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Title: Evaluation of Relationship between Laboratory, Electrodiagnostic, and Functional Parameters in Patients with Amyotrophic Lateral Sclerosis; A Cross Sectional Study

Authors: Marjan Zeinali[1], Mostafa Almasi Dooghaee[1], Mahsa Ziaee[1], Bahram Haghi Ashtiani[1,*]

1. *Neurology Resident at Department of Neurology, School of Medicine, Iran University of Medical Sciences, Tehran, Iran.*

*Corresponding Author: Bahram Haghi Ashtiani, Neurology Resident at Department of Neurology, School of Medicine, Iran University of Medical Sciences, Tehran, Iran. Email: bhaghi2000@yahoo.com

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ABSTRACT

Background: Amyotrophic lateral sclerosis (ALS) is an adult-onset motor neuron disease which leads to limb and/or bulbar muscle degeneration with a poor prognosis. Several demographic and biological factors have prognostic importance, but little data exist on the relationship between clinical, electrodiagnostic, and laboratory markers as predictors of disease progression. We aimed to assess the relationships between different aspects of clinical, electrodiagnostic, and laboratory features of ALS patients with their level of functioning.

Methods: We included 27 patients with ALS who were diagnosed within two years before enrolment. Clinical assessment and electrodiagnostic studies were done by a neurology resident. The motor unit number index (MUNIX) and compound motor action potential (CMAP) were used as measures of motor unit loss. Serum creatinine, urea, Albumin, and creatine kinase were measured as laboratory markers. We used the Persian version of ALS functional rating scale (ALS-FRS) as the main outcome measure. Data were analyzed using the SPSS software. Pearson's correlation coefficient was calculated to test for correlations.

Results: None of the demographic or laboratory parameters correlated with ALS-FRS. Patients with the onset of disease in the limbs had a higher MUNIX score compared to patients with a bulbar onset. Also, increased body mass index was associated with lower CMAP and MUNIX scores (p-value:0.02). Higher serum creatinine levels were significantly associated with higher lower limb MUNIX (p value:0.04). Higher lower limb MUNIX was in turn associated with higher lower limb functional score (ALS-FRS).

Conclusion: Decreased serum creatinine may possibly be an indicator of lower limb motor unit loss in patients with ALS. Also, MUNIX scores may be used as surrogates for ALS-FRS in ALS trials. Further research is needed to elucidate the clinical application of these findings.

Introduction

Amyotrophic lateral sclerosis (ALS) is the most common adult-onset motor neuron disease with an incidence of 2-3 cases per 100,000 per year (1). The average survival rate since the onset of symptoms is about 3-5 years (2). Prognosis is poor for most patients with ALS particularly when the diagnosis is delayed (3).

Several markers have been identified for the functional assessment of ALS patients, which include age (4-6), Symptoms of onset (7), BMI and nutrition status (8), and a number of biologic and laboratory markers such as creatine kinase serum level (9), serum creatinine (10), and serum albumin level (11). A better understanding of these factors helps physicians and patients make more informed decisions based on their shared treatment goals(12). Also, clinical trials for ALS will be more applicable and easier to conduct as more disease progression markers are validated as outcome measures. Electrodiagnostic measures are another important tool for the assessment of motor neuron diseases including ALS (13). Measuring the rate of motor units' loss is a good way to monitor disease progression (14). Electrodiagnostic measures such as the motor unit number index (MUNIX) can help determine the number of lost motor units (15-17).

Few studies have examined the relationship between the number of motor units and serologic biomarkers (e.g. CK, albumin and creatinine (Cr)). We aimed to study the relationship between the laboratory findings, the number of motor units (based on electrodiagnostic studies), and the patients functional scores.

Methods

We conducted a cross-sectional study of patients with ALS diagnosed within two years before the study onset. Diagnosis was confirmed by the AWAJI criteria which is a reliable criteria based on patient history, clinical examination, and electrodiagnostic testing. Patients with impaired renal function and high serum creatinine, those functionally impaired due to medical conditions as well as patients who refused to participate in the study were excluded.

Patients' demographic features, time from symptom onset to diagnosis, and primary presenting symptoms were recorded based on history taking and clinical interview. Laboratory investigations including serum creatine kinase (CK), Creatinine (Cr), blood urea nitrogen (BUN) and albumin were undertaken at the central laboratory of Firoozgar hospital upon enrollment. The motor unit number index (MUNIX) was calculated based on

electromyographic (EMG) assessment of four muscles: anterior tibialis, abductor digiti minimi, deltoid, and abductor policis brevis. In this method, compound muscle action potential (CMAP) amplitude and surface electromyographic interference patterns (SIP) are used to calculate MUNIX which is a measure for the size and number of motor units. An experienced neurologist in neuromuscular disorders conducted the electromyography. All assessments were done by the same neurologist using the same EMG machine.

The main outcome of this study was ALS functional rating scale (ALS-FRS) which is a measure of self-sufficiency and disease progression in ALS patients. It is a 10-item inventory covering functional domains including feeding, grooming, ambulation and communication, the Persian version of which has been validated for use in Persian-speaking ALS patients(18). Each item is rated zero to four based on patient's level of function. The scores were calculated and relevant clinical assessments were performed by a neurologist.

Statistical Analysis

Pearson's correlation coefficient was used to assess the association between demographic characteristics, clinical features, ALS-FRS, laboratory findings, and EMG findings. We used regression statistics to further assess for the strength of significant associations. All statistical analyses were performed using SPSS version 16 software. A p-value below 0.05 was considered significant.

Ethical Considerations:

This research received ethical approval from ethics committee at Iran University of Medical Sciences under the code: IR.IUMS.FMD.REC.1399.767

We adhered to the principals for medical research on human subjects in accordance with the Helsinki declaration. Informed consent was obtained from all of the included participants having clarified that all the elements of this research including clinical, electrodiagnostic, and laboratory assessments were part of the routine care of their illness without any additional costs for the patient.

Results

Patient characteristics

Twenty-seven patients were enrolled to this study. The mean age of patients was 57.8(±10.5) ranging from 36 to 77 years. The majority of patients (20/27) were men. Most patients presented primarily with limb symptoms (20/27(74.7%)) whereas 7 patients had a bulbar-onset form. The mean interval between ALS diagnosis to enrollment to the study was 8.5(±1.6) months. Clinical and laboratory features of the patients are presented in table 1.

Characteristics	Categories	Mean± SD/Number(%)
Age(years)		57.8±10.5
Sex	Man	20(74.7%)
	Woman	7(25.3%)
BMI		25.53±4.3
Disease-related Characteristics		
form of onset	Limb-onset	20(74.7%)
	Bulbar-onset	7(25.3%)
Time interval from diagnosis to enrollment(months)		8.5±1.6
CMAP	total	43.38±14.02
MUNIX	Upper-limb	347.0±227.8
	Lower-limb	153.7±89
	Total	751.3±324.04
ALS-FRS	Upper-limb	6.07±1.83
	Lower-limb	6.59±1.78
	Total	38.19±5.80
Laboratory Features		
Serum CPK		389.1(±386)
Serum Cr		0.97(±0.13)
BUN		31.93(±8.74)
Serum Albumin		4.62(±0.38)

Table 1 Characteristics and Laboratory features of study participants

Main Results

There was no relationship between demographic factors and the main study outcome (ALS-FRS). Serum albumin, creatinine, and CK did not correlate with total ALS-FRS scores (table 2). Higher serum creatinine correlated with higher lower limb MUNIX (P-Value: 0.04, Pearson correlation: 0.39).

Higher BMI was significantly associated with lower total as well as limb-specific MUNIX scores (p-value=0.01, Pearson's correlation coefficient=0.48). Furthermore, total CMAP was lower among patients with a higher BMI (p-value=0.02, Pearson's correlation coefficient=0.42). Patients with limb-onset form of ALS had higher total and upper-limb MUNIX scores compared to patients with a bulbar-onset form of the disease (P-Value=0.02, Pearson correlation=0.44).

	Total ALS-FRS		Total MUNIX		Total CMAP	
	Correlation Coefficient	P-Value	Correlation Coefficient	P-Value	Correlation Coefficient	P-Value
Serum BUN	0.13	0.51	0.17	0.37	0.09	0.62
Serum Creatinine	0.09	0.63	0.31	0.11	0.31	0.11
Serum Albumin	0.09	0.62	-0.08	0.67	-0.11	0.56
Serum CPK	0.15	0.45	-0.34	0.07	-0.31	0.11
BMI	0.07	0.70	-0.48	0.01	-0.42	0.02
Time interval from diagnosis to enrollment	-0.23	0.23	0.11	0.57	0.02	0.89
Age	-0.24	0.22	0.13	0.52	0.04	0.83
Limb-onset	0.27	0.16	0.51	<0.01	0.37	0.05

Table 2 The relationship between patient clinical features and level of functioning based on ALS-FRS, and electrodiagnostic indices assessed using Pearson's Correlation test

Total MUNIX scores, upper-limb MUNIX, and lower-limb MUNIX scores did not show a significant association with total ALS-FR scores. However, higher lower-limb MUNIX correlated with better lower limb level of functioning based on ALS-FRS (P-Value: 0.02, Pearson correlation: 0.44). The assessment of strength of this association using regression analysis indicated that for every 1-point increase in MUNIX, ALS-FRS increased 0.44 units.

	Total ALS-FRS	
	Pearson correlation	P-Value
Total MUNIX	**0.139**	0.490
Upper limb MUNIX	**0.034**	0.867
Lower limb MUNIX	**0.144**	0.472
Total CMAP	**0.295**	0.135

Table 3 The relationship between electrodiagnostic ALS features and patient level of functionin based on ALS-FRS according to Prson correlation calculations.

Discussion

Our findings can be discussed in the context of three different dimensions: the relationship between laboratory markers and patient level of functioning, laboratory markers in relation to electrodiagnostic markers of disease activity, and the relationship between clinical and demographic characteristics with laboratory and electrodiagnostic markers of disease activity.

Creatinine is a marker of normal muscle cell metabolism, and a decrease in its serum level indicates loss of muscle units(10, 19). We found no relationship between serum creatinine with patient functioning based on ALS-FRS. A 2019 meta-analysis showed that higher serum creatinine at baseline was associated with higher functional scores and that a sharper decline in serum creatinine correlated with more prominent decline in level of functioning(20). Another study that assessed for the relationship between plasma biomarkers and function in 355 well-phenotype ALS patients reported a consistent relationship between serum creatinine at all time-points during follow-up(21). These findings are inconsistent with our findings in that our study did not show any relationship between ALS-FRS scores and serum creatinine. The reason may be that we missed the association between Cr decline and level of functioning due to lack of longitudinal follow-up or the smaller number of participants in our study.

However, higher serum creatinine level correlated with higher lower limb MUNIX scores which reflect the number of active motor units in the lower limb. Moreover, we found that decreased lower limb MUNIX correlated with lower limb ALS-FRS scores. These findings are consistent with the notion that creatinine is related to active muscle mass(17, 22-24). Although, these findings were exclusively significant for the lower limb and total MUNIX was not related to serum creatinine. This may have been due to different clinical features of our patients or the small sample size.

Some studies have reported serum albumin decline as a prognostic factor and a predictor of the rate of disease progression(11, 25, 26); Interestingly, in some studies only the decline of albumin and not one-time serum albumin levels were associated with disease progression(27). This may be the reason why we did not find any association between serum albumin and functional scores in this cross-sectional study.

Creatine kinase has been a reliable and independent prognostic biomarker owing to its association with ALS-FRS scores in assessment of treatment outcomes in ALS patients (9, 28, 29). This enzyme is produced in greater quantities to provide energy for the metabolic stress imposed by ALS (28). In our study, the levels of this marker did not correlate with electrodiagnostic and functional indices.

In this study, patients with limb onset ALS, had a higher upper limb MUNIX and ALS-FRS functional score compared to patients with a bulbar onset; However, this was unrelated to serum creatinine, BUN, CPK, and level of functioning.

We also found that patients with higher BMI had lower MUNIX and CMAP scores; although, these factors were unrelated to ALS-FRS score and patient function. In contrast to our findings, most studies reported high BMI as a positive prognostic factor (11, 25). According to these studies, BMI and MUNIX score were not related and BMI was related to survival, functional score and overall prognosis. Since BMI does not exclusively represent muscle mass, fat-free mass (FFM) might be a more valid indicator of muscle bulk(11). A 2012 study stated that low BMI and malnutrition have a neurotoxic effect, highlighting the role of BMI in the prognosis of ALS patients(30). Another study found that in people with a BMI below 30, a higher initial BMI predicted a slower rate of function decline, but the relationship was reversed in patients with BMIs over 30(31). Although the patients in our study had a mean BMI of 25, s higher BMI was associated with lower MUNIX and CMAP and did not correlate with ALS-FRS.

Age, gender, and disease duration had no significant relationship with laboratory, electrodiagnostic and function findings.

Electrodiagnostic measures are common and reliable indicators of disease progression in ALS. The MUNIX index represents the number of active muscle units. In our study, cases with higher lower limb MUNIX score had also higher lower limb ALS-FRS function score. We did not find a significant relationship between other electrodiagnostic measures and functional scores. According to our results, the MUNIX score in the lower limb seems to be a more reliable measure for monitoring muscle function in these patients. A 2016 study concluded

that total MUNIX score had a significant correlation with ALS-FRS function score(23). The same findings were repeated in another study(32). Also, a decrease in MUNIX has been associated with a significant decrease in the ALS-FRS score (14, 23). Another study (33) found that the MUNIX score was significantly different between lower limb onset and upper limb onset patients. Also, MUNIX decreased more rapidly in patients whose lower limbs were involved at disease onset.

We investigated the relationship between electrodiagnostic measures and laboratory indices to find a predicting measure and to estimate the number of active motor units and the rate of its loss. As mentioned earlier, we found that higher serum creatinine levels, limb onset, lower BMI, and higher creatinine to BMI ratio were associated with higher MUNIX scores and, that represents more active motor units.

Our results as well as a review on the literature, suggest that biomarkers like creatinine, MUNIX score, BMI and can be exploited to estimate the severity of the disease, to assess and to monitor the function level in ALS patients. Also, our study showed that serum creatinine level and lower limb MUNIX score are significantly related, suggesting the benefit of creatinine as a minimally-invasive means to study the number of active motor units.

One of the strengths of our study was the simultaneous and multifaceted study of three aspects of disease activity together. However, the findings of this study are limited by small sample size, due to low prevalence of the ALS and pandemic condition, which could affect the results and let to underestimation of significant correlations between the parameters. Lack of prospective follow-up due to the challenges during the COVID-19 pandemic is the other limitation of the study. In addition, some of novel CSF and serum biomarkers which has popularized within past years for evaluation in patients with ALS, including high-titer GM1 ganglioside antibody, endocrine markers like thyroid hormones, infection markers and serum protein electrophoresis, are not included in this study.

Conclusion:

In this study, higher serum creatinine was associated with higher lower limb MUNIX; and lower limb MUNIX correlated with lower limb ALS-FRS. Although there was no direct relationship between serum creatinine and functional scores, the two were indirectly related. Our findings suggest that serum creatinine may be a reliable and versatile biomarker for disease progression in ALS. Our results also show that lower limb MUNIX may be an appropriate measure for level of functioning in the lower limb. Further research with a multidimensional

prospective design is needed to clarify the complex relationship between clinical and electrodiagnostic parameters with outcomes in ALS patients.

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