

Diagnostic Value of Serum Humanin-Like 3 Levels in Multiple Sclerosis Patients with Predominant Optic Nerve and Spinal Cord Involvement: A Preliminary Study

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ABSTRACT

Introduction: Our aim was to determine whether serum levels of humanin-like 3 (encoded by the MTRNR2L3 gene) may serve as a diagnostic biomarker for differentiation of NMOSD from relapsing remitting multiple sclerosis (RRMS) presenting with clinical features reminiscent of NMOSD.

Methods: Humanin-like 3 levels were measured by ELISA in sera of 30 RRMS patients, 10 NMOSD patients, 15 RRMS patients presenting predominantly with spinal cord and optic nerve attacks (MS-SCON) and 23 healthy controls.

Results: MS-SCON patients showed significantly higher humanin-like 3 levels than other groups. Receiver operating characteristic (ROC) curve

analysis showed that 5.26 ng/ml cut-off value of humanin-like 3 level discriminated MS-SCON from NMOSD by 66.7% sensitivity and 90% specificity. Humanin-like 3 levels did not correlate with demographic and clinical variables of MS and NMOSD.

Conclusion: Serum humanin-like 3 level might potentially be used as a biomarker in differential diagnosis of MS patients presenting with NMOSD-like features. Elevated humanin-like 3 levels of MS-SCON patients might be an indicator of increased stress on neuronal survival in this MS subgroup.

Keywords: Autoimmunity, CSF-1, humanin-like 3, multiple sclerosis, myelitis

Cite this article as: Küçükali Cİ, Sancar N, Yüceer Korkmaz H, Gündüz T, Kürtüncü M, Tüzün E. Diagnostic Value of Serum Humanin-Like 3 Levels in Multiple Sclerosis Patients with Predominant Optic Nerve and Spinal Cord Involvement: A Preliminary Study. Arch Neuropsychiatry 2026;63:1–245. doi: 10.29399/npa.29115

INTRODUCTION

Multiple sclerosis (MS) and neuromyelitis optica spectrum disorder (NMOSD) are both chronic autoimmune demyelinating disorders of the central nervous system (1). The core clinical features of NMOSD are long extensive spinal cord and optic nerve involvement. NMOSD is distinguished from other autoimmune demyelinating disorders by selective loss of astrocytes and subsequent demyelination mostly caused by anti-aquaporin-4 (Aqp-4) antibodies (2). Although most MS patients typically present with ovoid lesions located in periventricular white matter, cerebellum and brainstem, some may predominantly exhibit optic nerve and spinal cord involvement. These patients often have short spinal cord lesions, fulfill the clinical MS criteria and present with clinical, neuropsychological and immunological features that are similar to those of MS patients (3). Since a fraction of NMOSD patients may also exhibit short spinal cord lesions and periventricular white matter involvement, MS patients presenting with predominant optic nerve and spinal cord involvement (MS-SCON) may confound the differential diagnosis process, which prompts investigation of novel biomarkers for diagnosis of MS-SCON.

Highlights

- Humanin-like 3 is higher in MS-SCON patients than other MS or NMOSD cases.
- A 5.26 ng/ml cut-off value distinguishes MS-SCON from NMOSD with high specificity.
- Elevated levels may reflect neuronal stress and act as a diagnostic biomarker.

In our previous studies, we identified increased B cell expression levels of CCL4L2 and CCR4 and anti-astrocytic antibodies as distinguishing immunological features between MS-SCON and NMOSD. We also found significantly higher peripheral blood ratios of regulatory B cell and plasmablast subsets in MS-SCON patients (4).

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Received: 21.04.2025, **Accepted:** 28.05.2025, **Available Online Date:** 19.09.2025

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In a continued effort to describe additional biomarkers that might prove useful in discrimination of MS-SCON and NMOSD, in this study, we measured humanin-like 3 (encoded by the MTRNR2L3 gene) levels in sera of a group of patients with autoimmune demyelinating disorders. Humanin-like 3 belongs to a family of mitochondrial derived peptides involved in metabolism and cell survival (5).

We have recently shown reduced humanin-like 3 levels in sera of patients with Parkinson disease and restless legs syndrome and correlation between reduced humanin-like 3 levels and sleep dysfunction parameters, putatively emphasizing the neuroprotective role of this mediator (6). Recently, some members of the humanin-like peptide family has been offered as prognostic biomarker for MS (7). In this study, we aimed to investigate the possible utility of humanin-like 3 as a biomarker for the differentiation of NMOSD from MS presenting with clinical features reminiscent of NMOSD.

METHODS

Participants

This study includes 15 relapsing remitting MS (RRMS) patients with MS-SCON, that was determined as per previously described criteria (4, 8). Briefly, MS-SCON patients satisfied the revised McDonald's 2017 criteria for clinically definite MS (9), presented with at least one optic neuritis and one myelitis attack, displayed at least four attacks during the disease course, did not experience any long extensive transverse myelitis attacks, were negative for Aqp-4 and myelin oligodendrocyte glycoprotein (MOG) antibodies and did not satisfy the NMOSD criteria (10).

Age-gender matched control groups included 30 RRMS patients fulfilling the McDonald's 2017 criteria for clinically definite MS, 10 NMOSD patients (all positive for Aqp-4 and negative for MOG antibodies) fulfilling the relevant criteria (9, 10) and 23 healthy individuals (Table 1). RRMS patients also had a medical history of at least 4 attacks, had not experienced optic neuritis or myelitis attacks and were negative for Aqp-4 and MOG antibodies.

All included patients were in remission and none of the patients were under treatment with immunosuppressive or immunomodulation medications. The study was approved by the local ethics committee and written informed consent was obtained from all participants.

ELISA

Serum humanin-like 3 (Cusabio, China) levels were measured with ELISA kits according to the manufacturer's instructions and results were expressed as ng/ml.

Statistical analysis

Multiple-group comparisons were done by Kruskal-Wallis and post-hoc Dunn test. Correlation studies were conducted with Pearson or Spearman correlation tests, as required. Sensitivity and specificity for MS-SCON

diagnosis were calculated using receiver operating characteristic (ROC) curve analysis and cut-offs were selected using the Youden index. $p < 0.05$ was considered as statistically significant.

RESULTS

MS-SCON patients display significantly increased serum humanin-like 3 levels

MS-SCON patients showed trends towards higher serum humanin-like 3 levels than other groups ($p=0.012$). In pair-wise comparisons, the difference between MS-SCON and NMOSD patients attained statistical significance ($p<0.01$). None of the remaining pair-wise comparisons for humanin-like 3 levels were significant (Figure 1). Humanin-like levels were comparable among male and female MS-SCON patients ($p=0.594$) and MS-SCON patients with and without oligoclonal bands ($p=0.571$). Correlation analysis between humanin-like 3 levels versus age, EDSS, disease duration and age at onset of disease did not yield significant results ($p=0.199-0.820$; $R=0.004-0.124$) in MS-SCON patients, as well as the remaining study groups (not shown).

Potential diagnostic biomarker value of humanin-like 3

To assess the diagnostic sensitivity, specificity and the optimal cut-off values of serum humanin-like 3 levels that could be used in discrimination of MS-SCON and NMOSD, a ROC curve was constructed and the Youden index was calculated (Figure 2). The best area under curve value was obtained by serum humanin-like 3 level measurements at a cut-off value of 5.26 ng/ml. At this cut-off level, the diagnostic sensitivity of humanin-like 3 was 66.7%, specificity was 90% and area under curve was 0.873 ($p=0.002$, Table 2).

CONCLUSION

In this study, we found significantly increased serum levels of humanin-like 3 in MS-SCON patients. The difference between MS-SCON and NMOSD patients was more significant than other disease and healthy control groups suggesting that humanin-like 3 might be used in differential diagnosis of MS-SCON and NMOSD.

Humanin-like peptides have been studied only in a few autoimmune disorders such as type 1 diabetes mellitus (11). There are only a few studies on the significance and involvement of humanin-like peptides in MS and, to our knowledge, our study is the first to investigate humanin-like 3 levels in autoimmune demyelinating disorders.

Mitochondrial-derived peptides are encoded in the mitochondrial DNA and various mitochondrial microproteins, including humanin, mitochondrial open reading frame of the 12S rRNA type-c and humanin-like peptides have been identified, so far. These peptides are crucially involved in a variety of biological functions such as cell survival, anti-cancer defense, inflammation and metabolism by regulating mitochondrial function (11). Humanin-like peptides have also been shown to exhibit

Table 1. Clinical and demographic features of patients with MS-SCON

| | MS-SCON (n=15) | RRMS (n=30) | NMOSD (n=10) | HC (n=23) |
|----------------------------------|-------------------|----------------|-----------------|----------------|
| Age (\pm SD) | 34.0 \pm 9.4 | 33.5 \pm 8.6 | 35.7 \pm 7.1 | 32.7 \pm 8.1 |
| Gender (women/men) | 10 / 5 | 21 / 9 | 7 / 3 | 15 / 8 |
| Age at disease onset (\pm SD) | 24.3 \pm 7.4 | 25.4 \pm 7.2 | 24.2 \pm 9.1 | NA |
| Disease duration (\pm SD) | 10.2 \pm 7.1 | 8.0 \pm 6.4 | 11.2 \pm 6.8 | NA |
| EDSS (\pm SD) | 2.6 \pm 1.7 | 2.2 \pm 1.4 | 2.9 \pm 1.8 | NA |
| OCB (positive/negative) | 11 / 4 | 22 / 8 | 2 / 8 | NA |

SD: standard deviation; EDSS: expanded disability status scale; OCB: oligoclonal bands; HC: healthy controls; NA: not applicable.

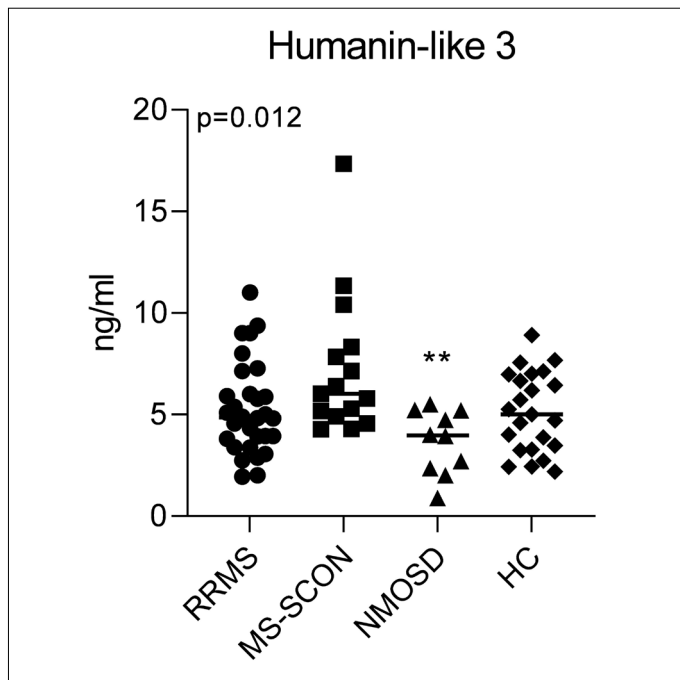


Figure 1. Serum levels of CSF-1 and humanin-like 3 in patients with MS-SCON, RRMS, NMOSD and healthy controls (HC). Horizontal lines indicate mean values. p values depicted on the upper left corner of the panels were obtained by Kruskal-Wallis test. ** $p < 0.01$ by Dunn's post-hoc test.

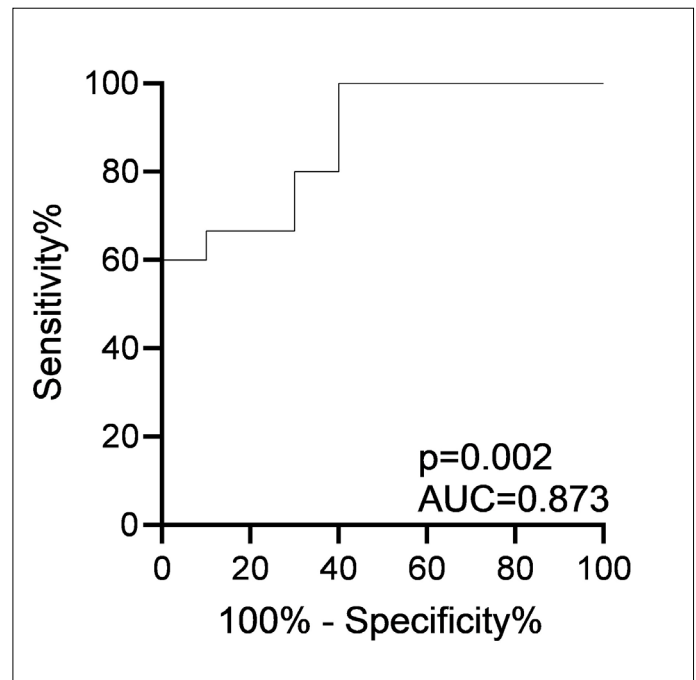


Figure 2. Receiver operation characteristics (ROC) curves for humanin-like 3 concentrations in sera of patients with MS-SCON and NMOSD, where the binary outcome is defined as MS-SCON vs. NMOSD.

Table 2. Sensitivity and specificity values of serum humanin-like 3 levels for the cut-off value proposed for discrimination of MS-SCON and NMOSD patients.

| | Cut-off value | Sensitivity % | 95% CI | Specificity % | 95% CI | LR | Youden index* | AUC, p value |
|------------------------------|---------------|---------------|----------------|---------------|----------------|-----|---------------|----------------|
| Serum humanin-like 3 (ng/ml) | > 5.26 | 66.7 | 41.7% to 84.8% | 90.0 | 59.6% to 99.5% | 6.7 | 56.7 | 0.873 0.002 |

CI: Confidence interval; LR: Likelihood ratio; AUC: Area under curve.

*Youden index was calculated by adding the sensitivity and specificity values and then subtracting 100 from that value.

neuroprotective properties via prevention of mitochondrial loss and to maintain synaptic integrity and neurotransmitter modulation (12).

Furthermore, humanin-like peptides have also been shown to attenuate disease pathology in diabetes, neurodegenerative disorders, cardiovascular diseases, cancer and macular degeneration (11, 12, 13). Humanin-like 3 has recently been shown to ameliorate gentamicin-induced oxidative stress and inflammatory gene overexpression, thereby conferring protection to hair cells involved in sensorineural hearing (14). Humanin-like 2 has been shown to demonstrate neuroprotective effects in models of macular degeneration, Parkinson and Alzheimer disease through regulation of mitochondrial and autophagic activity and activation of the anti-apoptotic signaling cascades (12).

The pathophysiology of MS comprises complex interactions of factors of inflammation, neurodegeneration and oxidative stress (15, 16). Humanin-like peptides are intimately involved in all these functions thus projecting as appealing targets of theranostic applications in MS. As a matter of fact, in a recent whole-transcriptome analysis study conducted on the peripheral blood mononuclear cells of MS patients, reduced humanin-like 8 expression levels have been associated with the presence of myelin lipid-specific oligoclonal IgM bands in the cerebrospinal fluid and increased clinical disease activity (7). Notably, humanin-like 8 is known to show neuroprotective and anti-apoptotic actions and is increased in brain specimens of patients with major depressive disorder (17).

Thus, our finding of altered serum humanin-like 3 levels in a subset of MS patients is in alignment with previous reports. Given the significant involvement of humanin-like peptides in inflammation, oxidative stress, apoptosis and neuroprotection, it is tempting to speculate that humanin-like 3 expression is increased in MS-SCON patients as a protective compensation measure to bring under control the ongoing neurodegeneration, a well-known component of MS pathophysiology. As a matter of fact, while mostly displaying spinal cord lesions in their clinical course, MS-SCON patients may show significant cognitive impairment, suggesting that disease activity proceeds in seemingly attack-free regions of the MS-SCON brain thus enhancing the overall disability (3). Our preliminary findings may also suggest that neurodegenerative mechanisms are more pronounced in patients with MS-SCON than patients with other autoimmune demyelinating disorders. Yet again, lack of significant correlation between EDSS and humanin-like 3 levels may argue against this speculation. Therefore, this assertion noticeably needs to be confirmed by studies conducted on larger MS cohorts.

In brief, our preliminary study provides some proof-of-concept evidence suggesting that humanin-like peptides may be involved in disease mechanisms of MS and may act as useful biomarkers of disease activity and differential diagnosis in MS patients. Our results also imply that humanin-like peptides are intimately involved in autoimmune disease mechanisms and the complex interplay between immune system functions and humanin-like peptides need to be studied in greater detail.

Ethics Committee Approval: Ethical approval was received from the Koç University Faculty of Medicine Clinical Research Ethics Committee on May 30, 2016. (Approval No: 2016.123.IRB2.077).

Informed Consent: Informed consents were obtained from patients to examine their blood samples for research purposes.

Peer-review: Externally peer-reviewed.

Author Contributions: Concept- ET, ÇİK; Design- ET, ÇİK; Supervision- ÇİK, HYK; Resource- ET, ÇİK; Materials- MK, TG; Data Collection and/or Processing- MK, TG, HYK, NS; Analysis and/or Interpretation- ET, NS; Literature Search- ET, HYK; Writing- ET, NS; Critical Reviews- ET, ÇİK.

Conflict of Interest: The authors declared that there is no conflict of interest.

Financial Disclosure: There is no funding associated with the work featured in this article.

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