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Hyperoxaluria-Associated Cytokines Dysregulation in Women with Recurrent Pyelonephritis

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ABSTRACT— The purpose of the study was to investigate the immune response in patients with recurrent pyelonephritis depending on the presence of hyperoxaluria.The observational cross-sectional study involved 64 women with recurrent pyelonephritis. The patients' immune response was evaluated by determination of serum concentrations of interleukins (IL) -4, -17, -18, -23, tumor necrosis factor-alpha (TNF-α) and monocyte chemoattractant protein 1 (MCP-1). Depending on the presence of hyperoxaluria (urinary oxalate excretion was more than 0.45 mmol per day), the patients were allocated into 2 groups: the women with hyperoxaluria were included to group I (n = 35) and the patients with normal levels of oxalate excretion were included to group II (n = 29). The control group consisted of 25 practically healthy donors.The mean age in the patient population was 31.6 ± 7.7. The average number of pyelonephritis recurrence was 6.4 ± 1.9 per year. We identified a moderate direct correlation between the levels of the urinary oxalate excretion and the number of pyelonephritis recurrences per year ($r = 0.71$, $p < 0.0001$) and the inverse strong correlation between oxaluria and GFR level ($r = 0.75$, $p < 0.0001$). The patients with hyperoxaluria had increased synthesis of the blood concentration of TNF-α, MCP-1, IL -4, -17 and 23. Our results have provided preliminary evidence that hyperoxaluria is associated with increased serum levels of IL-4, -17, -23, MCP-1 and TNF-α. The larger-scale studies are needed for further confirmation of our findings.

Keywords— hyperoxaluria, recurrent pyelonephritis, cytokines.

1. Introduction

Recurrent pyelonephritis is an important medical and social problem [1, 2]. Nephrolithiasis and preexisting nephrolithiasis states often coexist with recurrent pyelonephritis which may be caused by the destruction of the intestinal microbial composition [3].

The constant use of antibacterial medicine, including long-term antibiotic prophylaxis in patients with recurrent pyelonephritis, can disrupt the normal gut microbiota and leads to the destruction of O. formigenes with hyperoxaluria formation [4-6]. It would be logical to assume the formation of a so-called "closed circle": on the one hand, a disturbance of the intestinal microbiota and the development of dysbiosis are the main sources of the urinary tract infection, and, on the other hand, they lead to a disturbance of oxalate transport and acquired hyperoxaluria. In turn, crystal deposition in the proximal renal tubules causes chronic inflammation with fibrosis [7], and, it can be an independent risk factor for pyelonephritis recurrences. In this context, data on the cytokines response as one of the factors in the recurrent pyelonephritis formation appear to be well-founded.

However, 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 blood cytokines in patients with hyperoxaluria in the scientific literature yet. The evaluation of hyperoxaluria-associated systemic immune response in patients with recurrent pyelonephritis is relevant both in order to correct hyperoxaluria and reduce the frequency of disease recurrent.

The purpose
of the study was to investigate the immune response in patients with recurrent pyelonephritis depending on the presence of hyperoxaluria.

2. Materials and methods.

Study Design and Subjects.

This observational cross-sectional study was conducted at State Institution «Institute of Nephrology of the National Academy of Medical Sciences» in Kyiv, Ukraine between January 2016 and May 2019. The study was carried out within the framework of the Institute's research work: "The Study of the Intestinal Colonization Resistance Disorder Mechanisms and the Metabolic Functions of Gut Microbiota in Patients with Pyelonephritis as the Potential Risk Factors for Recurrent Pyelonephritis" (Domestic Trial Registration Number is 0116U000030). Writing informed consent was obtained from all the subjects participating in the study. The study protocol was confirmed by the Ethics Committee of the Institute.

Sixty-four women with recurrent pyelonephritis caused by E. coli or S. faecalis, non-stone formers were involved in the study according to such criteria: the presence of clinical signs of the disease (dysuria, frequent urination, increasing body temperature, pains, and severity in the bone-vertebral angle and others), leukocyturia and positive urine culture (at least 105 colony-forming units/mL of uropathogenic bacteria). The exclusion criteria were: a refusal of the patient to participate in the study, kidney stones, pregnancy and lactation, symptoms of urinary tract obstruction, decrease in glomerular filtration rate (GFR) < 60 mL/min/1.73 m2.

All of the patients underwent a complete evaluation including a detailed history, a physical examination, urine bacterial culture analysis and microscopic examination, routine blood tests.

Recurrent pyelonephritis in the patients was defined as 2 upper urinary tract infection episodes within 6 months or 3 or more episodes during the previous 12 months. Complicated pyelonephritis was diagnosed when the patient has anatomical, functional, or metabolic risk factors.

Depending on the presence of hyperoxaluria (urinary oxalate excretion was more than 0.45 mmol per day) [7], the patients were allocated into 2 groups: the women with hyperoxaluria were included to group I (n = 35) and the patients with normal levels of oxalate excretion were included to group II (n = 29). The control group consisted of 25 practically healthy donors.

The patients' immune response was evaluated by determination of serum concentrations of interleukins (IL) -4, -17, -18, -23, tumor necrosis factor-alpha (TNF-α) and monocyte chemoattractant protein 1 (MCP-1).

Sample collection and methods.

Whole blood tests were collected from the patients after an overnight fast during the time of their visit. Blood was collected using serum separator tubes. The tubes were immediately centrifuged at 2500 g for 10 minutes, and, the separated serum was stored at −80°C. Serum concentrations of IL -4, -17, -18, -23, TNF-α

and MCP-1 were detected using the STAT FAX-303 PLUS and ELISA kit (Diaclon, France; DRG, Germany; Ukrmedservice, Ukraine) according to the manufacturer protocols. The boundaries of normal values (reference range) were obtained based on the study results of 25 practically healthy individuals.

Routine biochemical blood tests including concentrations of urea and creatinine, serum albumin, C-reactive protein (CRP), glucose, electrolytes, and lipid profile parameters were carried out using an automatic analyzer "Flexor junior" (Netherlands). Hematological parameters of blood were determined using an "ABX Micros-60" (France).

GFR was calculated using the Chronic Kidney Disease Epidemiology Collaboration creatinine equation [8]. The analysis of 24-h urinary oxalate excretions was performed using suppressed ion chromatography. The urinary β2-microglobulin level in the participants was assayed using the beta-2-microglobulin ELISA kit (DRG).

Statistical analysis.

Analysis and all graphs were performed using MedCalc (Belgium). For the statistical analysis, we used the Student's t-test and nonparametric (U-test) Mann-Whitney taking into account the verification of indicators for a normal distribution with the Kolmogorov-Smirnov 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.

Categorical variables were expressed as proportions, and, chi-square tests were used for the comparison of 2 groups. Pearson's or Spearman's (as appropriate) correlation tests were used to evaluate relationships between biomarkers and the urinary oxalate excretion.

3. Result

The patients ranged in age from 18 to 52 years, with a mean age of 31.6 ± 7.7 years. The duration of the disease was from 6 months to 16 years (6.0 ± 4.1 yrs). The average number of disease recurrence was 6.4 ± 1.9 per year. Characteristics of the 64 enrolled patients depending on the presence of hyperoxaluria are provided in Table 1.

Table 1. Baseline characteristics of the study participants according to the oxaluria status

Clinical parameters	**Hyperoxaluric patients (Group I, n = 35)**	**Normo-oxaluric patients (Group II, n = 29)**	**P**
Age, years	33 ± 3.9	35.1 ± 7.8	0.1
Duration of the disease, years	5.8 ± 3.6	7.4 ± 3.0	0.39
Pyelonephritis recurrence rate per year	5.7 ± 3.7	3.4 ± 1.03	0.01
Chronic complicated pyelonephritis, %	37.0	41.0	0.71
Pyelonephritis caused by *E. coli*, %	54.3	60.7	0.58
Pyelonephritis caused by *S. faecalis*, %	45.7	39.3	0.6
Daily urinary oxalate excretion, mg	78.2 ± 7.4	43.8 ± 5.2	0.001
CRP, mg/L	20.8 [6.0-25]	18.6 [9.7-17.2]	0.29
GFR, mL/min/1.73 m2	68.9 ± 12.1	81.2 ± 13.6	0.0001
Urinary *β2*-microglobulin, μg/mL	50.0 ± 23	40.8 ± 15.5	0.03

White blood cell counts of blood, mm^3	12.2 [8.5-13.7]	11.75 [8.7-14.2]	0.66
Body temperature, ˚C	37.7 ± 2.1	38.1 ± 1.8	0.42
Costovertebral angle tenderness, %	78.9	85.7	0.49

The values are expressed as mean ± standard deviation (M ± SD) or as median and interquartile range (Me [Q25-Q75], or proportion (%). The values are compared between the groups by the t-test, Mann–Whitney U test and chi-square test as appropriate.
Abbreviations: CRP, C-Reactive Protein; GFR, glomerular filtration rate.

As evidenced in Table 1, the groups were identical in terms of age, nosology, and the etiological factors of the disease, its duration, and severity. However, the frequency of recurrent pyelonephritis during a year was significantly higher in the patients of group I. Moreover, the women with hyperoxaluria had lower GFR and higher urinary β2-microglobulin levels compared with the normal-oxaluric patients.

Moreover, we identified a moderate direct correlation between the levels of the urinary oxalate excretion and the number of pyelonephritis recurrences per year ($r = 0.71$, $p < 0.0001$) (Fig. 1). In contrast, we detected the inverse strong correlation between oxaluria and GFR level ($r = 0.75$, $p < 0.0001$), which is indicative of oxalate-induced renal damage (Fig. 2).

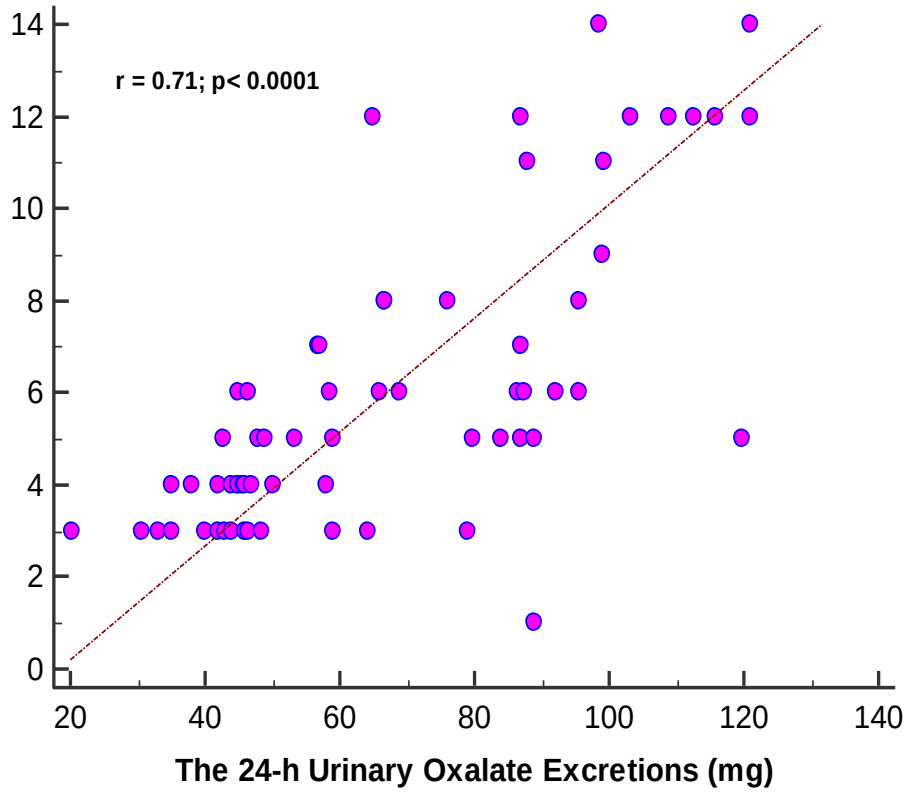

Fig. 1. The correlation between urinary oxalate excretions and the number of pyelonephritis recurrences per year.

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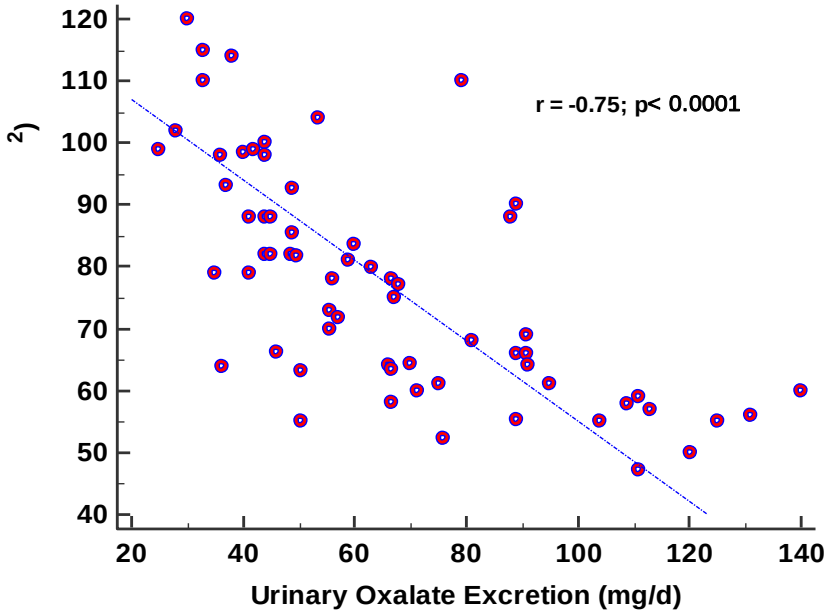

Fig. 2. The correlation between urinary oxalate excretions and GFR in patients with recurrent pyelonephritis.

In comparison with healthy donors, a significant increase in the content of serum MCP-1, TNF-α, IL-4, IL-17 and IL-23 was defined in all patients with recurrent pyelonephritis (Table 2).

Table 2. The comparative analysis of MCP-1 and serum cytokines in the patients with recurrent pyelonephritis and practically healthy donors

Indicator (pg/ml)		**Practically healthy donors (n = 25)**	**The patients with recurrent pyelonephritis (n = 64)**	**P**
MCP-1	Me [Q25-Q75]	96 [57-120]	211.7 [97.8-407.4]	0.0001
IL-4		16.1 [14-17]	59.6 [45.4-68.8]	<0.0001
IL-17		63 [37.9-85.2]	121.3 [94-123]	<0.0001
IL-18		27.6 [13.5-44.3]	39.5 [26.2-51.2]	0.4
IL-23		25 [16-36]	80.6 [60.8-113.1]	<0.0001
TNF-α	M ± SD	2.9 ± 2.3	19.2 ± 9.1	< 0.0001

The values are expressed as mean ± standard deviation (M ± SD) or as the median and interquartile range (Me [Q25-Q75]). The values are compared between the groups by the t-test and Mann–Whitney U test as appropriate.

Abbreviations: MCP-1, monocyte chemoattractant protein 1; IL, interleukin; TNF-α, tumor necrosis factor-alpha.

Moreover, the analysis of the immunological profile in the patients with recurrent pyelonephritis depending on the presence of hyperoxaluria demonstrated a high production of key inflammatory mediators (MCP-1, TNF-α, IL-4, -17 and -23) (Table 3).

Table 3. The comparative analysis of MCP-1 and serum cytokines in the patients with recurrent pyelonephritis depending on the presence of hyperoxaluria

Indicator (pg/ml)		**Group I (n = 35)**	**Group II (n = 29)**	**P**
MCP-1	Me [Q25-Q75]	325.2 [211-500]	121.4 [104-107.8]	0.0003
IL -4		62.2 [52.8-74.1]	44.5 [35.8-67]	0.019
IL -17		130.7 [101.3-231.2]	103.4 [77.5-133.9]	0.03
IL -18		40.9 [29-64]	27.3 [15.5-36.3]	0.061
IL -23	M ± SD	123.2 ± 17.1	80.98 ± 29.4	0.03
TNF-α		21.7 ± 7.6	10.8 ± 9.6	0.007

The values are expressed as mean ± standard deviation (M ± SD) or as the median and interquartile range (Me [Q25-Q75]). The values are compared between the groups by the t-test and Mann–Whitney U test as appropriate.

Abbreviations: MCP-1, monocyte chemoattractant protein 1; IL, interleukin; TNF-α, tumor necrosis factor-alpha.

In idition, a direct correlation between the daily oxalate excretion and blood levels of MCP-1 (r = 0.7, p = 0.0004) and IL-17 (r = 0.54, p = 0.03) in the patients with recurrent pyelonephritis was observed.

4. Discussion.

The problem of alteration of intestinal flora observed in chronic kidney disease or kidney stone formation and its effect on local and systemic immunity has been well described in previous studies [9-11]. But, specific reports on cytokines response in human subjects are limited. Unfortunately, at present, there are no studies devoted to the immunological response of hyperoxaluria conditions in modern scientific literature. To our knowledge, this study is the first cohort study to compare the immunological markers according to hyperoxaluria status in patients with recurrent pyelonephritis.

The main finding of the present study was the high production of key inflammatory mediators (IL-4, -17 and -23, MCP-1, TNF-α) in patients with hyperoxaluria. In addition to well-known scientific data, recent studies have indicated the fact that IL-4 has alternative and pro-inflammatory functions depending on the model of intestinal inflammation [12]. Experimental studies on the production of IL-4 by mononuclear cells, isolated from their own plate of the mucous membrane of the large intestine, demonstrated its ability to modulate local immune responses both under normal and inflammatory conditions [12]. The results of our own research have demonstrated the elevation of IL-4 in hyperoxaluria patients, which is an indirect sign of intestinal epithelium damage.

High levels of IL-17 and IL-23 production in the hyperoxaluria presence deserves special attention. Today, the leading role of intestinal microbiota in the support of the balanced status of T-regulatory cells /T-helper type 17 (Treg / Th17) has already been proven [10, 13, 14]. The differentiation of Th 17 from naive CD4+ T lymphocytes occurs in several stages: under the influence of IL-16 and IL-1, naive CD+ 4 T-lymphocytes are transformed into cells by precursors of Th 17 cells, and, subsequently, they mature in Th 17 under the influence of IL-23, which produce IL-17 and other inflammatory cytokines: TNF-α, IL-6, IL-8, MCP-1, as well as IL-23 [13]. Our findings demonstrated significantly high serum levels of IL-17 and IL-23 which might be one of the possible explanations for the hyperoxaluria condition in women with pyelonephritis.

MCP-1 plays an important role in attracting monocytes and macrophages in the blood to any type of inflammatory tissues including the intestine and kidneys [15, 16]. Until recently, it was thought that the main source of MCP-1 was epithelia at the site of inflammation. But, today, the experimental studies have proven the ability of MCP-1 production even by normal epithelial cells of the intestine and kidneys [16]. At the same time, overexpression of MCP-1 can also be the result of the interaction between renal epithelial cells and CaOx crystals after their sedimentation in the renal tubules [26]. Thus, the overproduction of this chemokine in women with recurrent pyelonephritis can be explained by the oxalate-induced inflammatory reaction of the kidneys [16].

Eventually, recent experimental studies have demonstrated the leading role of these cytokines in the pathogenesis of inflammatory bowel disease [17]. Furthermore, the expression of the intestinal epithelial cells of IL-17 cytokine family depended on synanthropic bacteria, and, namely: a decrease in the total microbiota in the adult mice after the administration of antibiotics resulted in an increased expression of IL-17 and IL -23 in the large intestine. It was suggested that synanthropic bacteria were active inhibitors of IL-23 and IL-17 [17, 18], and, this idea was indirectly confirmed by the results of our study.

Finally, we would like to focus on TNF-α which is an inflammatory cytokine implicated in various kidney diseases, including the formation of kidney stones [19]. Mulay SR et al [19] have demonstrated the TNF-α-mediated renal cell necroptosis that contributes to CaOx crystal-induced kidney pathology and, accordingly, is a fundamental mechanism for the initiation of nephrocalcinosis. At the same time, current clinical and experimental studies indicate that the gut microbiota contributes significantly to TNF-α production [19]. In support of these data, we found a significantly increased concentration of TNF-α in the patients with hyperoxaluria.

It is important to stress that our study has several limitations. First, it was a small sample size study performed in a single center; therefore, our findings only revealed associations. Second, the blood tests were measured only once. Third, we did not investigate the quantitative and qualitative composition of fecal microbiota and its relationship with cytokines response. Finally, we did not take into account the influence of dietary oxalate intake.

Despite its limitations, the strong association observed in the present study has indicated the potential power of hyperoxaluria in the development of immune responses.

5. Conclusions.

In conclusion, this study is the first to report the clinical importance of hyperoxaluria in patients with recurrent pyelonephritis. The blood cytokines concentration in patients with recurrent pyelonephritis and hyperoxaluria has not been scrutinized before. Our results have provided preliminary evidence that hyperoxaluria is associated with the increased serum levels of IL-4, -17, -23, MCP-1 and TNF-α. The larger-scale studies are needed for further confirmation of our findings.

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