Microstructure white matter changes and serum GFAP among different phenotypes of multiple sclerosis

By Elsayed Orief

WORD COUNT 5451
TIME SUBMITTED 04-MAR-2024 10:40AM
PAPER ID 107310500

Microstructure white matter changes and serum GFAP among different phenotypes of multiple sclerosis

Elsayed Orief [1], Samer Salama[1], Mohamed Elkhateeb[1], Ahmad El-morsy [2], Khaled Eltoukhy[1]

[1]Department of Neurology, Faculty of Medicine,Mansoura University, Mansoura, Egypt

[2]Department of Diagnostic Radiology, Faculty of Medicine, Mansoura University, Mansoura, Egypt

Samer Salama **ORCIDD ID**: 0000-0001-9510-0343

Ahmad El-morsy **ORCIDD ID**: 0000-0003-4333-8282

Corresponding author:

Samer Salama, MD, PhD

E-mail: samer_salama@mans.edu.eg

ABSTRACT

Background

Multiple sclerosis (MS) is an autoimmune disease that targets the central nervous system (CNS). A patient's degree of demyelination and axonal degeneration can only be roughly estimated based on clinical symptoms, neurochemical analyzes, or standard clinical MRI. Magnetic resonance diffusion tensor imaging (DTI) may provide more information on MS pathology than T1- and T2-weighted MRI alone. Glial fibrillary acidic protein (GFAP) uniquely found in astrocytes in the (CNS), non-myelinating Schwann cells in the peripheral nervous system (PNS), and enteric glial cells. GFAP is postulated to be a biomarker of astrocytic damage and reactive astrogliosis.

Methods

A total of 60 patients with MS was categorized into three equal groups according to The Multiple Sclerosis Progression Discussion Tool (MSProDiscuss tool): RRMS, SPMS, RRMS with high risk to become SPMS and 20 healthy controls. Baseline clinical characteristics and detailed medical and neurological history taking; time of onset of MS, delay in diagnosis, initial symptoms, relapses features and behavior, EDSS and disease modifying therapy. They were subjected to DTI-MRI and blood sampling for GFAP.

Results

DTI was able to differentiate between different MS phenotypes and was able to detect progression when we evaluated DTI changes in NAWM in different brain areas as low FA and high MD were associated with progression and increasing disability (p value =0.001). Serum GFAP Differ significantly between patients with SPMS or patient in transition, also, it was higher in patient in transition than RRMS or control group (P value <0.001). There was significant correlation between serum GFAP and DTI changes in NAWM as higher titres of GFAP were associated with lower FA and higher MD values in NAWM of frontal, temporal lobes, and CC body.

Conclusion

Serum GFAP in addition to DTI measurable microdamage in NAWM can give us wide scope of view about potential progression in MS pathology and related astrocytopathy.

Keywords: DTI, GFAP, RRMS, progression, multiple sclerosis

INTRODUCTION

Multiple sclerosis (MS) is a prevalent condition that primarily affects the brain and spinal cord and is recognized as one of the most prevalent non-traumatic disorders in young people. It impacts approximately 1 million people in the United States alone [1]. In the majority of MS patients, the disease initially manifests as episodes of localized or multifocal neurological deterioration, which improve on their own (known as relapsing-remitting MS or RRMS) [2]. From a pathological standpoint, these relapses are caused infiltration of by immune cells mainly macrophages, T lymphocytes and B lymphocytes which cause focal or multifocal inflammation and demyelination in both white and grey matters [3]. The majority of cases eventually progress to secondary progressive MS (SPMS), and in this stage neurodegeneration takes the upper hand over inflammation and the patient show gradual deterioration and accumulation of disability over time which mainly affecting mental function and ambulation. However, in a minority of MS patients, progression is evident from the onset of the disease, which is referred to as primary progressive MS (PPMS) [4].

However, it isn't easy to assess the degree of demyelination and axonal damage from just disease presentation, laboratory investigation, or standard conventional MRI. Magnetic resonance diffusion tensor imaging (DTI) can provide more information about MS pathology than routine conventional MRI alone. For example, a lower fractional anisotropy (FA) values were reported to be associated with MS although this finding appears not to be consistent [5]. This may depend on the certain site or may be due to progressive or reversible damage that occur over time. The reduced FA was verified as a universal indicator and observed in both non-affected white matter regions and specific areas with lesions [6].

Previously, it was commonly believed that astrocytes in multiple sclerosis (MS) lesions only became active after inflammation subsided and formed a glial scar. However, it is now recognized that astrocytes are actually involved at an early stage and have a substantial impact on lesion formation. They play a vital role in facilitating the entry of immune cells from the peripheral system into the central nervous system. Reactive astrocytes are present at the active borders of demyelinating lesions and can even extend into adjacent normal-appearing white matter (NAWM), suggesting that they are among the primary contributors to the progression of lesions [7].

Glial fibrillary acidic protein (GFAP) is present mainly in astrocytes within the central nervous system (CNS), non-myelinating Schwann cells in the peripheral nervous system (PNS), and enteric glial cells. GFAP is thought to serve as a biomarker for both astrocytic damage and reactive astrogliosis [8].

PATIENTS AND METHODS

Standard protocol approvals and patient consents

The Institutional Research Board – IRB Ethics Committee under the code of (MS.22.01.1843) approved the research protocol. A written informed consent was obtained from all participants according to the Declaration of Helsinki.

Study patients and protocols

A total of 60 patients with multiple sclerosis was categorized into three equal groups according to The Multiple Sclerosis Progression Discussion Tool (MSProDiscuss tool): RRMS, SPMS, RRMS with high risk to become SPMS and 20 healthy controls were recruited from the neurology outpatients' clinic in Mansoura University Hospital and outpatient clinic between September 2021 and September 2022. The exclusion criteria were patients with known neurological or non-neurological disease that can cause

disability that affect EDSS, cases with no informed consents or lost samples and general contraindication for MRI scan.

Baseline clinical characteristics and detailed medical and neurological history taking: time of onset of MS, delay in diagnosis, initial symptoms, relapses features and behavior, EDSS and disease modifying therapy.

Brain MRI was performed on a 1.5T Siemens Aera, Germany, and closed-configuration whole body scanner using a standard quadrature head coil. 3D fluid-attenuated inversion recovery (FLAIR) images were acquired. DTI was obtained using an echo-planner imaging sequence Mean values for fractional anisotropy (FA) and mean diffusivities (MD) were calculated both within the whole and segmented NAWM (frontal, parietal, temporal, occipital, cingulate and deep) using ROI approach (region of interest)

A blood sample was obtained from each patient, within 30 minutes, aliquots of plasma and serum underwent separation and were kept at -20°C for one year. Or store at 2-8°C for 6 months. If individual reagents are opened it is recommended that the kit be used within 1 month. Avoid repeated thaw cycles. In all patient and control groups serum glial fibrillary acidic protein (GFAP) was measured using GFAP ELISA kits. Wuhan, Hubei, China (430206) based on manufacturer guidelines.

STATISTICAL ANALYSIS

We used SPSS software, version 25, provided by SPSS Inc., for analyzing the data. Qualitative data were presented using numbers and percentages. Quantitative data were described differently depending on their distribution. For non-normally distributed data, we reported the median along with the minimum and maximum values. For normally distributed data, we reported the mean ± standard deviation. We tested the normality of the data using the Kolmogorov-Smirnov test. The significance level for our analysis was set at ≤ 0.05. To compare qualitative data between different groups, we employed appropriate statistical tests such as the Chi-Square test and Monte Carlo tests. For non-normally distributed data, we used the Mann Whitney U test when comparing two groups and the Kruskal Wallis test when comparing more than two groups. For normally distributed data, we used the Student t-test for comparing two independent groups. When comparing more than two independent groups, we employed the One-Way ANOVA test followed by the Post Hoc Tukey test for pairwise comparisons. To determine the strength and direction of a linear relationship between two non-normally distributed continuous variables and/or ordinal variables, we used the Spearman's rank-order correlation. The validity of continuous variables, including sensitivity and specificity, and the calculation of the optimal cutoff point, were assessed using the Receiver Operating Characteristics curve (ROC curve). Finally, we performed binary logistic regression using the

Stepwise/forward Wald/Enter technique to evaluate the impact of a combination of more than two independent variables on a dichotomous outcome.

RESULTS

The demographics and clinical characteristics of MS patients revealed that the studied subgroups had no statistically significant difference as regard age, sex, mean age of RRMS group is 31.75 years of patient in transition group is 35 years and patients in SPMS group is 35.9. Median time of disease onset is longer among SPMS group followed by patient in transition group and the least for RRMS group with statistically significant difference between them. No A statistically notable distinction observed among the groups under investigation in terms of delay in diagnosis and forms of initial symptoms presentation. Median number of relapses illustrates statistically significant higher median among SPMS group followed by patient in transition group and the least for RRMS group. A statistically significant association was also detected between types of recovery from relapse among studied groups.

All cases with RRMS shows complete recovery, 70% of patient in transition group show incomplete recovery, 25% complete recovery and 5% Initially complete then incomplete recovery and 80% of SPMS have incomplete recovery and 20% Initially complete then incomplete recovery. In addition, there was statistically significant higher median EDSS among SPMS group followed by patient in transition group and the least for RRMS group (6, 4 & 1 respectively). There is statistically significant higher mean GFAP among SPMS group followed by patient in transition group and RRMS group and the lowest median was detected for control group (Table 1).

Table (1): demographic characteristics of the studied groups

	RRMS N=20	Patient in transition N=20	SPMS N=20	Test of significance	Within group significance
Age / years	31.75±6.77	35.0±6.26	35.90±8.85	F=1.75 (p)=0.183	(p)1=0.1698 (p)2=0.08 (p)3=0.701
Sex					(p)1=0.507
Male	8(40.0)	6(30.0)	6(30.0)	MC=0.60	(p)2=0.507
Female	12(60.0)	14(70.0)	14(70.0)	(p)=0.741	(p)3=1.0
Time to onset of diseases (years)	1.75(0.25-7.0)	9.0(5.0-13.0)	15.0(5.0-22.0)	Kw=61.36 (p)<0.001*	(p)1<0.001* (p)2<0.001* (p)3<0.001*

Delay in diagnosis (years)	1.0(1.0-2.0)	3.0(1.0-8.0)	3.0(1.0-6.0)	Kw=1.59 (p)=0.223	(p)1=0.086 (p)2=0.170 (p)3=0.604
Initial symptom					
Motor	1(5.0)	3(15.0)	2(10)		(p)1=0.447
Cerebellar	0	2(10.0)	3(15)	MC=11.06	(p)2=0.924
Sensory	4(20.0)	2(10.0)	0	(p)=0.524	(p)3=0.505
Optic neuritis	9(45.0)	8(40.0)	6(30)		
Multifocal	3(15.0)	2(10.0)	5(25)		
Diplopia	0	1(5.0)	1(5)		
Number of relapses	1(1-5)	5(1-10)	9(3-15)	Kw=48.70 (p)<0.001*	(p)1<0.001* (p)2<0.001* (p)3<0.001*
last year Number					
0	0	0	15(83.3)	MC=58.45	(p)1<0.001*
1	18(94.7)	9(47.4)	3(16.7)	(p)<0.001*	(p)2<0.001*
2	1(5.3)	10(52.6)	0		(p)3<0.001*
Recovery					
Initially complete	0	1(5)	4(20)	MC=46.40	(p)1<0.001*
then incomplete	0	14(70)	16(80)	(p)<0.001*	(p)2<0.001*
Incomplete	20(100)	5(25)	0		(p)3<0.001*
Complete					
Prodiscuss					
Secondary	0	0	20(100)	Mc=114.28	(p)1<0.001*
progression	20(100)	1(5.0)	0	(p)<0.001*	(p)2<0.001*
RRMS	0	19(95)	0		(p)3<0.001*
High risk					
EDSS Median (range)	1(0-2)	4(2.5-5.0)	6(5-7)	KW=298.07 (p)<0.001*	(p)1<0.001* (p)2<0.001* (p)3<0.001*
GFAP Mean ± SD	0.365±0.08	0.645±0.087	2.72±0.49	F=406 (p)<0.001*	(p)1<0.001* (p)2<0.001* (p)3=0.424 (p)4<0.001* (p)5<0.001* (p)6<0.001*

MC: Monte Carlo test, *statistically significant, P1: difference between RRMS & Patient in transition group, p2: difference between RRMS &SPMS, P3: difference between patient in transition & SPMS, P4: Patient in transition group& SPMS group, P5: Patient in transition group& control, P6: SPMS & control group, KW: Kruskal Wallis test, F: One Way ANOVA test

First DMT was distributed as following; 40% of RRMS use Avonex, 30% naïve and 25% Betaferon and 5% dimethyl. For Patient in transition; 50% Betaferon, 20% naïve, 20% Avonex and 10% Gilynea. For SPMS group; 50% Avonex, 25% Gilynea and 25% Betaferon. Reasons for changing DMT was as following; one case in RRMS changed as it is ineffective, 9 cases in patient in transition group as it is ineffective and 15 cases in SPMS group as it is ineffective and one case because of its side effects.

Table (5): comparison of DTI between studied groups

	RRMS N=20 Mean ± SD	Patient in transition N=20 Mean ± SD	SPMS N=20 Mean ± SD	Control group N=20 Mean ± SD	Test of significance	Within group sig.	
Frontal							
FA (Left side)	0.611±0.071	0.430±0.02	0.301±0.04	0.808±0.06	F=369.6 (p)<0.001*	(p)1=0.001* (p)2=0.001* (p)4=0.001*	(p)5=0.001* (p)6=0.001*
MD (Left side)	0.839±0.02	0.951±0.02	1.42±0.09	0.748±0.02	F=676.17 (p)<0.001*	(p)1=0.001* (p)2=0.001* (p)3=0.001*	(p)4=0.001* (p)5=0.001* (p)6=0.001*
FA (Right side)	0.616±0.06	0.432±0.028	0.305±0.037	0.822±0.05	F=483.03 (p)<0.001*	(p)1=0.001* (p)2=0.001* (p)3=0.001*	(p)4=0.001* (p)5=0.001* (p)6=0.001*
MD (Right side)	0.836±0.02	0.954±0.02	1.38±0.14	0.833±0.09	F=191.94 (p)<0.001*	(p)1=0.001* (p)2=0.001* (p)4=0.001*	(p)5=0.001* (p)6=0.001*
Parietal							
FA (left side)	0.595±0.06	0.613±0.06	0.607±0.06	0.663±0.11	F=3.04 (p)=0.034*	(p)3=0.007* (p)5=0.042*	(p)6=0.024*
Temporal							
FA (Left side)	0.615±0.08	0.424±0.024	0.293±0.03	0.723±0.11	F=146.53 (p)<0.001*	(p)1=0.001* (p)2=0.001* (p)3=0.001*	(p)4=0.001* (p)5=0.001* (p)6=0.001*
MD (Left side)	0.838±0.016	0.959±0.02	1.40±0.096	0.745±0.02	F=656.32 (p)<0.001*	(p)1=0.001* (p)2=0.001* (p)3=0.001*	(p)4=0.001* (p)5=0.001* (p)6=0.001*
FA (Right side)	0.636±0.05	0.429±0.02	0.325±0.12	0.849±0.05	F=194.46 (p)<0.001*	(p)1=0.001* (p)2=0.001* (p)3=0.001*	(p)4=0.001* (p)5=0.001* (p)6=0.001*
MD (Right side)	0.839±0.02	0.965±0.07	1.44±0.15	0.752±0.02	F=254.62 (p)<0.001*	(p)1=0.001* (p)2=0.001* (p)3=0.002*	(p)4=0.001* (p)5=0.001* (p)6=0.001*
Corpus callosum							
FA	0.611±0.07	0.433±0.028	0.303±0.036	0.808±0.059	F=362.78 (p)<0.001*	(p)1=0.001* (p)2=0.001* (p)3=0.001*	(p)4=0.001* (p)5=0.001* (p)6=0.001*
MD	0.826±0.03	0.954±0.02	1.42±0.18	0.806±0.129	F=122.51 (p)<0.001*	(p)1=0.001* (p)2=0.001* (p)4=0.001*	(p)5=0.001* (p)6=0.001*

F: One Way ANOVA test, *statistically significant, P1: difference between RRMS & Patient in transition group, p2: difference between RRMS &SPMS, P3: difference between RRMS & control group, P4: Patient in transition group& SPMS group, P5: Patient in transition group& control, P6: SPMS & control group.

Table (5) illustrates A statistically notable distinction observed among the groups under investigation regarding FA assessed at left & right frontal lobes with the highest mean value is control group followed by RRMS, Patient in transition and SPMS, within group significance demonstrates statistically notable distinction between each of the studied groups except between RRMS & control group for left side. Also, A statistically notable distinction observed among the

groups under investigation regarding MD at left & right frontal lobes with the lowest mean value is detected for control group followed by RRMS, Patient in transition and SPMS, within group significance demonstrates statistically significant difference between each of the studied groups except between RRMS & control group for right side. Parietal lobe study revealed A statistically notable distinction observed among the groups under investigation in terms of FA assessed at left side with the highest mean value is control group followed by patient in transition, SPMS and RRMS, within group significance demonstrates statistically notable distinction between each of the studied groups except between RRMS & control group (p=0.007), between patient in transition & control group (p=0.04) and between SPMS & control group (p=0.02).

In addition, there is A statistically notable distinction observed among the groups under investigation regarding FA assessed at left & right frontal lobes with the highest mean value is control group followed by RRMS, Patient in transition and SPMS, within group significance demonstrates statistically significant difference between each of the studied groups. Also, A statistically notable distinction observed among the groups under investigation in terms of MD at left & right temporal lobes with the lowest mean value is detected for control group followed by RRMS, Patient in transition and SPMS, within group significance demonstrates statistically significant difference between each of the studied groups except between RRMS & control group for right side.

Corpus callosum DTI study revealed A statistically notable distinction observed among the groups under investigation in terms of FA with the highest mean value is control group followed by RRMS, Patient in transition and SPMS, within group significance demonstrates statistically significant difference between each of the studied groups. Also, A statistically notable distinction observed among the groups under investigation regarding MD with the lowest mean value is detected for control group followed by RRMS, Patient in transition and SPMS, within group significance demonstrates statistically significant difference between each of the studied groups except between RRMS & control group

	Transition group		**RRMS group**		**SPMS**	
			GFAP			
	r	p	r	p	r	p
Frontal FA (left side)	-0.330	0.156	-0.193	0.414	-0.07	0.770

MD (left side)	-0.162	0.496	-0.287	0.220	0.230	0.330
FA (right side)	-0.596	0.006*	0.185	0.436	-0.121	0.612
MD (right side)	-0.307	0.187	0.172	0.468	-0.187	0.430
Parietal						
FA (left side)	0.071	0.768	-0.380	0.098	0.369	0.110
MD (left side)	0.136	0.568	**-0.492**	**0.027***	**0.275**	**0.241**
FA (right side)	0.223	0.345	-0.102	0.670	0.138	0.562
MD (right side)	-0.264	0.261	-0.01	0.967	0.174	0.464
Temporal						
FA (left side)	0.416	0.07	0.084	0.724	-0.211	0.372
MD (left side)	0.049	0.836	-0.083	0.729	0.207	0.381
FA (right side)	-0.168	0.479	-0.386	0.093	-0.003	0.990
MD (right side)	0.187	0.429	0.308	0.186	-0.552	0.012*
Occipital						
FA (left side)	0.014	0.954	0.328	0.158	0.038	0.873
MD (left side)	-0.031	0.898	0.122	.610	-0.045	0.851
FA (right side)	0.201	0.397	-0.164	0.490	-0.062	0.794
MD (right side)	-0.004	0.986	-0.063	0.791	0.035	0.883
CC						
FA	-0.357	0.123	-0.245	0.298	-0.064	0.790
MD	-0.372	0.106	-0.037	0.878	-0.098	0.681

r: Spearman correlation coefficient, *statistically significant

Table (8): correlation between GFAP & clinical and DTI Parameters

RRMS group had statistically significant negative correlation between GFAP and MD at left side assessed for parietal lobe (r= -0.492, p=0.027). For Patient in transition group; a statistically significant positive correlation was detected between GFAP and age of the studied cases (r=0.470, p=0.036), also statistically significant negative correlation is detected between GFAP & FA at right side assessed for frontal lobe (r=-0.596, p=0.006). FOR SPMS group; a statistically significant positive correlation was detected between GFAP and EDSS (r=0.575, p=0.008), also statistically significant negative correlation is detected between GFAP & MD at right side assessed for temporal lobe (r=-0.552, p=0.012).

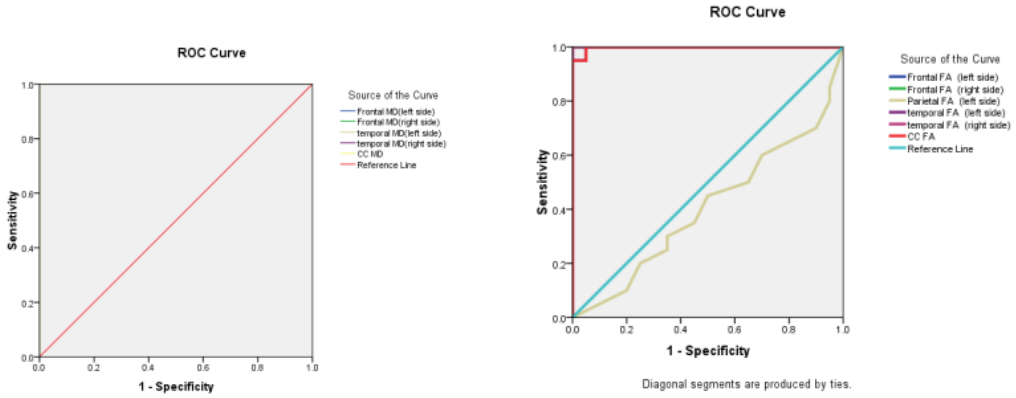

Figure (3): ROC curve of MD in differentiating RRMS & Patient in transition group, FA in differentiating RRMS & Patient in transition group

Table (10) Area under roc curve for FA, MD assessed for frontal lobe in differentiating between RRMS & Patient in transition group is excellent ranging from 0.998 to 1, the best detected cut off points for FA (left side), MD (left side), FA (right side) &MD (right side) are 0.484 with sensitivity 100% & specificity 95%, 0.865 with sensitivity 95.0% & specificity 100%, 0.506 with sensitivity 100% & specificity 100% & 0.863 with sensitivity 90% & specificity 100%.

Area under roc curve for FA, MD assessed for frontal lobe in differentiating between RRMS & Patient in transition group is excellent 1.0, the best detected cut off points for FA (left side), MD (left side), FA (right side) &MD (right side) are 0.484 with sensitivity 100% & specificity 100%, 0.863 with sensitivity 95.0% & specificity 100%, 0.528with sensitivity 100% & specificity 100% & 0.927 with sensitivity 100% & specificity 90%.

Area under roc curve for FA, MD assessed for corpus callosum in differentiating between RRMS & Patient in transition group is excellent ranging from 0.998 to 1, the best detected cut off points for FA &MD are 0.484 with sensitivity 100% & specificity 95% and 0.860 with sensitivity 95.0% & specificity 100%.

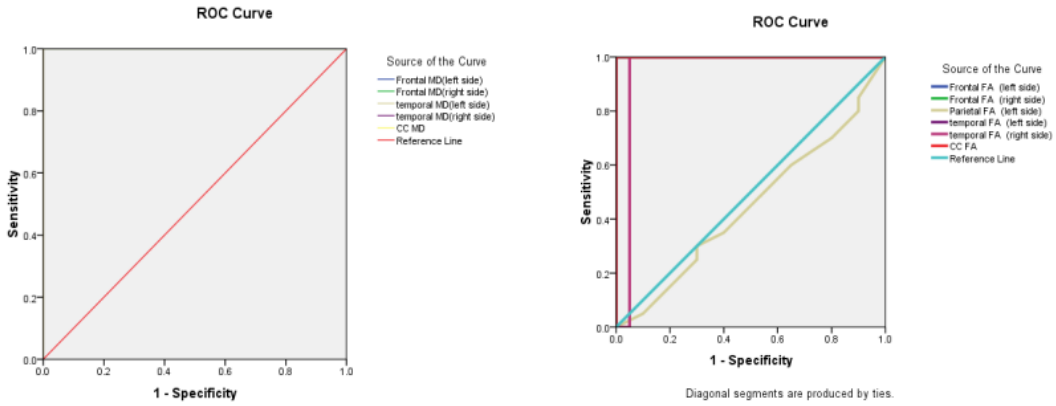

Figure (5): ROC curve of MD in differentiating between RRMS &SPMS, MD in differentiating between RRMS &SPMS

Table (11) Area under roc curve for FA, MD assessed for frontal lobe in differentiating between RRMS & SPMS is excellent 1.0, the best detected cut off points for FA (left side), MD (left side), FA (right side) &MD (right side) are 0.429 with sensitivity 100% & specificity 100%, 1.007 with sensitivity 100.0% & specificity 100%, 0.436 with sensitivity 100% & specificity 100% & 1.03 with sensitivity 100% & specificity 100%.

Area under roc curve for FA, MD assessed for temporal lobe in differentiating between RRMS & SPMS group is excellent ranging from 0.950 to 1.0, the best detected cut off points for FA (left side), MD (left side), FA (right side) &MD (right side) are 0.426 with sensitivity 100% & specificity 100%, 1.007 with sensitivity 100.0% & specificity 100%, 0.466 with sensitivity 100% & specificity 95% & 1.05 with sensitivity 100% & specificity 90%.

Area under roc curve for FA, MD assessed for corpus callosum in differentiating between RRMS & SPMS group is excellent 1.0, the best detected cut off points for FA &MD are 0.429 with sensitivity 100% & specificity 100% and 1.03 with sensitivity 100.0% & specificity 100%.

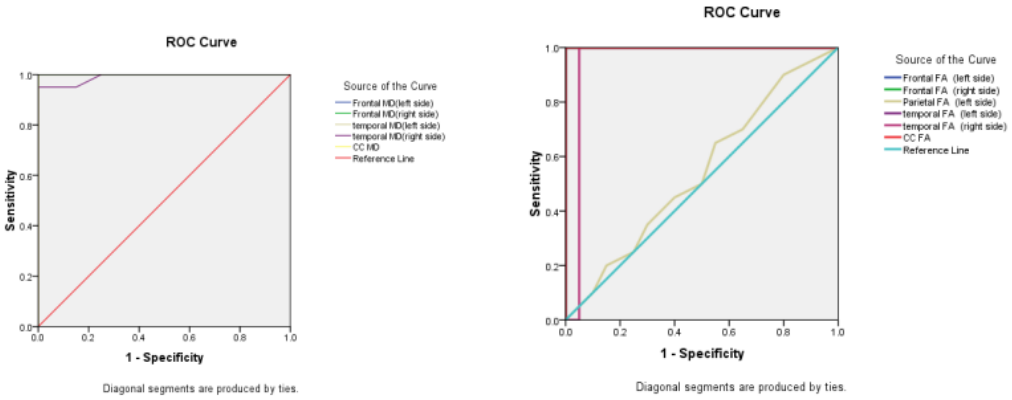

Figure (7): ROC curve of MD in differentiating SPMS from patient in transition groups, MD in differentiating SPMS from patient in transition groups

Table (12) Area under roc curve for FA, MD assessed for frontal lobe in differentiating between SPMS from patient in transition groups is excellent 1.0, the best detected cut off points for FA (left side), MD (left side), FA (right side) &MD (right side) are 0.375 with sensitivity 100% & specificity 100%, 0.985 with sensitivity 95.0% & specificity 100%, 0.399 with sensitivity 95% & specificity 100% & 0.982 with sensitivity 100% & specificity 100%.

Area under roc curve for FA, MD assessed for temporal lobe in differentiating between SPMS from patient in transition groups is excellent ranging from 0.950 to 1.0, the best detected cut off points for FA (left side), MD (left side), FA (right side) &MD (right side) are 0.373 with sensitivity 100% & specificity 100%, 1.06 with sensitivity 100.0% & specificity 100%, 0.385 with sensitivity 100% & specificity 95% & 0.979 with sensitivity 85% & specificity 90%.

Area under roc curve for FA, MD assessed for corpus callosum in differentiating between SPMS from patient in transition groups is excellent 1.0, the best detected cut off points for FA &MD are 0.375 with sensitivity 100% & specificity 100% and 0.979 with sensitivity 100.0% & specificity 100%.

Table (13): binary logistic regression for predictors of studied cases

	β	P value	Odds ratio (95%CI)
GFAP	19.99	0.003*	48.37(11.29-150.6)
Left frontal FA	-40.79	0.003*	Undefined

Left frontal MD	1077.58	0.976	Undefined
Right frontal FA	-598.62	0.984	undefined
Right frontal MD	88.41	0.994	undefined
Lt parietal MD	-29.467	.999	Undefined
Rt parietal FA	23.934	1.000	undefined
Rt parietal MD	-204.550	.998	undefined
Lt temporal FA	-10.978	1.000	Undefined
Lt temporal MD	134.072	.999	undefined
Rt temporal FA	-148.399	.999	undefined
Rt temporal MD	73.863	.999	Undefined
LT occipital FA	-1.867	.912	.155 (Undefined)
LT occipital MD	-6.358	.672	.002(Undefined)
RT occipital FA	-31.154	.265	.000(Undefined)
RT occipital MD	-4.942	.812	.007(Undefined)
CC.FA	-57.094	.036*	.00001(0.001-0.023)
CC.MD	6.201	.5[illegible]	493.262(Undefined)

Overall % predicted=97.5%

Binary logistic regression for predictors of studied cases demonstrates that increase in GFAP, decrease in left frontal FA, decrease in right occipital MD are statistically significant predictors of cases with the overall percent predicted is 97.5%. Table (13)

DISCUSSION

Multiple sclerosis (MS) is a chronic, inflammatory, disease affecting brain and spinal cord and involve two processes, demyelination and neurodegeneration, MS is a multifactorial, immunological disease that is caused by complex interaction between genetic and environmental factors.[9] According to of MS, most of RRMS patients progress to SPMS after about 10 to 20 years but, the speed of Progression is widely variable [10], and a small percentage of patients (i.e., referred to as 'benign MS' will never progress to SPMS.**[11]**

Many patients face a period of uncertainty when it comes to diagnosing the shift from relapsing-remitting multiple sclerosis (RRMS) to secondary progressive multiple sclerosis (SPMS), which can extend for as long as three years. In numerous instances, individuals with RRMS experience ongoing deterioration in their condition over the long term, even in the absence of relapses. These patients experience a gradual accumulation of progression over time during the early stages of RRMS, yet they are not officially diagnosed with SPMS.**[12]**

In multiple sclerosis (MS), the neurodegenerative processes begin early in the relapsing-remitting phase (RRMS) of the disease. However, these processes remain inactive or "silent" until the compensatory mechanisms of the central nervous system (CNS) become depleted, which is often associated with the concept of "brain reserve."**.[13]** Likewise, no particular symptoms were identified as distinctive markers for the various stages of the disease. However, it was noted that the frequency, duration, and overall impact of symptoms on quality of life were more pronounced during the phase of secondary progressive multiple sclerosis (SPMS)**.[14]**

Neurodegeneration is considered the key characteristic of the progression of multiple sclerosis (MS) and usually causes sever irreversible axonal damage, atrophy, and the formation of scar tissue in chronic lesions. Glial fibrillary acidic protein (GFAP), an important intermediate cytoskeletal protein found in astrocytes, is recognized as the structural basis for astrogliosis and is the primary protein component in chronic MS lesions. Our research findings support this notion by demonstrating heightened levels of GFAP in the blood serum of patients experiencing disease progression, whether they have secondary progressive MS (SPMS) or are in a transitional phase, in comparison to patients with relapsing-remitting MS (RRMS) and the control group.[15]

In a 2023 study led by Xiaotong Jiang, the clinical significance of serum glial fibrillary acidic protein (sGFAP) levels as a potential biomarker for disability progression in multiple sclerosis (MS) was investigated. The study focused specifically on the secondary progressive MS (SPMS) phenotype in the absence of acute inflammation. The research involved a retrospective analysis of longitudinal data obtained from the Phase 3 ASCEND trial. The trial included participants with SPMS (n = 264) who did not experience relapse or show signs of inflammatory activity on MRI at the beginning of the study and throughout its duration. Prognostic and dynamic analyses were conducted using various measurements, including sGFAP, serum neurofilament (sNfL), T2 lesion volume, Expanded Disability Status Scale (EDSS), Timed 25-Foot Walk (T25FW), 9-Hole Peg Test (9HPT), and composite confirmed disability progression (CDP). Linear and logistic regressions, along with generalized estimating equations, were employed for these analyses.[16]

The findings of the study indicated a significant link between the initial levels of sGFAP concentration, the concentrations of sNfL, and the volume of T2 lesions. However, when

examining the changes in EDSS, T25FW, 9HPT, or CDP, either no correlations or weak correlations were observed with sGFAP concentration. This implies that serum GFAP may serve as an indicator of silent progression, which is not yet clinically evident.

DTI (diffusion tensor imaging) has been extensively studied in the context of multiple sclerosis (MS), and most of these studies have reported positive results regarding the ability of DTI to detect microstructural changes in normal-appearing white matter (NAWM).

Supporting our findings, a study conducted by A. Pokryszko-Dragan et al. in 2018 investigated 50 patients with relapsing-remitting MS (37 women, 13 men, average age 36.4 years) and 27 controls matched for age and sex. The study employed DTI to measure fractional anisotropy (FA) and apparent diffusion coefficient (ADC) values in the corpus callosum (CC), thalami (TH), and middle cerebellar peduncles (MCP). Disability measures such as Expanded Disability Status Scale (EDSS), MS Functional Composite (MSFC), Symbol Digit Modalities Test (SDMT), and Fatigue Severity Scale (FSS) were utilized to assess the participants. The study compared DTI indices between MS patients and controls, as well as examined correlations between these indices and disability measures within the MS group. The results demonstrated a significant reduction in fractional anisotropy (FA) and an increase in apparent diffusion coefficient (ADC) in the corpus callosum (CC) and thalami (TH) of MS patients compared to controls. DTI indices within the CC and TH were significantly correlated with the Symbol Digit Modalities Test (SDMT) score, while within the TH and middle cerebellar peduncles (MCP), they were correlated with the Manual Dexterity measure of the Multiple Sclerosis Functional Composite (MSFC). These findings are consistent with previous research that focused on the corpus callosum and used DTI, which also reported positive outcomes. Although the earlier study specifically examined relapsing-remitting MS (RRMS), it indirectly demonstrated the potential of DTI in detecting disease progression, as indicated by the significant correlation between DTI indices within the CC and TH and the SDMT score, a commonly used measure for assessing progression.[17]

Multiple studies were done to test the ability of serum GFAP and DTI in diagnosis and assessment of MS and as a simple lab test, GFAP, the correlation between serum GFAP level and DTI changes in different MS phenotypes has been studied, but to our knowledge only few studies have focused on this correlation.

In 2021, a study conducted by Maija Saraste and colleagues aimed to investigate the relationship between increased levels of serum glial fibrillary acidic protein (GFAP) and pathological factors in the normal-appearing white matter (NAWM) of multiple sclerosis (MS) patients using diffusion tensor imaging (DTI). The study involved 62 MS patients with a median age of 49.2 years. DTI-MRI scans were performed to measure various DTI parameters, such as mean fractional anisotropy (FA), mean diffusivity (MD), axial diffusivity (AD), and radial diffusivity (RD), in both the entire NAWM and segmented NAWM regions. Blood samples were also analyzed for GFAP levels. The study found a significant correlation between elevated serum GFAP levels and decreased FA values in the overall NAWM, as well as in the frontal, temporal, and cingulate NAWM regions. Additionally, higher MD and RD were observed in the frontal NAWM. Multiple regression analysis, accounting for sex and disease-modifying treatment, confirmed that increased GFAP levels were associated with lower FA values specifically in the frontal and cingulate NAWM regions. These findings suggest a connection between elevated GFAP levels in MS patients and changes in DTI parameters, characterized by reduced FA and increased MD and RD, particularly in the frontal and cingulate NAWM regions.[8]

CONCLUSION

Serum GFAP in addition to DTI measurable microdamage in NAWM can give us wide scope of view about potential progression in MS pathology and related astrocytopathy.

Ethics approval and consent to participate:

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. The study was accepted by IRB, Faculty of Medicine, Mansoura University under the code number of MS.22.01.1843.

An informed written consent was taken from each participant involved in this study prior to the conduct of any study-related activities.

All data obtained from participants were confidential and were not used outside the study. The patients had the rights to withdraw from the study at any time without giving any reason.

Consent for publication: Not applicable

Availability of data and material:

The datasets generated and or analyzed during the current study are not publicly available due to current Mansoura University regulations and Egyptian legislation but are available from the corresponding author on reasonable request and after institutional approval.

Competing interest:

All Authors declare that they have no conflict of interest.

Funding: This study was this research was supported by the Department of Neurology, Faculty of Medicine at Mansoura University.

All authors have read and approved the final manuscript.

Acknowledgement: The authors acknowledge subjects for their participation and cooperation in this study.

References

1. Wallin, M.T., et al., *The prevalence of MS in the United States: a population-based estimate using health claims data.* Neurology, 2019. **92**(10): p. e1029-e1040.
2. Housley, W.J., D. Pitt, and D.A. Hafler, *Biomarkers in multiple sclerosis.* Clinical immunology, 2015. **161**(1): p. 51-58.
3. Frohman, E.M., M.K. Racke, and C.S. Raine, *Multiple sclerosis—the plaque and its pathogenesis.* New England Journal of Medicine, 2006. **354**(9): p. 942-955.
4. Lassmann, H., J. Van Horssen, and D. Mahad, *Progressive multiple sclerosis: pathology and pathogenesis.* Nature Reviews Neurology, 2012. **8**(11): p. 647-656.
5. Temel, Ş., et al., *Diffusion tensor magnetic resonance imaging in patients with multiple sclerosis and its relationship with disability.* The neuroradiology journal, 2013. **26**(1): p. 3-17.
6. Herbert, E., et al., *Fractional anisotropy of white matter, disability and blood iron parameters in multiple sclerosis.* Metabolic brain disease, 2018. **33**: p. 545-557.
7. Ponath, G., C. Park, and D. Pitt, *The role of astrocytes in multiple sclerosis.* Frontiers in immunology, 2018. **9**: p. 217.
8. Saraste, M., et al., *Increased serum glial fibrillary acidic protein associates with microstructural white matter damage in multiple sclerosis: GFAP and DTI.* Multiple Sclerosis and Related Disorders, 2021. **50**: p. 102810.
9. Pitt, D., et al., *Toward precision phenotyping of multiple sclerosis.* Neurology-Neuroimmunology Neuroinflammation, 2022. **9**(6).
10. Scalfari, A., et al., *The natural history of multiple sclerosis, a geographically based study 10: relapses and long-term disability.* Brain, 2010. **133**(7): p. 1914-1929.
11. Skoog, B., et al., *A representative cohort of patients with non-progressive multiple sclerosis at the age of normal life expectancy.* Brain, 2012. **135**(3): p. 900-911.

12. Cree, B.A., J. Mares, and H.-P. Hartung, *Current therapeutic landscape in multiple sclerosis: an evolving treatment paradigm.* Current opinion in neurology, 2019. **32**(3): p. 365-377.
13. Lassmann, H., *Pathogenic mechanisms associated with different clinical courses of multiple sclerosis.* Frontiers in immunology, 2019. **9**: p. 3116.
14. Inojosa, H., D. Schriefer, and T. Ziemssen, *Clinical outcome measures in multiple sclerosis: a review.* Autoimmunity reviews, 2020. **19**(5): p. 102512.
15. Mey, G.M., K.R. Mahajan, and T.M. DeSilva, *Neurodegeneration in multiple sclerosis.* WIREs Mechanisms of Disease, 2023. **15**(1): p. e1583.
16. Jiang, X., et al., *Glial fibrillary acidic protein and multiple sclerosis progression independent of acute inflammation.* Multiple Sclerosis Journal, 2023. **29**(9): p. 1070-1079.
17. Pokryszko-Dragan, A., et al., *Diffusion tensor imaging findings in the multiple sclerosis patients and their relationships to various aspects of disability.* Journal of the Neurological Sciences, 2018. **391**: p. 127-133.