The Correlation between Uric Acid Levels and Amyotrophic Lateral Sclerosis

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Abstract **Introduction** Uric acid (UA) may act as an antioxidant in ALS pathophysiology. This study examined whether serum UA concentration correlates to clinical course in the ALS patients. **Methods** We measured serum UA levels in 51 sporadic ALS patients, and compared them to 51 age-and-gender-matched healthy subjects. We analyzed the correlation between serum UA levels, illness duration, and disease "course factors" (defined as "disease duration" over "terminal time") in the ALS patients. **Results** The mean serum UA levels in the ALS groups were higher than the control groups in both genders. Serum UA levels were inversely correlated with the disease "course factors" in the ALS male patients. **Discussion** The elevation of serum UA concentration in ALS patients may be related to oxidative stress. The reasons that serum UA levels inversely correlated with ALS "course factors" include: muscle wasting, poor nutrition, reduced antioxidant reserve, or increased UA consumption in end-stage patients.

Keywords: amyotrophic lateral sclerosis, uric acid, oxidative stress, SOD1, TDP-43

1. Introduction

The pathophysiology of amyotrophic lateral sclerosis (ALS) has been postulated to be related to oxidative stress from glutamate-mediated excitotoxicity, especially since the discovery of Cu/Zn superoxide dismutase (SOD) gene mutations in familial ALS and TDP-43 aggregation in sporadic ALS patients [1]. Recent findings suggest that glutamate-mediated NO overproduction and the subsequent generation of peroxynitrite play an important role in the pathogenesis of ALS [1]. Glutamate-mediated excitotoxicity is associated with adenosine triphosphate degradation, which is reflected by increased levels of extracellular hypoxanthine and xanthine and the generation of oxygen radicals. As xanthine is degraded to uric acid, it has been shown that elevated levels of both xanthine and uric acid in the cerebrospinal fluid (CSF) are related to glutamate-mediated excitotoxicity in some neurological disorders [1].

Uric acid (UA) has the ability to react with peroxynitrite and stimulate the expression of extracellular SOD which may confer additional antioxidant protection, and has therapeutic action in mouse model of experimental autoimmune encephalomyelitis (EAE) by inactivating ONOO(-), preventing blood-brain barrier breakdown, and reducing neuroimmune responses [1]. It also stabilizes ascorbate, possibly by forming complexes with iron ions, and scavenges nitrogen radicals [2].

There is a correlation between serum urate and CSF urate in humans with an intact blood-brain barrier, and the CSF urate concentration is approximately 7~10% that of plasma [3,4]. Considering an intact blood-brain barrier in ALS patients and healthy persons, a comparison of serum UA levels may be able to reflect the CSF UA levels in these two groups.

We hypothesized that the concentration of uric acid would be elevated in ALS patients, and may have a correlation to the disease course or severity. Therefore, we aimed to determine whether the concentration of serum UA level correlated to the clinical parameters and disease course in ALS patients.

2. Methods

2.1. Study Population

We enrolled patients diagnosed with ALS at National Cheng Kung University Hospital and Mackay Memorial Hospital, from 1996/9 to 2006/9. All subjects met the EI Escorial World Federation of Neurology criteria for the diagnosis of ALS. We excluded the patients with chronic kidney disease, diabetes mellitus, gout, and those who were receiving any medications that would affect serum UA levels.

2.2. Data Collection

We retrospectively reviewed chart records for serum UA data measured at the time of diagnosis, during hospitalization, and outpatient follow-up. The onset time of ALS, the illness duration at the time of blood sampling, and illness terminal time (meaning the time of death from the disease) were recorded. We also collected serum UA data of 51 age- and gender-matched healthy subjects (the control group).

2.3. Data Analysis

We use the matched-pairs t-test to compare the serum UA data of the ALS patients at the time they were diagnosed to the serum UA data of the control group. Using serum UA levels from the ALS patients at different durations of illness, we performed regression analysis to correlate serum UA levels with illness duration, disease stage, and progression rate. We definite "illness duration/terminal time" as "course factors", and also correlated serum UA levels with the course factors.

3. Results

Fifty-one ALS patients (38 male patients, 13 female patients) with serum UA data at the time of diagnosis from two medical centers were included in our analysis. All of these patients were in the early stage of ALS (according to the definition by the Augmentative and Alternative Communication Institute, AAC) at the time they were diagnosed. We divide the ALS patients and control group into male and female groups, and compared the serum UA levels separately. The average serum UA level in the male ALS patients was 6.489mg/dL compared to 5.961mg/dL in the male controls (Table 1), and 5.508mg/dL in the female ALS patients compared to 5.054mg/dL in the female controls (Table 2). There were significantly higher mean serum UA levels in the ALS patients compared to the control group, both in males and females.

Table 1. Comparing average UA level of ALS male patients to health male group

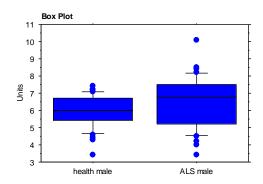| One Sample t-test Hypothesized mean = 0				
	Mean	DF	t-Value	P-Value
health male	5.961	37	39.429	< .0001
ALS male	6.489	37	26.535	< .0001

Figure 1. The graph shows higher average UA level of ALS male patients comparing to health male group. Y-axis represents UA level (unit: mg/dL)

Table 2. Comparing average UA level of ALS female patients to health female group

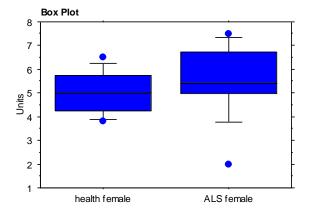| One Sample t-test Hypothesized mean = 0				
	Mean	DF	t-Value	P-Value
health female	5.054	12	20.296	< .0001
ALS female	5.508	12	13.745	< .0001

Figure 2. The graph shows higher average UA level of ALS female patients comparing to health female group. Y-axis represents UA level (unit: mg/dL)

Using serum UA data from the ALS patients at different durations of illness, we correlated the serum UA data to illness duration and "course factors" (i.e. illness duration/terminal time). As the disease progressed, serum UA levels gradually declined, eventually reaching below the normal range of serum UA levels in the controls when the patients progressed to the terminal stage. The average serum UA level in late stage male ALS patents was about 3mg/dL. In the male ALS patients, the serum UA level was inversely correlated with the course factors (R^2: 0.677) (Table 3), but not with the illness duration (R^2: 0.166) (Table 4). In the female ALS patients, because the sample size was too small, the tendency of UA levels was not significantly correlated with either illness duration (Table 5) or course factors (Table 6).

Table 3. The correlation between UA levels and "course factors" (duration/terminal) in ALS male patients

Regression Summary UA male vs. M Duration/Terminal	
Count	42
Num Missing	19
R	.823
R Squared	.677
Adjusted R Squared	.669
RMS Residual	1.105

ANONA Table UA male vs. M Duration/Terminal					
	DF	Sum of Squares	Mean Square	F-Value	P-Value
Regression	1	102.339	102.339	83.803	< .0001
Residual	40	48.847	1.221		
Total	41	151.186			

Regression Plot

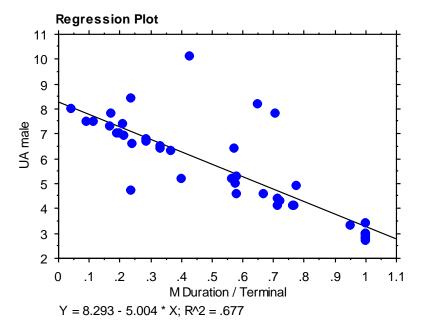Y = 8.293 - 5.004 * X; R^2 = .677

Figure 3. The graph indicates the UA levels decline with "course factors" (duration/terminal) in ALS male patients (r^2=0.677). X-axis represents the "course factors" (duration/terminal) while UA levels were measured. Y-axis represents UA levels (unit: mg/dL)

Table 4. The correlation between UA levels and illness duration in ALS male patients

Regression Summary UA male vs. M Duration	
Count	61
Num Missing	0
R	.408
R Squared	.166
Adjusted R Squared	.152
RMS Residual	1.661

ANONA Table
UA male vs. M Duration

	DF	Sum of Squares	Mean Square	F-Value	P-Value
Regression	1	32.491	32.491	11.772	.0011
Residual	59	162.840	2.760		
Total	60	195.331			

Table 5. The correlation between UA levels and illness duration in ALS female patients

Regression Summary UA male vs. F Duration	
Count	16
Num Missing	0
R	.264
R Squared	.070
Adjusted R Squared	.004
RMS Residual	1.652

ANONA Table
UA male vs. F Duration

	DF	Sum of Squares	Mean Square	F-Value	P-Value
Regression	1	2.874	2.874	1.053	.3222
Residual	14	38.220	2.730		
Total	15	41.094			

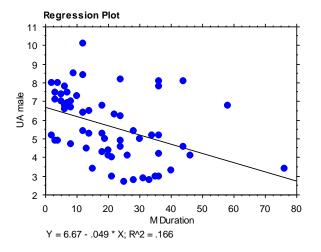**Regression Plot**

Y = 6.67 - .049 * X; R^2 = .166

Figure 4. The graph shows no significant correlation between UA levels and illness duration in ALS male patients. X-axis represents the durations while UA levels were measured. Y-axis represents UA levels (unit: mg/dL)

Regression Plot

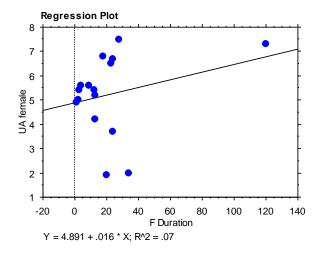Y = 4.891 + .016 * X; R^2 = .07

Figure 5. The graph shows no significant correlation between UA levels and illness duration in ALS female patients. X-axis represents the durations while UA levels were measured. Y-axis represents UA levels (unit: mg/dL)

Table 6. The correlation between UA levels and "course factor" (duration/terminal) in ALS female patients

Regression Summary UA male vs. Some of F Duration/Terminal	
Count	7
Num Missing	9
R	.809
R Squared	.654
Adjusted R Squared	.585
RMS Residual	1.095

ANONA Table
UA female vs. Some of F Duration/Terminal

	DF	Sum of Squares	Mean Square	F-Value	P-Value
Regression	1	11.353	11.353	9.467	.0276
Residual	5	5.996	1.199		
Total	6	17.349			

Regression Plot
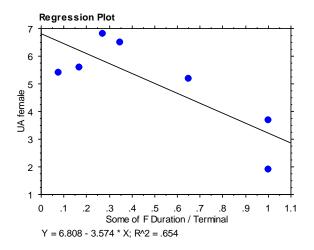
$$Y = 6.808 - 3.574 * X; R^2 = .654$$

Figure 6. The graph indicates the UA levels decline with "course factor" (duration/terminal) in ALS female patients ($r^2 = 0.654$). X-axis represents the "course factor" (duration/terminal) while UA level was measured. Y-axis represents UA level (unit: mg/dL)

4. Discussion

According to the AAC Institute, the symptoms of early stage ALS include limb weakness and loss of muscle mass, while ambulatory and most daily functions are preserved. In this study, we found that in patients with the early stage of ALS, serum UA levels were significantly higher compared to the controls. However, as the disease progressed, serum UA levels decreased, and in patients with late stage ALS, the serum UA levels were significantly lower than the normal range. The difference in serum UA levels between the early and late stage was 3.5mg/dL in average.

We found that the serum UA level of male ALS patients was not correlated to the duration of disease, but was correlated with the course factors of disease. Using regression analysis, we divided the full course of male ALS patients into three intervals: 1) course factors (illness duration/terminal time) less than 0.25; 2) course factors between 0.25~0.75; and 3) course factors greater than 0.75. The average serum UA level of male ALS patients in the first interval was above 6mg/dL, and below 4mg/dL in the last interval. At the end point of the disease course (meaning illness duration/terminal time = 1), the serum UA levels of the male ALS patients were below the lower limit of normal values. Regression analysis of the female

ALS patients did not show a significant correlation between UA levels and course factors; however we found that there was a similar tendency in the UA levels of the female patients as with the male patients, according to the limited data.

In subjects without ALS, the strongest correlates of serum urate level were male sex, obesity, and arterial hypertension [2]. Use of thiazide diuretics or nonsteroidal anti-inflammatory drugs, and cigarette smoking are known to increase urate levels [2]. However, the relationship between serum urate level and ALS progression in our study was independent from these factors.

According to our results, although the average serum UA level in the early stage ALS patients was higher than in the controls, we found that the prevalence rates of gout and cardiovascular events were not elevate compared to the controls. This suggests that the elevation of serum UA may be a reactive process to the increasing oxidative stress in early stage ALS patients.

Many studies have reported a relationship between UA level and free radical/oxidant scavengers. Some studies have also demonstrated a correlation between serum UA concentration and certain disorders of the central nervous system, including multiple sclerosis (MS) and Parkinson's disease (PD), and strongly suggest that the antioxidant effect of urate is important in protecting against MS and PD [2,5,6,7,8,9,10]. Unlike MS and PD, in which serum UA levels are low from the beginning and do not change much through the course of disease, the UA levels in ALS patients are higher in the early stage and then decrease through the course. The UA levels reach the lowest point in the terminal stage of ALS, and are far lower than in MS and PD patients.

Many studies on the pathogenesis of ALS have focused on the properties of SOD1 mutations, which contribute to about 20% of familial ALS (FALS) cases. It has been proved that mutations in the SOD1 gene cause toxic gains of function of abnormal Cu/Zn SOD enzyme, lead to increase in peroxidase activity in the FALS patients [11,12]. Mutant SOD1 may also accept peroxynitrites or hydrogen peroxide as a substrate, and thereby catalyze the nitration of tyrosine residues of SOD1 and hydroxyradicals [13], increasing oxidative stress in the FALS patients.

Recent studies have placed emphasis on the accumulation of abnormal TDP-43 protein in the majority of sporadic ALS cases. Chronic excitotoxicity, oxidative stress, proteasome dysfunction and endoplasmic reticulum stress mechanistically induce mislocalization, phosphorylation and aggregation of TDP-43 [14]. Mutant TDP-43 can induce mitochondrial dysfunction, oxidative damage and nuclear accumulation of nuclear factor E2-related factor 2 [15]. Studies on abnormal TDP-43-expressing cells have also shown markedly increased markers of oxidative stress, apoptosis, and necrosis [16].

Urate, the product of xanthine oxidation, is a good reductant and effective at preventing peroxynitrite from nitrating the tyrosine residues of proteins, thereby preventing the inactivation of cellular enzymes and modification of the cytoskeleton [17]. The ability of UA to preferentially react with peroxynitrite has been shown to aid in substantial antioxidant benefits, including the stabilization of endothelial nitric oxide synthase (eNOS) activity [6]. UA can also chelate metal ions such as iron

and copper, converting them to poorly reactive forms unable to catalyze free-radical reactions[18]. In vitro and in vivo studies suggest that UA also exerts an antioxidant effect on neurons [8,9,12,19].

Serum uric acid can be readily increased by diet, particularly fatty meats rich in purines, alcohol (especially beer and hard liquor) and fructose. Low-fat dairy products have the opposite effect, which may be mediated by the ability of lactose (and galactose) to activate UA transferase [6,17]. Milk has been found to lower serum UA levels by increasing UA excretion by the kidneys [17,18]. High consumption of meat, a food rich in purine, may cause hyperuricemia [17,20]. Exercise increases serum UA levels and decreases urinary excretion of UA. The inverse correlation between subsequent serum UA levels and disease stages may have been caused by muscle wasting, decreased exercise, and low meat intake of the ALS patients. Reduced serum UA levels may represent a constitutive loss of protection against nitric oxide and its oxidizing congeners, and reduced antioxidant reserve. In addition, increased UA consumption as a oxidant scavenger may also have influenced the serum UA levels as the disease progressed. Furthermore, the rate of progression to the primary clinical end point declined with increasing levels of baseline serum urate.

Overall, it seems unlikely that the inverse relationship between UA levels and ALS progression was due to confounding by known factors. We analyzed the data in a number of ways, including controlling for confounding factors, such as age, gender, kidney disease, and medications that influence UA levels.

The strength of our study is that we collected UA data at different time points in the same patients, enabling us to analyze the correlation between UA levels and disease course. This correlation has not been studied previously by any other articles in the literature. The tendency of UA levels through the disease course is a significant finding in studying the mechanism of ALS.

The limitations of this study include that we did not monitor or restrict the daily diet of our ALS patients. In addition, we did not follow up and adjust the patients' body mass index data, and the effect of riluzole treatment was not considered. To determine whether or not riluzole or other experimental medications increase serum UA levels, double-blind prospective placebo controlled studies are needed with a greater number of patients.

Abbreviations

ALS:	amyotrophic lateral sclerosis
UA:	uric acid
SOD:	superoxide dismutase
CSF:	cerebrospinal fluid
EAE:	experimental autoimmune encephalomyelitis
AAC:	the Augmentative and Alternative Communication Institute
MS:	multiple sclerosis
PD:	Parkinson's disease
FALS:	familial amyotrophic lateral sclerosis
TDP-43:	TAR DNA-binding protein-43
eNOS:	endothelial nitric oxide synthase

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