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Assessment of axonal injury in multiple sclerosis: combined analysis of serum light-chain neurofilaments and diffusion tensor imaging

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ABSTRACT

Background Multiple sclerosis (MS) is a chronic neuroinflammatory condition characterised by demyelination and axonal damage in the central nervous system. Diffusion tensor imaging (DTI) enables non-invasive investigation of microstructural white matter alterations, while serum neurofilament light chain (NFL) holds promise as a fluid biomarker of axonal injury.

Objectives To use DTI and serum NFL measurements to evaluate white matter pathology in patients with MS and explore the relationship between in vivo imaging and biochemical indicators of axonal damage.

Methods 41 patients with relapse-remitting MS and 41 age-matched healthy controls underwent brain MRI including DTI acquisition. Serum samples were analysed for NFL concentrations using ELISA. Region of interest analysis was conducted to derive DTI metrics including fractional anisotropy, mean diffusivity, axial diffusivity and radial diffusivity. Correlational analyses were used to explore the associations between the imaging and biochemical indices.

Results Patients exhibited significantly elevated serum NFL levels and altered DTI metrics compared with controls, indicative of axonal/myelin pathology. DTI parameters were positively correlated with serum NFL concentration (p value<0.0001). Visual analogue scale scores demonstrated a significant positive relationship between DTI metrics and NFL, validating their potential as radiological and fluid-based markers of symptom severity.

Conclusions Combined DTI and serum NFL measurements may enhance the evaluation of axonal injury in MS by providing complementary in vivo and biochemical perspectives. The corresponding changes observed between the modalities support their utility as non-invasive biomarkers reflecting pathophysiological processes and clinical status in MS. Larger validation cohorts are needed to determine the clinical applicability.

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ Multiple sclerosis (MS) is characterised by demyelination and axonal damage in the central nervous system. While serum neurofilament light chain (NFL) levels can indicate axonal injury, diffusion tensor imaging (DTI) can provide in vivo assessment of white matter pathology. The relationship between these imaging and biochemical markers of axonal damage in MS has not been fully elucidated.

WHAT THIS STUDY ADDS

⇒ This study found that MS patients exhibit elevated serum NFL levels and altered DTI metrics compared with healthy controls, indicating axonal and myelin pathology. Importantly, the DTI parameters were positively correlated with serum NFL concentrations, supporting their utility as complementary non-invasive biomarkers of disease processes in MS.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ The combined assessment of DTI and serum NFL may enhance the evaluation of axonal injury in MS by providing both in vivo imaging and biochemical perspectives on neurodegeneration. Larger validation studies are needed to determine the clinical applicability of these biomarkers for MS prognosis and treatment monitoring.

INTRODUCTION

Multiple sclerosis (MS) is a predominant autoimmune disorder affecting the central nervous system in young adults. This disease is characterised by inflammation of the central nervous system, resulting in demyelination, axonal damage and degenerative alterations.[1] Axonal injury is the primary driver of the progression and stability of neurological deficits in MS.[2] The diagnosis of MS is determined by a combination of factors, including a thorough medical history, neurological clinical examination and MRI of the brain and spinal cord. Furthermore, elevated levels of CXCL13 and neurofilaments in the cerebrospinal fluid (CSF) may indicate a more unfavourable prognosis for patients.[3 4] Neurofilament light chain (NFL) is a vital component of the neuronal cytoskeleton and plays a critical role in axonal growth, stability and intracellular transport.[5] Neurofilaments or their degenerative products are released into the extracellular fluid following axonal

injury and subsequently detected in the CSF and blood. The levels of neurofilaments in the serum and CSF are correlated with relapses, neurological impairment, MRI lesion load and treatment outcomes in MS patients, as shown by Reinert *et al*.[6] The assessment of neurofilament levels as a biochemical indicator of axonal damage holds promise for application in prognostication, diagnosis and disease progression tracking.[7] Conventional MRI is regarded as the gold standard technique for MS diagnosis, in addition to clinical examination. However, there is only a poor link between patient disability during recurrence and clinical signs on conventional MRI. Additionally, conventional MRI only offers qualitative information and cannot distinguish between demyelination and axonal damage.[8 9] Axonal damage, demyelination and a combination of both can be distinguished using diffusion tensor imaging (DTI), an advanced MRI method that assesses the diffusion of water molecules in various directions. This is done by modelling quantities such as the axial diffusivity (AD, diffusion along axons), radial diffusivity (RD, diffusion across axons and myelin) and fractional anisotropy (FA, which reflects the directionality of water molecule diffusion) of the brain fibres.[9 10] With this imaging technique, brain plaque physiology and pathophysiology can be estimated using quantitative image data. Given the high incidence of MS, it is necessary to investigate plaque physiology using a reliable imaging method in conjunction with a biochemical marker to enhance disease treatment and prognosis for relapse. The aim of this investigation was to assess axonal damage using DTI in patients with MS and to quantify serum NFL levels as a biochemical indicator of axonal damage. To ascertain whether there is a connection between these in vivo imaging and biochemical indicators of axonal pathology in MS, this study examined the relationship between DTI-derived metrics of lesions and serum NFL levels. Determining the precise relationship between DTI and NFL will shed light on the potential of each method to be used as a non-invasive biomarker of neurodegeneration and disease progression for MS patient prognosis and treatment response monitoring.

METHODS

Patients

This study examined 41 patients with relapsing-remitting MS and 41 healthy individuals from the same age group who were recruited by a highly skilled neurologist. Of the 41 patients with relapsing-remitting MS, 32 patients (78%) were receiving disease-modifying therapies (DMTs) at the time of study enrolment. The DMTs included interferon-β-1a (n=12), glatiramer acetate (n=8), dimethyl fumarate (n=6) and fingolimod (n=6). Nine patients (22%) were treatment-naïve or had discontinued previous therapies for at least 6 months prior to study inclusion. Specific criteria were established to account for the influence of ageing, neurological disorders and other conditions on NFL and DTI parameters. These criteria ensured that the patients had no other neurological disorders apart from MS, no history of trauma or claustrophobia.

Neurology and NFL assessment

In this study, the patients underwent a comprehensive examination by a board-certified neurologist. The classification of MS is determined by analysing clinical symptoms, disease severity and type. Furthermore, the degree of degradation and severity of the clinical symptoms were assessed using a valid scale. Specifically, the visual analogue scale (VAS) was used to measure pain scores in both quantitative and qualitative forms. Serum NFL chain levels were quantified using an ELISA, following the manufacturer's protocol (figure 1). Blood samples (5 mL) were collected from both patients and controls, which were then subjected to centrifugation for 5 min to obtain plasma. The plasma samples were subsequently incubated in the ELISA kits for 1 hour at room temperature. A specific antigen was added to the samples, followed by incubation with a horseradish peroxidase-conjugated anti-IgG antibody. The samples were read using a microplate reader to obtain the readings.

MRI acquisition

Acquisitions were performed using a 1.5T scanner (GE MRI Signa Explorer) equipped with a 16-channel

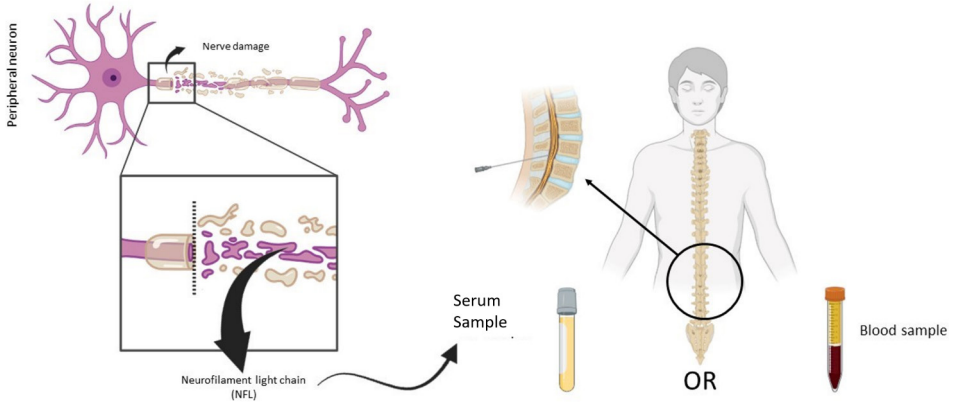

Figure 1 Overview of the neurofilament light (NFL) protein extraction and quantification workflow from plasma samples of multiple sclerosis patients.

Table 1 MRI parameter

Sequence	3D-T1 sagittal	T2-FLAIR	DTI
FOV (mm)	256	240	230
TR (ms)	5.9	10 000	10 000
TE (ms)	Min	120	70
FA	6	90	90
Voxel size (mm^3)	1×1×1	0.9×0.9	2×2×2
Matrix	256×256	256×256	112×112
ST (mm)	1	5	2

DTI, diffusion tensor imaging; FA, flip angle; FLAIR, fluid attenuated inversion recovery; FOV, field of view; ST, slice thickness; TE, time of echo; TR, time repetition.

phased-array head coil. The initial step involved obtaining a 3D-T1 SPGR, T2-fluid attenuated inversion recovery image (TI=2450 ms) to detect lesions. Following this, the axial DTI sequence was performed with the following parameters: b-value=1000 s/mm^2, and 33 diffusion encoding gradients (b-value=0, #3; b-value=1000, #30). The imaging parameters for the three sequences are listed in table 1.

Image processing

On obtaining the images, we transferred the data to a personal computer and prepared for the subsequent steps. DTI data were pre-processed using Explore DTI V.4.8.6 in MATLAB R2018a, incorporating signal drift, motion, eddy currents and susceptibility corrections, along with b-matrix rotation.[11] Visual inspection was conducted to identify plaque lesions, followed by utilisation of a region of interest (ROI) approach to derive FA, mean diffusivity (MD), RD and AD measurements from selected regions of interest within the patient group and the corresponding regions in healthy controls (HCs, figure 2).

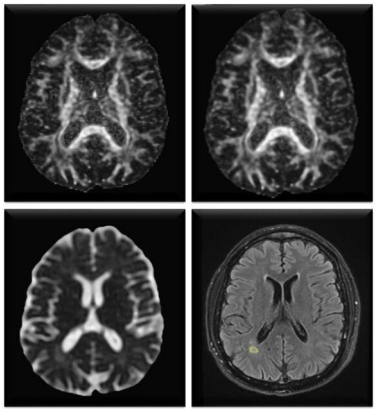

Figure 2 From left to right, the figures display the raw diffusion tensor imaging fractional anisotropy (FA) image, as well as the derived FA and mean diffusivity parametric maps, alongside the fluid attenuated inversion recovery image. These images were obtained following preprocessing to correct for signal drift, subject motion, eddy currents and susceptibility artefacts associated with spin echo/echo planar imaging acquisition. A representative region of interest (yellow circle) positioned to correspond with a lesion on another image is shown. An identical preprocessing pipeline was employed for healthy control participants for comparative purposes.

Table 2 Clinical demographics of the subjects in the study

Variable	MS (n=41)	HC (n=41)
Male/female	13/28	17/24
Age (years)	33.5±7.9	32.3±6.9
Max/min	45/18	45/20
Disease duration (years)	5.8±4.2	
VAS	4.9±2.26	

HC, healthy control; MS, multiple sclerosis; VAS, visual analogue scale.

Statistical analysis

All statistical analyses were performed using SPSS V.6. The demographic characteristics of the study participants (sex, age and disease duration) and their VAS scores were reported as descriptive statistics (mean and SD for continuous variables; maximum and minimum for age variable). DTI and NFL parameters are presented as mean and SD by ROI and compared between the MS and HC groups. In this study, we used the Kolmogorov-Smirnov test, which is a common test for checking the normality of data in diverse research. The statistically significant DTI metrics, NFL and VAS were correlated in the MS and HC groups using Spearman's correlation coefficient.

RESULTS

Patient demographics

This study included 41 patients with MS who were matched to 41 HCs based on age and sex. Of the 41 patients with MS, 28 were female, accounting for 68.3% of the group, while 13 were male, accounting for 31.7%. The control group consisted of 58.5% female individuals and 41.5% male individuals. The average age of the patients with MS involved in the study was approximately 33 years, with a minimum age of 19 years and a maximum age of 45 years. In contrast, the mean age of the control group was approximately 32 years. The VAS score mean of the MS group and HC group was 9.4 and 3.1, respectively. The demographic characteristics and clinical assessments of both the MS and HC groups are summarised in table 2.

NFL serum levels and DTI

The findings showed that, with the exception of FA, the means of the other factors examined were significantly higher in the study group than in the control group. There was also a statistically significant difference between the mean FA, MD, RD, AD and serum neurofilament levels in MS patients and the control group ($p<0.05$) (table 3).

In addition, the results showed that there was no statistically significant difference between the mean values of

Table 3 Relationships between diffusion tensor imaging-derived metrics and serum neurofilament light chain levels

Metric	MS	HC	t	P value
NFL (pg/mL)	30.61±13.53	7.9±1.64	10.673	0.0001
FA	0.3±0.089	0.74±0.059	26.058	0.0001
MD (mm^2/s)	1.13±0.148	0.598±0.04	22.443	0.0001
AD (mm^2/s)	1.65±0.146	1.14±0.09	18.915	0.0001
RD (mm^2/s)	1.05±0.216	0.046±0.04	17.341	0.0001

AD, axial diffusivity; FA, fractional anisotropy; HC, healthy control; MD, mean diffusivity; MS, multiple sclerosis; NFL, neurofilament light; RD, radial diffusivity.

FA, MD, RD, AD and NFL in either the research group for men or women.

NFL and DTI correlations with VAS

The study conducted a correlation analysis between DTI-derived metrics and NFL with disease duration and the VAS in MS patients. The results indicated that there was no significant correlation between disease duration and FA, MD, RD, AD or NFL ($p>0.05$). However, there was a significant correlation between the VAS and DTI and NFL, and the positive correlation coefficients indicated a direct and significant relationship between the VAS and the aforementioned parameters. Consequently, as DTI-derived metrics and serum NFL levels increased, the VAS score increased significantly (table 4).

DISCUSSION

The role of NFL as a biomarker has been extensively studied in MS, Alzheimer's disease, frontotemporal dementia, amyotrophic lateral sclerosis, atypical Parkinsonian disorders and traumatic brain injury.[12] NFL levels track relapsing-remitting MS and demyelinating disorders.[7 13] Other neurological evaluations, biomarkers and imaging should be used in addition to NFL to maximise its diagnostic usefulness.[14] The concentration of CSF NFL increases in MS and during the initial clinical episode.[13] Similar findings have been reported for the serum NFL levels.[8]

MRI lesion load, recurrence and neurological impairment in conventional MRI have been demonstrated to correlate with elevated blood neurofilament levels.[15] Nevertheless, neurofilament release in the blood and serum as well as neuronal myelin structural breakdown can also be affected by neurodegenerative disorders[16] and ageing.[17]

Using DTI and serum NFL, we examined axonal alterations in white matter lesions in patients with MS and compared them with those in HCs.

Table 3 displays the quantitative features of serum NFL levels and DTI metrics for the two study groups. Only the mean FA value was lower in the patients than in the controls. The mean values of MD, RD, AD and NFL were found to be greater in patients than in controls. This difference was observed for both the minimum and maximum values of the parameters under investigation,[16] which is consistent with previous studies.[18–20]

Given the concept of FA (which denotes anisotropy, or being anisotropic, in voxel diffusion and determines the ellipsoid diffusion shape, scaling from 0 to 1), it is reasonable for the patient group's mean FA value to be lower than that of the control groups. The numerical FA value deviates from the maximal ellipsoid state[1] towards sphere-like behaviour owing to anisotropic diffusion being lost in the voxel and the predominance of isotropic diffusion as a result of axonal and myelin damage (0). The outcomes of our study verified this procedure. Mean MD, reflecting overall diffusion within a voxel, was higher in patients validating oedema associated with lesions. RD reflecting mean diffusion perpendicular to the main ellipsoid axis also validates the oedema associated with the lesions. A higher mean AD in the patient group, partially reflecting diffusion along the main ellipsoid axis, revalidated the presence of significant oedema.

Overall, the above metrics indicate axonal and myelin damage in MS lesions accompanied by oedema, and the ability of DTI to detect both axonal injury and oedema

Table 4 Correlations between diffusion tensor imaging-MRI indices and serum NFL concentrations with VAS and disease duration

Variable	Statistics	FA	MD	AD	RD	NFL
Disease duration	Correlation coefficient	0.171	−0.125	−0.144	−0.212	0.019
	Significance level	0.284	0.437	0.370	0.183	0.905
VAS	Correlation coefficient	0.924	0.703	0.792	0.893	0.913
	Significance level	0.0001	0.0001	0.0001	0.0001	0.0001

AD, axial diffusivity; FA, fractional anisotropy; MD, mean diffusivity; NFL, neurofilament light; RD, radial diffusivity; VAS, visual analogue scale.

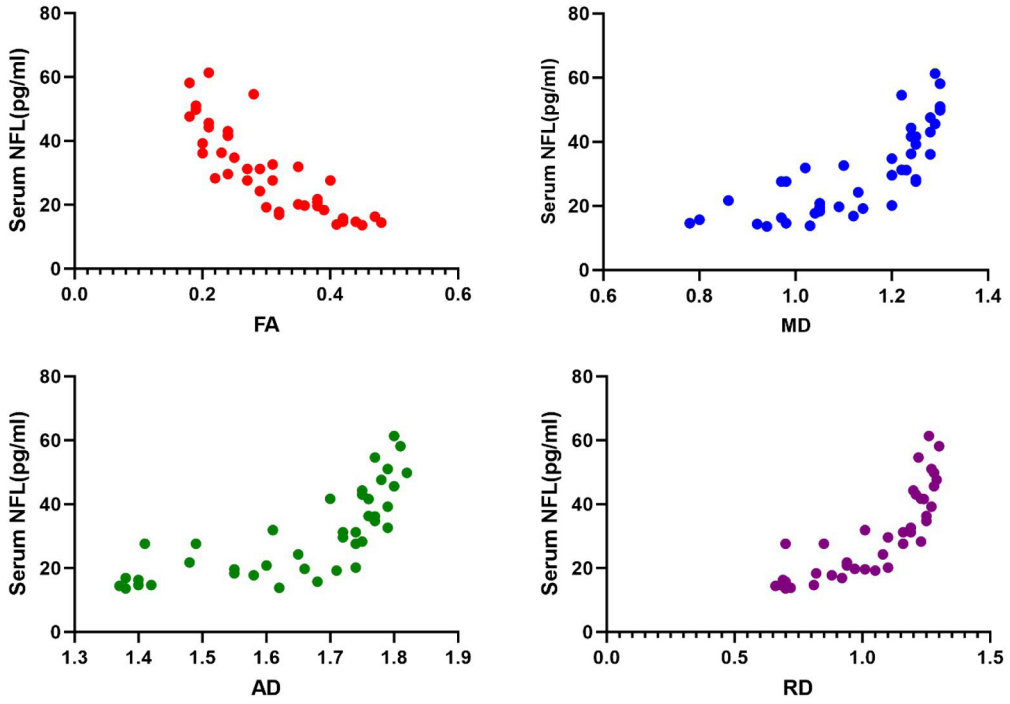

Figure 3 Correlation showing associations between derived diffusion tensor imaging parameters and serum levels of neurofilament light (NFL) protein in multiple sclerosis patients. AD, axial diffusivity; FA, fractional anisotropy; MD, mean diffusivity; RD, radial diffusivity.

separately or in combination. Significantly higher and meaningful serum NFL levels in the patient group strongly validated neuronal myelin and axonal degradation in patients with MS and supported the role of NFL as a reliable marker for diagnosis and tracking the progression of the disease.

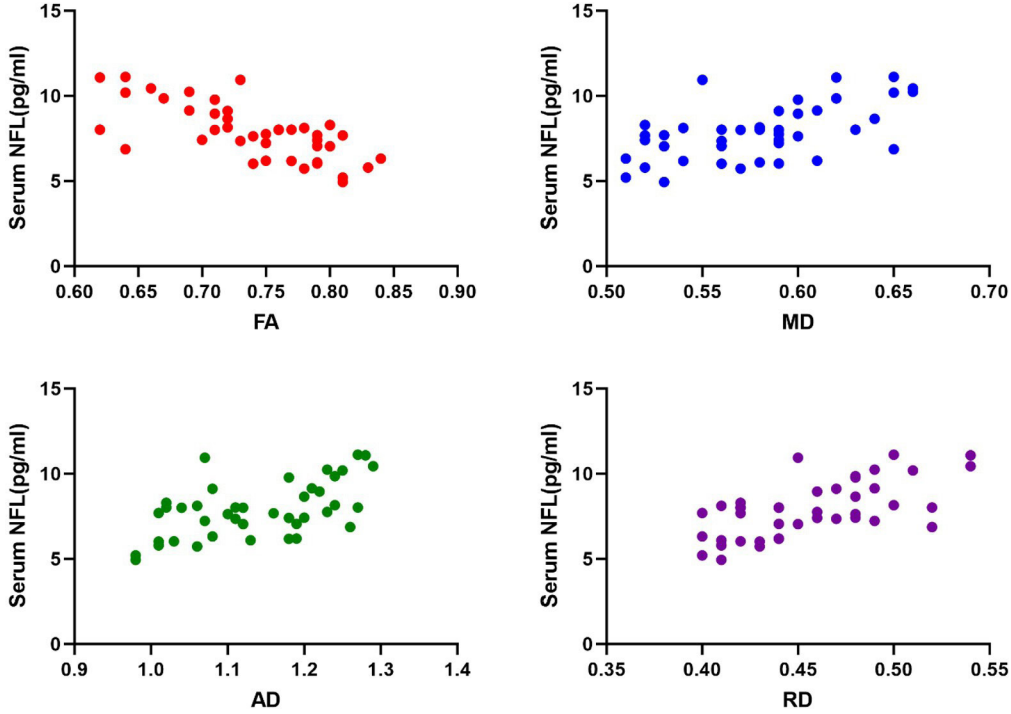

Figure 4 Correlation showing associations between derived diffusion tensor imaging parameters and serum levels of neurofilament light (NFL) protein in healthy controls. AD, axial diffusivity; FA, fractional anisotropy; MD, mean diffusivity; RD, radial diffusivity.

DTI-metrics and serum NFL levels changes in MS

Significant correlations were found between DTI-metrics and blood NFL levels in both the patient and control groups according to the findings of the correlation analysis (table 3). Increasing serum NFL levels were positively correlated with MD, AD and RD but negatively correlated with FA (p value<0.0001). Considering the explanations regarding increases and decreases in DTI-metrics and their definitions, this result also reconfirms the reliability and confidence of DTI-metrics and serum NFL as diagnostic biomarkers for MS. Furthermore, according to the results showing no statistically significant differences in mean FA, MD, RD and AD parameters and serum neurofilament levels between males and females in both study groups, it can be concluded that axonal degradation in MS is not sex-dependent (figures 3 and 4).

Correlation of VAS with DTI-metrics and serum NFL

The presence and intensity of pain are frequently crucial diagnostic factors in a wide range of neurological disorders.[1 21 22] An increase in DTI metrics and serum NFL levels was significantly correlated with an increase in VAS, indicating a positive and meaningful link between VAS and the variables listed in table 4. These findings indicate the validity of serum NFL and DTI characteristics as diagnostic biomarkers of MS as well as the use of VAS as a generalisable scale for symptom severity evaluation and MS diagnosis. Additionally, using Pearson's correlation coefficient, we assessed the relationship between age and the other metrics in this study. The results showed no meaningful correlation between patient age and the studied parameters, but a meaningful positive correlation was observed between control individuals' age and DTI and serum NFL parameters, except for FA, meaning parameter means increased with age (p value<0.0001). Because meaningful changes were observed in patient parameters and serum NFL levels attributable to disease factors, parameter changes with age could not be assessed. However, in controls, age-related alterations were evaluated and validated according to table 4 results, which show that blood/serum NFL levels and neuronal (myelin and axonal) degradation are age-dependent and increase with age, consistent with previous pertinent investigations.[23]

In this study, DTI was conducted in 33 diffusion encoding directions; however, conducting it in a higher number of directions (64 or beyond) in future research could potentially yield more extensive outcomes. Since CSF neurofilament level analysis has limitations, this study depended on serum neurofilament analysis. However, including both assessments (CSF and serum), along with DTI scans, could increase the overall completeness of the study. Serum NFL analysis was performed using ELISA due to constraints, using the SIOMA method if conditions allowed would likely lead to more accurate results. Larger sample sizes are necessary to determine DTI parameter Cut-off values. We suggest that future studies obtain these values and correlate them with clinical symptoms to gain a better understanding of outcomes.

CONCLUSION

The results of this study suggest that patients with MS have significantly increased serum NFL levels, which is consistent with previous research. The study also found that changes in DTI parameters (FA, MD, RD and AD) were significant and independent of sex, and remained stable throughout the course of the disease. However, it is important to note that serum NFL levels may also increase with age in healthy elderly individuals, making it less reliable as a biological marker of MS in older patients. Based on these findings, it is recommended that DTI alongside serum NFL measurement may be useful in investigating axonal injury in MS patients, particularly in younger individuals.

Contributors MJ and IA were responsible for the design of MRI acquisition, analysis and interpretation of data. MS handled NFL processing works and analysis. ME and MJ wrote the manuscript and conducted the statistical analysis. IA was responsible for the concept and design, administration, ensuring the integrity and accuracy of the data analysis, and supervision. All authors contributed to the article, and the submitted version was approved by all. IA acted as guarantor.

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Competing interests None declared.

Patient consent for publication Consent obtained directly from patient(s).

Ethics approval This study involves human participants and the local research ethics committee (Isfahan University of Medical Sciences) has approved the study with ID: IR.MUI.MED.REC.1400.223. The participants provided written informed consent prior to the MRI scan.

Provenance and peer review Not commissioned; internally peer reviewed.

Data availability statement Data are available upon reasonable request.

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