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Original Article

Association Between Serum Inflammatory Biomarkers and Disease Severity in Motor Neuron Disease

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ABSTRACT

Background: Motor neuron disease (MND) progression is increasingly linked to systemic neuroinflammation, yet comprehensive data on conventional inflammatory markers within South Asian populations remains limited. This study evaluated the relationship between serum inflammatory biomarkers and functional disease severity in a Bangladeshi MND cohort. **Methods & Materials:** A hospital-based cross-sectional study was conducted with 80 adult MND patients. Functional severity was classified as mild, moderate, or severe using the Amyotrophic Lateral Sclerosis Functional Rating Scale-Revised (ALSFRS-R). The study analyzed serum levels of C-reactive protein (CRP), interleukin-6 (IL-6), tumor necrosis factor-alpha (TNF-α), erythrocyte sedimentation rate (ESR), neutrophil-to-lymphocyte ratio (NLR), and albumin. Multivariable logistic regression was utilized to identify independent predictors of severe disease while adjusting for demographic and clinical variables. **Results:** The cohort had a mean age of 55.8 ± 10.7 years, with 58.8% being male. Serum concentrations of CRP, IL-6, TNF-α, ESR, and NLR showed a significant progressive increase as disease severity worsened (all $p < 0.001$). Conversely, serum albumin significantly decreased with escalating disease severity ($p = 0.002$). All measured inflammatory markers exhibited significant inverse correlations with ALSFRS-R scores, with IL-6 showing the strongest correlation ($r = -0.58$, $p < 0.001$). Multivariable analysis confirmed that higher levels of CRP, IL-6, TNF-α, and NLR were independently associated with increased odds of severe MND, whereas serum albumin lost its independent predictive significance. **Conclusion:** Elevated systemic inflammatory biomarkers are strongly correlated with increased functional severity in MND. CRP, IL-6, TNF-α, and NLR serve as independent predictors of severe illness, underscoring the role of systemic immune activation and highlighting their potential as accessible, low-cost markers for disease monitoring and prognostication.

Keywords: *Motor Neuron Disease, Amyotrophic Lateral Sclerosis, Inflammatory Biomarkers, ALSFRS-R, Neuroinflammation, Systemic Inflammation, Neutrophil-to-Lymphocyte Ratio (NLR).*

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INTRODUCTION

A collection of progressive neurodegenerative conditions known as motor neuron disease (MND) are characterized by motor neuron death. The most prevalent and severe adult-onset type of the disease is amyotrophic lateral sclerosis (ALS) leading muscular weakening, atrophy, and stiffness that finally affects respiratory and bulbar function [1]. The majority of patients pass away from respiratory failure three to five years after the beginning of symptoms due to the disease's unrelenting course [2].

Globally, the prevalence of ALS is 5.05 per 100,000 people and the pooled incidence is around 1.65 per 100,000 person-years. Asia and South America are said to have a significantly lower disease burden than Europe, North America, and Oceania [3]. Previous meta-analyses yielded similar results, estimating a global incidence of 1.59 and prevalence of 4.42 per 100,000 [4]. As populations age, the number of ALS cases worldwide is predicted to increase from approximately 223,000 in 2015 to nearly 377,000 by 2040, with the largest relative increase anticipated in developing nations [5]. There is still a dearth of data from Bangladesh in particular. A hospital-based series revealed that ALS was responsible for 61% of MND patients, with a male-to-female ratio of 1.79:1 and a mean age at onset of 53 years, aligning with international series [6].

The Revised ALS Functional Rating Scale (ALSFRS-R), a 12-item tool covering the bulbar, fine motor, gross motor, and respiratory domains, is typically used to track functional decline in ALS patients. It is still the most popular outcome measure in both clinical practice and clinical trials [7]. These markers may be useful for prognostication, patient stratification, and treatment response monitoring.

It is becoming more well acknowledged that neuroinflammation causes motor neuron loss in ALS rather than just being a result of it. There is increasing evidence that the peripheral immune system interacts directly with this central process, with the circulation of monocytes, T lymphocytes, and cytokines crossing a compromised blood-spinal cord barrier to affect motor neuron survival. Post-mortem and imaging studies have revealed activation of microglia and reactive astrocytes in affected regions of the spinal cord and motor cortex [8]. Peripheral blood markers of inflammation have gained interest as more accessible surrogates of this process because direct sampling of the central nervous system is intrusive and impractical for everyday usage.

In ALS cohorts, a number of these markers have been investigated. Tumor necrosis factor-alpha (TNF-α), TNF receptor 1, interleukin-6 (IL-6), interleukin-1beta, and interleukin-8 blood concentrations were significantly higher in ALS patients than in controls, according to a meta-analysis of 25 studies [9]. Furthermore, a longitudinal UK cohort found that TNF-α and IL-6 were the most consistently elevated cytokines, with IL-6 closely correlated with C-reactive protein (CRP) and increased as patients' necrosis [10].

This study sought to assess the relationship between serum inflammatory biomarkers and disease severity measured by the ALSFRS-R, among patients with motor neuron disease. It also sought to find out whether these biomarkers remained independently associated with severe disease after adjusting for pertinent demographic and clinical variables.

METHODS & MATERIALS

Study design and setting

A hospital-based observational cross-sectional study was conducted among patients diagnosed with motor neuron disease (MND). The study was designed to

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evaluate the association between circulating inflammatory biomarkers and disease severity, with functional impairment assessed using the Amyotrophic Lateral Sclerosis Functional Rating Scale-Revised (ALSFRS-R).

Study population and participant selection

Patients with a clinical diagnosis of MND who attended the neurology or relevant specialist outpatient and inpatient services during the study period were considered for inclusion. Participants were eligible if they were adults aged 18 years or older, had a confirmed diagnosis of MND, and were able to undergo clinical assessment and provide a blood sample for biomarker analysis. Patients with active infection, known autoimmune or systemic inflammatory disease, malignancy, severe hepatic or renal disease, or other conditions likely to substantially influence circulating inflammatory biomarker levels were excluded. Patients receiving systemic corticosteroids or other immunomodulatory treatment were also excluded. A total of 80 eligible patients were included in the final analysis.

Clinical assessment and data collection

Demographic and clinical information was collected using a structured data collection form. Information included age, sex, body mass index (BMI), smoking history, family history of MND, disease duration, and the presence of bulbar involvement. Disease duration was calculated from the time of initial symptom onset or diagnosis, as applicable, to the date of assessment.

Functional disease severity was assessed using the ALSFRS-R, a validated 12-item scale evaluating functional impairment in areas including speech, swallowing, salivation, fine motor activities, gross motor activities, mobility, and respiratory function. Each item is scored from 0 to 4, giving a total score ranging from 0 to 48, with lower scores indicating greater functional impairment. For the present analysis, participants were categorized into mild, moderate, and severe disease groups according to their ALSFRS-R scores. The same severity classification was used throughout the analysis.

Assessment of serum inflammatory biomarkers

Venous blood samples were collected from each participant under standardized conditions. Serum was separated according to standard laboratory procedures and analyzed for inflammatory biomarkers. The primary inflammatory biomarkers evaluated were C-reactive protein (CRP), interleukin-6 (IL-6), and tumor necrosis factor-alpha (TNF-α). Routine inflammatory indices, including erythrocyte sedimentation rate (ESR) and the neutrophil-to-lymphocyte ratio (NLR), were also assessed. Serum albumin was measured as an additional clinical and nutritional marker.

CRP was expressed in mg/L, IL-6 and TNF-α in pg/mL, ESR in mm/hour, and albumin in g/dL. NLR was calculated by dividing the absolute neutrophil count by the absolute lymphocyte count obtained from the complete blood count.

Outcome measures

The primary outcome was the association between serum inflammatory biomarker concentrations and MND disease severity. Disease severity was primarily represented by the ALSFRS-R score. Secondary analyses evaluated differences in inflammatory biomarker concentrations across mild, moderate, and severe disease categories and determined whether inflammatory biomarkers were independently associated with severe disease.

Statistical analysis

Data were analyzed using appropriate statistical software. Continuous variables were expressed as mean ± standard deviation (SD) or median with interquartile range (IQR), while categorical variables were presented as frequency and percentage. Differences between disease-severity groups were assessed using appropriate parametric or non-parametric tests, and categorical variables were compared using the chi-square or Fisher's exact test. Correlations between inflammatory biomarkers and ALSFRS-R scores were evaluated using Pearson's or Spearman's correlation coefficient, as appropriate. Multivariable logistic regression was performed to identify factors independently associated with severe disease, with adjusted odds ratios (ORs) and 95% confidence intervals (CIs) reported. A two-sided p-value <0.05 was considered statistically significant.

Ethical considerations

The study was conducted in accordance with the principles of the Declaration of Helsinki. Ethical approval was obtained from the appropriate institutional ethics committee before commencement of the study. Written informed consent was obtained from all participants before clinical assessment and blood sample collection. Participant information was kept confidential and used exclusively for research purposes.

RESULTS

Baseline characteristics of the study participants

A total of 80 patients with motor neuron disease (MND) were included in the analysis. The mean age of the participants was 55.8 ± 10.7 years, and 47 (58.8%) were male. The mean BMI was 23.6 ± 3.2 kg/m². The median disease duration was 18 months (IQR, 10–30 months). Based on the ALS Functional Rating Scale-Revised (ALSFRS-R), 29 (36.3%) participants had mild disease, 31 (38.8%) had moderate disease, and 20 (25.0%) had severe disease.As shown in *Table I*, the mean ALSFRS-R score for the total study population was 31.4 ± 7.1. The mean ALSFRS-R scores were 38.2 ± 2.1, 31.8 ± 2.7, and 21.2 ± 3.4 among participants with mild, moderate, and severe disease, respectively ($p < 0.001$). Disease duration increased progressively with disease severity, with median durations of 12 months (IQR, 7–18), 19 months (IQR, 12–29), and 28 months (IQR, 18–42) in the mild, moderate, and severe groups, respectively ($p < 0.001$). Bulbar involvement was present in 28 (35.0%) participants overall and was more frequent among those with severe disease (50.0%) than among those with mild disease (20.7%), although the difference was not statistically significant ($p = 0.091$). No significant differences were observed among the severity groups in terms of age, sex, BMI, smoking history, or family history of MND.

Table I

Baseline demographic and clinical characteristics of the study participants according to disease severity.

Characteristics	Total (n=80),n(%)	Mild (n=29),n(%)	Moderate (n=31),n(%)	Severe (n=20),n(%)	p-value
Age, years	55.8 ± 10.7	53.1 ± 10.2	56.4 ± 10.8	59.1 ± 10.5	0.139
Male sex	47 (58.8)	16 (55.2)	19 (61.3)	12 (60.0)	0.880
BMI, kg/m²	23.6 ± 3.2	24.1 ± 3.0	23.7 ± 3.4	22.8 ± 3.2	0.374
Disease duration, months	18 (10–30)	12 (7–18)	19 (12–29)	28 (18–42)	<0.001
ALSFRS-R score	31.4 ± 7.1	38.2 ± 2.1	31.8 ± 2.7	21.2 ± 3.4	<0.001
Bulbar involvement	28 (35.0)	6 (20.7)	12 (38.7)	10 (50.0)	0.091
Smoking history	18 (22.5)	5 (17.2)	7 (22.6)	6 (30.0)	0.566
Family history of MND	7 (8.8)	2 (6.9)	3 (9.7)	2 (10.0)	0.914

Values are presented as mean ± SD, median (IQR), or frequency (%), as appropriate.

Serum inflammatory biomarkers according to disease severity

The serum inflammatory biomarker profile according to disease severity is presented in *Table II*. There was a significant progressive increase in serum CRP concentration across the severity groups. Mean CRP levels were 2.8 ± 1.4 mg/L in patients with mild disease, 4.1 ± 2.0 mg/L in those with moderate disease, and 6.3 ± 3.1 mg/L in those with severe disease ($p < 0.001$).

Similarly, serum IL-6 levels increased significantly with increasing disease severity, from 3.9 ± 1.8 pg/mL in the mild group to 5.6 ± 2.4 pg/mL in the moderate group and 7.8 ± 3.5 pg/mL in the severe group ($p < 0.001$). Mean TNF-α concentrations also increased significantly across the three severity categories, with values of 8.7 ± 2.9 pg/mL, 10.9 ± 3.6 pg/mL, and 13.6 ± 4.8 pg/mL in the mild, moderate, and severe groups, respectively ($p < 0.001$). The mean NLR was 2.12 ± 0.67 in the mild group, 2.71 ± 0.91 in the moderate group, and 3.48 ± 1.26 in the severe group, demonstrating a significant increase with disease severity ($p < 0.001$). ESR showed a similar pattern, increasing from 18.4 ± 7.2 mm/hour in mild disease to 24.7 ± 9.5 mm/hour in moderate disease and 31.2 ± 12.4 mm/hour in severe disease ($p < 0.001$). In contrast, serum albumin concentration decreased significantly with increasing disease severity, from 4.02 ± 0.34 g/dL in the mild group to 3.86 ± 0.38 g/dL in the moderate group and 3.62 ± 0.41 g/dL in the severe group ($p = 0.002$).

Table II

Serum inflammatory biomarkers according to disease severity.

Biomarker	Mild (n=29)	Moderate (n=31)	Severe (n=20)	p-value
CRP, mg/L	2.8 ± 1.4	4.1 ± 2.0	6.3 ± 3.1	<0.001
IL-6, pg/mL	3.9 ± 1.8	5.6 ± 2.4	7.8 ± 3.5	<0.001
TNF-α, pg/mL	8.7 ± 2.9	10.9 ± 3.6	13.6 ± 4.8	<0.001
NLR	2.12 ± 0.67	2.71 ± 0.91	3.48 ± 1.26	<0.001
ESR, mm/hour	18.4 ± 7.2	24.7 ± 9.5	31.2 ± 12.4	<0.001
Serum albumin, g/dL	4.02 ± 0.34	3.86 ± 0.38	3.62 ± 0.41	0.002

Correlation between inflammatory biomarkers and disease severity

The correlations between serum inflammatory biomarkers and ALSFRS-R scores are shown in *Table III*. Significant inverse correlations were observed between ALSFRS-R score and all measured inflammatory biomarkers. Serum IL-6 demonstrated the strongest inverse correlation with ALSFRS-R score ($r = −0.58$, $p < 0.001$), followed by CRP ($r = −0.52$, $p < 0.001$) and TNF-α ($r = −0.49$, $p < 0.001$).

NLR ($r = −0.45$, $p < 0.001$) and ESR ($r = −0.42$, $p < 0.001$) were also significantly inversely correlated with ALSFRS-R score. Thus, higher inflammatory biomarker concentrations were associated with lower ALSFRS-R scores, indicating greater functional impairment. In contrast, serum albumin showed a significant positive correlation with ALSFRS-R score ($r = 0.36$, $p = 0.001$), suggesting that lower albumin concentrations were associated with greater disease-related functional impairment.

Table III

Correlation between serum inflammatory biomarkers and ALSFRS-R score.

Biomarker	Correlation coefficient (r)	p-value
CRP	−0.52	<0.001
IL-6	−0.58	<0.001
TNF-α	−0.49	<0.001
NLR	−0.45	<0.001
ESR	−0.42	<0.001
Serum albumin	0.36	0.001

Independent association between inflammatory biomarkers and severe disease

The independent association between inflammatory biomarkers and severe MND was assessed using multivariable logistic regression, with severe disease as the outcome. The analysis was adjusted for age, sex, BMI, smoking history, and disease duration. As shown in *Table IV*, higher serum CRP, IL-6, TNF-α, and NLR levels were independently associated with increased odds of severe disease.

For every 1 mg/L increase in serum CRP, the odds of severe disease increased by 31% (adjusted OR [aOR] 1.31, 95% CI 1.09–1.57; $p = 0.004$). Similarly, each 1 pg/mL increase in serum IL-6 was associated with a 28% increase in the odds of severe disease (aOR 1.28, 95% CI 1.10–1.49; $p = 0.002$). TNF-α was also independently associated with severe disease (aOR 1.16, 95% CI 1.04–1.30; $p = 0.008$), while each unit increase in NLR was associated with higher odds of severe disease (aOR 1.52, 95% CI 1.08–2.14; $p = 0.017$).

Disease duration was also independently associated with severe disease (aOR 1.04, 95% CI 1.01–1.07; $p = 0.011$). In contrast, age, male sex, and serum albumin were not independently associated with severe disease in the adjusted model.

Table IV

Multivariable logistic regression analysis of factors associated with severe MND.

Variable	Adjusted OR	95% CI	p-value
Age, per year	1.03	0.99–1.07	0.142
Male sex	1.18	0.46–3.04	0.728
Disease duration, per month	1.04	1.01–1.07	0.011
CRP, per 1 mg/L	1.31	1.09–1.57	0.004
IL-6, per 1 pg/mL	1.28	1.10–1.49	0.002
TNF-α, per 1 pg/mL	1.16	1.04–1.30	0.008
NLR, per unit	1.52	1.08–2.14	0.017
Albumin, per 1 g/dL	0.58	0.29–1.15	0.119

Overall, the findings demonstrated a consistent relationship between systemic inflammatory activity and MND severity. Patients with severe disease had higher concentrations of CRP, IL-6, TNF-α, ESR, and NLR and lower serum albumin levels than patients with milder disease (Table 2). Furthermore, higher inflammatory biomarker concentrations were associated with lower ALSFRS-R scores (Table 3) and remained independently associated with severe disease after adjustment for potential confounding variables (*Table IV*).

DISCUSSION

In patients with motor neuron disease (MND), the current study assessed the correlation between functional severity and systemic inflammatory biomarkers. Serum levels of C-reactive protein (CRP), interleukin-6 (IL-6), tumor necrosis factor-alpha (TNF-α), erythrocyte sedimentation rate (ESR), and the neutrophil-to-lymphocyte ratio (NLR) all significantly increased as functional impairment worsened in the mild, moderate, and severe disease categories. Serum albumin showed an adverse connection, sharply declining as the severity of the illness increased. Additionally, even after controlling for clinical and demographic factors such age, sex, body mass index, and length of disease, multivariable analysis verified that elevated levels of CRP, IL-6, TNF-α, and NLR remained independently related with severe MND.

These results are in strong agreement with international research highlighting the part systemic neuroinflammation plays in the development of ALS and MND. ALS patients had considerably higher peripheral blood concentrations of TNF-α and IL-6 than healthy controls, according to a thorough meta-analysis by Hu et al. [9]. Longitudinal cohort data from the UK revealed IL-6 as a continually increased cytokine that closely tracks with CRP and illness progression, which is consistent with our finding that IL-6 strongly corresponds with functional decline [10]. In a similar vein, our cohort's NLR prognostic utility is consistent with reliable global data. Higher NLR is linked to lower pulmonary function, faster functional decline, and shorter life, according to population-based research from France and Italy [11, 12].

Additionally, a large Chinese cohort showed that a higher rate of ALSFRS-R decrease is independently predicted by systemic immune-inflammation indices, especially the NLR [2]. Our results are consistent with research conducted in northern India that demonstrated a positive correlation between the duration of the disease and higher blood TNF-α [14]. When contrasting our results with particular findings in different cohorts, significant differences become apparent despite these general commonalities. Although our univariate analysis revealed a significant inverse relationship between serum albumin and ALSFRS-R scores, our adjusted multivariable model did not show an independent association between albumin and severe disease [5]. In contrast, decreased albumin at diagnosis independently predicted shorter life in a large population-based Italian registry, indicating systemic inflammatory activity [13].

Additionally, an Indian study found that circulating levels of some neuroinflammatory markers, like monocyte chemoattractant protein-1, were surprisingly lower in ALS patients than in controls, indicating that not all inflammatory proteins circulate in the same direction, despite the fact that our study shows a uniform rise of conventional pro-inflammatory markers with severity of the disease [15]. Lastly, rather than thorough inflammatory profiles, previous published research from Bangladesh has mostly concentrated on metabolic and nutritional markers, such as ferritin, uric acid, and homocysteine [16]. Thus, by demonstrating that traditional, inexpensive inflammatory indicators independently correlate with MND severity in a South Asian context, our work makes a significant contribution to the regional literature.

CONCLUSION

In conclusion, this study shows a strong correlation between increased functional severity in patients with motor neuron disease and elevated systemic inflammatory biomarkers, particularly C-reactive protein, interleukin-6, tumor necrosis factor-alpha, erythrocyte sedimentation rate, and the neutrophil-to-lymphocyte ratio (NLR). Importantly, even after controlling for important clinical and demographic confounding variables including disease duration, elevated levels of CRP, IL-6, TNF-α, and NLR were revealed to be independent predictors of severe illness. Serum albumin did not maintain its independent predictive significance in the multivariable model, despite its initial inverse correlation with disease severity. These results demonstrate the potential of using these readily available, inexpensive peripheral blood markers for disease severity monitoring, patient stratification, and prognostication within the South Asian population and support the important role of systemic immune activation in the progression of MND.

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