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Evaluation of the role of serum uric acid in patients with multiple sclerosis; An observational case–control study

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Objectives: To evaluate the role of uric acid (UA) in patients with multiple sclerosis (MS) as an investigational marker in true relapse events.
Methods: 108 patients with relapsing–remitting and secondary progressive MS, compared to case–control of about 120 patients (manifested other neurological diseases, OND) who were included in this study which was conducted from March 2008 to July 2009 at Baghdad Teaching Hospital multiple sclerosis clinic. A study protocol sheet was done and filled from the patient's database in the MS clinic.
Results: In the overall MS group, serum UA levels were lower than in controls, the difference did reach statistical significance ($p = 0.01$). Serum UA was found to be lower in patients during relapse than when they were in remission. The mean serum UA level from patients after 1 month of follow up shows an inverse correlation with MS type, age, and EDSS score, and positive correlation with gender and clinical activity, but none of these correlations reach statistical significance.
Conclusion: The question whether reduced serum UA level in MS is a primary deficit or an epiphenomenon remains open. Despite the fact that UA level was lower in clinically documented active patients, a general mean UA decrease is evident also in clinically and MRI inactive MS patients as compared to OND.
Keywords: Multiple sclerosis, uric acid, nitric oxide, peroxynitrite.

Introduction

Multiple sclerosis (MS) is a disease of myelin, the insulating cover around the nerves of the central nervous system (CNS: brain, optic nerves, and spinal cord) that becomes damaged in MS. MS most commonly begins in young adulthood and affects about twice as many women as men.[1] Uric acid (UA) is a diprotic acid with pKa1=5.4 and pKa2=10.3.[2]

There are now at least three independent mechanisms by which UA can interact with the immune system. The induction of the nitric oxide synthase isoform (NOS-2) in the CNS commonly associated with cells of the macrophage–monocyte lineage is a characteristic feature of experimental allergic encephalomyelitis (EAE). Moreover, production of the free radical nitric oxide (NO) in CNS tissue of mice has been correlated with the development of clinical signs of the disease.[3,4] Peroxynitrite (ONOO2), a potent oxidant that is formed by the rapid combination of NO with superoxide (O22), can be formed in an inflammatory response[5] and can cause a variety of toxic effects, including lipid peroxidation[6] and tyrosine nitration.[7,8] It has been shown that treatment with UA, a naturally occurring compound that selectively binds and inactivates peroxynitrite,[9] inhibits the onset of clinical disease in an acute, aggressive form of mouse EAE, a result expected only if peroxynitrite is the more toxic molecule.[10] It is proposed that soluble products generated from infiltrating immune cells and glial cells contribute to the damage to myelin and oligodendroglia in MS.[11,12] When NO and superoxide are formed simultaneously, they may react to form the powerful oxidant peroxynitrite (ONOO–) at a rate constant which is three times faster than that at which superoxide dismutase scavenges superoxide.[13] Extensive peroxynitrite activity has also been identified during early stages of EAE.[14] UA is a strong peroxynitrite scavenger. The administration of UA to treat EAE in mice has been shown to produce a strong beneficial effect.

It has been confirmed that NO and its toxic metabolite peroxynitrite inhibit components of the mitochondrial respiratory chain leading, if damage is severe enough, to a cellular energy deficiency state.[15] It has been demonstrated that both NO and peroxynitrite may possibly have a role in the process of demyelination by inducing oligodendrocyte death[16] and through damage of the myelin sheath by inducing lipid peroxidation.[17] Moreover, NO donors have been shown to cause reversible conduction block in both normal and demyelinated axons of the central and peripheral nervous systems.[18,19] Beneficial results of treatment with UA have been observed in experimental pneumococcal meningitis (PN)[20] and in focal brain injury in rats.[21] By virtue of its potent oxidant activity, PN is believed to be responsible for the majority of damage to the CNS attributed to NO.[22] Since observations on EAE may apply to MS one could expect that levels of UA, a natural scavenger of PN able to penetrate and preserve the blood–brain barrier integrity,[23] may be abnormal in MS patients. Regardless of clinical status, MS patients have significantly lower serum UA levels than controls.[19,24] One study indicated that UA reduction is strictly linked to clinical and MRI activity in MS patients.[25] It is not known whether UA reduction is primarily linked to MS or simply represents an epiphenomenon.

Patients and methods

Blood samples were collected from 108 patients retrospectively diagnosed by revised McDonald criteria having definite MS. 94 patients from 108 patients had the relapsing–remitting (RR) type of the disease, 14 secondary progressives (SP). The patients selected randomly from the outpatient register of our Neurology Clinic Baghdad Teaching Hospital at Medical City for the period from March 2007 through July 2008.

The control population: we recruited 120 patients admitted to the neurological ward of Baghdad Teaching Hospital

at Medical City the period from March 2007 through July 2008. There were 64 men and 56 women with other neurological diseases (ONDs) and clinical diagnosis (excluded gout). Our patients (cases and control) were sent for serum UA in fasting state. The cases with MS were followed up after one month to be another second reading of serum UA. Only 47 patients' serum UA results were documented in the protocol sheet. Serum UA levels of cases and controls were performed by the same laboratory of Baghdad Teaching Hospital, using a commercially available kit. Normal ranges of UA, according to the Baghdad Teaching Hospital at Medical City laboratory standardization, were 3.0–7.0 mg/dl.

Results

In the overall MS group, serum UA levels were lower than in controls, the difference did reach statistical significance (p = 0.01) as shown in Table 1.

There were 25 patients (from 108 patients) with clinically definite MS and their serum showed lower levels of UA (<3mg/dl) compared to sera from controls with OND (4 patients from 120 patients) as shown in Table 1. 18 patients from 25 patients were RRMS while 7 patients were SPMS as shown in Table 2.

14 patients from 18 patients who had low serum UA were in clinically active disease (on relapse) and all patients with SPMS were in relapse (7 patients) as shown in Table 3.

14 patients from 18 patients with EDSS equal or more than 3.5 and all patients with SPMS with EDSS >3.5 as shown in Table 4. In addition, in 47 RRMS patients in whom we performed repeated serum UA levels after 1-month analyses, we observed an increase in serum UA level during remission and decrease during relapse as shown in Table 5.

The mean serum UA level from patients after 1 month of follow up shows inverse correlation with MS type (-0.17), age of the patients (-0.239), and EDSS score (-0.21), and positive correlation with gender (0.11) and clinical activity (0.09); but none of these correlations reach statistical significance. We found a significant positive correlation of mean serum UA concentration before and after 1 month of follow up of those patients (p = 0.0001) as shown in Table 6.

Discussion

In the present study, we observed lower levels of UA in sera from patients with clinically active MS than in sera from clinically inactive MS patients or controls with OND. Additionally, in 47 RRMS patients in whom we performed repeated serum UA levels after 1-month analyses, we observed an increase in serum UA level during remission and decrease during relapse. We also found lower serum UA concentrations in the overall MS group than in the OND group, but the difference was statistically significant. UA levels in sera from MS patients have been recently reported in two studies.[10,26] One found serum UA levels to be significantly lower in MS patients than in patients with OND,[10] while the other observed no such difference.[26] However, the correlation between UA levels

Table 1. **The percentage of low serum UA in the cases and control enrolled in the study.**

Number of patients	Number of patients with serum uric acid >3mg/dl	Number of patients with serum uric acid <3mg/dl	*p*-value
Cases (108)	83(77%)	25(23%)	0.01
Control (120)	116(97%)	4(3%)	0.05*

* Not significant

Table 2. **Relation between types of MS with the level of serum uric acid who their serum uric acid below 3 mg/dl.**

MS type with number of patients	Number of patients with serum uric acid <3mg/dl	Number of patients with serum uric acid >3mg/dl
RRMS (94)	18 (19.1%)	76 (80.9%)
SPMS (14)	7 (50%)	7 (50%)
Total (108)	(25)	(83)

Table 3. **The relation between types of MS who low serum uric acid with active diseases (relapsing).**

MS type with no. of patients	No. of patients in remission	No. of patients in relapsing
RRMS 18	4 (22%)	14 (78%)
SPMS 7	0 (0%)	7 (100%)

Table 4. **The relation between MS types with EDSS patients who their serum uric acid below 3 mg/dl.**

MS type with no. of patients uric acid below 3 mg/dl	No. of patients with EDSS less than 3.5	No. of patients with EDSS equal or more than 3.5
RRMS (18)	4 (22.2%)	14 (77.8%)
SPMS (7)	0	7 (100%)

Table 5. **The mean serum UA concentration was lower in the MS 47 cases (3.77) than in the same group (4.22) after one month of follow up, the difference is statistically significant (P < 0.01).**

Uric acid	First visit	After one month
Mean	3.77	4.22
S.D.	0.98	0.77
95% C.I.	3.48 - 4.06	3.99 - 4.44

Table 6. **Correlation of the UA of 47 patients of the cases after 1 month of follow up with different variables.**

Correlation between	R-value	*p*-value
UA and age in years	–0.239	0.106*
UA and duration of illness	–0.197	0.184*
UA and no. of relapses	–0.288	0.05
UA and EDSS score	–0.205	0.168*
UA and clinical activity	0.091	0.542*
UA and MS type	–0.186	0.21*
UA before and after 1 month	0.543	0.01

* Not statistically significant

and clinical measures of MS was not tested in these studies. Constantinescu et al.[27] reported elevation in the mean serum UA levels in MS patients after 6 months of treatment with glatiramer acetate, suggesting that beneficial effect of this drug is based on elevation in UA,[27] as a natural scavenger of peroxynitrite. We found significantly lower values of serum UA in SPMS patients than in controls, while significant differences were observed neither between RR or PPMS and controls nor between the different clinical subtypes of MS. However, after performing multivariate linear regression analyses, we concluded that low values of UA in SPMS were not due to the independent effect of SP disease course. Our RRMS and SPMS patients may have had lower serum UA concentrations due to the relative predominance of female gender, active disease, and longer disease duration in this subgroup of patients since we found a strong inverse correlation between these variables and serum UA concentration. Inverse correlation between serum UA concentration and female gender may be one of the factors that influence female predominance in MS. As noted above, we found significantly lower concentrations of UA in sera from patients with a clinically active disease than in those with clinically inactive disease or controls. Moreover, we found an independent effect of disease activity on serum UA in MS patients by the multivariate regression analyses. Another study depend on MRI which resulted lower values were also observed in patients with Gd-DTPA enhancement on brain MRI as a sign of MRI disease activity in comparison with those without active lesions, but the difference did not reach statistical significance, perhaps due to the small number of patients in whom brain MRI with Gd-DTPA injection was performed. Since Gd-DTPA enhancement in MRI of the brain in MS is associated with inflammation.[28] Whether the reduction in UA level in patients with active MS is a cause or a consequence of disease activity in MS remains uncertain. It may be speculated that patients with active MS have an intrinsically reduced antioxidant reserve which contributes to the development of CNS inflammation and tissue damage in MS, or that CNS inflammation in the active phase of the disease leads to the consumption of UA as a scavenger. Hooper et al.[29] recently reported that UA protects the integrity of the blood–CNS barrier in mice with EAE such that inflammatory cell migration into CNS tissues is reduced. However, the same study showed that exogenously administered UA penetrates the already compromised blood–CNS barrier and blocks peroxynitrite-mediated tyrosine nitration and apoptotic cell death within the areas of inflammation in the spinal cord in EAE, speaking in favor of the latter notion. Although inflammation and demyelination are central features in MS, recent observations from pathological studies and magnetic resonance spectroscopy have led to the hypothesis that axonal damage is responsible for a significant proportion of the clinical phenomena and irreversible neurological impairment in this disease.[30,31] Axonal damage, represented by decreases in brain *N*-acetylaspartate concentrations in magnetic resonance spectroscopy studies, was shown to progress over time[32] and to be correlated with clinical disability.[33,34] Serial observations[10,19,24] indicated that low UA levels are possibly a primary feature of MS patients. Instead, another study suggested that UA levels serve as a marker of MS activity, being inversely correlated with disease activity and duration.[25] In the present study, we obtained confirmatory evidence for an association of low serum UA levels with MS, even though some control neurological diseases (such as epilepsy and stroke) were potentially related to hyperuricemia. Drulovic and colleagues[25] found confirmatory evidence that patients with active RR and SP forms of MS have significantly lower UA levels than controls. On the other hand, in the overall group (including MS patients with clinically inactive forms of MS) serum UA levels did not differ significantly from those of controls, although a strong tendency was evident ($p = 0.068$). It may be observed that the two study populations (MS and OND) of Drulovic et al.[25] differed in terms of numerosity (240 and 104, respectively) and that about 30% of their control patients had seizures. Since carbamazepine and valproic acid can decrease serum UA levels,[35] they may possibly have lowered the mean UA level in the OND population. In their study, comparison of UA levels in patients stratified according to MS course and disability showed a significant difference. This observation may favor the view that, although a concomitant disease activity-related effect is present, low UA levels might represent a primary "MS-specific" deficiency. In fact, despite the fact that the UA level was lower in clinically documented active patients, therefore partially confirming Drulovic et al's[25] results, a general mean UA decrease is evident also in clinically and MRI inactive MS patients as compared to OND. Therefore, the question whether reduced UA level in MS is a primary deficit or an epiphenomenon related to its oxidation by PN and free radicals remains open, although the two alternative hypotheses are not mutually exclusive and concomitance of either facts is likely. In the latest metabolic stage, UA is transformed into allantoin which does not have PN scavenging activity. In other pathological conditions, such as myocardial infarction[36] and chronic lung disease of preterm infants[37] or during muscle metabolic stress induced by intense exercise,[38] serum allantoin is a useful early predictor of the subsequent free radicals generation. To our knowledge, no study has assessed allantoin levels in MS patients so as to determine whether UA is primarily deficient or secondarily reduced by virtue of its protective role against oxidant compounds. Investigations aimed at determining such an *in vivo* surrogate marker of free radical production in MS are clinically relevant.

Conflict of Interest

None

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