи газовой хроматографии – масс-спектрометрии и динамического светорассеивания до и после курса комплексной противорецидивной терапии

Group of patients	Total bacterial load, CFU	Size of THP complexes, nm
Recurrent urolithiasis (n = 12)		
before treatment	62715 ± 3054	1518.4 ± 12.3
after treatment	6317 ± 239	177.6 ± 6.4
Control group (n = 35)	2362 ± 112	111.4 ± 4.8



Scheme of complex anti-relapse therapy
Схема комплексной противорецидивной терапии

CONCLUSION

Structural failure of THP – the primary stabilizer of the colloidal properties of urine – in patients with recurrent urolithiasis is associated with an extremely high total bacterial load and an imbalance of the urine microbiota. Hence, in the context of metaphylaxis measures, it is necessary to employ means and methods aimed at regulating and restoring the balance of the urinary tract microbiota and reducing the total bacterial load, which would increase the uromodulin stability and improve the quality of the metaphylactic measures undertaken.

REFERENCES

1. Human Microbiome Project Consortium. Structure, function and diversity of the healthy human microbiome. *Nature*. 2012;486(7402):207-214. https://doi.org/10.1038/nature11234.
2. Human Microbiome Project Consortium. A framework for human microbiome research. *Nature.* 2012;486(7402):215-221. https://doi.org/10.1038/nature11209.
3. Romero V, Akpinar H, Assimos DG. Kidney stones: a global picture of prevalence, incidence, and associated risk factors. *Rev Urol*. 2010;2(2-3):86-96.
4. Каприн А.Д., Аполихин О.И., Сивков А.В., и др. Анализ уронефрологической заболеваемости и смертности в Российской Федерации за 2003–2013 г. // Экспериментальная и клиническая урология. – 2015. – № 2. – С. 4–12. [Kaprin AD, Apolihin OI, Sivkov AV, et al. Analysis of uronephrological morbidity and mortality in Russian Federation for 2003-2013. *Experimental and clinical urology*. 2015;(2):4-12. (In Russ.)]
5. Turney BW, Reynard JM, Noble JG, Keoghane SR. Trends in urological stone disease. *BJU Int.* 2012;109(7):1082-1087. https://doi.org/10.1111/j.1464-410X.2011.10495.x.
6. Гаджиев Н.К., Малхасян В.А., Мазуренко Д.А., и др. Мочекаменная болезнь и метаболический синдром. Патофизиология камнеобразования // Экспериментальная и клиническая урология. – 2018. – № 1. – С. 66–75. [Gadzhiev NK. Malhasjan VA, Mazurenko DA, et al. Urolithiasis and metabolic syndrome. The pathophysiology of stone formation. *Experimental and clinical urology*. 2018;(4):66-75. (In Russ.)] https://doi.org/10.29188/2222-8543-2018-9-1-66-75.
7. Аль-Шукри С.Х., Слесаревская М.Н., Кузьмин И.В. Литолитическая терапия уратного нефролитиаза // Урология. – 2016. – № 2. – С. 23–27. [Al-Shukri SKh, Slesarevskaya MN, Kuzmin IV. Litholytic therapy for urate nephrolithiasis. *Urologiia*. 2016(2): 23-27 (In Russ.)]
8. Саенко В.С., Газимиев М.А., Песегов С.В., Аляев Ю.Г. Мочекаменная болезнь. Часть 5. Лекарственные средства, применяемые в медикаментозной метафилактике мочекаменной болезни // Урология. – 2019. – № 3. – С. 156–164. [Saenko VS, Gazimiev MA, Pesegov SV, Alyaev YuG. Urinary stone disease. Part V. Drugs used for metaphylaxis of urinary stone disease. Urologiia. 2019(3):156-164. (In Russ.)]. https://dx.doi.org/10.18565/urology.2019.3.156-164.
9. Аль-Шукри С.Х., Слесаревская М.Н., Кузьмин И.В. Современные подходы к патогенезу, лечению и метафилактике уратного уролитиаза // Эффективная фармакотерапия. – 2019. – Т. 15. – № 16. – С. 46–50. [Al-Shukri SKh, Slesarevskaya M, Kuzmin I. Modern approaches to pathogenesis, treatment and metaphylaxis of urate urolithiasis. *Effektivnaja farmakoterapija*. 2019;15(16):46-50. (In Russ.)]. https://dx.doi.org/10.33978/2307-3586-2019-15-16-46-50.
10. Голощапов Е.Т., Лукичев Г.Б., Игнашов Ю.А. Особенности нарушения гемостаза и фибринолиза при различных клинических формах мочекаменной болезни // Урологические ведомости. – 2013. – Т. 3. – № 1. – С. 8–11. [Goloshchapov ET, Lukichev GB, Ignashov JuA. Features of hemostasis and fibrinolysis disorders in various clinical forms of urinary stone disease. *Urologicheskie vedomosti.* 2013;3(1):8-11. (In Russ.)]
11. Голощапов Е.Т., Глазунова Е.В., Савенкова Т.А. Профилактика камнеобразования при двустороннем рецидивирующем нефролитиазе // Нефрология. 2008. – Т. 12. – № 3. – С. 89–94. [Goloshchapov ET, Glazunova EV, Savenkova TA. Profilaktika kamneobrazovanija pri dvustoronnem recidivirujushhem nefrolitiaze. *Nefrologija.* 2008;12(3):89-94. (In Russ.)]
12. Гусакова Д.А., Калинченко С.Ю., Камалов А.А., Тишова Ю.А. Факторы риска развития мочекаменной болезни у больных с метаболическим синдромом // Экспериментальная и клиническая урология. – 2013. – № 2. – С. 61–64. [Gusakova DA, Kalinchenko SYu., Kamalov AA, Tishova YuA. Risk factors for the development of the urolithiasis in patients with the metabolic syndrome. *Experimental and clinical urology*. 2013;(2):61-64. (In Russ.)]
13. Чухловин А. Б., Эмануэль Ю. В., Напалкова О. В., и др. Роль локальных инфекций в генезе мочекаменной болезни // Нефрология. – 2011. – Т. 15. – № 3. – С. 11–17. [Chuhlovin AB, Jemanujel' YuV, Napalkova OV, et al. Rol' lokal'nyh infekcij v geneze mochekamennoj bolezni. *Nefrologija.* 2011;15(3):11-17. (In Russ.)]
14. Голощапов Е.Т., Четвериков А.В., Белозеров Е.С. Инфекционный фактор в генезе мочевого камнеобразования // Урологические ведомости. – 2016. – Т. 6. – № 4. – С. 21–27. [Goloshchapov ET, Chetverikov AV, Belozerov ES. The infectious factor in the genesis of urinary stone formation. *Urologicheskie vedomosti.* 2016;6(4):21-27. (In Russ.)]. https://doi.org/10.17816/uroved6421-27.
15. Аль-Шукри С.Х., Голощапов Е.Т., Эмануэль Ю.В., Горбачев М.И. Белок Тамма – Хорсфалла — потенциальный маркер ранних стадий мочекаменной болезни и рецидивного камнеобразования // Урологические ведомости. – 2012. – Т. 2. – № 1. –

С. 26–28. [Al-Shukri SKh, Goloshchapov ET, Emanuel YuV, Gorbachev MI. Tamm-Horsfall protein – potential marker of early stages urolithiasis and stone recurence // *Urologicheskie vedomosti.* 2012;2(1):26-28. https://doi.org/10.17816/uroved2126-28.

16. Zasloff M. Antimicrobial peptides, innate immunity and the normally sterile urinary tract. *J Am Soc Nephrol*. 2007;18(11):2810–2816.
17. Голощапов Е.Т. Прогнозирование и доклиническая диагностика риска мочевого камнеобразования с использованием биофизических технологий // Урологические ведомости. – 2016. – Т. 6. – № 2. – С. 11–15. [Goloshchapov ET. Prediction and preclinical diagnosis of the risk of urinary stone formation using biophysical technologies. *Urologicheskie vedomosti.* 2016;6(2):11-15. (In Russ.)]. https://doi.org/10.17816/uroved6211-15.
18. Дутов В.В., Буймистр С.Ю., Русанова Е.В. Изменение микрофлоры мочи у пациентов с мочекаменной болезнью // Урология. – 2018. – № 6. – С. 32–36. [Dutov VV, Buymistr SYu, Rusanova EV. The changes in urine microflora in patients with urinary stone disease. *Urologiia*. 2018(6):32-36. (In Russ.)]. https://dx.doi.org/10.18565/urology.2018.6.32-36
19. Четвериков А.В., Голощапов Е.Т., Белозеров Е.С. Микробиом мочи и его влияние на стабильность коллоидных свойств у больных уролитиазом // Ульяновский медико-биологический журнал. – 2019. – № 4. – С. 74–81. [Chetverikov AV, Goloshchapov ET, Belozerov ES. Urinary microbiome and its effect on colloidal stability in patients with urolithiasis. *Ulyanovsk Medico-Biological Journal.* 2019;4:74-81. (In Russ.)]. https://doi.org/10.34014/2227-1848-2019-4-74-81.

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