Research Article

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Intestinal Colonization Resistance and the Cytokine Response Associated with Hyperoxaluria in the Patients with Recurrent Pyelonephritis

Natalia Stepanova[*1], Victoria Driianska[2], Natalia Stashevska[1], Georgy Drannik[2] and Mykola Kolesnyk[1]

[1]*Department of Nephrology and Dialysis, Institute of Nephrology of the National Academy of Medical Sciences, Kyiv, Ukraine*

[2]*Laboratory of Immunology, Institute of Nephrology of the National Academy of Medical Sciences, Kyiv, Ukraine*

[*]Corresponding author
Natalia Stepanova, State Institution, Institute of Nephrology of the National Academy of Medical Sciences, Kyiv, Ukraine, Tel: +38 044 225 93 77; E-mail: nmstep88@gmail.com.

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Abstract

Introduction and Aims: *Hyperoxaluria and the violation of intestinal colonization resistance can be trigger factors in the formation of recurrent pyelonephritis. The aim of our study was to investigate the intestinal colonization resistance and the serum cytokines concentration in the patients with recurrent pyelonephritis depending on the presence of hyperoxaluria.*

Materials and Methods: *The observational cross-sectional study involved 70 women with recurrent pyelonephritis caused by E. coli or S. faecalis, non-stone formers. The state of the patients' intestinal colonization resistance was evaluated by bacteriological study of feces, determination of secretory IgA (sIg A) and IgA against lipopolysaccharide (LPS) of gram-negative bacteria in saliva and levels of interleukins (IL) -4 - 17, -23 and monocytic chemotactic protein-1 (MCP-1) in the serum.*

According to the presence of hyperoxaluria, the women were allocated into two groups: the first group of the patients (n = 38) had hyperoxaluria (> 44mg in 24 hours) and the second one (n = 32) didn't have any hyperoxaluria. The samples of faces had been collecting during the presence of clinical symptoms of pyelonephritis before starting the antibiotic therapy. The cytokines concentrations were analyzed using ELISA and STAT FAX-303 PLUS (Diaclon, France; DRG, Germany; Ukrmedservice, Ukraine).

All the statistical analyses were performed using MedCalc.

Results: *Microbiological studies of the colon microflora showed a lower content of Lactobacillus spp. in 53/70 (76%) in the patients with recurrent pyelonephritis. The blood levels of IL-4, IL-17, IL-23 and MCP-1 in the women with hyperoxaluria were significantly higher compared with the non-hyperoxaluria patients: 62.2 [52.8-74.1] vs 44.5 [35.8-67] pg/ml (P=0.019), and 130.7 [101.3-231.2] vs 103.4 [77.5-133.9] (P=0.03), 123.2±17.1 vs 80.98±29.4 (P=0.03) and 325.2 [211-500] vs 121.4 [104-107.8] (P=0.0003), respectively. The saliva levels of sIg A and IgA against LPS were significantly higher in patients of the first group: 298 ± 104 vs 150.1 ± 79.3 (P<0.0001) and 0.353 ± 0.16 vs 0,211 ± 0.09 respectively.*

In addition, we identified a moderate direct correlation between the blood level of IL-17 in the patients with recurrent pyelonephritis and daily excretion of oxalate: R=0.54, P=0.03.

Conclusions: *The deficit of Lactobacillus spp. violates the immune response and oxalate metabolism with formation of hyperoxaluria. The blood cytokines concentration in the patients with recurrent pyelonephritis and hyperoxaluria has not been scrutinized before. The further studies are needed to determine the role of these cytokines in the progression of urolithiasis in hyperoxaluric conditions.*

Introduction and Aims

Hyperoxaluria often associates with recurrent pyelonephritis which may be caused by destruction of the Oxalobacter formigenes colonies in the intestinal tract [1-3]. Antibacterial treatment and long-term antibiotic prophylaxis can disrupt the normal flora of the gastrointestinal tract and lead to violate of oxalate metabolism [4-7]. In this way, hyperoxaluria and the violation of intestinal colonization resistance can be trigger factors in the formation of recurrent pyelonephritis. But, there are a limited number of studies that have analyzed the effects of gut microbiota composition on the systemic inflammation and formation of hyperoxaluria in the patients with recurrent pyelonephritis.

Crystal-cell interaction has been reported as one of the most crucial steps in urinary stone formation [8-9]. Many clinical studies have demonstrated the leading role of inflammation on renal damage associated with CaOx crystals and the cellular and molecular mechanisms involved in the genesis of these processes [8-10,11]. But, their basic part has been examined *in vivo* by inducing hyperoxaluria in rats [7-12,13].

Recent experimental studies have demonstrated the leading role of IL-17 and IL-23 in the pathogenesis of inflammatory bowel disease [8-12,14]. Moreover, the expression of intestinal epithelial cells cytokine IL-17 family depend on synanthropic bacteria, namely: the reduction of the total microbiota in the adult mice after the administration of antibiotics has resulted in increased expression of IL-23 and -17 in the colon, suggesting that the synanthropic bacteria are active inhibitors of IL-23 and IL-17 [12]. Although, it should be noted that there have not been any descriptions of the results of clinical studies on the content of pro- and anti-inflammatory cytokines in the blood and urine in the patients with hyperoxaluria in the scientific literature yet.

The purpose of the present study was to investigate the intestinal colonization resistance and the serum cytokines concentration in the patients with recurrent pyelonephritis depending on the presence of hyperoxaluria.

Materials and Methods

The observational cross-sectional study involved 70 women with recurrent pyelonephritis caused by *E. coli* or *S. faecalis*, non-stone formers. The mean age in the patient population was 21-48 yrs (32.3 ± 8.2). Duration of the disease was from 0.5 to 18 years (mean duration 7.6 ± 5.4 yrs). The average of urinary oxalate excretion was 91.9 ± 22.7 mg/d. Recurrent pyelonephritis was defined as 2 upper urinary tract infection episodes within 6 months or 3 or more episodes during the previous 12 months.

The Local Ethics Committee approved the study protocol.

According to the presence of hyperoxaluria, the women were allocated into two groups: the first group of the patients (n = 38) had hyperoxaluria (> 44 mg in 24 hours) and the second one (n = 32) didn't have any hyperoxaluria. The control group consisted of 15 conditionally healthy donors.

The state of the patients' intestinal colonization resistance was evaluated by bacteriological study of feces, determination of secretory IgA (sIg A) and IgA against lipopolysaccharide (LPS) of gram-negative bacteria in saliva and levels of interleukins (IL) -4 - 17, -23 and monocytic chemotactic protein-1 (MCP-1) in the serum. The samples of faces had been collecting during the presence of clinical symptoms of pyelonephritis before starting the antibiotic therapy.

The concentration of interleukins (IL) -4, -17, -23 and MCP-1 were analyzed in the blood of 40 women using an ELISA and STAT FAX-303 PLUS (Diaclon, France; DRG, Germany; Ukrmedservice, Ukraine). The analysis of 24-h urinary oxalate excretions was performed by suppressed ion chromatography.

For the statistical analysis, we used the Student's t-test, nonparametric (U-test) Mann-Whitney and Pearson's rank correlation test. The average values (M) and standard deviation (SD) or the median (Me) and interquartile ranges [Q25; Q75] were calculated according to a normal distribution. All the statistical analyses were performed using MedCalc.

Results

Microbiological studies of the colon microflora showed a lower content of *Lactobacillus spp.* in 53/70 (76%) in the patients with recurrent pyelonephritis. The increasing level of opportunistic bacteria (*Klebsiella pn., Proteus, E. coli* with altered enzymatic properties) was determined in 34/70 (49%) of the women. In general, all of the examined patients had the gut dysbiosis. But, we have to note, that only 28/70 (40%) of them had clinical signs of gut dysbiosis.

The comparative analysis of the fecal microbiota composition depending of the presence of hyperoxaluria identified a significant decrease of bifidobacteria (10 [5-900] vs 800 [10-1000] CFU/g, p = 0.03) and lactobacteria (0.14 [0.1-0.5] vs 4 [0.2-20] CFU/g, p = 0.0001) in the women of the first group. Moreover, in the patients with the deficit of intestine *lactobacillus spp.*, we observed the high level of hyperoxaluria (110.8 ± 39 vs 55.5 ± 29 mg/day, p < 0.0001) and significant increase of episodes of frequent pyelonephritis recurrences (5.8 ± 3.8 vs 3.1 ± 2.9 per year, p < 0.0001).

The quantitative content of *Lactobacillus spp.* in the patients' intestine was significantly correlated with the level of daily urinary oxalate excretion (R=- 0.72; P < 0.0001). That is, the less the number of *Lactobacillus spp.* in the composition of intestinal in the women was, the more the levels of hyperoxaluria occurred (Figure 1).

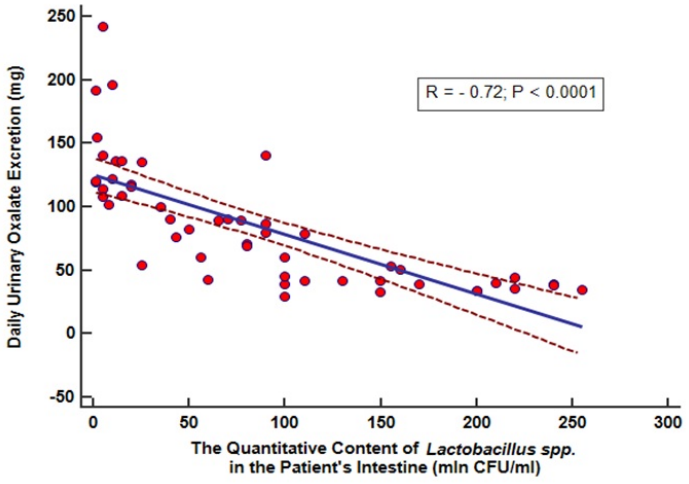

Figure 1: The correlation between the levels of the urinary oxalate excretion and the content of *Lactobacillus spp.* in the intestine.

All the patients had increased synthesis of sIg A and IgA against LPS of gram-negative bacteria in the saliva, as well as the concentration of the MCP-1, IL-4, IL-17 and IL-23 in the serum (Table 1).

Markers (pg/ml)	The conditionally healthy donors (n = 15)	The patients (n = 70)	P
The saliva markers M ± 2SD			
sIg A (mg/L)	278.4 ± 98.7	138.8 ± 77	<0,0001
IgA against LPS (CAU)	0.056 ± 0.02	0.32 ± 0.19	<0.0001
The serum markers Me [Q25-Q75]			
MCP-1 (pg/mL)	96 [57-120]	211.7 [97.8-407.4]	0.0001
IL-4 (pg/mL)	16.1 [14-17]	59.6 [45.4-68.8]	<0.0001
IL-17 (pg/mL)	63 [37.9-85.2]	121.3 [94-123]	<0.0001
IL-23 (pg/mL)	25 [16-36]	80.6 [60.8-113.1]	<0.0001

Table 1: The comparative analysis of the studied saliva and blood markers in the patients with recurrent pyelonephritis and conditionally healthy donors. sIg A: Secretory IgA, LPS: Lipopolysaccharide, CAU: Conditional Absorbance Units.

The saliva levels of sIg A and IgA against LPS, and the blood levels of MCP-1, IL-4, IL-17 and IL-23 in the women with hyperoxaluria were significantly higher compared with the non-hyperoxaluria patients (Table 2).

Markers (pg / ml)	Group 1 (n = 38)	Group 2 (n = 32)	P
The saliva markers M ± SD			
sIg A (mg/L)	298 ±104	150.1 ± 79.3	<0.0001
IgA against LPS (CAU)	0.353 ± 0.16	0.211 ± 0,09	<0.0001
The serum markers Me [Q25-Q75]			
MCP-1 (pg/mL)	325.2 [211-500]	121.4 [104-107.8]	0.0003
IL-4 (pg/mL)	62.2 [52.8-74.1]	44.5 [35.8-67]	0.019
IL-17 (pg/mL)	130.7 [101.3-231.2]	103.4 [77.5-133.9]	0.03
IL-23 (pg/mL), M ± SD	123.2 ± 17.1	80.98 ± 29.4	0.03

Table 2: The comparative analysis of the saliva and blood markers between the patient's groups. sIg A: secretory IgA, LPS: lipopolysaccharide, CAU: conditional absorbance units.

Moreover, the blood levels of IL-4 and IL-17 in the women with the deficit of *Lactobacillus spp.* in the gut (n = 53) were significantly higher compared with the deficit-free patients (n = 17): 61.45 [47.8-69.7] vs 47.05 [36.1-67.9] pg/ml (P=0.04), and 126.8 [98.7-217] vs 108.4 [76-143] pg/ml (P=0.01), respectively (Figure 2).

In addition, we identified a moderate direct correlation between the blood level of IL-17 in the patients with recurrent pyelonephritis and the daily oxalate excretion (r=0.54, p=0.03; Figure 3).

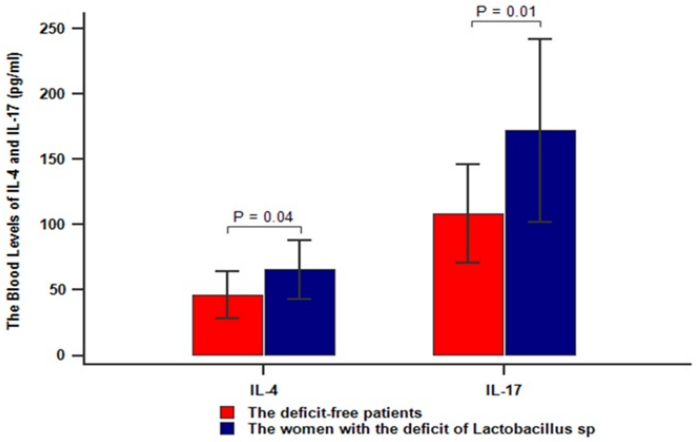

Figure 2: The blood levels of IL-4 and IL-17 in the women with recurrent pyelonephritis.

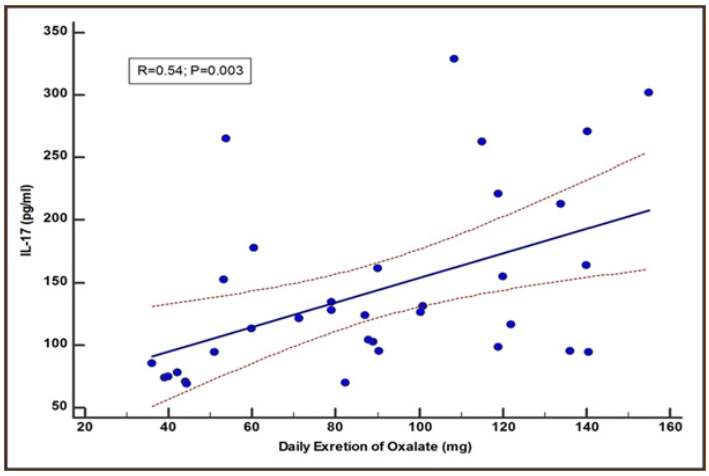

Figure 3: The correlation between the levels of the urinary oxalate excretion and serum IL-17.

Conclusions

The status of intestinal colonization resistance of the patients with recurrent pyelonephritis and hyperoxaluria is characterized by a decrease in the content of indigenous microflora. The deficit of *Lactobacillus spp.* violates the immune response and oxalate metabolism with formation of hyperoxaluria. Thus, it is possible to conclude that it leads to a decrease in the immunological properties of the gut microbiota and may be one of the causes of hyperoxaluria. The blood and urinary cytokines concentration in the patients with recurrent pyelonephritis and hyperoxaluria has not been scrutinized before. The further studies are needed to determine the role of these cytokines in the progression of urolithiasis in hyperoxaluric conditions.

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