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Exploring the Role of Neurofilament Light Chain and Some Non-coding RNAs in Different Demyelinating Diseases

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ABSTRACT

Background: Multiple sclerosis (MS) and Neuromyelitis Optica (NMO) are demyelinating diseases. Even though they both share many clinical characteristics, their treatments may vary because most MS-modifying medications are not only ineffective for NMO but also have the potential to exacerbate the condition. Thus, it is better to investigate novel biomarkers that could help differentiate between them. **Methods:** Blood samples were collected from all participants; serum was separated for determination of NfL. While total RNA was extracted from the whole blood for cDNA synthesis, qPCR was performed and the expression levels of miR-7 and ciRS-7 were calculated. **Results:** Neurofilament light chain levels (NfL) were significantly elevated only in MS patients and SP patients in comparison to healthy control group ($P < 0.05$ and $P < 0.005$ respectively), and in comparison to NMO group ($P < 0.05$ and $P < 0.005$ respectively), while decreased in NMO group compared to healthy controls group. ROC curve analysis indicated that NfL could distinguish between SP and healthy control (AUC of 0.936 ($p < 0.0001$)), NMO (AUC of 0.93 ($p < 0.0001$)) and RR (AUC of 0.81 ($p < 0.001$)). Also, differentiating NMO from MS by AUC of 0.752 ($p < 0.01$). CiRS-7 achieved significant upregulation in RR compared to control group ($p < 0.05$) and between NMO and RR ($p < 0.05$). ROC curve analysis for ciRS-7 showed AUC of RR and healthy controls ($p < 0.05$, AUC = 0.619) and between NMO and RR ($p < 0.05$, AUC = 0.635). The results showed non-significant correlations between miR-7, ciRS-7 expressions and NfL level. **Conclusion:** The present studies revealed the possibility of using NfL as diagnostic and monitoring tool for MS and suggested that NfL may serve as a more reliable biomarker for distinguishing between MS and NMO and improving diagnostic precision.

Keywords: Multiple sclerosis (MS), Neuromyelitis Optica (NMO), demyelinating diseases, Neurofilament light chain (NfL), ciRS-7, MiR-7

INTRODUCTION

Demyelinating diseases are idiopathic disorders characterized by myelin loss that occurs on a background

of inflammation such as (anti-MOG) syndromes, acute disseminated encephalomyelitis (ADEM) and acute hemorrhagic leukoencephalitis (AHL)^{1, 2}. Multiple sclerosis (MS) is the most significant and widespread

chronic inflammatory demyelinating disease of the central nervous system (CNS), which typically affects individuals between the age of 20–40 years old³. Relapsing remitting (RR), secondary progressive (SP), and primary progressive (PP) are the three main types of MS from which, RR, the most common kind of the disease, where periods of neurological dysfunction (relapses) alternate with times of clinical stability (remissions), most patients eventually progress to SP, where neurological deterioration is persistent. However, PP is unique in that it progresses over time after the disease's onset and represents ~15% of people with MS^{4, 5}. A series of pathobiological events, ranging from focal lymphocytic infiltration and microglia activation to demyelination and axonal degradation, are indicative of the etiology of multiple sclerosis⁶. Diagnosis of MS is based on McDonald's criteria that depend on the clinical characteristics of the attacks, including the patient's medical history and examination results. The principles of dissemination in space (DIS) and dissemination in time (DIT) serve as the foundation for the diagnosis. There isn't just one laboratory test used for MS diagnosis. Based on clinical observations and investigational support as Magnetic resonance imaging (MRI), Cerebrospinal fluid (CSF) & visually evoked potentials (VEPs), the diagnosis is confirmed^{7, 8}. On the other hand, Neuromyelitis Optica (NMO), previously identified as Devic's syndrome, is an autoimmune illness of the central nervous system that was formerly limited to the optic nerve and spinal cord, in order to broaden the definition of NMO and encompass a larger range of clinical symptoms, the term NMO spectrum disorders (NMO-SD) was introduced⁹. The water channel aquaporin-4 (AQP4) is the target antigen of autoimmunity in NMO and antibodies against this antigen cause damage to astrocytes. Myelin and oligodendrocytes are the NMO main targets, but no specific target to multiple sclerosis has been found yet¹⁰. However, in 2004 the discovery of serum aquaporin4-IgG (AQP4-IgG) or (NMO-IgG) which was found almost exclusively in patients with NMO but not in MS patients, facilitate the differentiation between NMO and MS^{11, 12, 13}. Although (AQP4-IgG) is used for NMO diagnosis, some patients still show negative or undetermined antibody. Moreover, AQP4-IgG cannot estimate clinical disease activity¹⁴. Despite NMO and MS clinical similarities, they may differ in treatment, as most MS modifying drugs are not only ineffective in NMO but may cause disease exacerbation. Therefore, it is preferable to explore new biomarkers which may aid in differential diagnosis of both MS and NMO^{15, 16}. The cytoskeletal elements of neurons called neurofilaments are more prevalent in axons, they serve to preserve the axons' size, shape, and caliber in addition to providing structural support. The triplet of neurofilaments classified according to molecular weight—neurofilament light chain (~68 kDa) (NfL),

neurofilament medium chain (~145 kDa) (NfM), and neurofilament heavy chain (~200 kDa) (NfH)¹⁷. Neurofilament light chain (NfL), has become a blood biomarker for neuronal injury across a broad range of neurologic disorders, after injury to both peripheral and central neurons, NFL is released into the blood and cerebrospinal fluid (CSF)¹⁸. There are some non-coding RNAs that affect level of NfL as microRNA -7(miR-7) (the mature miR-7 homology for human called (Homo sapiens; hsa-miR-7) [19]. It was found that miR-7a in mice affects expression of NfL in neuropathic pain²⁰. MicroRNA-7 (miR-7) exhibits limited spatiotemporal expression both throughout development and during maturity. Mature miR-7 is produced by three distinct genes in both humans and mice, demonstrating unexpected redundancy and the significance of this miRNA in controlling essential cellular functions and organ differentiation and development, miR-7 has pathogenic function in diseases of mammals, especially those of the brain, heart, endocrine pancreas, skin and cancer²¹. MicroRNAs are affected by another type of non-coding RNA called circular RNAs (circRNAs). CircRNAs are more stable than linear RNAs(miRNAs) as their closed structures increase their resistance to exonuclease digestion and their accumulation in body fluids and tissues. Disruptions in the expression of circRNAs have been related to many human diseases, including cancers, cardiovascular diseases, immune diseases and demyelinating diseases²². One of circRNAs that affect miR-7 is ciRS-7, it was the first circRNAs identified miRNA sponge, also known as (CDR1as) which was generated from the cerebellum degeneration-related protein 1 antisense transcript (CDR1as), has >70 conserved binding sites for miR-7 and so represents an effective miR-7 sponge that affects miR-7 target gene activity²³. There isn't any study done yet to reveal its role in MS nor NMO.

The aim of present study is to determine the expression level of circular RNA (ciRS-7), its sponge miRNA (miR-7) and concentration level of neurofilament light chain (NfL) in demyelinating diseases (MS and NMO), explore the axis and their role in differential diagnosis between MS subtypes, NMO and also between MS subgroup and evaluate the possible correlation between the axis (miR-7, ciRS-7 and NfL).

MATERIAL AND METHODS

Study participants

The current study comprised 252 participants, were divided into three groups. Group I (Healthy controls) comprised of 70 healthy volunteers (26 males and 44 females, mean age [34.47 ± 1.457 years]), Group II (NMO group) comprised of 38 patients (9 males and 29 females, mean age [36.26 ± 1.843 years]) and Group

III(MS group) comprised of 144 patients (37 males and 107 females) and classified into two subgroups according to disease pattern: (RR subgroup) comprised of 74 patients (12 males and 62 females, mean age $[31.28 \pm 1.085 \text{ years}]$) and (SP subgroup) comprised of 70 subjects (25 males and 45 females, mean age $[38.11 \pm 1.308 \text{ years}]$), all subjects were matched for age, sex, and ethnicity. Healthy controls were recruited from Cairo University Hospitals Blood Bank with MS and NMO patients specifically drawn from El Kasr Al-Ainy Multiple Sclerosis Unit (KAMSU) in Cairo, Egypt. MS Diagnosis was confirmed using the 2017 McDonald's Criteria²⁴, while NMO diagnosis (whether seropositive or seronegative for AQP4-IgG) was confirmed by the International Panel for NMO Diagnosis in 2015²⁵. All subjects were given written informed consent. The study was conducted in accordance with the regulations and recommendations of the Declaration of Helsinki with approval ethical committee from faculty of pharmacy Helwan university under number (Approval No. 01H2022 9/2/2022).

Laboratory measurements

Blood samples were collected from all participants and divided into two aliquots. The first aliquot was placed in plain tubes for serum separation and stored at -80°C for subsequent analysis and determination of serum NfL levels. The second aliquot was placed in 0.5M EDTA containing tubes and stored at -20°C for determination of the expression level of both miR-7 and ciRS-7.

Determination of serum NfL level

The serum levels of NfL were measured using enzyme-linked immunosorbent assay (ELISA) by commercial kits, Human NEFL (Neurofilament, Light Polypeptide) ELISA kit (Catalog No: E-EL-H0741) according to the manufacturer's procedure.

Polymerase chain reaction (PCR)-based analyses

Total RNA was extracted from the blood samples using The GENEzol™ TriRNA Pure Kit (Catalog number: GZX050, GZXD050, provided by Geneaid, Taiwan). The extracted RNA was then utilized for cDNA synthesis using ThermoScientific™ Revertaid™ First strand cDNA synthesis kit (Catalog number: K1622, provided by Thermo Fisher Scientific™, USA).

Real-time qPCR was performed using HERAPLUS SYBR® Green qPCR Kit (WF1030800X Kits). The expression levels of ciRS-7 and miR-7 were calculated as ΔCt method with GAPDH and U6 serving as internal controls for ciRS-7 and miR-7, respectively. The sequences of the primers were used listed in Table (1).

Statistical analysis

Data was analyzed and figures were plotted using GraphPad Prism software version 9.0 (La Jolla, CA, USA). The expression levels of ciRS-7 and miR-7 were quantified using the ΔCt method. The serum levels of NfL were analyzed by ANOVA, followed by Tukey's test for multiple comparisons. The distribution of data was evaluated for normality using the Shapiro-Wilk test. Differences in the expression levels of these biomarkers between Neuromyelitis Optica (NMO) and Multiple Sclerosis (MS) patients were assessed using the unpaired t-test. To evaluate the effect of disease status and other variables on biomarker expression, two-way ANOVA followed by Tukey's post hoc test was employed. Spearman's rank correlation coefficient was used to determine the relationships between the expression levels of ciRS-7, miR-7, and NfL. Receiver Operating Characteristic (ROC) curves were constructed to assess the diagnostic and prognostic potential of these biomarkers. p-value of < 0.05 was considered statistically significant.

Table 1. Sequences of the primers used in qPCR transcriptomics analysis (5' to 3').

RNA	Forward	Reverse
hsa-miR-7	GTTGGCCTAGTTCTGTGTGGA	GGCAGACTGTGATTTGTTGTCG
ciRS-7	CCTGGGCTCCTCGCCTGACC	TCTCTCTGCCCTCAGCCTTGCC
U6	GCTTCGGCAGCACATATACTA	CGAATTTGCGTGTGCATCCTTG
GAPDH	GGAGCGAGATCCCTCCAAAT	GGCTGTTGTCATACTTCTCATGG

Table 2. Demographic and clinical characteristics data of all the subjects studied

Parameters	Healthy Control N=70	MS N=144 (RR=74, SP=70)	NMO N=38	P-value
Age (min-max)	(18 – 70)	(18 - 60)	(18 - 53)	P>0.05
(Mean ± SEM) (Yrs)	(34.5± 1.5)	(35± 0.92)	(36.3± 1.8)	
Sex				P>0.05
Male N (%)	26 (37 %)	37 (26%)	9 (24%)	
Female N (%)	44 (63%)	107 (74%)	29 (76%)	
Disease Onset				
(Min-Max)		(12 - 48)	(16 - 50)	P<0.01
(Mean ± SEM) (Yrs)		(26.04± 0.81)	(31.15± 1.681)	
OCB				P <0.0001
Positive	-	139 (97%)	-	
Negative	-	5 (3%)	29 (76.3%)	
Not done	70 (100%)	-	9 (23.7%)	
Aquaporin- 4				P <0.0001
Positive	-	-	31 (81.5%)	
Negative	-	44 (30.6%)	7 (18.5%)	
Not done	70 (100%)	100(69.4%)	-	
EDSS (Mean ± SEM)	Not done	4.5 ± 0.18	4.6 ± 0.34	P>0.05

SEM: Standard Error of Mean, MS: Multiple Sclerosis, NMO: Neuromyelitis Optica, N: Number, OCB: Oligoclonal Bands. EDSS: Expanded Disability Status Scale. Numerical data were done by independent t tests. Categorical data were compared by Chi square test (χ^2) P values <0.05 are considered significant

RESULTS

The demographic and clinical characteristics data of the studied groups

The results of the demographic and clinical characteristics data between the different studied groups showed that there was no significant difference between the studied groups regarding age ($P>0.05$) and sex ($P>0.05$), the disease onset in neuromyelitis Optica (NMO) (31.15 ± 1.681) was significantly higher than multiple sclerosis (MS) (26.04 ± 0.81) with ($P<0.01$). Oligoclonal Bands (OCB) showed significant difference between diseased groups (NMO (29 (76.3%) negative and 9 (23.7%) not done) and MS (139 (97%) positive and 5 (3%) negative)) with ($P <0.0001$). Aquaporin- 4 revealed a significant difference between diseased groups (NMO (31 (81.5%) positive and 7 (18.5%) negative) and MS (44 (30.6%) negative and 100(69.4%) not done)) with ($P <0.0001$). Expanded Disability Status Scale (EDSS) was found to be non-significant between

diseased groups (NMO (4.6 ± 0.34) and MS (4.5 ± 0.18)) with ($P>0.05$), listed in table (2).

Serum neurofilament light chain (NfL) Levels in the different studied groups

The present study revealed that serum NfL levels were increased across MS patient (10.77 ± 5.933) and its subgroups (RR (8.303 ± 5.284) and SP (13.51 ± 5.504)) compared to healthy controls (6.392 ± 1.433) and not found any change in NMO patient group (6.300 ± 2.196) than control group. It showed significant increase only in MS patients and SP patients in

comparison to healthy control group ($P<0.05$ and $P<0.005$ respectively, as shown in (Figure 1.A), and also significant elevation in serum NfL levels in MS patients and SP patients in comparison to NMO group ($P<0.05$ and $P<0.005$ respectively, as shown in (Figure 1.B). Also, it showed significant increase in serum NfL levels in SP patients in comparison to RR group ($P<0.05$) (Figure 1.C). To assess the potential of serum NfL as a biochemical biomarker, Receiver operating curve (ROC) curve analysis was performed, as illustrated in Figure. 2, studying area under the curve (AUC) demonstrated serum NfL's robust ability to distinguish both SP and MS from healthy with (AUC) values of 0.94 ($p < 0.0001$) and 0.73 ($p < 0.05$), respectively (Figures. 2C–2D). Additionally, NfL showed potential effect in differentiating NMO from MS, as evidenced by an AUC of 0.752 ($p < 0.01$, Figure 2.G), aids in differentiating NMO from SP, as evidenced by an AUC was 0.93 ($p < 0.0001$, Figure 2.F) and could differentiate RR from SP as AUC was 0.81 ($p < 0.001$, Figure 2.H).

Expression rate of miR-7 in the different studied groups

MiR-7 is known to target NfL in neuropathic pain in mice [20]. To address the role of miR-7 and its potential as a differential marker between NMO and MS, the expression of miR-7 was quantified across various groups: MS, NMO, RR, SP, and healthy controls counterpart.

Unfortunately, the present study showed no statistical differences in plasma miR-7 expression levels in NMO (5.330 ± 1.838), RR (5.426 ± 2.280), SP (5.555

± 1.631), MS (5.533 ± 1.964) patients and control group (5.711 ± 2.637), $p > 0.05$, Figure 3)

To evaluate the diagnostic and prognostic potential of miR-7, ROC curve analysis was performed. As shown in Figure 4, miR-7 can't differentiate between healthy controls and NMO ($p > 0.05$, AUC = 0.53, Figure 4.A), RR ($p > 0.05$, AUC = 0.56, Figure 4.B), SP ($p > 0.05$, AUC = 0.55, Figure 4.C) and MS ($p > 0.05$, AUC = 0.56, Figure 4.D), and also between MS and NMO by AUC=0.54 ($p > 0.05$, figure 4G). Additionally, comparisons between RR and SP ($p > 0.05$, AUC = 0.51, figure 4H) were calculated.

Expression rate of ciRS-7 in different groups studied

CiRS-7, which is known to act as a sponge for miR-7 and subsequently promote NfL expression, was assessed across MS, NMO, RR, SP, and healthy control groups counterpart to evaluate its role to differentiate between MS and NMO. The current study showed elevation in Plasma ciRS-7 expression levels in NMO (4.518 ± 2.342), RR (5.215 ± 1.986), SP (4.627 ± 1.713) and MS (4.849 ± 1.908) patients than healthy control group (4.316 ± 1.915), without revealing any significant differences ($p > 0.05$, figures 5A,5C,5D), but achieved significant upregulation only with RR compared to control group ($p < 0.05$, figure 5B). Additionally, comparisons between SP and NMO did not yield significant results ($p > 0.05$, figure 5F), but achieved significant upregulation with RR compared to NMO ($p < 0.05$, figure 5E).

ROC for ciRS-7 analysis was performed and showed no significance in all studied groups; NMO ($p > 0.05$, AUC = 0.5115, figure 6 A), SP ($p > 0.05$, AUC = 0.548, figure 6 C) and MS ($p > 0.05$, AUC = 0.569, figure 6 D) compared to healthy control group. Also, between NMO and MS ($p > 0.05$, AUC = 0.511, figure 6 G), but AUC of RR showed significant difference compared to healthy controls ($p < 0.05$, AUC = 0.619 figure 6 B). Also, ciRS-7 could differentiate between NMO and RR with AUC = 0.635 ($p < 0.05$, figure 6 E). But it could not differentiate between NMO and SP with AUC = 0.54 ($p > 0.05$, figure 6 F) and MS with AUC = 0.57 ($p > 0.05$, figure 6 G). ROC analysis for ciRS-7 showed AUC between RR and SP was 0.58 ($p > 0.05$, figure 6 H).

Correlation between expression rate of miR-7, ciRS-7 and NfL level

The Spearman correlation analysis was performed to assess the relationship between miR-7, ciRS-7 and NfL expressions. The results showed non-significant correlations between miR-7 and ciRS-7 expression rate ($r = -0.2052$, $p > 0.05$, Figure 7 A), also revealed non-significant correlations between serum NfL levels and miR-7 expression rate ($r = -0.22$, $p > 0.05$, Figure 7 B) and between serum NfL levels and ciRS-7 expression rate ($r = 0.017$, $p > 0.05$, Figure 7 C).

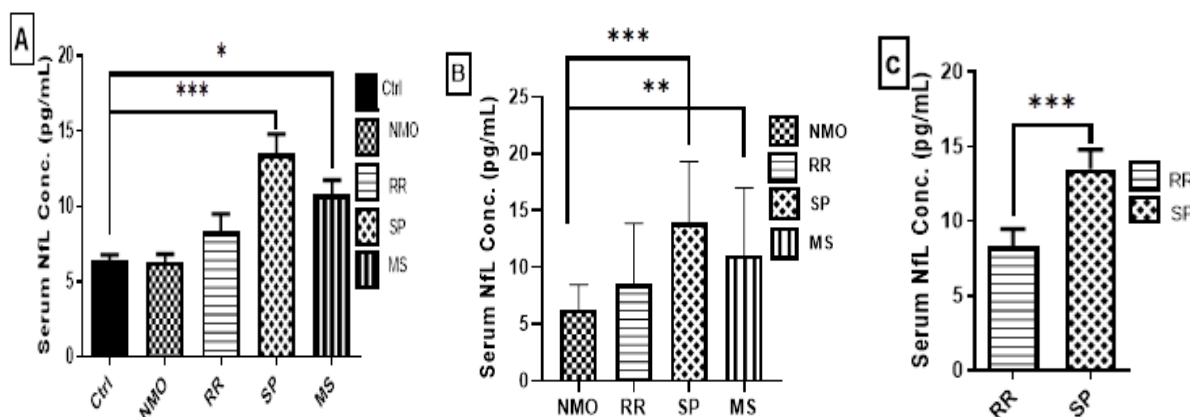


Figure 1. Analysis of serum NfL Concentrations in Different Studied Groups. (A) Serum NfL levels in different groups (Ctrl, RR, SP, MS, NMO). (Ctrl, RR, SP, MS, NMO). **(B)** Serum NfL concentrations Comparison of each studied group with NMO group (RR, SP, MS, NMO). **(C)** Comparison of Serum NfL levels between Relapse Remitting Multiple Sclerosis (RR) and Secondary Progressive Multiple Sclerosis (SP). Statistical significance is indicated as follows: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.005$. All values are presented as mean $\pm$ SEM.

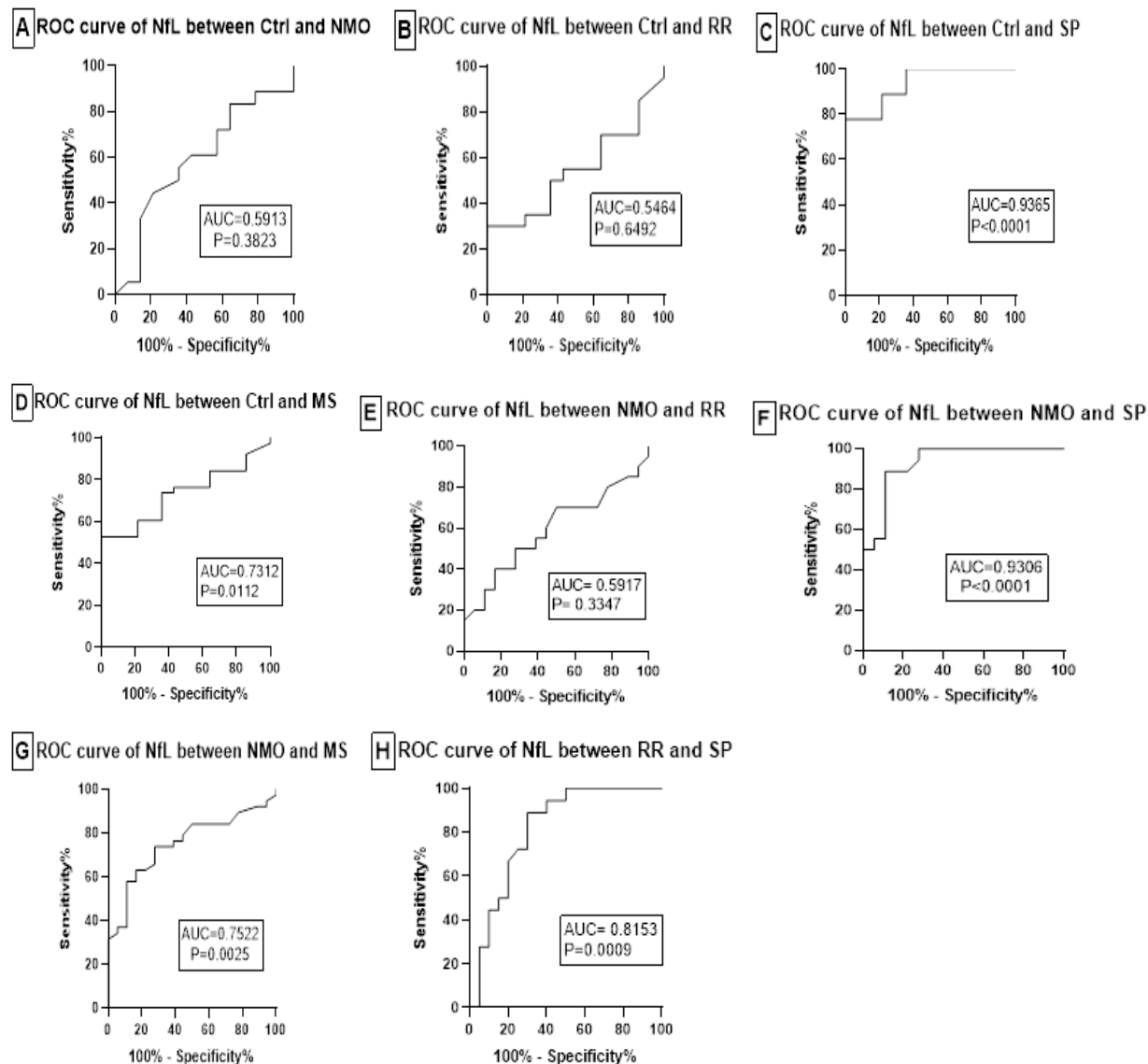


Figure 2. Analysis of NfL receiver operating characteristic (ROC) curves for NfL Expression levels in different studied groups. (A) ROC curve for Ctrl vs. Neuromyelitis Optica (NMO), (B) ROC curve for Ctrl vs. Multiple Sclerosis (MS), (C) ROC curve for Ctrl vs. Relapse Remitting Multiple Sclerosis (RR), (D) ROC curve for Ctrl vs. Secondary Progressive Multiple Sclerosis (SP), (E) ROC curve for Neuromyelitis Optica (NMO) vs. Multiple Sclerosis (MS), (F) ROC curve for Neuromyelitis Optica (NMO) vs. Relapse Remitting Multiple Sclerosis (RR), (G) ROC curve for Neuromyelitis Optica (NMO) vs. Secondary Progressive Multiple Sclerosis (SP), (H) ROC curve for Relapse Remitting Multiple Sclerosis (RR) vs. Secondary Progressive Multiple Sclerosis (SP). The area under the curve (AUC) values for each comparison are displayed above the respective curves, indicating the diagnostic accuracy of NfL expression for each condition. AUC values represent the ability of NfL to distinguish between the specified groups, with higher AUC values indicating better diagnostic performance. Statistical significance is indicated as follows: $p < 0.05$.

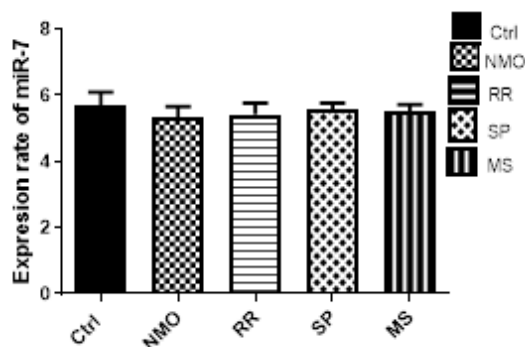


Figure 3. Analysis of ΔCT of miR-7 in Different Studied Groups. Expression rate of miR-7 in different groups (Ctrl: Control, NMO: Neuromyelitis Optica, RR: Relapsing-Remitting, SP: Secondary Progressive, MS: Multiple Sclerosis), All values are presented as mean $\pm$ SEM.

DISCUSSION

MS and NMO are demyelinating diseases of the CNS that have long been in the news, with a wide range of clinical manifestations and caused by a peripheral autoimmune attack. Immune cells interacting with CNS antigens cause neuroinflammation and then destruction of myelin sheath²⁶.

The current study aimed to study the role of Neurofilaments light chain (NfL), miR-7 and ciRS-7 in differentiation between MS, NMO and subgroups of MS (RR and SP). Neurofilaments light chain considered the cytoskeletal elements of neurons and its levels indicate neural damage in different neural conditions, that have demyelinating destruction²⁷.

The present study revealed that serum NfL levels were increased across MS patient groups (RR, SP, and MS) compared to healthy controls and showing significant increase in MS patients and SP patients in comparison to both healthy control and NMO group. While its level in NMO patient group didn't reveal any difference compared to healthy control.

Current finding is concomitant with kuhle et al who demonstrated that the CSF and serum NfL concentration is increased in RR than control, it tend to be higher in patients with relapsing-remitting MS (RR) with a recent relapse than in patients with clinically stable RR^{28, 29}, meaning that level of serum NfL depends on activity of disease and which was proved by highly significant upregulation with SP subgroup, so it can be used as prognostic or monitoring tool for these types of disorders (particularly for differentiating between RR and SP.). Also, Disanto et al showed that the CSF and serum NfL concentration is increased in MS than control³⁰.

Some studies were done on serum samples revealed high NfL level in patients with Alzheimer Disease (AD) than in health controls³¹, increased NfL levels in Parkinson's disease (PD) patients compared with healthy controls and NfL was significantly elevated in patients in advanced stage compared to patients in the early stages³².

In our current study, the results revealed significant lowering of serum NfL concentrations in NMO patients compared to MS patients, suggesting that NfL may serve as a more reliable biomarker for distinguishing between these conditions and improving diagnostic precision. This finding contradicts with previous study that showed that CSF NfL concentration was higher in NMO than in MS³³. This conflict may be due to the difference in type of sample used, activity of disease or sample size.

miR-7 has been shown to target NfL in neuropathic pain in mice [20]. Also has role in CNS disorders such as PD³⁴ and schizophrenia³⁵.

In the present study, miR-7 showed no statistical differences between healthy controls and NMO or MS subtypes. Some studies revealed miR-7 downregulated in NMO patients³⁶, and other neurodegenerative illnesses, including Parkinson's disease, schizophrenia, Alzheimer's disease, and multiple sclerosis (MS) and have been linked to brain tissue inflammation^{34, 35, 37, 38}, these findings disagree with Matar et al. who showed that miR-7 was upregulated in Lebanese RRMS patients in comparison to controls, this difference in results may due to use circulating exosomal miRNAs (differ from serum sample)³⁹.

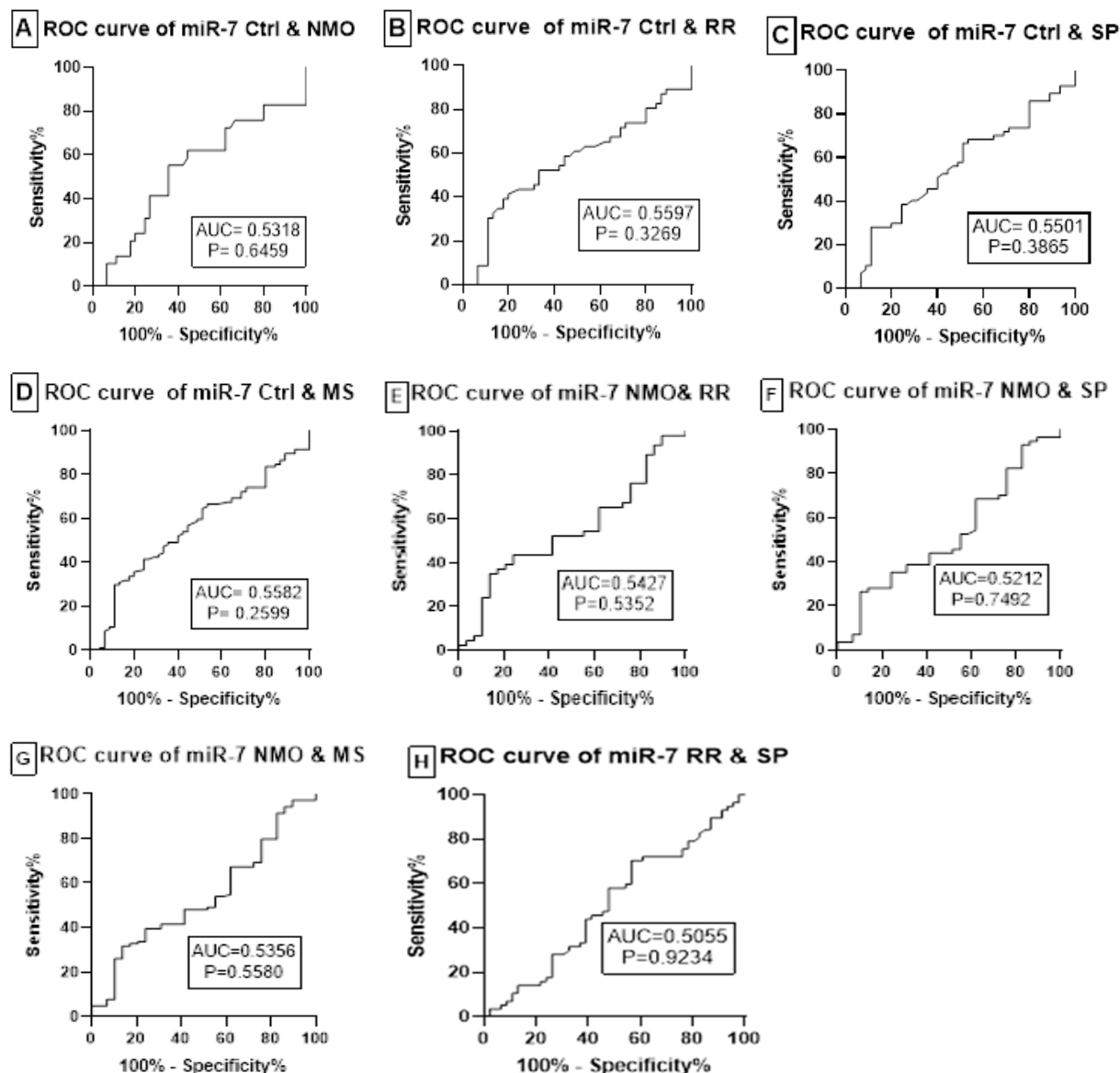


Figure 4. Analysis of Receiver Operating Characteristic (ROC) Curves for miR-7 Expression rate, in Different Studied Groups. (A) ROC curve for Ctrl vs. Neuromyelitis Optica (NMO), (B) ROC curve for Ctrl vs. Multiple Sclerosis (MS), (C) ROC curve for Ctrl vs. Relapse Remitting Multiple Sclerosis (RR), (D) ROC curve for Ctrl vs. Secondary Progressive Multiple Sclerosis (SP), (E) ROC curve for Neuromyelitis Optica (NMO) vs. Multiple Sclerosis (MS), (F) ROC curve for Neuromyelitis Optica (NMO) vs. Relapse Remitting Multiple Sclerosis (RR), (G) ROC curve for Neuromyelitis Optica (NMO) vs. Secondary Progressive Multiple Sclerosis (SP), (H) ROC curve for Relapse Remitting Multiple Sclerosis (RR) vs. Secondary Progressive Multiple Sclerosis (SP). The area under the curve (AUC) values for each comparison are displayed above the respective curves, indicating the diagnostic accuracy of miR-7 expression rate for each condition. AUC values represent the ability to distinguish between the specified groups, with higher AUC values indicating better diagnostic performance. Statistical significance is indicated as $p < 0.05$.

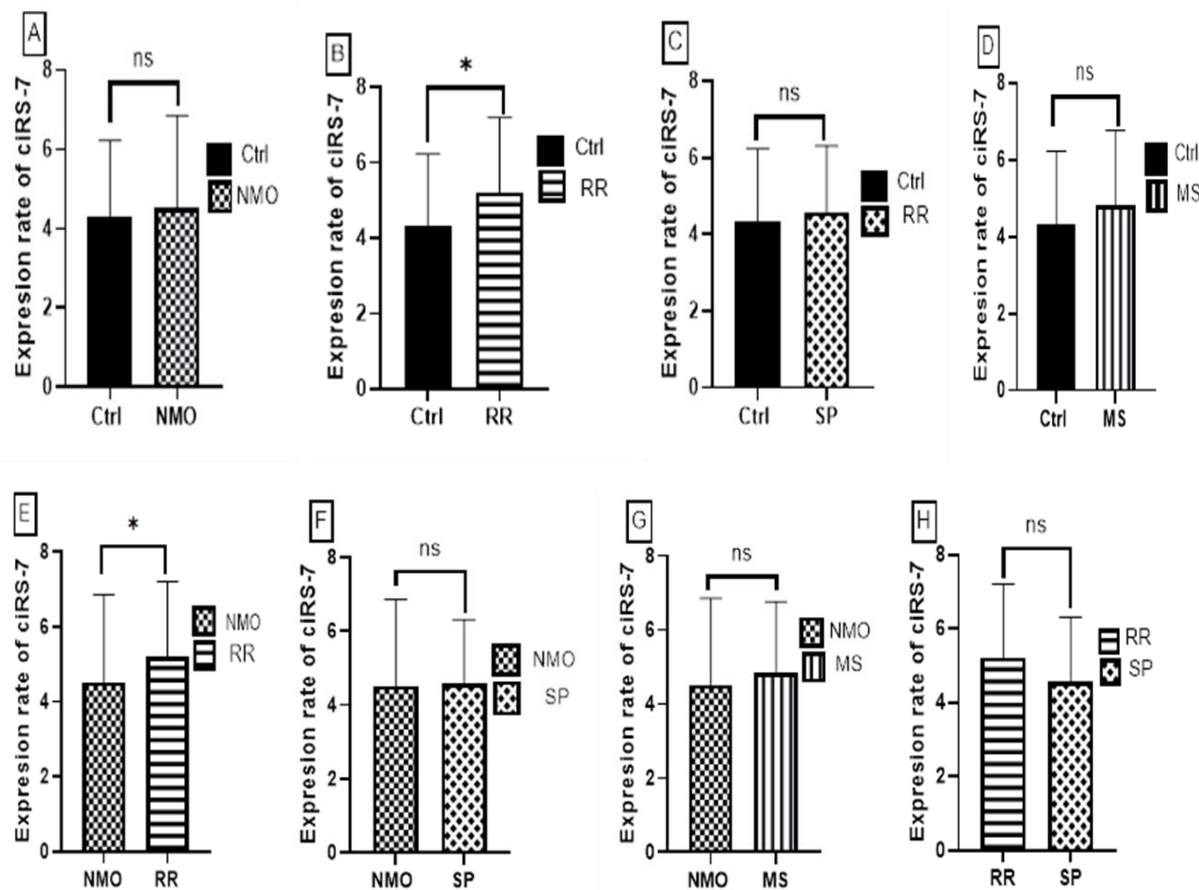


Figure 5. Analysis of Δ^{CT} of ciRS-7 Expression rate in Different Studied Groups. (A) Comparison of Expression rate of ciRS-7 between Control(Ctrl) and Neuromyelitis Optica (NMO), (B) Comparison of Expression rate of ciRS-7 between Ctrl and Relapse Remitting Multiple Sclerosis (RR), (C) Comparison of Expression rate of ciRS-7 between Ctrl and Secondary Progressive Multiple Sclerosis (SP), (D) Comparison of Expression rate of ciRS-7 between Ctrl and Multiple Sclerosis (MS), (E) Comparison of Expression rate of ciRS-7 between NMO and Relapse Remitting Multiple Sclerosis (RR), (F) Comparison of Expression rate of ciRS-7 between NMO and Secondary Progressive Multiple Sclerosis (SP), (G) Comparison of Expression rate of ciRS-7 between Neuromyelitis Optica (NMO) and Multiple Sclerosis (MS), (H) Comparison of Expression rate of ciRS-7 between Relapse Remitting Multiple Sclerosis (RR) and Secondary Progressive Multiple Sclerosis (SP), Unpaired t test is used. Statistical significance is indicated as follows: * $p < 0.05$. All values are presented as mean $\pm$ SEM.

In present study, ciRS-7 showed upregulation in NMO, SP subtype and MS groups with no significant value in comparison to healthy controls, but the significant upregulation observed in the RR group compared to both healthy controls and NMO. It notably demonstrated the ability to differentiate RR from both healthy controls and NMO. The significant upregulation observed in the RR group suggests its potential as a differential diagnostic marker for RR and differentiated from NMO. However, ciRS-7 did not exhibit prognostic utility for predicting progression to SPMS in MS patients.

The present study is considered the first study to detect the role of ciRS-7 in both MS and NMO. Satoh et al. studied ciRS-7 on prion diseases and showed that ciRS-7 expression level is upregulated and so may play role in the pathogenesis of prion diseases. Hypothetically, given the putative roles of ciRS-7 in gene regulation, its upregulation in prion diseases may be associated with changes in protein folding and processing involved in pathogenesis of the diseases^{40, 41}, while Liu et al. showed that ciRS-7 was downregulated in Alzheimer's Disease(another demyelinating disease) through inhibition of NF- κ B signaling pathway⁴².

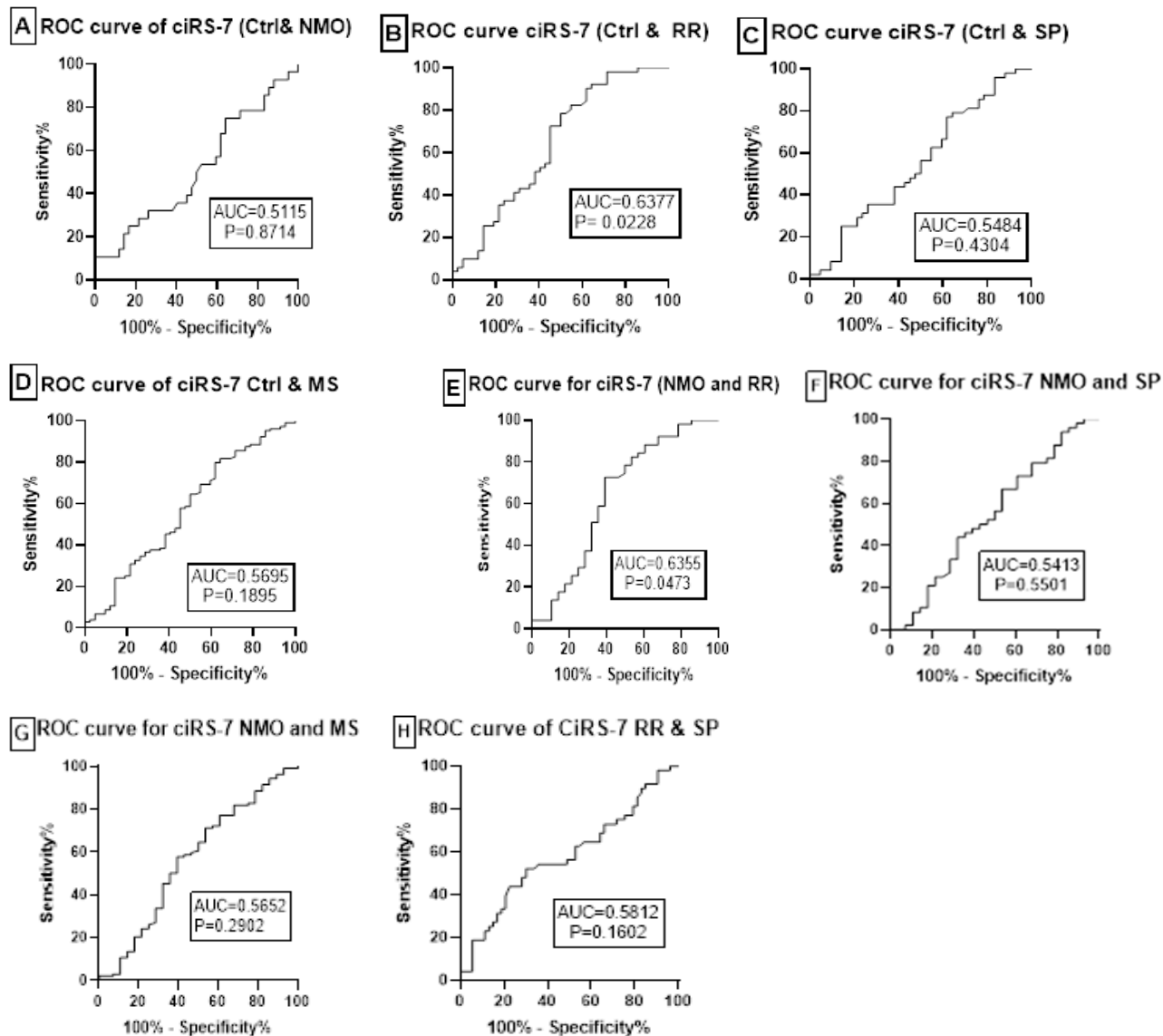


Figure 6. Analysis of receiver operating characteristic (ROC) curves for ciRS-7 expression rate in different studied groups. (A) ROC curve for Ctrl vs. Neuromyelitis Optica (NMO). (B) ROC curve for Ctrl vs. Multiple Sclerosis (MS). (C) ROC curve for Ctrl vs. Relapse Remitting Multiple Sclerosis (RR). (D) ROC curve for Ctrl vs. Secondary Progressive Multiple Sclerosis (SP). (E) ROC curve for Neuromyelitis Optica (NMO) vs. Multiple Sclerosis (MS), (F) ROC curve for Neuromyelitis Optica (NMO) vs. Relapse Remitting Multiple Sclerosis (RR), (G) ROC curve for Neuromyelitis Optica (NMO) vs. Secondary Progressive Multiple Sclerosis (SP), (H) ROC curve for Relapse Remitting Multiple Sclerosis (RR) vs. Secondary Progressive Multiple Sclerosis (SP). The area under the curve (AUC) values for each comparison are displayed above the respective curves, indicating the diagnostic accuracy of ciRS-7 expression for each condition. AUC values represent the ability to distinguish between the specified groups, with higher AUC values indicating better diagnostic performance. Statistical significance is indicated as $p < 0.05$.

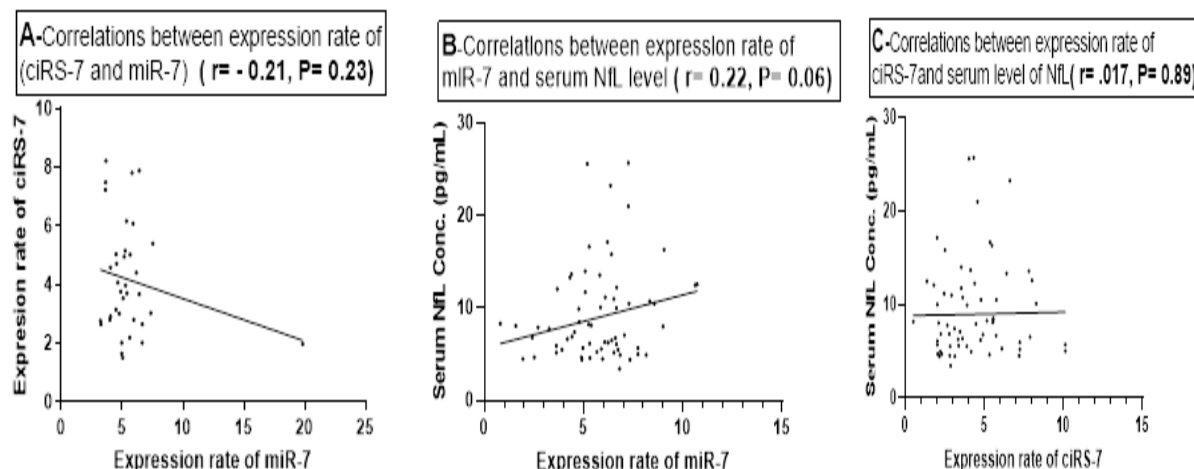


Figure 7. Spearman Correlation Between MiR-7, CiRS-7 Expression rate and NfL Concentration in Different Groups.

A previous study in neuropathic pain showed inverse correlation between serum level of NfL and MiR-7 expression²⁰, unfortunately this relation was not observed in this study again. The present study considered the first one that detects correlation between serum NfL concentration and ciRS-7 expression, and the study revealed non-significant correlation between them.

Despite the current study that showed inverse expression profiles of miR-7 and ciRS-7, it did not reach statistical significance in spearman correlation analysis, this may be attributed to small sample size.

Many studies revealed the inverse correlation between noncoding RNAs, deficits in ciRS-7-mediated 'sponging events' resulted in excess free miRNA-7, which triggered selective down-regulation of miRNA-7-sensitive mRNA targets such as the gene that encodes UBE2A⁴³. Furthermore, transfection with miR-7 induced more efficient repression of α -synuclein SNCA (The abnormal expression and aggregation of (SNCA), which is present in Lewy bodies, is regarded as a distinctive marker in Parkinson's disease) in the empty HeLa cell line that did not express ciRS-7, suggesting that ciRS-7 may play a role in modulating SNCA through a miR-7-dependent pathway. These results also suggested a possible sponge effect between ciRS-7 and miR-7 in vitro⁴⁴.

CONCLUSION

The present studies revealed the possibility of using NfL as diagnostic and monitoring tool for MS and suggested that NfL may serve as a more reliable biomarker for distinguishing between MS and NMO and

improve diagnostic precision. Remarkably, ciRS-7 exhibited notable diagnostic ability, effectively distinguishing RR from healthy controls and its diagnostic strength extends to differentiate NMO from RR.

Abbreviations:

MS	Multiple sclerosis
NMO	Neuromyelitis Optica
RR	Relapsing remitting MS
SP	secondary progressive MS
NfL	Neurofilament light chain
ADEM	acute disseminated encephalomyelitis
AHL	acute hemorrhagic leukoencephalitis
CNS	central nervous system
PPMS	primary progressive MS
DIT	dissemination in time
DIS	dissemination in space
VEPs	Visually evoked potentials
NMO-SD	NMO spectrum disorders
AQP4	water channel aquaporin-4
NF-M	neurofilament medium chain
NF-H	neurofilament heavy chain
MiR-7	MicroRNA-7
CDR1as	cerebellum degeneration-related protein 1 antisense transcript
ELISA	enzyme-linked immunosorbent assay
OCB	Oligoclonal Bands.
EDSS	Expanded Disability Status Scale
AD	Alzheimer Disease
PD	Parkinson's disease
UBE2A	Ubiquitin-conjugating enzyme E2 A
SNCA	Alpha-synuclein

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Competing Interests

The authors declare that they have no relevant financial or non-financial competing interests to disclose.

Author Contributions

Conceptualization, SA, MH and HT; Formal Analysis, HS, HT; Investigation, HS, MH, HT & SA; Resources, HS.; Writing – Original draft, HS, HT, SA; Writing – Review & Editing, HS, MH, HT, SA. Supervision, SA, HT & MH.

Ethics Approval

This study was conducted in accordance with the principles of the Declaration of Helsinki and was approved by the Scientific Research Ethics Committee of the Faculty of Pharmacy, Helwan University under number (Approval No. 01H2022 9/2/2022).

Consent to Participate

All participants provided written informed consent to participate in the study.

Data Availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author upon reasonable request.

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Not applicable for that section

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