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DIFFERENCES AND INTERSECTIONS IN RENAL BIOPSY FINDINGS IN PATIENTS WITH ASYMPTOMATIC HYPERURICEMIA AND GOUT

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Abstract

The aim of this study is to establish the association between asymptomatic hyperuricemia and gout with morphological and immunofluorescent changes in the renal biopsy. A total of 64 patients, 46 with asymptomatic hyperuricemia and 18 with gout were included in this retrospective study. Renal biopsy findings, clinical and laboratory data were analyzed by using medical documentation. We did not find a significant difference in the distribution of chronic renal failure between the two groups. In the gout group, the proportion of patients with nephrolithiasis was higher ($p < 0.001$), and the presence of erythrocyturia was more common ($p = 0.047$). The percentage of damaged glomeruli ($p = 0.249$) and the distribution of mesangial proliferation ($p = 0.536$) was similar in the groups. The proportion of patients with interstitial fibrosis $> 50\%$ was significantly higher in the gout group ($p = 0.032$), but no difference was observed in the distribution of tubular atrophy $> 50\%$ ($p = 0.183$). In subjects not receiving urate-lowering therapy, serum uric acid levels were comparable in the different stages of tubular atrophy and interstitial fibrosis. We found that in both asymptomatic hyperuricemia and gout, there is a deposition of immune deposits subepithelially, subendothelially, in the mesangium and in the vessel walls. In gout, the kidneys are affected to a much greater extent. We consider that not only the increased serum uric acid is important, but also monosodium urate crystals deposited in the renal interstitium causing a chronic inflammatory process followed by fibrosis. We suggest that the activation of the innate

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immune system by soluble uric acid and crystals with the subsequent development of a proinflammatory state in the body has led to the activation of complement and deposition of immune deposits in the kidneys.

Key words: gout, hyperuricemia, renal morphological changes, kidney immune deposits

Introduction. From 1890, hyperuricemia has been associated with chronic urate nephropathy [1], in which urate deposits are found in the interstitium where they cause moderate inflammation and fibrosis. In a study conducted before the era of urate-lowering drugs, 20 to 60% of patients with gout had mild to moderate renal impairment [2].

The antioxidant activity of uric acid has led many experts to perceive hyperuricemia as an adaptive response of the body, preventing from oxidative stress due to age, neoplasia or cardiovascular disease [3].

The risk of crystallization is the main consequence of the increased concentration of urate in the urine. Excessive urinary uric acid excretion causes acute urate nephropathy, nephrolithiasis and gouty nephropathy. From 25 to 40% of patients with gout have renal dysfunction. Renal damage is expressed in varying degrees of arteriosclerosis, glomerulosclerosis and interstitial fibrosis, often with the deposition of urate crystals in the outer medulla [4]. However, data on whether immune deposits are found in the renal biopsy in these conditions are quite insufficient.

In the present study, we aimed to establish the relationship between the disease (asymptomatic hyperuricemia and gout) with morphological and immunofluorescent changes in the renal biopsy.

Patients and methods. In this retrospective study, the renal biopsy findings, clinical and laboratory data of a total of 64 patients, 46 with asymptomatic hyperuricemia and 18 with gout, hospitalized in the Clinic of Nephrology at the University Hospital "St. Ivan Rilski" for the period 2013–2020 were analyzed. By using the medical documentation the data were analyzed by a rheumatologist and a nephrologist. Patients with hyperuricemia did not have a gouty arthritis clinic. Subjects with gout were diagnosed before hospitalization. For hyperuricemia, we considered serum uric acid levels above the upper reference limit (above 404.5 µmol/l for men and above 356.9 µmol/l for women).

The other clinical and laboratory indicators that were evaluated were: smoking; arterial hypertension taken at systolic blood pressure $\geq$ 140 mmHg, diastolic $\geq$ 90 mmHg or treatment with antihypertensive drugs; diabetes mellitus, based on physician's diagnosis and/or documented use of insulin and/or oral hypoglycemic agents; dyslipidemia, based on elevated fasting lipid levels or low high density lipoprotein cholesterol (HDL) and/or documented use of lipid-lowering agents; reduced glomerular filtration rate taken at estimated glomerular filtration rate (eGFR) $<$ 90 ml/min calculated by the Cockroft-Gault formula, chronic kidney failure (CKF) was accepted by K/DOQI 2002 guidelines [5]. Total protein, serum

albumin, blood urea nitrogen (BUN), erythrocyte sedimentation rate (ESR), 24-hour proteinuria, the presence of erythrocytes and uric acid crystals in the urine, as well as ultrasound data for nephrolithiasis were all reported. The therapy carried out by the patients was also taken into account. Individuals were defined as having suffered a cardiovascular event if they had coronary artery disease, cerebrovascular disease and/or peripheral artery disease [6]. Subjects with Systemic Connective Tissue Diseases or Vasculitis, as well as those with neoplastic processes were excluded.

The renal biopsy was analyzed by a pathologist who was unfamiliar with the patients' clinical and laboratory data. The number of damaged glomeruli, mesangial proliferation (without expansion; with expansion, expansion with proliferation) was assessed. The stage of tubular atrophy and interstitial fibrosis was determined as follows: $< 25\%$; $> 25\%$ and $< 50\%$; $> 50\%$. By applying direct immunofluorescence the localization of the different types of immune deposits is described.

Statistical analyses were performed by using statistical software SPSS version 13 (SPSS Inc., Chicago, IL, USA). The distribution of the quantitative variables was tested with the One-Sample Kolmogorov–Smirnov test. Normally distributed data were presented as Mean ± SD, while abnormally distributed data were given as Median or interquartile range (IQR). Categorical variables were presented as number (n) or percentage (%). Comparisons of two independent groups were conducted by t-test and Mann–Whitney test. In cases with more than two groups comparisons were done by the test of ANOVA. Correlation analyses were performed by the Spearman correlation coefficient. Statistical significance of the results is assumed at $p < 0.05$.

Results. Gout patients were predominantly male ($p = 0.021$). In the distribution of dyslipidemia and CKF no significant difference was observed ($p = 0.055$, $p = 0.085$). Individuals with diabetes mellitus were less in the gout group than in the asymptomatic hyperuricemia group ($p = 0.010$). Patients with gout receiving Febuxostat were markedly more than patients with hyperuricemia ($p = 0.001$). The proportion of subjects treated with corticosteroids was significantly higher in the gout group ($p = 0.017$). We found no difference in the distribution of individuals treated with NSAIDs ($p = 0.122$) and aspirin ($p = 1.000$). In the gout group, the proportion of patients with nephrolithiasis was significantly higher ($p < 0.001$), and the presence of erythrocyturia was more common ($p = 0.047$), while the distribution of uric acid crystals in the urine was equal ($p = 0.822$), (Table 1).

Neither in the group of asymptomatic hyperuricemia nor in the group of gout we estimated a correlation between the serum level of uric acid and the level of hemoglobin, creatinine and 24-hour proteinuria (Fig. 1).

Between the two groups there was no difference in the percentage of damaged glomeruli ($p = 0.249$) and in the distribution of mesangial proliferation

Table 1

General characteristics of the patients

Index	Asymptomatic hyperuricemia ($n = 46$)	Gout ($n = 18$)	p
Age (years) /mean(SD)/	50.61 (13.67)	55.94 (10.18)	139.000
Sex (males) /n(%)/	27 (58.7)	16 (88.9)	**0.021**
Disease duration (years) /median(IQR)/	0.00 (0.00)	7.00 (13.00)	**< 0.001**
Serum uric acid (µmol/l) /mean(SD)/	484.67 (97.42)	506.72 (143.21)	0.483
BUN (mmol/l) /mean(SD)/	9.69 (4.85)	11.26 (6.48)	0.349
Total protein (g/l) /mean(SD)/	65.85 (8.21)	66.31 (7.49)	0.903
Serum albumin (g/l) /mean(SD)/	43.16 (5.39)	41.69 (3.92)	0.333
eGFR (ml/min) /median(IQR)/	63.16 (55.59)	44.24 (26.05)	0.138
Proteinuria (g/24 h) /median(IQR)/	1.00 (2.00)	1.50 (4.00)	0.416
ESR (mm/h) /mean(SD)/	29.05 (24.01)	29.40 (23.5)	0.961
Dyslipidemia /n(%)/	21 (45.7)	13 (72.2)	0.055
BMI (>30 kg/m^2) /n(%)/	9 (19.6)	2 (11.1)	0.714
Arterial hypertension /n(%)/	42 (91.3)	18 (100)	0.570
Patients suffered a cardiovascular event /n(%)/	10 (21.7)	3 (16.7)	0.743
Patients with diabetes mellitus /n(%)/	21 (45.7)	2 (11.1)	**0.010**
Patients with chronic kidney disease /n(%)/	24 (70.6)	10 (100.0)	0.085
Smokers /n(%)/	13 (28.3)	9 (50.0)	0.176
Alcohol users /n(%)/	1 (3.2)	1 (16.7)	0.302
ACE-inhibitors /n(%)/	22 (48.9)	7 (38.9)	0.472
Ca-antagonists /n(%)/	25 (56.8)	11 (61.1)	0.756
Beta-blockers /n(%)/	24 (54.5)	9 (50.0)	0.745
Diuretics /n(%)/	22 (50.0)	8 (44.4)	0.691
Lipid lowering therapy /n(%)/	18 (40.0)	8 (44.4)	0.746
Allopurinol /n(%)/	8 (18.2)	3 (16.7)	0.887
Febuxostat /n(%)/	3 (6.7)	8 (44.4)	**0.001**
Benzbromarone /n(%)/	0 (0.0)	1 (5.6)	0.286
Oral antidiabetic therapy /n(%)/	20 (44.4)	4 (22.2)	0.101
Insulin /n(%)/	4 (8.9)	0 (0.0)	0.317
Aspirin /n(%)/	9 (33.3)	2 (40.0)	1.000
Corticosteroid /n(%)/	2 (4.4)	5 (27.8)	**0.017**
NSAIDs /n(%)/	2 (4.4)	3 (17.6)	0.122
Nephrolithiasis /n(%)/	5 (10.9)	11 (61.1)	**< 0.0014**
Erythrocyturia /n(%)/	18 (39.1)	12 (66.7)	**0.047**
Urinary uric acid crystals /n(%)/	14 (30.4)	6 (33.3)	0.822

Legend: BUN – blood urea nitrogen; eGFR – estimated glomerular filtration rate; ESR – erythrocyte sedimentation rate; BMI – body mass index; NSAIDs – non-steroidal anti-inflammatory drugs.

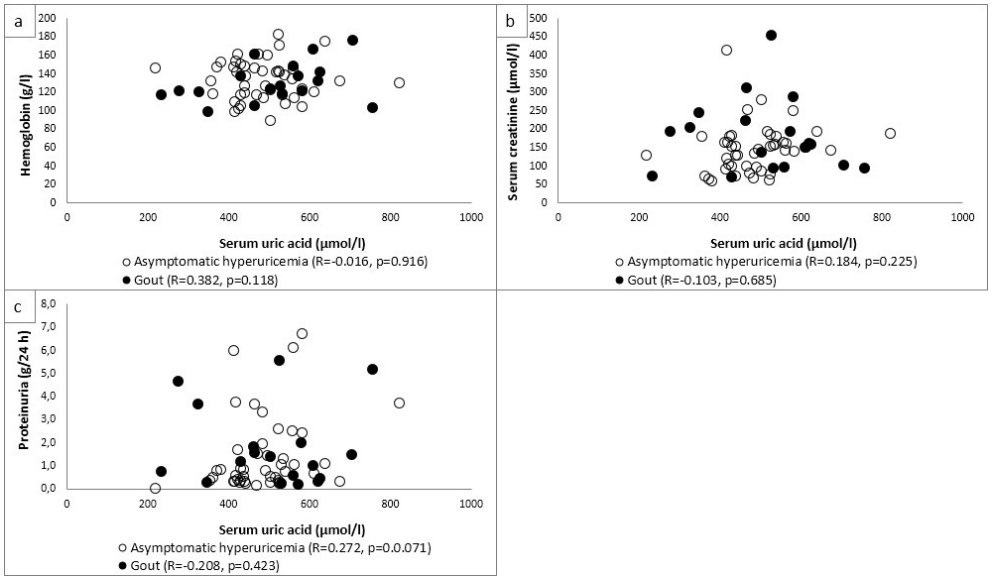

Fig. 1. Correlation analysis of serum uric acid and hemoglobin, creatinine and 24-hour proteinuria

($p = 0.536$). The proportion of patients with interstitial fibrosis > 50% was significantly higher in the gout group (33.3% vs. 6.5%, $p = 0.032$). In the groups the distribution of tubular atrophy involving more than 50% of the tubules was similar ($p = 0.183$). There was a tendency serum uric acid levels to be highest in patients with proliferation compared to those who had only expansion without proliferation and subjects with neither expansion nor proliferation (mean ± SD; 574.67 ± 99.40 vs. 494.67 ± 64.41 vs. 500.50 ± 81.52 μmol/l, $p = 0.056$). In individuals not treated with allopurinol, febuxostat or benzbromarone, serum uric acid levels were comparable among those with degenerative changes of the tubules, tubular atrophy < 25%; tubular atrophy > 25% and < 50% and atrophy engaging more than 50% of the tubules. The same result was observed in individuals with interstitial fibrosis < 25%; > 25% and < 50% and interstitial fibrosis > 50%, (Fig. 2). No correlation was found to exist between disease duration and interstitial fibrosis ($r = 0.222$; $p = 0.187$).

In the group of asymptomatic hyperuricemia, IgA deposits were found in nine of the patients. Three of them did not have diabetes. IgA deposits were located subepithelially, subendothelially and mesangially. Only two of the patients in this group had IgG deposits. One of the patients was diabetic, and the deposits were located subepithelially and subendothelially. Thirty-two subjects with asymptomatic hyperuricemia had IgM deposits, 14 of them were without diabetes. In 24 of the subjects the deposits were found in the subepithelium, while in ten of the patients were registered simultaneously in the subepithelium and in the

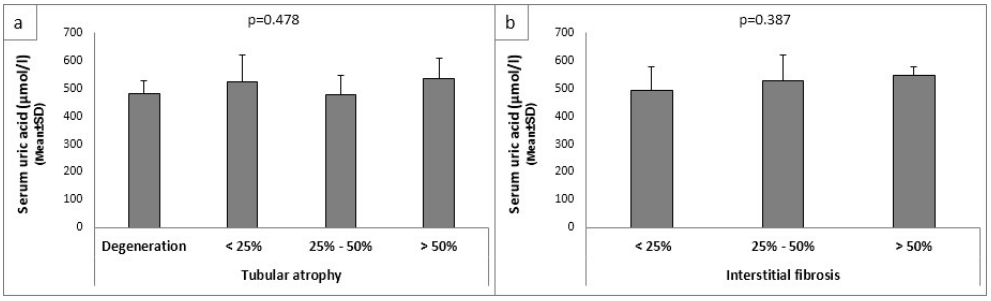

Fig. 2. Summary statistical characteristics of uric acid and ANOVA test results depending on tubular atrophy and interstitial fibrosis in patients not treated with allopurinol, febuxostat or benzbromarone

mesangium. A pure mesangial location was present in two patients, and only one had a subendothelial location. C1q deposits were observed in only eight of the individuals with asymptomatic hyperuricemia, three of whom were non-diabetic. In this group C3 deposits were present in 39 of the subjects, 20 of them did not have diabetes mellitus. In 16 patients deposits only in the vessel walls were reported. Deposits only in the glomeruli had 13 individuals. Deposits simultaneously in the vessel walls and in the glomeruli were established in ten subjects.

In the gout group, only two of the patients had diabetes. C3 deposits were registered in 13 of the subjects. In four individuals deposits were found in the vessel walls, in one patient had glomerular localization, and in one mixed. IgG deposits were detected in only two of the patients, in one of them their deposition was in the glomerular basement membrane. IgM deposits were present in 13 patients, in two their location was mesangial, while in five was subepithelial. IgA deposits were found in four patients. In one of the individuals the deposits were in the vessel walls, while in the others they were located mesangially and subepithelially.

From the data used, we observed that the most common histological diagnoses in patients with asymptomatic hyperuricemia were: Hypertensive nephropathy, which was registered in 22 of the patients; Focal-segmental glomerulosclerosis, which was found in 20 of the patients; Chronic tubulointerstitial nephritis present in 12 of the subjects, and Diabetic glomerulosclerosis, which we established in six individuals. The most common histological diagnoses in patients with gout were: Hypertensive nephropathy present in seven of the patients; Focal segmental glomerulosclerosis in six patients, and Chronic tubulointerstitial nephritis in 5 patients.

Discussion. Analyzing renal biopsy findings, clinical and laboratory data in patients with asymptomatic hyperuricemia and gout, we found that there was no difference between these two groups in the number of damaged glomeruli and in the distribution of mesangial proliferation. Nephrolithiasis, erythrocyturia, and

the proportion of individuals with advanced interstitial fibrosis were more common in the gout group. In subjects not receiving urate-lowering therapy, serum uric acid levels were similar in the varying stages of tubular atrophy and interstitial fibrosis. On the other hand, no correlation was established between disease duration (asymptomatic hyperuricemia or gout) with interstitial fibrosis. Another important finding of the present study is that in both groups there is a deposition of immune deposits subepithelially, subendothelially, in the mesangium and in the vessel walls. In the literature we have not found data about the deposition of immune deposits in the kidneys in subjects with asymptomatic hyperuricemia and gout. Autopsy studies in individuals with gout have revealed that urate crystals are deposited in the renal tubules and interstitium, where they cause inflammation with a giant cell reaction [4, 7]. The formation of uric acid crystals in the kidneys induces tubular obstruction and granulomatous inflammation with macrophages and T-cell infiltration leading to interstitial fibrosis and arteriosclerosis [8]. Arterial and arteriolar nephrosclerosis are typical [9]. Further, monosodium urate crystals activate the innate immune system, activate the NLRP3 inflammasome and cause inflammation [10]. There is evidence that soluble uric acid also acts as a dangerous signal [11]. Uric acid is an important endogenous stimulus of the innate immune response. Data show that both the crystallized form and the soluble uric acid are natural dangerous molecules that send signals via the NLRP3 inflammasome to the cells of the innate immune system [12, 13].

Studies in human renal biopsy findings have shown that serum uric acid levels are associated with interstitial inflammation/fibrosis and tubular atrophy independently of deposited crystals [14, 15]. Uric acid induces endothelial and mesenchymal damage in tubular cells, which was more pronounced in animals with renal impairment [16]. The mechanisms of its development are complex. The prooxidative properties of uric acid and mitochondrial dysfunction with subsequent synthesis of chemotaxis factors, oxidants and activation of RAAS are of major importance. Hyperuricemia stimulates the release of alarmines from endothelial cells through mechanisms that include calcium mobilization and activation of Toll-like receptor pathways [17]. On these data is based the assumption that soluble uric acid is responsible for the development of tubulointerstitial damage. It has been suggested that blood flow to the tubulointerstitial area may be reduced by uric acid-induced vasculopathy leading to ischemia in this area. Even more, hyperuricemia is also an independent risk factor for the development of glomerulosclerosis [14]. Chronic hyperuricemia results in glomerular hypertrophy/hypertension, afferent arteriolar sclerosis, macrophage infiltration, vasoconstriction and chronic ischemia. Morphological changes typical of mesangiocapillary glomerulonephritis develop, with increased mesangial cell and matrix numbers, focal segmental glomerulosclerosis, basement membrane thickening, and chronic tubulointerstitial damage progressing to terminal renal failure [18]. Investigations in animal models have shown that macrophageal and lymphocyte infiltration in the interstitium increases with the

level of hyperuricemia. The infiltration of these cells decreases with the introduction of xanthine oxidase inhibitors [13, 19]. These results further emphasize the need for early normalization of serum uric acid.

We did not observe a difference in the distribution of chronic renal failure between patients with asymptomatic hyperuricemia and gout. The distribution of proteinuria and the number of damaged glomeruli were also similar in the groups. Interstitial fibrosis $> 50\%$, erythrocyturia and nephrolithiasis were more common in patients with gout. On the other hand, in individuals not receiving urate-lowering therapy, serum uric acid levels are similar in the different stages of tubular atrophy and interstitial fibrosis, suggesting that monosodium urate crystals deposited in the interstitium may be involved in the pathophysiological process. In addition to activating intracellular NLRP3 inflammasomes and inducing the synthesis of IL-1β and IL-18, they also stimulate the expression of ICAM-1 in human mesangial cells and monocytes [20]. The crystals maintain a chronic pro-inflammatory state in the body, which most likely has led to advanced interstitial fibrosis in patients with gout. Based on the data from this retrospective study, we could suggest that gout is more deleterious to the kidneys than asymptomatic hyperuricemia. Future prospective investigations are needed to confirm this assumption.

We found that in both stages of the disease there is a deposition of immune deposits in different renal structures. We hypothesize that activation of the innate immune system by soluble uric acid and monosodium urate crystals led to complement activation with subsequent deposition of immune deposits in the kidneys. In the literature there are few reports examining renal biopsy findings in patients with asymptomatic hyperuricemia and gout. The present study is a step forward in the search for the main differences in renal biopsy between these two conditions. The results further support the need for early prevention and early initiation of urate-lowering therapy.

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