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Serum Uric Acid Concentration in Patients with Cerebrovascular Disease (Ischemic Stroke and Vascular Dementia)

Nafija Serdarevic[1,2], Adina Elena Stanciu[3], Lejla Begic[4], Snezana Uncanin[2,5]

[1]Institute for Clinical Biochemistry and Immunology University of Sarajevo Clinics Center, Sarajevo, Bosnia and Herzegovina

[2]Faculty of Health Studies, University of Sarajevo, Bosnia and Herzegovina

[3]Institute of Oncology Bucharest, Department of Carcinogenesis and Molecular Biology. Bucharest, Romania

[4]Department of Biochemistry, Faculty of Pharmacy, University of Tuzla, Tuzla, Bosnia and Herzegovina

[5]Clinic of Nephrology, University of Sarajevo Clinics Centre, Sarajevo, Bosnia and Herzegovina

Corresponding author: prof. Nafija Serdarevic, PhD. Institute for Clinical Biochemistry and Immunology University of Sarajevo Clinical Center, Faculty of Health Studies, Sarajevo, Bosnia and Herzegovina Phone: (+387) 33 444 113, Fax: (+387) 33-444-113. E-mail: serdarevicnafija@yahoo.com. ORCID ID. https://orcid.org/0000-0001-7977-9819.

ABSTRACT

Introduction: Significance of serum uric acid (UA) in cerebrovascular disease still remains controversial. UA is most abundant natural antioxidant in human plasma. Its antioxidant properties might protect against free radical damage, thereby reducing the risk of oxidative stress-related cognitive impairment and dementia. **Aim:** In our investigation, we determine the level of UA in 100 male patients diagnosed with the first ischemic brain stroke (blood samples were collected during the acute phase and post-acute phase), 100 male patients diagnosed with vascular dementia and 100 male healthy volunteers (control group). **Methods**: UA was determined using DIMENSION LxR automatic analyzer. Measurement of UA concentration was based on an enzymatic method (range 208-428 μmol/L). **Results:** The prevalence of hyperuricemia among ischemic stroke and vascular dementia patients was 30% and 8%, respectively. Serum UA concentration was higher 7 and 14 days after the stroke compared to the acute phase (24-48 hours after hospitalization) and these concentrations were significantly higher than those measured in the control group. UA levels measured at 24-48 hours after the first symptoms of ischemic stroke were strongly correlated with those measured after 7 days of treatment ($r = 0.79$, $p = 0.001$) or after 14 days ($r = 0.839$, $p = 0.0049$). No significant differences were found between ischemic stroke and vascular dementia groups. **Conclusion:** UA concentrations were higher in ischemic stroke and vascular dementia groups than in controls. UA increase may reflect vascular atherosclerosis and tissue hypoxia. UA monitoring in patients with cerebrovascular disease is essential, because UA is more harmful than protective.

Keywords: Uric acid, ischemic brain stroke, vascular dementia.

1. INTRODUCTION

Stroke is the third most common cause of death worldwide, mortality rate being in acute phase higher than 20%. It is defined as a syndrome of rapidly developing clinical signs of focal or global neurological disturbance, with symptoms lasting for more than 24 hours or longer or leading to death, with no apparent cause other than of vascular origin. Recent studies indicated that higher concentrations of uric acid (UA) are involved in various vascular diseases (1, 2). UA (pKa 5.8) is distributed throughout the extracellular fluid compartment as sodium urate and cleared from the plasma by glomerular filtration. Around 90% of filtered UA is reabsorbed from the proximal renal tubule, while active secretion into the distal tubule by an ATPase-dependent mechanism contributed to overall clearance. Serum UA concentration within the population has a typical reference range (95% CI) of 120-420 μmol/L (3, 4). Hyperuricemia is an abnormally high level of UA in the blood. In humans, the upper end of the normal range is 360 μmol/L (6 mg/dL) for women and 400 μmol/L (6.8 mg/dL) for men. Xanthine oxidase activity and UA synthesis are increased *in vivo* under ischemic conditions and elevated serum UA may act as a marker of underlying tissue ischemia. UA crosses dysfunctional endothelial cells and accumulates as crystals within atherosclerotic plaques.

The crystals may contribute to local inflammation and plaque progression (5, 6). An association between raised serum UA concentration and increased cerebrovascular risk has been recognized for over 50 years, although a causal relationship has not been clearly established.

However, many studies have shown that UA may have beneficial

effects, considering its anti-oxidant and neuroprotective capacity (7-9). We are faced with the paradox of UA: on the one hand, UA is a powerful anti-oxidant, leading to a decrease in free radicals, and on the other hand, UA acts as a pro-oxidant giving rise to an increase in free radicals, endothelial vascular dysfunction, inflammation and atherosclerosis. Generally, the harmful pro-oxidant effects predominate over the beneficial anti-oxidant effects, except in the central nervous system, where the beneficial anti-oxidant actions seem to prevail.

Regarding the neuroprotective effects, it is still unclear whether UA has a protective effect in neurologic degenerative diseases such as dementia. Dementia is one of the most commonly seen neurological diseases that have a significant impact on public health. There are several types of dementia among which Alzheimer's disease and vascular dementia are the two major types. Patients with Alzheimer's disease have a lower level of UA and a higher serum urate level, associated with reduced rate of cognitive decline in patients with mild cognitive impairment (10). The age- and sex-adjusted analyses showed no clear association between serum uric acid levels and the risk of dementia, or cognitive function later in life (11, 12). Growing evidence supports the neuroprotective effect of UA administration after brain ischemia (9).

As can be easily seen, the significance of UA in cerebrovascular disease still remains controversial.

2. AIM

The aim of our study was to investigate the serum UA levels in patients with ischemic stroke and vascular dementia and the association between endogenous UA in the two types of cerebrovascular diseases.

3. MATERIAL AND METHODS

Patients and study protocol

In the present study, we investigated a cohort of 200 male patients divided into two groups: patients with ischemic stroke (median age 73.12 years, IQR: 66-81 years) and vascular dementia (median age 73.74 years, IQR: 69-80 years), compared to 100 male subjects of the control group (median age 69.74 years, IQR: 65-75 years). Criteria for inclusion of patients with stroke and vascular dementia were: male subjects, age over 65 years, diagnosis of first ischemic stroke determined by computerized tomography (CT), presence of vascular dementia identified using (CT) and nuclear magnetic resonance (NMR), "*Hachinski ischemic score*" greater or equal to 7 for patients with vascular dementia, two weeks treatment in hospital for patients with ischemic stroke, ischemic brain stroke in patients with vascular dementia in the last three to six years. Subjects with a history of vascular disease, angina, myocardial infarction, peripheral artery disease, active infections, neoplasms, leukemias, myeloma, tumor lysis syndrome, gout, renal (Glomerular Filtration Rate <90 mL/min/1.73m^2) or liver disease, thyroid dysfunction, lactic acidosis, chronic obstructive pulmonary disease and excessive alcohol consumption were excluded from the study groups. Patients with prolonged starvation or other ketoacidosis states, which can lead to more than 20% increase in uricemia, were excluded too.

Inclusion criteria for the 100 subjects in control group were as follows: healthy male subjects and age over 65 years. None of the controls was receiving specific lipid-lowering treatment (statins or fibrate). Exclusion criteria for the control group were: history of drug use that affects the level of UA (corticosteroid, colchicine, and allopurinol), or that interfere with creatinine (catecholamines, levodopa, α-methyldopa, rifampicin, cephalosporins and calcium dobesilate).

The blood samples were collected in the morning before the first meal and after 12 hours of fasting. For the group of 100 patients diagnosed with the first ischemic brain stroke, blood samples were taken during the acute phase (the first 24-48 hours of hospitalization) and in post-acute phase (after 7 and 14 days of hospitalization). Only men were included in the study groups (patients and control) to avoid intersex variations. The study was done respecting ethical standards in the Helsinki Declaration and was approved by the Clinic Center University of Sarajevo ethics committee. Enrolled patients and controls signed an informed consent.

Measurement of uric acid

UA was determined using DIMENSION LxR automatic analyzer from Siemens Company. UA absorbs light at 293 nm is converted by uricase to allantoin, which is nonabsorbing at 293 nm. The change of absorbance at 293 nm, due to the disappearance of UA, is indirectly proportional to the concentration of UA in the sample and is measured using a bichromatic endpoint technique. The interferants reported with uricase/peroxidase methods include hemolysis, theophylline metabolites, catecholamines, methylene blue, sulfasalazine, gentisic acid, and hydralazine or other reducing substances.

Measurement of creatinine and glomerular filtration rate

The serum creatinine concentrationwas determined by kinetic method. Reference interval for serum creatinine was 0.72-1.25 mg/dL for adult male. The exclusion criteria were the following: patients treated with corticosteroids and blockers of the distal tubular creatinine secretion such as cimetidine, trimethoprim and cefoxitin. Creatinine was determined using ARCHITECT I 8200 SR automatic analyzer from Abbott Company. Creatinine determination is based on a kinetic method with alkaline picrate. At an alkaline pH, creatinine in the sample reacts with picrate to form a creatinine-picrate complex. The rate of increase in absorbance at 500 nm due to the formation of this complex is directly proportional to the concentration of creatinine in the sample. The GFR was estimated using MDRD equation.

MDRD equation:

$GFR = 32788 \times Scr^{-1.154} \times age^{-0.203} \times constant$

Statistical analysis

The collected data were statistically processed, using the descriptive and statistical methods, with SPSS version 16.0, Med Calc and Microsoft Office Excel 2007 SP2. The statistical significance of the differences between the groups was tested using Mann-Whitney and

t-test on independent samples. The correlation analysis used Pearson test. All P-levels were two-tailed, and statistical significance was defined if $p < 0.05$.

4. RESULTS

In our study hyperuricemia was indicated as serum UA concentration higher than 428 μmol/L. The mean levels of UA in patients and control groups are presented in Figure 1. As can be seen from Figure 1, during the fourteen-day monitoring period, in patientswith ischemicstroke, UA levels were higher than in control group. Serum UA concentration increased with 16.6 μmol/L after 7 days of hospitalization (340 +/- 107.42 μmol/L), and with 30.8 μmol/L after 14 days (354.2 +/- 134.89 μmol/L), comparedto the concentration ofUAmeasured in the first24-48 h of hospitalization (323.4 +/- 108.77 μmol/L). In the period of post-acutephase,between 7and 14 days ofhospitalization,the concentration ofUAincreased with 14.2 μmol/L. Also, serum UA level was significantly higher in vascular dementia group compared to the control group (321.25 +/- 85.75 μmol/L *vs.* 263 +/- 62.5 μmol/L).

Figure 2 and 3 presents the relationship between patients with ischemic stroke in acute and post-acute phase

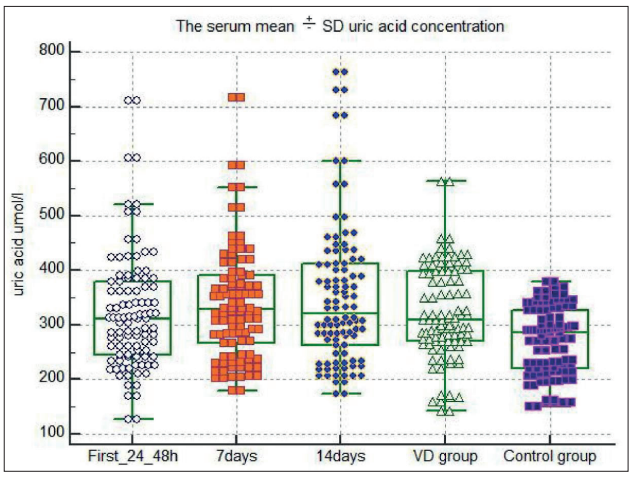

Figure 1. Serum uric acid concentrations in ischemic stroke, vascular dementia and control groups.

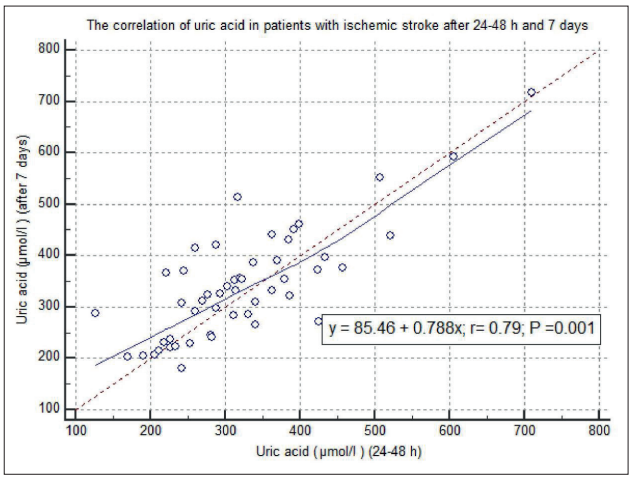

Figure 2. The results of correlation serum uric acid in patients with ischemic stroke in acute and post-acute phase (24-48 h) vs (after 7 days), p<0.05.

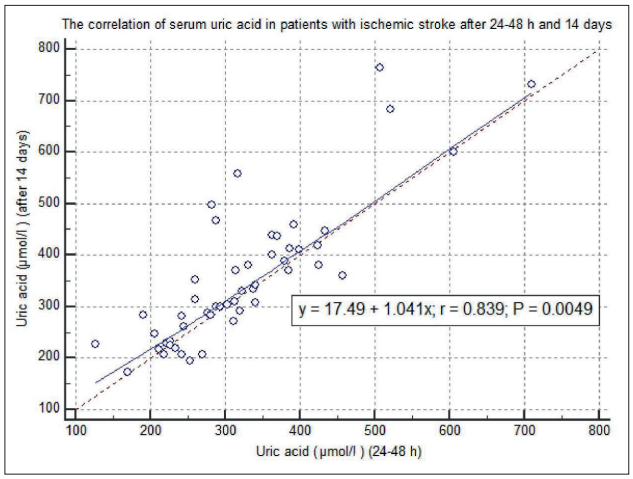

Figure 3. The results of correlation serum uric acid in patients with ischemic stroke in acute and post-acute phase (24-48 h) vs (after 14 days) p<0.05.

	Ischemic stroke 24-48 h	Ischemic stroke 7 days	Ischemic stroke 14 days	Vascular dementia group	Control group
Creatinine (mean concentration mg/dL)	0.885	0.874	0.870	0.864	0.872
SD	0.208	0.2159	0.2366	0.192	0.152
GFR (mean concentration)	90.4	91.7	92.2	91.9	91.9
SD	0.2	0.4	0.8	1.0	0.7

Table 1. Mean serum creatinine concentration and glomerular filtration rate in patients and control groups.

in serum UA levels. UA concentrations measured at 24-48 hours after the first symptoms of ischemic stroke were strongly correlated with those measured after 7 days of treatment ($r = 0.79$, $p = 0.001$) or after 14 days ($r = 0.839$, $p = 0.0049$). There was no significant correlation between UA concentrations measured after 7and 14 days of treatment ($p > 0.05$).

The serum concentration of creatinine was in the reference range in all study and control groups. GFR levels were also in the reference range in all groups. GFR levels were > 90mL/min/1.73m^2. All the results are presented in Table 1.

Mann-Whitney test was used to compare the study and control groups. The results are presented in Table 2. For $\alpha = 5\%$, there was a significant difference between the concentration of UA in the acute and post-acute phase of ischemic stroke and control group. No statistically significant difference was found between acute and post-acute phase of ischemic stroke groups and vascular dementia group or between vascular dementia and control groups. Interestingly, there was a significant difference between the concentration of UA in vascular dementia group and control group.

5. DISCUSSION

The main findings obtained through our study are as follows: (i) the prevalence of hyperuricemia among stroke patients was 30% and among vascular dementia patients

Mann-Whitney test	Ischemic stroke vs control group (24-48 h) (n = 100)	Ischemic stroke vs control group 7 days (n = 100)	Ischemic stroke vs control group 14 days (n = 100)	Ischemic stroke vs vascular dementia (24-48 h) (n = 100)	Ischemic stroke vs vascular dementia 7 days (n = 100)	Ischemic stroke vs vascular dementia 14 days (n = 100)	VD vs control group (n=100)
Mann-Whitney U	3632.0	3004.0	3090.0	1183.5	1161.0	1148.0	925.0
Z	3.343	4.877	4.667	0.458	0.614	0.700	2.241
p	0.0008*	< 0.0001*	< 0.0001*	0.646	0.539	0.484	0.025*

Table 2. The results of Mann-Whitney test between patients and control groups in serum uric acid concentration..
*** Statistic significant difference for p<0.05**

was 8%; (ii) serum UA concentration was higher 7 and 14 days after the stroke compared to the acute phase (24-48 hours after hospitalization) and these concentrations were significantly higher than those measured in the control group; (iii) no significant differences were found between ischemic stroke and vascular dementia groups; (iv) significant differences were found between vascular dementia and control group.

Our results were very carefully verified for quality control (precision, reproducibility, recovery). Also, all patients and controls had a normal kidney function, GFR levels being higher than 90mL/min/1.73m^2.

Serum UA levels measured in ischemic stroke patients were similar to those previously measured by Lamani et al. (14) (24 hours after stroke: 323.4 +/- 108.77 μmol/L*vs.*272.4 +/- 26 μmol / L; 7 days after stroke: 340 +/- 107.42 μmol/L*vs.*282.3 +/- 32 μmol/L) and in controls (263 +/- 62.5 μmol/L vs. 246.8 +/- 25 μmol/L). Moreover, we observed that the average concentration of UA gradually increased from acute to post-acute phase of ischemic stroke (from 24 hours to 7 days and 14 days thereafter). In the post-acute phase, after 14 days of the first symptoms of ischemic stroke, about 30% patients had hyperuricemia. Iranmanesh et al. (15) showed that 13.0% of patients from the stroke group had hyperuricemia. Similarly, Lamani et al. (14)have found that 46.7% of patients with stroke had an increased level of UA in comparison with controls. Actually, in our study, UA levels measured in acute and post-acute phase of patients with ischemic stroke were significantly higher than those measured in controls. As opposed to our results, Varga et al. showed that after 72 hours of the first symptoms of ischemic stroke, UA level was not higher in patients than in controls (16).

Our findings confirm previous scientific evidence who argue that UA is associated with a high risk of both hemorrhagic and ischemic stroke, acting as a proinflammatory molecule (11, 17, 18). A direct role for hyperuricemia in the clinical course of cerebrovascular disease has been suggested by the links between elevated UA and increased production of oxygen free radicals. Elevated production of reactive oxygen species, particularly superoxide anion occurs in ischemia and is accompanied by drop in concentration of tissue antioxidants. The post-stroke increase of UA in our group of patients with ischemic stroke could be explained as a feedback phenomenon of oxidative stress after neurons injury. Also, increased uric acid levels may be associated with increased platelet adhesiveness, and this effect could potentiate thrombus formation (19). Despite the overwhelming evidence of previous epidemiological studies, which showed that the patients with hyperuricemia have a higher risk for stroke incidence and mortality compared to controls (17, 18), there are authors who continue to come up with contradictory explanations. Paradoxically, these authors have demonstrated the positive effect of the increase in UA concentration, explaining this effect through anti-oxidant capacity of UA. Studies in human cells showed a significant increase in serum-free radical scavenging capacity from baseline during UA infusion and this could explain its neuroprotective effects. Chammorro et al. (20). noticed that in patients with acute ischemic stroke there is a 12% increase in the odds of good clinical outcome for each milligram per deciliter increase of serum UA. Amaro et al. (21)showed that the increased levels of UA are associated with a better outcome in patients treated with reperfusion therapies. Ming Jin et al. (12) suggested the possibility that individuals with elevated UA levels, who survived to stroke follow-up, might have had more 'cognitive reserve' than those with high baseline UA levels who did not reach follow-up for various reasons. These findings support the fact that high UA concentration could be neuroprotective and has antioxidant properties against free radical damage in the setting of an acute stroke (22). UA increase may be a compensatory measure to counteract the oxidative stress and vascular damage related to ischemic stroke (23). As a proof, addition of physiological concentrations of UA in *in vitro* models of ischemic neuronal injury has shown to protect hippocampal neurons against cell death (24).

Regarding vascular dementia, it was a significant difference between the concentration of UA in the group with vascular dementia and control group. However, there was no significant difference between the acute and post-acute phase of ischemic stroke and vascular dementia groups. The several studies showed that increased UA might reduce the risk for progression of dementia in subjects with underlying impaired cognitive function (25, 26). A lower serum UA may also reflect a reduction in serum antioxidants in human plasma and can scavenge numerous oxidants *in vitro* including superoxide, hydroxyl radicals, and peroxynitrite. When the UA reacts with peroxynitrite will also generate free radicals in the process including triuretcarbonyl and aminocarbonyl radicals. In contrast, Dawson et al. (27) could not find an independent association of UA levels with functional

outcome at 3 months. It is possible that the protective effect of UA to be unmasked: higher levels of UA were probably associated with a lower risk of dementia and better cognitive function later in life.

The main limitation of our study is that we do not know the concentration of UA in brain tissue. Another limitation is that we have not measured the indicators of oxidative stress. Also, details on blood pressure measurements were not systematically collected during the study.

6. CONCLUSION

Despite these intriguing differences between studies, the findings of our study support the fact that UA is a poor prognostic marker among ischemic stroke and vascular dementia patients. This affirmation is reinforced by the fact that the UA concentrations were higher in ischemic stroke and vascular dementia groups than in controls. Moreover, the fact that no significant difference has been found between ischemic stroke and vascular dementia groups, means that UA growth has another source of origin and another mechanism of release. Thus, UA increase in cerebrovascular disease may reflect vascular atherosclerosis or tissue hypoxia. UA monitoring in patients with ischemic stroke and vascular dementia is essential, because UA is more harmful than protective. Further studies are needed to confirm these findings.

- **Author's contribution:** Each author gave substantial contribution to the conception or design of the work and in the acquisition, analysis and interpretation of data for the work. Each author have role in drafting the work and revising it critically for important intellectual content. Each author gave final approval of the version to be published and they agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.
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