J Neurol Neurosci ©
13 (10) 2022 : 001-005 • RESEARCH ARTICLE

Potential of Serological Markers for Evaluating Neurological Function and Progression Rate of Amyotrophic Lateral Sclerosis

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SUMMARY

Objective: The present study was to investigate the potential of creatinine, uric acid, creatine kinase, total cholesterol, triglyceride, HCY (Homocysteine), and cystatin C for predicating neurological function and progression rate of amyotrophic lateral sclerosis.

Methods: All enrolled ALS patients were given corresponding serological tests at the initial diagnosis. The Revised ALS Functional Rating Scale (ALSFRS-R) and Disease Progression Rate (DPR) were evaluated. The detected indexes in blood tests included creatinine, uric acid, creatine kinase, total cholesterol, triglycerides, homocysteine, and cystatin C. Data analysis was performed by SPSS 22.0 statistical software.

Results: There were significant differences in creatinine, uric acid, creatine kinase, total cholesterol, HCY and cystatin C between the two groups ($P<0.05$). The levels of uric acid and creatinine of ALS group were lower than those of the control group, and the levels of other test indicators were higher than that in the control group.

The results from correlation analysis demonstrated that there was a significant positive correlation between ALSFRS-R and creatinine ($P<0.01$, $r=0.567$); There was negative significant correlation between DPR and creatinine ($P<0.01$, $r=-0.408$). The correlations of DPR with triglyceride and total cholesterol were significantly negative correlated ($P<0.05$, $r=-0.201,-0.210$ respectively). The remaining indexes did not show any correlation with ALSFRS-R and DPR.

Conclusion: Uric acid and creatinine of ALS patients were lower than that in healthy people. There were significant metabolic abnormalities in ALS patients 2. Creatinine level is an independent risk factor affecting ALSFRS-R. The creatinine and total cholesterol levels are also the independent risk factors affecting DPR. Creatinine and total cholesterol levels could be used as reliable indicators to evaluate the ALSFRS-R and DPR of ALS patients.

Keywords: Amyotrophic lateral sclerosis; Biomarkers; Creatinine; Uric acid; Blood lipid

Abbreviations: ALS: Amyotrophic Lateral Sclerosis; ALSFRS-R: ALS Functional Rating Scale; DPR: Disease Progression Rate; FVC: Forced Vital Capacity; CK: Creatine Kinase; HCY: Homocysteine

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Word count: 3,479 **Figures:** 01 **Tables:** 02 **References:** 22

Date of Submission: 25 April, 2022, Manuscript No. IPJNN-22-12753; **Editor assigned:** 28 April, 2022, PreQC No. P-12753 (PQ); **Reviewed:** 13 May, 2022, QC No. Q-12753; **Revised:** 20 October, 2022, Manuscript No. R-12753 (R); **Published:** 29 October, 2022

INTRODUCTION

Amyotrophic Lateral Sclerosis (ALS), also known as Lou Gehrig's disease, is a progressive neurodegenerative disorder characterized by loss of motor neurons in motor cortex, brainstem, and spinal cord that results in muscle weakness and atrophy, spasticity, compromised speech, swallowing, and breathing. There is currently no effective treatment for ALS that can reverse the progression of the disease. The main possible treatments include drug therapy, stem cell transplantation, momentum transplantation, gene therapy, respiratory support, and nutritional management. The prognosis of ALS is poor, and the rate of disease progression varies greatly. Rapidly progressing patients may involve the respiratory system within a few months due to respiratory failure, requiring assisted ventilation or even death, and slower progressing ones can even survive for 10 years or longer. Since the vast majority of ALS is fatal, it is very important to assess the prognosis, which can help patients plan their lives better.

Previous studies have confirmed that biological markers can be used as indicators for early diagnosis and prognosis of ALS. The purpose of this experiment is to explore the relationship between serum markers and amyotrophic lateral sclerosis function score and disease progression rate, which will play significant role in early diagnosis and effective treatment.

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Objective

Enrollment criteria: ALS patients were selected from the first confirmed patients admitted to the neurology clinic and inpatient department from January 2015 to August 2020. The enrolled 103 patients (60 males and 43 females) were all met the EI Escorial amyotrophic lateral sclerosis clinical diagnostic criteria revised in 2000 by the Motor Neuron Disease Group of the World Federation of Neurology [1]. The average age of the patients is (58.95 ± 9.98) years old. EMG examination is performed on all patients.

The enrollment criteria are as follows: There are three conditions that the patients must be met: Firstly, the evidence of lower motor neuron injury must be found through clinical, electrophysiological or neuropath logical examination secondly, evidence of upper motor neuron lesion must be confirmed by clinical examination. Finally, the upper and lower motor neuron lesions spread from one part to other parts gradually based on medical history or examination. There are

are two conditions that must be excluded at the same time: At first, patient suffering from other diseases that cause upper and lower motor neuron lesions is proven through electrophysiology or pathology. Secondly, neuroimaging proves that the patient has other diseases lead to clinical manifestations of upper and lower motor neuron diseases and electrical physiological changes.

Exclusion criteria:

- The patients suffering from acute cardiovascular and cerebrovascular events, stroke, acute coronary syndrome.
- Those who also suffer from other neurodegenerative diseases, such as multiple system atrophy, Parkinson's disease, front temporal dementia, etc.
- Those who also suffer from renal insufficiency or gout. The renal function is evaluated by the glomerular filtration rate. When the glomerular filtration rate is less than 90 ml/min, it means renal insufficiency.
- Those with mental symptoms and cognitive impairment who cannot cooperate with the test; Patients with severe liver and kidney disease or malignant tumors or thyroid disease or patients who have taken drugs that affect monitoring indicators in the past 3 months (such as lipid-lowering drugs, diuretics, folic acid, B vitamins, etc.).
- The patients who have a confirmed ALS in their family or similar symptoms but undiagnosed patients.

Healthy control group: 90 healthy volunteers had a physical examination in the physical examination center of our hospital during the same period. Patients with mental disorders, severe liver and kidney diseases, thyroid diseases, and malignant tumors were excluded, and those who had taken drugs that affected the observed indicators in the past 3 months were also excluded (such as lipid-lowering drugs, diuretics, folic acid, B vitamins, etc.). There were 52 males and 38 females, aged 35-77 (59.34 ± 7.71) years old. There was no age and gender bias between the control group and the ALS patient group.

MATERIALS AND METHODS

Detection of serum markers: 2 ml of peripheral venous blood was collected from all patients on an empty stomach for at least eight hours in the morning of outpatient or admission, after centrifugation at 1000 r/min for 5 minutes; the serum was separated and detected by the Roche modular P-900 automatic biochemical analyzer.

ALSFRS-R scores: The ALSFRS-R revised in 2000 was used to evaluate ALS patients function at the first visit. ALSFRS-R is a 12 items rating scale covering four functional areas: fine motor, gross motor, bulbar and respiratory function. Each project contains five levels (0=cannot perform task to 4=normal function). These 12 items involve activities of daily living: speech, salivation, swallowing, writing, food preparation, dressing, bed activities, walking, climbing stairs, breathing difficulties, sitting breathing, and mechanical ventilation. The total score ranges from 0-48 [2].

Disease progression rate: The patient's functional scores at the first diagnosis and the time from the onset of symptoms to clinical diagnosis (calculated in months) were recorded. The progression rate of amyotrophic lateral sclerosis disease (DPR)=(48-ALSFRS-R at the first diagnosis)/first onset to time of clinical diagnosis (months) [3].

Statistical analysis

All data were analyzed by SPSS 22.0. The mean and standard deviation were used to describe the uric acid level, which conforms to the normal distribution. Two independent sample t-tests were used to compare the two groups. The data of creatinine, creatine kinase, triglycerides, total cholesterol, HCY, and cystatin C are not In line with normal distribution, which were described by the median and quartile, and the two independent sample rank sum tests were used for comparison between the two groups; The correlation analysis was used to analyze the correlation between ALSFRS-R or DPR and the above mentioned serological indicators; linear regression analysis were performed for those with obvious correlation with ALSFRS-R and DPR.

RESULTS

- Comparison of serum index (creatinine, uric acid, creatine kinase, total cholesterol, triglycerides, HCY, cystatin C) levels between the ALS group and the control group (**Fig. 1**).
- The correlation analysis of creatinine, uric acid, creatine kinase, total cholesterol, triglycerides, homocysteine, and cystatin C levels with ALSFRS-R and DPR, respectively. And the linear regression analysis on indicators that had obvious correlation with ALSFRS-R and DPR (**Tab. 1 and Fig. 2**).

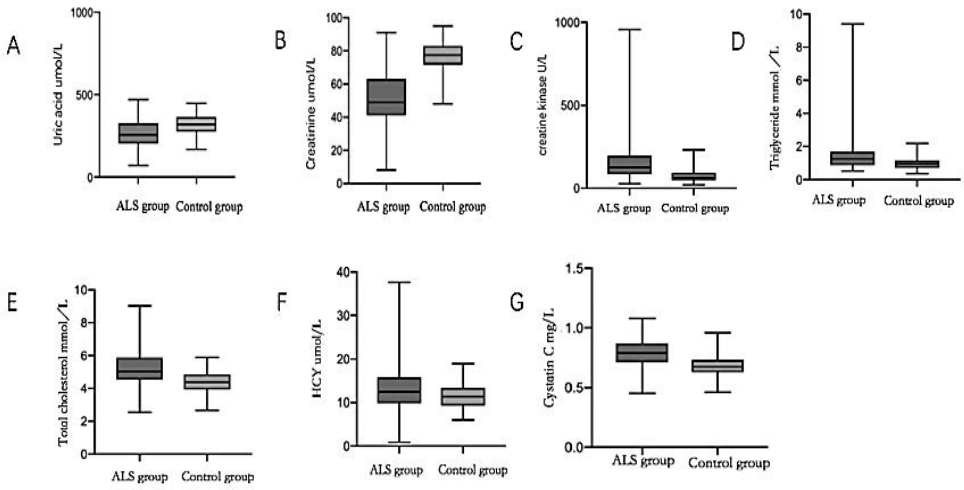

Fig. 1. Comparison of serum index levels between the ALS group and the control group. There are significant differences in the levels of creatinine, uric acid, creatine kinase, total cholesterol, triglycerides, HCY, and cystatin C of the two groups (P<0.05). The levels of uric acid and creatinine in the ALS group were significantly lower than those in the control group (P<0.05, Fig. 1A-B). The levels of creatine kinase, triglyceride, total cholesterol, blood HCY and cystatin C in the ALS group were significantly higher than those in the control group. (P<0.05, Fig. 1C-G).

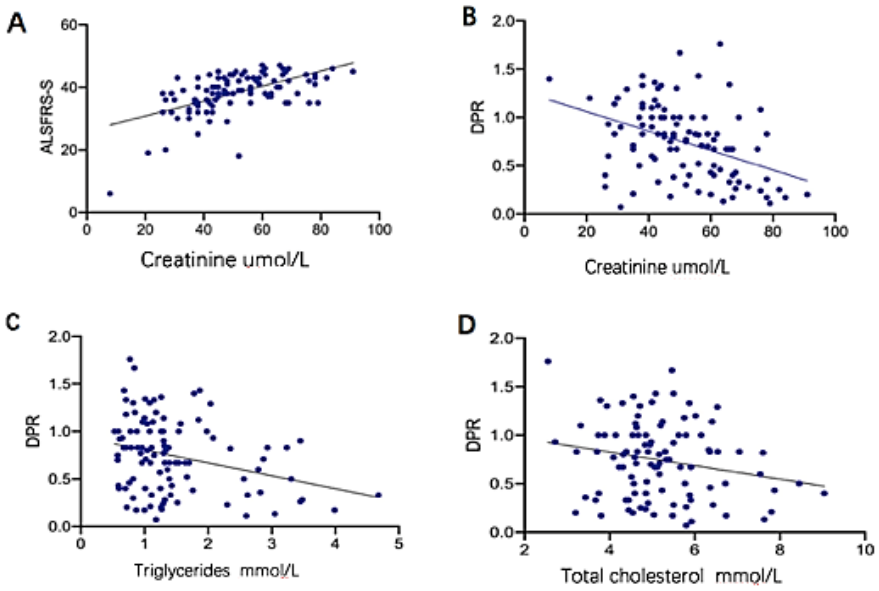

Fig. 2. Linear regression of creatinine and ALSFRS-R, creatinine, total cholesterol, triglycerides and DPR, respectively. There is a significant positive correlation between creatinine level and ALSFRS-R ($P<0.05$). The ALSFRS-R score increased with the creatinine level ($P<0.05$, Fig. 2A). As shown in Fig. 2B and Fig. 2D, creatinine and total cholesterol are independent risk factors affecting DPR ($P<0.05$), and DPR decreases with the increase of creatinine and total cholesterol levels.

Tab. 1. Correlation analysis between ALSFRS, DPR and serological markers.

	ALSFRS-S		DPR	
	r	P	r	P
Creatinine	0.567**	0	-0.408**	0
Creatine kinase	-0.061	0.542	-0.097	0.332
Uric acid	0.07	0.482	-0.102	0.305
Triglyceride	0.075	0.452	-0.201*	0.042
Total cholesterol	0.012	0.904	-0.210*	0.033
HCY	0.007	0.947	0.088	0.378
Cystatin C	0.04	0.689	-0.052	0.604

** At the 0.01 level (two-tailed), the correlation is significant;
*At the 0.05 level (two-tailed), the correlation is significant.

As shown in **Tab. 1**, ALSFRS-R has a very significant correlation with creatinine level ($P<0.01$), and the correlation coefficient is 0.567 (positive correlation); DPR has a very obvious correlation with creatinine level ($P<0.01$), and the correlation is -0.408 (negative correlation); There is a significant correlation between DPR and triglyceride levels/total cholesterol levels ($P<0.05$), and the cor-relation coefficients are -0.201 and -0.210, respectively, which are negatively correlated. The residual indexes had no correlation with ALSFRS-R and DPR.

DISCUSSION

ALS is a disease characterized by irreversible neuronal necrosis. The overall prognosis of the disease is poor. At present, there is no method for early diagnosis and effective treatment. Previous animal experiments have confirmed that biomarkers may be used as an indicator for early diagnosis and prognosis of ALS. In this study, we explored the difference between the serum indicators of ALS patients and the control group, hoping to find indicators that may prompt the diagnosis and prognosis of ALS, so as to provide a basis for further experiments.

Creatinine can assess the functional status and muscle strength of the patients, predict the disease progression rate and prompt prognosis: The blood creatinine is converted from creatine. The creatinine level is a direct reflection of the creatine pool, which reflects the mitochondrial function of muscles [4]. A Japanese study showed that patients with ALS had lower serum creatinine levels compared with healthy controls. In addition, the ALS function rating scale and the rate of decrease in Forced Vital Capacity (FVC) are negatively correlated with serum creatinine levels [5]. ALSFRS-R and FVC are effective indicators for evaluating limb function and respiratory function in patients with ALS, respectively. The results of Van showed that there is a strong

correlation between muscle strength and serum creatinine level. In this study, we observed a smaller variation of serum creatinine decline rate in patients than that of ALSFRS-R, suggesting that the rate of creatinine decline is a more stable predictor of ALS disease progression than ALSFRS-R, and it is more effective in patients with limb symptoms. The results of a large study in Hua xi Hospital showed that the serum creatinine of ALS patients was significantly lower than that of the healthy control group after adjusting for BMI and age, and those patients with low serum creatinine levels were more likely to develop severe motor disorders and low BMI values [6]. But they thought that there was no significant difference in serum creatinine level among ALS patients with different disease sites, and there is no relationship between the serum creatinine level and the survival time of ALS patients [7]. Chio, et al. suggested that lower creatinine levels were closely associated with poorer clinical function at diagnosis (clinical function is evaluated by ALSFRS-R score and FVC). His study showed that serum creatinine is related to survival, and the predictive sensitivity of serum creatinine to mortality is similar to FVC, ALSFRS-R score and age. Patin, et al. suggested that the creatinine level of patients with limb disease was lower than that of patients with medulla oblongata disease. The difference still exists after excluding confounding factors such as gender and weight [8,9]. All the above studies indicated that blood creatinine may serve as an accurate measure of muscle quality, and can evaluate limb function, disease progression rate and prognosis. In this study, the creatinine levels of the ALS group were significantly lower than that of the healthy control group. The results of multi-variate analysis showed that: creatinine level is an independent risk factor for ALSFRS-R and DPR. Higher creatinine level indicates better limb function and slower disease progression within a certain range. Serum creatinine is a cheap and easy to obtain biomarker with good reproducibility, so it can be used extensively and repeatedly in clinical practice to evaluate the limb function and prognosis of patients with ALS.

Higher blood lipids indicate better limb function and slower disease progression: Studies have shown that ALS patients have a higher probability of dyslipidemia compared with the general population. Two studies from the United Kingdom and Germany found that the prevalence of hypercholesterolemia and hypertriglyceridemia in ALS patients was significantly increased [10,11]. Some researchers believe that hyperlipidemia is a protective factor for ALS. After muscle denervation, the body's efforts to regenerate nerves lead to an increase in energy demand, and the increased demand is supplemented by lipids, which can also explain the decrease of blood lipid levels in patients with advanced disease progression. And the total body fat decreased. In this study, the blood total cholesterol and triglyceride levels of the ALS group were significantly higher than those of the control group. However, only the total cholesterol level was significantly correlated with the disease progression rate after correlation analysis. As the total cholesterol level increased, the disease progression rate decreased. The presence of dyslipidemia in ALS patients is increasingly recognized. The exact cause of hyper-lipidemia in ALS is still uncertain: It may be a compensatory mech-anism, lipids are the preferred energy source for skeletal muscle, and the regeneration of denervated muscles may lead to increased energy demand in ALS. It is currently believed that higher blood lipids may be related to better limb function and slower progression. In ALS patients, lipid-lowering drugs should be used carefully, especially statins, which may aggravate muscle damage.

Uric acid reduced the damage to neurons caused by oxidative stress: Uric Acid (UA) is the main end product of human purine metabolism. As a natural antioxidant, it can remove superoxide and reduce neuronal death caused by oxidative stress. The study of Bakshi, et al. showed that urate significantly reduced the death of motor neuron cells induced by hydrogen peroxide in an astrocyte-dependent manner [12]. A study in Japan showed that the serum UA level of ALS patients was significantly higher than that of the healthy control group, and that the serum UA level was positively correlated with BMI, ALSFRS-R score and creatinine level, and negatively correlated with DPR [13]. Paganoni, et al. reported that when creatinine and BMI were controlled, serum uric acid level was an important predictor of ALS survival rate and dysfunction [14]. A prospective study observed that people with higher serum uric acid levels than their age and sex-matched control group have a slightly lower risk of ALS in the future [15]. The meta-analysis results of Zhang, et al. showed that the serum UA level of ALS patients was significantly lower than that of the control group, and the serum UA level of ALS patients was negatively correlated with the risk of death. Nicholson, et al. increased the blood UA level by intravenous infusion of inosine in 25 ALS patients [16,17]. The results showed that the biomarkers of oxidative were significantly reduced, and ALSFRS-R was improved after treatment. Although the results of various studies are partially contradictory, most of the current experimental results believe that uric acid as an antioxidant can reduce the damage of oxidative stress to neurons. So it is a protective factor for ALS. In this study, the level of uric acid in the ALS group was significantly lower than that in the control group, but there was no correlation with ALSFRS-R and DPR in the multivariate correlation analysis. Further clinical trials are needed to determine whether uric acid can reduce neuronal loss, improve limb function and extend life span of ALS patients.

Creatine kinase, homocysteine, and cystatin C are future research directions: Creatine Kinase (CK) is an enzyme that catalyzes the reversible conversion of creatine and provides energy for muscle contraction. Linkhart, et al. believes that the degeneration of in-nervated motor neurons leads to increased muscle cell membrane permeability and CK release. CK levels may be related to the severity of lower motor neuron loss and muscle atrophy in patients with ALS. Tai, et al. found that the serum CK level was associated with the persistent low F wave of the median nerve in the EMG, which confirmed that the CK level in ALS patients was related to the severity of lower motor nerve loss. In this study, 31 of 103 (30%) ALS patients had moderately elevated serum CK (<1000 U/L), The CK level of the ALS group was significantly higher than that of the control group. However, no correlation was observed between the CK level and the patient's physical function score and disease progression rate. It may be related to the small sample size and the lack of long-term follow-up of patients. At present, the mechanism of the increase in CK levels in ALS patients is not very clear. Larger sample studies and more animal experiments are needed to further clarify [18,19].

Homocysteine (HCY) is a sulfhydryl containing amino acid produced by the demethylation of methionine. A Japanese small sample double-blind clinical trial of 24 ALS patients found that short-term (4 weeks) high-dose (0.5 mg/d) administration of vitamin B12 effectively improved the compound motor action potential on the electromyogram of ALS patients, suggesting that the loss of spinal cord motor neurons is improved [20]. In our study, the serum HCY level of the ALS group was significantly higher than that of the control group, and there was no significant correlation between ALSFSR and DPR and HCY levels in the correlation analysis. A cohort study on ALS patients is needed to get a more precise conclusion. However, we believe that HCY may be one of the factors that cause neuronal damage in ALS. Even if

ALS cannot be cured, the treatment of reducing HCY still has a positive meaning and it is an important part of the treatment of ALS.

Cystatin C is an endogenous cysteine protease inhibitor, which plays an important role in the repair of the nervous system after injury and disease. The neuroprotective activity of CysC includes inducing autophagy in nerve cells, promoting the degradation of misfolded or unfolded proteins, and preventing the accumulation of abnormal mutant proteins [21]. A longitudinal study analysis showed that the level of CysC in the cerebrospinal fluid of fast-progresses was lower, while those with slower progress showed an increasing trend of CysC levels, and higher cerebrospinal fluid CysC levels suggested slower progress [22]. In this study, the blood CysC level of the ALS group was significantly higher than that of the control group, and the correlation analysis had nothing to do with the functional score and disease progression rate. Further researches were needed to determine whether there is a correlation between cerebrospinal fluid and CysC in the blood and whether increasing the level of CysC in the blood can improve the function, delay the progression, and improve the prognosis of ALS patients.

CONCLUSION

In summary, this study performed a statistical comparison of creatinine, uric acid, creatine kinase, total cholesterol, triglycerides, HCY, cystatin C and other indicators in the blood of ALS patients, and found that the serum uric acid and creatinine levels of ALS patients is lower than healthy people, and the levels of creatine kinase, triglycerides, total cholesterol, HCY and CysC are higher than healthy people. Patients with ALS have obvious metabolic abnormalities. Higher levels of creatinine, blood lipids and uric acid usually indicate better physical function, slower disease progression, and better prognosis. However, this study has some shortcomings. The ALS team has not yet conducted any human intervention and randomized controlled studies. Our next study is to conduct a randomized controlled study of every indicator that seems to be meaningful in this trial. As for the indicators that have not been obtained in this experiment, animal experiments need to be further validated to discover their role in the diagnosis, treatment and prognosis of ALS.

Amyotrophic Lateral Sclerosis (ALS) is a devastating condition with an estimated mortality of 30,000 patients a year worldwide. The median reported survival time since onset ranges from 24 to 48 months. Therefore, early diagnosis and treatment of ALS are important. Serum markers may be a promising method to predict the occurrence, diagnosis, and progress of ALS. The application of serum markers to the development of ALS drugs will greatly improve our ability to correctly test drugs in clinical trials and ultimately find a treatment for ALS. At present, the field of ALS serum markers is still an active research field with great hope.

ACKNOWLEDGEMENTS

We would like to thank all patients in this study and Neurological Sciences for providing this excellent platform for researchers to communicate and learn.

AUTHOR'S CONTRIBUTIONS

DLK designed the experiment plan and directed the thesis writing. XWC, MW, YYL, WZ were in charge of the whole experiment process and thesis writing. JRL participated in the design of the experiment. We would like to thank the patient and his families for their participation in this study.

CONFLICT OF INTREST

The authors report no conflicts of interest in this work.

ETHICAL APPROVAL

None.

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