5. Ghimire S, Banks C Castelino RL, Jose MD, Zaidi STR. Medication adherence assessment practices in dialysis settings: A survey of renal nurses' perceptions. Journal of clinical nursing. 2019 Feb; 28(3-4): 528–537.
6. Ghimire S, Castelino RL, Jose MD, Zaidi STR. Medication adherence perspectives in haemodialysis patients: a qualitative study. BMC Nephrol. 2017 May 22; 18(1):167. DOI: 10.1186/s12882-017-0583-9.
7. Jing Jin, Sklar GE, Min Sen Oh V, Cheun Li S. Factors affecting therapeutic compliance: A review from the patient′s perspective. Ther Clin Risk Manag. 2008 Feb; 4(1):269–86. DOI: 10.2147/tcrm.s1458.
8. Owsiany MO, Hawley CE, Paik JM. Differential Diagnoses and Clinical Implications of Medication Nonadherence in Older Patients with Chronic Kidney Disease: A Review. Drugs Aging. 2020 Dec; 37(12):875–884. DOI: 10.1007/s40266-020-00804-8.
9. Parker K, Bull-Engelstad I, Aasebø W, Von der Lippe N, Reier-Nilsen M, Os I, Stavem K. Medication regimen complexity and medication adherence in elderly patients with chronic kidney disease. Hemodial Int. 2019 Jul; 23(3):333–342. DOI: 10.1111/hdi.12739.
10. Tangkiatkumjai M, Walker DM, Praditpornsilpa K, Boardman H. Association between medication adherence and clinical outcomes in patients with chronic kidney disease: a prospective cohort study. ClinExpNephrol. 2017 Jun; 21(3):504–512. DOI: 10.1007/s10157-016-1312-6.
11. Verma B, Singh A, Bishnoi JS, Mishra AK. Adherence to Medications in Chronic Kidney Disease: Prevalence, Predictors and Outcomes. International Journal of Current Research and Review. 2018 Oct;10(19):14–19. DOI: http://dx.doi.org/10.31782/IJCRR.2018.10193

Стаття надійшла 19.08.2020 р.

DOI 10.26724/2079-8334-2021-3-77-77-81
UDC 616.34-002:616.61/62-002

O.A. Kyrian, I.A. Derkach[1], A.E. Dorofeyev[2], M.M. Rudenko[3]
Poltava State Medical University, Poltava, [1] Truskavets City Hospital, Truskavets
[2] P.L. Shupyk National Healthcare University of Ukraine, Kyiv
[3] O.O. Bogomolets National Medical University, Kyiv

CHANGES IN THE INTESTINAL MICROBIOTA IN PATIENTS WITH ULCERATIVE COLITIS AND IRRITABLE BOWEL SYNDROME COMBINED WITH UROLITHIASIS

e-mail: hel_kirjan@i.ua

The paper is devoted to the study of changes in the intestinal microbiome in patients with urolithiasis that occurred against the background of ulcerative colitis and irritable bowel syndrome. It was found that about 20 % of patients with urolithiasis have symptoms of irritable bowel syndrome, with more than half of the stones consisting of oxalates. In the study of fecal microflora in patients with the method of sequencing of the 16S gene of ribosomal RNA, compared with healthy, a significant decrease in Firmicutes, Faecalibacterium prausnitzii, Akkermansia muciniphila and the Firmicutes/Bacteroidetes ratio was revealed. The detected changes in the microflora can be associated both with direct changes in the intestine, inherent in nonspecific ulcerative colitis and irritable bowel syndrome, and with more frequent use of uroseptics/antibiotics in this category of patients.

Key words: urolithiasis, fecal microflora, phylotypes, intestinal diseases

О.А. Кир'ян, І.А. Деркач, А.Е. Дорофєєв, М.М. Руденко

ЗМІНИ КИШКОВОЇ МІКРОБІОТИ У ПАЦІЄНТІВ ІЗ НЕСПЕЦИФІЧНИМ ВИРАЗКОВИМ КОЛІТОМ ТА СИНДРОМОМ ПОДРАЗНЕНОГО КИШЕЧНИКА І СЕЧОКАМ'ЯНОЮ ХВОРОБОЮ

Робота присвячена вивченню змін кишкового мікробіома у пацієнтів із сечокам'яною хворобою, що виникла на тлі неспецифічного виразкового коліту та синдрому подразненого кишечника. Виявлено, що близько 20 % хворих із сечокам'яною хворобою мають симптоми синдрому подразненого кишечника, причому більше половини каменів складалися з оксалатів. При вивченні фекальної мікрофлори у хворих методом секвенування гена 16S рибосомальної РНК, порівнюючи із здоровими, виявлено достовірне зменшення Firmicutes, Faecalibacterium prausnitzii, Akkermansia muciniphila і співвідношення Firmicutes/Bacteroidetes. Виявлені зміни мікрофлори можуть бути пов'язані як з безпосередніми змінами в кишці, властивими неспецифічному виразкового коліту і синдрому подразненого кишечника, так і з більш частим використанням уросептиків/антибіотиків у даної категорії хворих.

Ключові слова: уролітіаз, фекальна мікрофлора, філотипи, захворювання кишечника

The work is a fragment of the research project "Features of the course, prognosis and treatment of comorbid conditions in diseases of internal organs, taking into account genetic, age and gender aspects", state registration No. 0118U004461

Intestinal microbiota changes are one of the leading links in the pathogenesis of both inflammatory and functional intestinal diseases. The intestinal microbiome has a direct effect on the development of inflammation, disruption of the permeability of the intestinal mucosal barrier and, as a consequence, the appearance of extraintestinal manifestations and complications. In addition, the combined pathology of internal organs and systems largely depends on the condition and permeability of the intestinal mucosal

barrier. Therefore, changes in the microbiome of the colon are now considered as a trigger factor in the development and progression of many diseases [1].

Today, almost 15 % of the world's adult population has kidney stones – kidney stone disease (KSD). In addition, urolithiasis has tended to increase over the past 50 years. During this period, the number of patients with KSD increased fourfold [4]. Ukraine is also no exception. The annual incidence of KSD is about 60 thousand people. It is well known that urolithiasis is a polyetiological disease that depends on climatic conditions, age, sex, genetic predisposition, diet, infections, as well as the presence of comorbidities (diabetes, obesity, hypertension). Moreover, the chemical composition of stones depends on a combination of risk factors [3, 4].

Inflammatory and functional diseases of the intestine are among the risk factors for KSD, which can have a significant impact [1]. According to our data and those of other authors [2, 6], the post-infectious irritable bowel syndrome (PI-IBS) is combined with KSD in more than 40 % of cases. In ulcerative colitis (UC), very often such kidney damage as KSD is considered as extraintestinal manifestations of the primary disease [1]. Oxalobacter formigene, which is able to destroy oxalates in the intestinal lumen and reduce their absorption, was one of the first microorganisms found to reduce the risk of KSD developing [5, 9]. However, further studies have not confirmed the leading importance of this bacterium in the pathogenesis and treatment of urolithiasis [8, 11]. Therefore, more attention is currently paid to the study of changes in the number of other representatives of the intestinal microbiota. An increase in Bacteroides with a decrease in Prevotella, Enterobacter, Dorea, Faecalibacterium, Lactobacillus, Akkermansia muciniphila, Faecalibacterium prausnitzii was found in patients with KSD. That is, there is a decrease in microorganisms capable of destroying oxalates and an increase in those that cause inflammation [5, 7, 9, 11].

If the treatment of clinically manifest KSD is primarily minimally invasive urological interventions, then the problem of recurrence prevention requires further development and differentiated approach [10, 12] to the management of such patients.

The purpose of the study was to establish disorders of the gut microflora in patients with ulcerative colitis and irritable bowel syndrome with concomitant urolithiasis.

Methods and materials. To achieve this purpose, we surveyed and analyzed more than 9,000 patients with KSD, who were treated in inpatient departments of Poltava, Kyiv and Lviv regions. Of these, more than 2,000 patients had intestinal pathology. 1,951 patients showed symptoms of irritable bowel syndrome (IBS) of various subtypes, and 112 were diagnosed with UC. Patients were initially admitted to the Department with attacks of renal colic. Different approaches were used to treat urolithiasis depending on the localization of calculi. If calculi were detected in the kidney and/or upper third of the ureter, percutaneous nephrolithotripsy with antegrade ureterolithotripsy was used, and if localized in its middle or lower third, retrograde contact ureterolithotripsy or ureterolithoextraction was used. The chemical composition of urinary stones was spectrophotometrically studied in all patients. A combination of KSD and IBS was found in 1,053 (53.9 %) patients and oxalate stones were found in 72 (64.3 %) patients with UC. We selected 116 patients with unilateral single oxalate stones in combination with IBS and 70 patients with UC and KSD for further in-depth microbiological examination. In addition, in 50 patients with UC and 60 patients with IBS without urolithiasis, a colon microbiome was examined. Polymerase chain reaction studies were performed to determine the main types of fecal microbiota. 40 healthy representatives of the Ukrainian population were examined as a control group for assessing the intestinal microbiota.

The chemical composition of the removed stones was studied on the UR-20 device (Carl Zeiss, Jena, Germany) in the KBr Matrix (potassium, bromide) in the frequency range 4000-400 cm^{-1} by determining the number, position and intensity of absorption bands in the infrared spectra of the studied samples. Quantitative determination of different fecal bacterial taxa was performed by qPCR using primers targeting the 16S rRNA gene specific for Firmicutes, Actinobacteria, Bacteroidetes, Akkermansia muciniphila and Faecalibacterium prausnitzi, as well as universal primers. The quantity and quality of DNA was measured by NanoDrop ND-8000 (Thermo Scientific, USA). Samples of fresh faeces of each patient were placed in a special container. Within 10 minutes after defecation, an aliquot of faeces was taken, which was immediately frozen and stored at -20 °C until DNA isolation by the phenol-chloroform method according to the protocol. DNA was eluted in 200 μl elution buffer. Genotyping was performed by polymerase chain reaction using the primer structure and temperature cycle parameters. Statistical analysis was performed using Office Excel 2016 (Microsoft Corporation, USA), using analytical programs, t – Student's criteria, (probability value $p<0.05$).

The results of the study and their discussion. According to the obtained data, women predominated among the examined patients, which is more typical for patients with irritable bowel syndrome, than for patients with urolithiasis (table 1).

Table 1

Characteristics of the examined patients with IBS and UC + urolithiasis

	IBS and KSD		UC and KSD	
	N=116	%	N=70	%
M/F	45/71	39/61	31/39	44/56
Age (years)	45.2	±2.9	49.8	±2.7
Oxalate stones	61	52.6	46	65.7
Phosphate Oxalate stones	55	47.4	24	34.3
Percutaneous nephrolithotripsy + antegrade ureterolithotripsy	68	58.6	45	64.3
Retrograde ureterolithotripsy/ureterolithoextraction	48	41.4	25	35.7

Thus, in the group of patients with IBS and KSD, the disease was detected in women in 61 % of cases (men – 39 % of cases), in the group of UC and KSD – in 56 % of cases (men – 44 %, respectively). The age groups of the examined patients were comparable. The average age of patients in both study groups did not have a significant difference and was 45.2±2.9 years in patients with different subtypes of IBS and KSD, 49.8±2.7 years in the group of patients with UC and KSD ($p>0.05$). According to the chemical composition, both oxalate and mixed oxalate-phosphate stones were detected in patients with IBS with the same frequency. According to the data obtained in patients with IBS, oxalate stones were found in 61 (52.6 %) patients, and oxalate-phosphate – in 55 (47.4 %) patients. In contrast to patients with ulcerative colitis, who had oxalate stones in two-thirds of cases – 46 (65.7 %) patients and only a third – oxalate-phosphate stones – 24 (34.3 %) patients. More frequent formation of oxalate stones in patients with UC may be associated with more pronounced changes in the content of intestinal microbiota phylotypes, due to the effect on the permeability of the intestinal mucosal barrier and its reparative properties, which requires further study. Among the methods of treating stones, percutaneous nephrolithotripsy was more often used, which was associated with a large number of patients with concretions in the kidneys and upper third of the urinary tract.

Phylotypes of the gut microbiota are essential for the normal functioning of the human body. The appearance of imbalances and differences in the composition and ratio of the main phylotypes of the gut microflora can play a key role in the occurrence of diseases, including KSD. In-depth study of changes in the intestinal microbiota will allow to identify the mechanisms of development of various pathological conditions and prescribe appropriate therapy. To study the features of disorders of changes in the intestinal microflora and identify the relationship with the possible impact on the increased risk of urolithiasis in patients with irritable bowel syndrome and ulcerative colitis with urolithiasis, a study of the main phylotypes of the fecal microbiota was performed. When studying the percentage of phylotypes of fecal microorganisms in patients with combined pathology, the following features were found (table 2).

Table 2

Phylotypes of fecal microorganisms in patients with UC and IBS combined with KSD

Type of bacteria	Healthy people (n=40)	UC without KSD (n=50)	UC + KSD (n=70)	IBS without KSD (n=60)	IBS + KSD (n=116)
Firmicutes	37.2	26,1*	24,2*	32.1	28,8 *
Faecalibacterium prausnitzii	8.4	5,2*	2,9*●	7.7	4,1 *●
Bacteroidetes	40.5	37.2	35,9*	40.8	43.8
Actinobacteria	13.7	30,5*	29,1*	19.9	19,5 *
Akkermansia muciniphila	1.9	2.0	2.1	1.5	1,2 *
Others	6.7	6.2	10,8*●	7.2	7.9

Note: * – significant differences compared to the group of healthy individuals $p<0.05$ ● significant differences when comparing a group of patients with the presence and absence of KSD $p<0.05$

According to the data obtained, significant changes in the intestinal microbiome were found in patients with UC, regardless of the presence of urolithiasis. At the same time, in patients with intestinal pathology and KSD, these disorders were more significant, especially in patients in the group with ulcerative colitis. Thus, when analyzing the percentage of the main phylotypes of intestinal microflora

in patients with UC, a significant decrease in Firmicutes was noted – 26.1 % in patients without KSD and more pronounced with urolithiasis – 24.2 % (37.2 % – in healthy individuals) and Bacteroidetes – 37.2 % in the absence of KSD and reliably with urolithiasis detection – 35.9 % (40.5 % – healthy individuals), against the background of Actinobacteria growth in both groups with UC (13.7 % – control group of healthy individuals) ($p<0.05$). At the same time, in patients with isolated UC, the percentage of other representatives of the intestinal microflora, mainly Proteobacteria, was within the normal range – 6.2 % (6.7 % in healthy people). In patients with UC + KSD, these bacteria were significantly increased –10.8 % of the fecal microflora content. In addition, patients with UC and KSD showed a significant decrease in one of the main representatives of the butyrate-producing regulatory flora, gram-positive anaerobic bacterium Faecalibacterium prausnitzii. Thus, in the group of patients with non-specific ulcerative colitis, who did not have KSD, this phylotype was detected in 5.2 % of the intestinal microflora. When urolithiasis was detected, the frequency of detection of Faecalibacterium prausnitzii significantly decreased, up to 2.9 % of the intestinal microflora content, compared with healthy people and patients with UC without urolithiasis ($p<0.05$). This phenomenon can be considered as an additional burden on the intestinal mucosal barrier with a decrease in its reparative properties and an increase in the permeability of the mucous membrane of the colon. When analyzing the data obtained in the percentage of the Akkermansia muciniphila phylotype, which regulates the permeability of the intestinal mucosal barrier and can modulate the immune response of patients with UC both with urolithiasis and without KSD in comparison with healthy individuals, no significant difference was determined.

When analyzing data on the content of phylotypes in the intestinal microbiota of patients with different subtypes of IBS who did not have urolithiasis, comparing with a group of healthy individuals, no significant difference was found. Thus, the percentage of Firmicutes of intestinal microflora was 32.1 % (37.2 % in healthy individuals), Bacteroidetes was detected in 40.8 % of the microbiota content (40.5 % in healthy individuals), Faecalibacterium prausnitzii was detected in 7.7 % of the microbiota content in patients with IBS (8.4 % in healthy people). The content of phylotypes of Actinobacteria and Akkermansia muciniphila bacteria in patients with IBS without urolithiasis, as well as other intestinal microflora, also did not significantly differ from the group of healthy individuals ($p>0.05$).

In comparison with practically healthy residents of Ukraine, patients with oxalate urolithiasis, which was formed against the background of IBS, have significant changes in the percentage of intestinal microflora. Patients with IBS and KSD showed a significant decrease in the content of Firmicutes – 28.8 % (37.2 % in healthy individuals) and such a representative as Faecalibacterium prausnitzii – 4.1 % (8.4 % healthy individuals), which also had a significant difference with the group of IBS patients without KSD – 7.7 %, which confirms the special role of this phylotype in the development of the disease. In addition, patients with irritable bowel syndrome and urolithiasis had significantly reduced levels of one of the most important protective microorganisms, the phylotype Verrucomicrobia – Akkermansia muciniphila – 1.2 % (1.9 % healthy individuals) and had the lowest rates, compared with all the studied groups and the ratio Firmicutes/Bacteroidetes. At the same time, a significant increase in the proportion of Actinobacteria and an insignificant increase in Bacteroidetes were observed. Phylotypes of other intestinal microflora in the group of patients with IBS and KSD were also determined more frequently, unreliably compared to the group of healthy individuals and IBS without urolithiasis ($p>0.05$).

Consequently, patients with the most common functional and inflammatory bowel diseases, such as irritable bowel syndrome and ulcerative colitis, who had a combination of the disease with urolithiasis, show more pronounced changes in the intestinal microbiome, both in comparison with the healthy population and in comparison with patients with isolated IBS and UC. Detection of disorders in the content of the main phylotypes of commensal intestinal bacteria, such as Faecalibacterium prausnitzii, which is one of the main butyrate-producing bacteria, Firmicutes, Akkermansia muciniphila, the ratio of Firmicutes/Bacteroidetes in patients with IBS and UC and urolithiasis, allows to assume the similarity of the mechanisms of disease development and determines the further path in the diagnostic search and therapeutic correction of the detected abnormalities.

Discussion. According to our study, almost 20 % of Ukrainian patients with KSD have symptoms of IBS, which is twice as common as in people without urolithiasis, and more than half of the stones consisted of oxalates. This is due to common pathogenetic mechanisms [1, 6]. UC with KSD was less common, but it should be noted that the prevalence of non-specific ulcerative colitis is 10-12 times lower than that of IBS. The examined patients with IBS and UC were more likely to complain of diarrhea, which can lead to dehydration and changes in urine volume and pH. Another reason for stone formation in

intestinal pathology can be an altered intestinal microbiota. When studying the fecal microflora by sequencing the 16S ribosomal RNA gene, we found changes in the ratio of the main phylotypes of microorganisms.

Compared to healthy patients, the group of patients showed a significant decrease in Firmicutes, Faecalibacterium prausnitzii, Akkermansia muciniphila and the Firmicutes/Bacteroidetes ratio. These microorganisms have a protective effect on the intestine, are able to normalize the metabolic processes of the macroorganism and reduce the level of oxalates [5, 7]. In addition, microbiome changes were more intense in patients with UC and IBS in whom KSD was formed. The revealed deviations in the content of colon microflora in patients with a combination of intestinal pathology and ICD can be associated with both direct changes in the intestine, characteristic of UC and IBS, and with more frequent use of uroseptics/antibiotics in this category of patients [13]. Exposure to the intestinal microbiome with the use of drugs and diet can be a promising method of treatment and prevention of urolithiasis.

Conclusions

1. Symptoms of IBS were detected in 21.7 % patients with KSD and UC - in 1.2 % of cases.

2. Patients with UC and IBS have pronounced changes in the fecal microbiota, which were more significant in patients with KSD combination.

3. In the fecal microbiota of these patients, the amount of Actinobacteria is significantly increased and the content of Firmicutes, Faecalibacterium prausnitzii, Akkermansia muciniphila is reduced, which may reduce the destruction of oxalates and increase the risk of KSD.

References

1. Dorofeev AE, Rudenko NN, Derkach IA, Chichula YuV Zabolevaniya kishechnika i pochki. Gastroenterologiya. 2015 57 (3):101–105. [in Russian]

2. Erdem E, Akbay E, Sezgin O, Doruk E, Canpolat B, Cayan S. Is there a relation between irritable Bowel syndrome and urinary stone disease? Dig Dis Sci. 2005 Mar; 50(3):605–608. DOI:10.1007/s10620-005-2482-3.

3. Ferraro PM, Taylor EN, Gambaro G, Curhan GC. Dietary and Lifestyle Risk Factors Associated with Incident Kidney Stones in Men and Women. J Urol. 2017 Oct; 198(4):858–863. DOI: 10.1016/j.juro.2017.03.124.

4. Khan SR, Pearle MS, Robertson WG, Gambaro G, Canales BK, Doizi S, et al. Kidney stones. Nat Rev Dis Primers. 2016 Feb 25; 2:16008. DOI: 10.1038/nrdp.2016.8.

5. Lee JA, Stern JM. Understanding the Link Between Gut Microbiome and Urinary Stone Disease. Curr Urol Rep. 2019 Mar 22; 20(5):19. DOI: 10.1007/s11934-019-0882-8.

6. Lei WY, Chang CY, Wu JH, Lin FH, Hsu Chen C, Chang CF, et al. An Initial Attack of Urinary Stone Disease Is Associated with an Increased Risk of Developing New-Onset Irritable Bowel Syndrome: Nationwide Population-Based Study. PLoS One. 2016 Jun 23;11(6):e0157701. DOI: 10.1371/journal.pone.0157701.

7. Millán Rodríguez F, Sabiote Rubio L, Girón Nanne I, Sánchez Martín F, Emiliani E, Angerri Feu O. The relationship between calcium oxalate lithiasis and chronic proinflammatory intestinal dysbiosis pattern: a prospective study. Urolithiasis. 2020 Aug; 48(4):321–328. DOI: 10.1007/s00240-020-01181-y.

8. Milliner D, Hoppe B, Groothoff J. A randomised Phase II/III study to evaluate the efficacy and safety of orally administered Oxalobacter formigenes to treat primary hyperoxaluria. Urolithiasis. 2018 Aug; 46(4):313–323. DOI:10.1007/s00240-017-0998-6.

9. Sadaf H, Raza SI, Hassan SW. Role of gut microbiota against calcium oxalate. Microb Pathog. 2017 Aug; 109:287–291. DOI: 10.1016/j.micpath.2017.06.009.

10. Suryavanshi MV, Bhute SS, Jadhav SD, Bhatia MS, Gune RP, Shouche YS. Hyperoxaluria leads to dysbiosis and drives selective enrichment of oxalate metabolizing bacterial species in recurrent kidney stone endures. Sci Rep. 2016 Oct 6; 6:34712. DOI: 10.1038/srep34712.

11. Ticinesi A, Milani C, Guerra A, Allegri F, Lauretani F, Nouvenne A, et al. Understanding the gut-kidney axis in nephrolithiasis: an analysis of the gut microbiota composition and functionality of stone formers. Gut. 2018 Dec; 67(12):2097–2106. DOI: 10.1136/gutjnl-2017-315734.

12. Ticinesi A, Nouvenne A, Chiussi G, Castaldo G, Guerra A, Meschi T. Calcium Oxalate Nephrolithiasis and Gut Microbiota: Not just a Gut-Kidney Axis. A Nutritional Perspective. Nutrients. 2020 Feb 20; 12(2):548. DOI: 10.3390/nu12020548.

13. Zampini A, Nguyen AH, Rose E, Monga M, Miller AW. Defining Dysbiosis in Patients with Urolithiasis. Sci Rep. 2019 Apr 1; 9(1):5425. DOI: 10.1038/s41598-019-41977-6.

Стаття надійшла 14.08.2020 р.