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Comparative study of the effects on the microbiota and leukocyturia of fresh and canned Naftussya water

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Background. Balneotherapy has its rightful place in the arsenal of means of treatment of chronic. The universally recognized gold standard of balneofactors is Naftussya bioactive water. The issue of the loss of its healing properties by Naftussya water after extraction from the subsoil, the possibility of its preservation and transportation remain debatable and relevant. The purpose of this study is to compare the impact of fresh and canned water on the microbiota of feces and urine in patients with chronic pyelonephritis. **Materials and Methods**. The object of clinical-physiological observation were residents of the city of Truskavets' (21 men aged 24-67 years and 8 women 33-76 years) with chronic pyelonephritis in remission. The subject of the study were the leukocyturia and bacteriuria levels and components of microbiota of feces. **Results**. Weekly use of fresh Naftussya water causes an increase in the reduced content of probiotics in the microbiota and a decrease in the increased content of conditionally pathogenic microflora, which is accompanied by a reduction of bacteriuria and leveling of leukocyturia. The beneficial effect on the listed parameters of canned Naftussya water is less pronounced, but the differences are statistically insignificant. **Conclusion**. The obtained results provide grounds for continuing research into the effects of canned Naftusya water on the body of urological patients.

Keywords: Naftussya bioactive water, chronic pyelonephritis, urinary syndrome, microbiota.

INRODUCTION

Balneotherapy has its rightful place in the arsenal of means of treatment of chronic pyelonephritis [1]. The universally recognized gold standard of balneofactors is Naftussya bioactive water. The issue of the loss of its healing properties by Naftussya water after extraction from the subsoil, the possibility of its preservation and transportation remain debatable and relevant [11,14,16-18]. The purpose of this study is to compare the impact of fresh and canned water on the microbiota of feces and urine in patients with chronic pyelonephritis.

MATERIALS AND METHODS

The object of clinical-physiological observation were residents of the city of Truskavets' (21 men aged 24-67 years and 8 women 33-76 years) with chronic pyelonephritis in remission. The day before, samples of morning urine and feces was collected, in which was determined the leukocyturia and bacteriuria levels and components of microbiota respectively. Unified methods are applied.

Urinary syndrome was assessed by quantitative and quantitative-qualitative [12] levels of bacteriuria and leukocyturia. To qualitatively assess the manifestations of pyelonephritis, a single-point IL Popovych's [5,12] scale, built on the basis EC Harrington's desirability function [6], was used.

In particular, bacteriuria over 10^6 CFU/mL is quantified at 0,9 points (strongly expressed), within $(0,3\div1,0)\bullet10^6$ CFU/mL – 0,715 p (more than average, but not strong), 10^5 CFU/mL – 0,5 p (moderately expressed), $(0,2\div0,5)\bullet10^5$ CFU/mL – 0,285 p (weakly expressed), $(0,01\div0,1)\bullet10^5$ CFU/mL - 0,1 p (very weak), less than $0,01\bullet10^5$ CFU/mL - 0 p (absent). Leukocyturia over $60\bullet10^3$/mL - 0,715 p, within $(20\div60)\bullet10^3$/mL – 0,5 p, $(4\div20)\bullet10^3$/mL – 0,285 p, $(2\div4)\bullet10^3$/mL – 0,1 p, less than $2\bullet10^3$/mL – 0 p.

The inclusion criteria were the presence of pronounced urinary syndrome (bacteriuria: 0,285÷0,715 points; leukocyturia: 0,1÷0,5 points) with preservation of functional renal reserve (>10%), previously assessed by the AI Gozhenko method [4].

Every day, 6 patients were examined, who were divided into two groups, approximately equal in terms of gender, age and severity of urinary syndrome. After the initial testing, the members of the main group received a weekly course of balneotherapy [16] with fresh Naftussya water (taken directly from the field), instead, for the experimental group, Naftussya water, previously preserved in anaerobic conditions, was used. On the second day after the end of the drinking course, repeated testing was carried out.

Normal (reference) values of variables are taken from the database of the Truskavetsian Scientific School of Balneology.

For statistical analysis used the software package "Statistica 6.4".

RESULTS AND DISCUSSION

Both groups were equal in terms of gender (4 women each), age (M±SD: 52±15 and 50±10 years), body mass index (26,8±4,2 and 27,8±3,3 kg/m^2) as well as of the initial symptoms of urinary syndrome (Table 1) and components of the microbiota of feces (Table 2).

Table 1. Comparative characteristics of the symptoms of urinary syndrome

		Naftussya water used for balneotherapy		Student's Statistics		Reference value	
Symptons of the urinary syndrome		Fresh (n=15)	Canned (n=14)	t	p	Mean	SD
Bacteriuria actual, lg CFU/mL	Before After Change	2,20±0,12*** 1,10±0,24*** -1,10±0,22***	2,01±0,11*** 1,22±0,25*** -0,79±0,20***	1,14 0,35 1,04	>0,2 >0,5 >0,5	0	0,98
Bacteriuria qualitative, points	Before After Change	0,51±0,05*** 0,22±0,05*** -0,29±0,06***	0,48±0,04*** 0,28±0,06*** -0,20±0,05***	0,49 0,75 1,18	>0,5 >0,5 >0,2	0	0,24
Leukocyturia actual, lg L/mL	Before After Change	3,61±0,14*** 3,14±0,14 -0,46±0,20*	3,51±0,18*** 3,35±0,13* -0,16±0,17	0,43 1,04 1,15	>0,5 >0,5 >0,2	3,00	0,21
Leukocyturia qualitative, points	Before After Change	0,21±0,04*** 0,08±0,03* -0,14±0,05**	0,19±0,05*** 0,13±0,03*** -0,06±0,06	0,38 1,19 1,04	>0,5 >0,2 >0,5	0	0,15

Note. Significant deviations from the norm as well as changes are indicated by stars (p<0,05*, <0,01**, <0,001***).

Table 2. Comparative characteristics of the microbiota of feces

		Naftussya water used for balneotherapy		Student's Statistics		Reference value	
Bacteria		Fresh (n=15)	Canned (n=14)	t	p	Mean	Cv/ SD
Lactobacilli, lg CFU/g	Before After Change	5,36±0,36*** 6,85±0,35** +1,49±0,54**	5,41±0,36*** 6,37±0,35*** +0,96±0,47*	0,10 0,98 0,75	>0,5 >0,5 >0,5	8,10	0,179
Bifidobacteria, lg CFU/g	Before After Change	4,76±0,32*** 6,02±0,29** +1,26±0,45**	4,91±0,29*** 5,64±0,27*** +0,73±0,38	0,35 0,96 0,90	>0,5 >0,5 >0,5	6,94	0,164
Escherichia coli common, lg CFU/g	Before After Change	8,18±0,07*** 8,31±0,07*** +0,12±0,09	8,15±0,07*** 8,26±0,07*** +0,11±0,10	0,31 0,47 0,12	>0,5 >0,5 >0,5	8,66	0,045
E. coli hemolytic, %	Before After Change	29±11** 8±7 -21±15	39±12** 14±7 -25±15	0,56 0,62 0,16	>0,5 >0,5 >0,5	0	25
E. coli attenuated, %	Before After Change	77±5*** 50±7*** -27±8**	74±5*** 65±6*** -9±7	0,42 1,63 1,70	>0,5 >0,05 >0,05	17	1,0
Klebsiela&Proteus, %	Before After Change	19±4*** 10±3** -9±6	19±6*** 11±3** -8±6	0,03 0,16 0,06	>0,5 >0,5 >0,5	0	11

Adhering to the Truskavetsian Scientific School's analytical algorithm, the actual/raw parameters were normalized by recalculation by the equations:

$Z = 4•(V – N)/(Max – Min) = (V – N)/SD = (V/N – 1)/Cv$, where

V is the actual value; N is the normal (reference) value; SD and Cv are the standard deviation and coefficient of variation respectively.

Fig. 1 visualizes how a weekly intake of fresh Naftussya water causes an increase in the significantly reduced content in the microbiota of feces/gut of classical probiotics [13] to the lower normal zone. The beneficial effect is less noticeable in relation to the total content of Escherichia coli. Instead, the marginally increased content of the E. coli strain capable of

hemolysis is almost completely normalized. However, the extremely high content of the E. coli strain with weakened enzymatic activity remains in the pessimistic zone. Finally, the less elevated content of conditionally pathogenic Klebsiela&Proteus decreases only to the upper normal zone.

The physiological benefit of the described effects on the microbiota is confirmed by their combination with a significant reduction of bacteriuria and leveling of leukocyturia as markers of pyelonephritis.

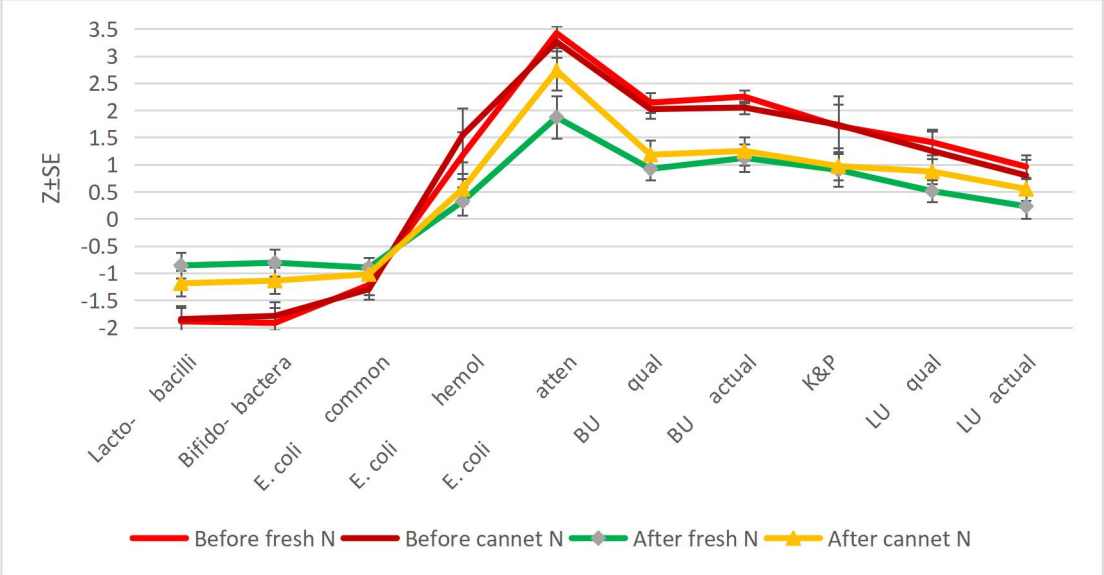

Fig. 1. Profiles of normalized parameters of microbiota and leukocyturia in patients with chronic pyelonephritis before and after a course of balneotherapy with fresh and canned Naftussya water

The effects of Naftussya water are manifested even more clearly in direct differences between individual final and initial values (Fig. 2). At the same time, it can be seen that the effect of canned Naftussya water on the registered parameters is somewhat weaker, but the differences are statistically insignificant.

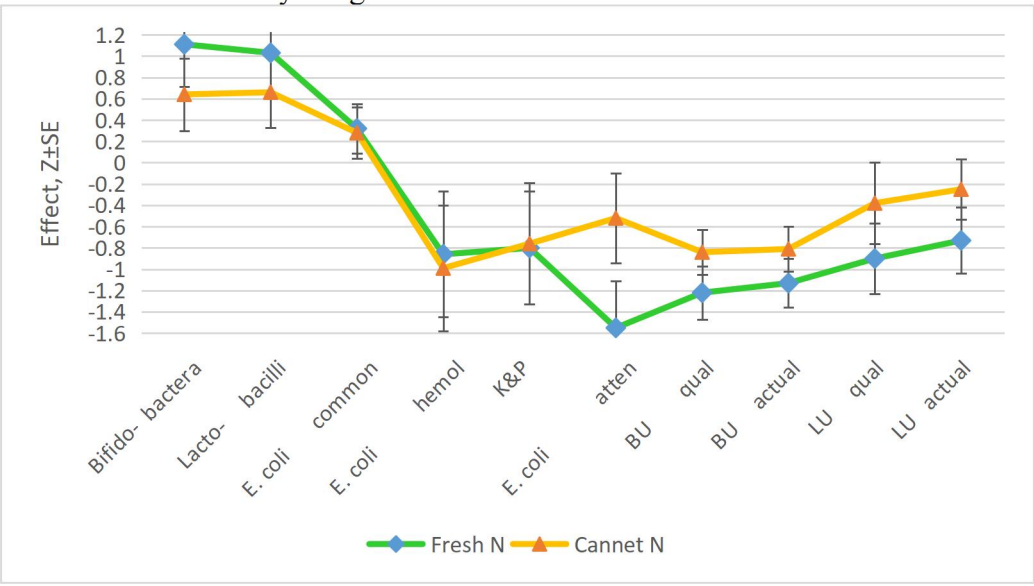

Fig. 2. Profiles of changes in normalized parameters of microbiota and leukocyturia in patients with chronic pyelonephritis after a course of balneotherapy with fresh and canned Naftussya water

From the above, one gets the impression that among the organic substances of Naftussya water [3,8] prebiotics are present.

Prebiotics are a group of biological nutrients that are capable of being degraded by microflora in the gastrointestinal tract, primarily Lactobacilli and Bifidobacteria. When prebiotics are ingested, either as a food additive or as a supplement, the colonic microflora degrade them, producing short-chain fatty acids, which are simultaneously released in the colon and absorbed into the blood circulatory system. Prebiotics boost the proliferation of gut microbes [2,7,10].

In May 2019, the International Scientific Association for Probiotics and Prebiotics (ISAPP) convened a panel of nutritionists, physiologists and microbiologists to review the definition and scope of synbiotics. The panel updated the definition of a synbiotic to "a mixture comprising live microorganisms and substrate(s) selectively utilized by host microorganisms that confers a health benefit on the host". The panel concluded that defining synbiotics as simply a mixture of probiotics and prebiotics could suppress the innovation of synbiotics that are designed to function cooperatively. Requiring that each component must meet the evidence and dose requirements for probiotics and prebiotics individually could also present an obstacle. Rather, the panel clarified that a complementary synbiotic, which has not been designed so that its component parts function cooperatively, must be composed of a probiotic plus a prebiotic, whereas a synergistic synbiotic does not need to be so. A synergistic synbiotic is a synbiotic for which the substrate is designed to be selectively utilized by the co-administered microorganisms. This Consensus Statement further explores the levels of evidence (existing and required), safety, effects upon targets and implications for stakeholders of the synbiotic concept [15].

This passage perfectly overlaps the well-known fact that about 1/3 of the mass of organic matter in Naftussya water is the products of biotransformation by its native microbes of organic matter leached from the aquifer [3,9,17].

The obtained results provide grounds for continuing research into the effects of canned Naftusya water on the body of urological patients.

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ACCORDANCE TO ETHICS STANDARDS

Tests in patients are conducted in accordance with positions of Helsinki Declaration 1975, revised and complemented in 2002, and directive of National Committee on ethics of scientific researches. During realization of tests from all participants the informed consent is got and used all measures for providing of anonymity of participants.

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