MiR-150, MiR-155, NFL, and YKL-40 as Potential Biomarkers among Egyptian Multiple Sclerosis Patients

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Abstract:

Background and Objectives: Multiple sclerosis (MS) is a disabling neurodegenerative disease. Diagnosis of MS is often difficult, and the currently used biomarkers are not well correlated with the disease due to diverse phenotypes. So, blood-derived biomarkers that can identify and discriminate MS phenotypes and detect progression may be fortunate.

Methods: Expression levels of miR-150 and miR-155 by qRT-PCR, NFL, and YKL-40 levels by ELISA were all evaluated in the serum of 30 MS patients (23 RRMS and 7 SPMS) and 30 HCs, pairwise comparison between groups and correlation with EDSS were conducted. ROC curve analysis was carried out to examine the diagnostic and discriminative potential of the biomarkers.

Results: Levels of the assayed biomarkers were significantly increased in whole MS patients, RRMS, and SPMS patients compared to HCs with high diagnostic accuracy by ROC curve analysis. Regarding comparing RRMS vs SPMS, only the NFL showed differential levels.
Single biomarker analysis by the ROC curve showed that NFL, miR-155, and YKL-40 are potential discriminative biomarkers with the best performance for the NFL.
Combined biomarker investigation showed that adding YKL-40 alone or combined YKL-40 and miR-155 to NFL increased the specificity**.** In addition, adding miR-150 to NFL decreased the sensitivity of NFL alone but increased its specificity.
MiR-155 and NFL were correlated with EDSS in the whole MS group. MiR-150, miR-155, and YKL-40 showed a correlation in SPMS.

Conclusion: Serum biomarkers miR-150, miR-155, NFL, and YKL-40 may be potential biomarkers in MS diagnosis, discriminating MS subtypes.

Keywords: MicroRNA (miRNA), miR-150 (miRNA-150), miR-155 (miRNA-155), neurofilament light chain (NFL), chitinase 3-like-1(CHI3L1, YKL-40), biomarkers.

Running title: MiR-150, MiR-155, NFL, and YKL-40 as Potential Biomarkers for Multiple Sclerosis.

Abbreviations: **AUC**=Area under the Curve; **CHI3L1**= Chitinase3-like-1; **CI**=Confidence Interval; **CSF**=Cerebrospinal fluid; **EDSS**= Expanded disability status scale; **ELISA**= Enzyme-linked Immunosorbent Assay; **HC**= Healthy control; **MiR (MiRNA)**= MicroRNA; **MRI**= Magnetic Resonance Imaging; **MS**=Multiple sclerosis; **NFL**= Neurofilament-Light Chain; **qRT-PCR**=Quantitative reverse transcription-PCR; **R**=range; **ROC**= receiver operating characteristic; **RRMS**=Relapsing remitting multiple sclerosis; SD=standard deviation; **SPMS**= Secondary progressive multiple sclerosis; **Th**=T helper.

Introduction

Multiple sclerosis (MS) is a central nervous system autoimmune disease characterized by axonal damage, demyelination, and localized inflammation. The etiology of MS is still unknown (1). MS has significant societal and economic effects despite its comparatively modest prevalence rates. The reported prevalence of multiple sclerosis (MS) in Egypt was 14.1/100,000 in a prior meta-analysis study conducted in several referral centers (2). The course of the disease is highly variable, and the heterogeneity of its phenotype is not correlated with the biomarkers currently in use (3). Moreover, Secondary Progressive MS (SPMS) is usually diagnosed retrospectively after irreversible disability, and this can take many years in many patients (4). Therefore, it's imperative to discover novel, targeted biomarkers that can aid in differentiating between MS phenotypes, forecast the course of the disease, and provide a more precise correlation with the degree of disability (5).

MicroRNAs (miRNAs) are short, non-coding RNAs that regulate gene expression at a post-transcriptional level. They, including microRNA-150 (miR-150), are implicated in the pathophysiology of many human diseases, including autoimmune disorders such as MS. They can alter proteins related to myelination, gliosis, and neurogenesis (6, 7). One of the most studied miRNAs in the MS disease pathogenesis is MicroRNA-155 (MiR-155); it has a proinflammatory effect that disturbs the blood-brain barrier permeability, mediating demyelination and neurodegeneration (8). Neurofilament light chain (NFL) is a cytoskeletal protein of axons and neurons in the CNS and peripheral nervous system (9). A recent meta-analysis review highlights the NFL as a biomarker of neuroinflammation and brain atrophy in the progressive MS phenotype (10). Chitinase3-like-1 (CHI3L1), also known as YKL-40, belongs to the glycosyl hydrolase family of chitinases and is shown to be involved in various neurodegenerative conditions, including MS (11).

Serum miR-150, miR-155, serum NFL, and YKL-40 are potential biomarkers that have shown positive, inconclusive results in the detection of progression, severity, and transition from relapsing-remitting MS (RRMS) into SPMS (12-15). In our study, we aimed to investigate the correlation between measured biomarkers with different MS phenotypes, disability status, and prognosis.

Patients and Methods

Patients

In this case-control study, we recruited 30 MS patients (23 RRMS and 7 SPMS) from the Neurology Department, Assiut University Hospital, Assiut, Egypt, from January 2021 to March 2022, and were diagnosed according to the revised McDonald Criteria 2017 (16). Thirty age- and sex-matched healthy volunteers from the Medical Biochemistry Department, Assiut University, were included as controls. The patient underwent a detailed history taking and a complete neurological assessment, including the Expanded Disability Status Scale (EDSS) assessment and magnetic resonance imaging (MRI) of the brain and spinal cord. Patients with other neurological, malignancy, cardiac, renal, or hepatic diseases were excluded.

Ethical Approval

The study protocol conformed to the guidelines outlined in the Helsinki Declaration and was approved by the Faculty of Medicine Ethics Committee of Assiut University, Egypt (**IRB:** 17101160).

Consent to Participate

Every participant in the study gave their informed consent.

Samples

Three ml of venous blood were collected from all patients and healthy controls in a plain tube, where coagulation was allowed to happen at room temperature for 10 min, blood samples were centrifuged within 2 hours of sampling at 3000 rpm for 15 min, and serum was stored in –80°C until further use.

Analytical Methods

Real-time PCR for estimation of miRNA-150 and miRNA-155

MiRNA extraction was done using the MiRNeasy Mini kit (QIAGEN, cat. no. 217004, Germany) according to the manufacturer's protocol; all steps were performed in an RNase-free environment. The miRNA concentrations in the eluate were measured using a NanoDrop spectrophotometer (EPOCH, BioTek Instruments Inc., USA). Poly A polymerase enzyme kit (NEB, New England, cat. no. M0276S) was used to increase the poly-A tail of small non-coding miRNA. 10 µL of total RNA was used per sample for reverse transcription into single-stranded cDNA with Applied Biosystems High-Capacity cDNA Reverse Transcription Kit (cat. no. 4368814) using Thermal cycler (Biometra). Under the sterilized condition, qPCR was prepared using Thermo Scientific Maxima SYBR Green/ROX qPCR Master Mix (2X), two sets of reactions were performed in the same way except for the primers added, U6-snRNA acted as an internal control, and the sequences of the primers were as follow: miR-150 forward, 5′-GGGTCTCCCAACCCTTGTA-3′ and reverse, 5′-CAGTGCGTGTCGTGGAGT-3′ (17); miR-155 forward, 5′-CGGCGCTTAATGCTAATCGTGATAG-3′ and reverse, 5′- GTGCAGGGTCCGAGGT-3′; U6 forward, 5′-GCTTCGGCAGCACATATACTAAAAT-3′ and reverse, 5′-CGCTTCACGAATTTGCGTGTCAT-3′ (18). The primers were obtained from (Invitrogen, UK). Following the manufacturer's instructions, a 20µl reaction mix containing 3µl of the corresponding cDNA was prepared for each sample. The thermal cycler (Applied Biosystems Step One Plus™ Real-Time PCR Systems, California, USA) was programmed to perform a Two-step cycling protocol, hot start step at 95°C for 10-min, initial denaturation for 15 s at 95º C, annealing and extension for 60 s at 60º C for each, in 40 cycles. Applied Biosystems Step One PlusTM software was utilized to analyze the data after the reactions, transforming the obtained cycle threshold (CT) values into relative quantities using the Comparative Ct (ΔΔCt) method. The acquired amounts were then normalized against U6-snRNA as a housekeeping gene. Fold change was computed as $2^{-\text{delta delta Ct}}$ to obtain the relative expression.

Enzyme-linked Immunosorbent Assay (ELISA) for investigation of NFL and CHI3L1

NFL and CHI3L1 were measured in serum using a double-antibody sandwich technique in line with the guidelines provided by the manufacturer. Test kits were supplied by Shanghai Sun Red Biological Technology Co., Ltd., cat. No. 201-12-5767 for NFL kit and cat. No. 201-12-2064 for CHI3L1 kit.

Statistical Analysis

The data was analyzed with IBM-SPSS 24.0 (IBM-SPSS Inc., Chicago, IL, USA). Descriptive statistics: Calculations were made for means, standard deviations, medians, ranges, frequencies, and percentages. If applicable, the Kolmogorov-Smirnov or Shapiro-Wilk tests were used to determine whether continuous variables were normal. Test of significance: The chi-square/Fisher's exact test was employed to assess the extent of variation in the frequency distribution across several groups. Student t-test / Mann-Whitney U analysis was used to compare the means/medians of dichotomous data. The ANOVA test was calculated to test the mean differences of data that follow normal distribution for continuous variables with more than two categories. The median difference between non-normally distributed groups was compared using an independent sample Kruskal-Wallis test, and a post-hoc test was calculated with Bonferroni corrections. The correlation between the variables was analyzed using correlation analysis (Spearman's rank correlation). ROC curve was provided to investigate the various markers′ diagnostic performance and their combination for MS disease prediction, analyzed as the area under the curve (AUC), standard error (SE), and 95% CI. A

significant p-value was considered when it was <0.05.

Results

Demographic and Clinical Data of the participants

Our study included 30 MS patients and 30 healthy controls. In the MS group, 23 had RRMS (76.7%) with a mean age (30.96 ± 8.1) years, and 7 had SPMS (23.3) with a mean age (35.86 ± 9.8) years. Most of the recruited participants were females. **Table 1** summarizes the research groups' overall demographic and clinical characteristics.

Table 1: Demographic and clinical characteristics of patients and healthy controls

	RRMS (n = 23)	SPMS (n = 7)	HC (n = 30)	P-value
Age/year				
• Mean ± SD	30.96 ± 8.1	35.86 ± 9.8	32.10 ± 8.1	= 0.415* NS
• Median (R)	30 (14-48)	35 (22-55)	32 (17-55)	
P-value**	RR vs. HC =0.629	SP vs. HC =0.296	RR vs. SP =0.186	
Sex				
• Female	15 (65.2%)	6 (85.7%)	21 (70%)	= 0.585*** NS
• Male	8 (34.8%)	1 (14.3%)	9 (30%)	
Disease Duration/months				
• Mean ± SD	25.16 ± 5.9	45.34 ± 8.5		= 0.238# NS
• Median (R)	12 (0-156)	12 (6-132)		
EDSS				
• Mean ± SD	2.65 ± 0.7	4.57 ± 0.3		< 0.001$
• Median (R)	2.5 (1-5)	4.5 (4-5)		

RRMS=relapsing remitting multiple sclerosis, SPMS=secondary progressive multiple sclerosis, HC=healthy control, SD=standard deviation, R= range, EDSS=expanded disability status scale, NS=not significant
*One-way ANOVA test was used to compare the difference in Mean between groups
**Post-hoc test was used for pairwise comparison using Tukey's Correction
*** The Chi-square test was used to compare the proportion difference between groups
# Independent Sample t-test was used to compare the difference in Mean between groups
$Mann-Whitney U test was used to compare the difference in Median between groups

Expression patterns of miRNAs and levels of sNFL and sYKL-40 among the studied groups (**Table 2**)

The medians of miR-150 and miR-155 expression levels in serum were significantly higher in whole MS patients and in RRMS and SPMS compared to HC. No significant difference was detected in serum expression of miR-150 or miR-155 between RRMS and SPMS.

Median sNFL concentration was significantly higher in MS patients and in RRMS and SPMS than in HC. A significantly higher level of sNFL median concentration was observed in SPMS compared to RRMS ($p < 0.001$).

The median YKL-40 concentration was significantly higher in MS patients and in RRMS and SPMS than in HC. However, no significant difference was detected between the two subtypes.

Table 2: Levels of measured biomarkers in different groups

	MS (n = 30)	RRMS (n = 23)	SPMS (n = 7)	HC (n = 30)	P-value
Serum miR-150 Fold Change					
Mean ± SD	10.45 ± 7.3	9.81 ± 11.9	12.56 ± 16.2	1.11 ± 0.5	
Median (R)	5 (2-51.5)	5.7 (2.1-51.6)	3.9 (2.6-42.8)	1.1 (0.2-2.2)	
P-value**	**MS vs. HC <0.001**	**RR vs. HC <0.001**	**SP vs. HC =0.002**	RR vs. SP =0.445	**< 0.001***
Serum miR-155 Fold Change					
Mean ± SD	15.75 ± 11.7	13.29 ± 12.1	23.86 ± 21.9	1.11 ± 0.5	
Median (R)	10 (2.5-76.5)	7.4 (2.4-44.3)	12.5 (5.5-76.6)	1.0 (0.3-2.1)	
P-value**	**MS vs. HC <0.001**	**RR vs. HC <0.001**	**SP vs. HC <0.001**	RR vs. SP =0.511	**< 0.001***
sNFL Concentration (ng/L)					
Mean ± SD	45.04 ± 14.6	33.58 ± 11.1	82.70 ± 18.7	17.53 ± 2.4	
Median (R)	33.5 (22-135)	33 (22-67)	101 (31-135)	19 (8-36)	

P-value**	**MS vs. HC <0.001**	**RR vs. HC =0.002**	**SP vs. HC <0.001**	**RR vs. SP <0.001**	**< 0.001***
sYKL-40 Concentration (ng/ml)					
Mean ± SD	75.73 ± 20.1	74.01 ± 12.3	81.43 ± 15.7	38.96 ± 13.4	
Median (R)	69 (51-172)	68 (51-165)	71 (54-172)	44 (6-55.5)	
P-value**	**MS vs. HC <0.001**	**RR vs. HC <0.001**	**SP vs. HC <0.001**	RR vs. SP =0.468	**< 0.001***

MS=multiple sclerosis, RRMS=relapsing remitting multiple sclerosis, SPMS=secondary progressive multiple sclerosis, miR= microRNA, sNFL=serum neurofilament light chain, SD=standard deviation, R=range.
***Kruskal-Wallis test was used to compare the median difference between groups**
****Post-hoc test was used for pairwise comparison using Tukey's Correction**

Correlation of measured biomarkers with EDSS and disease duration among patient groups (Table 3, Figure 1,2)

The MS group had a significant moderate positive correlation between EDSS and NFL and miR-155 levels. MiR-150, miR-155, and YKL-40 also significantly positively correlated with EDSS in the SPMS group.

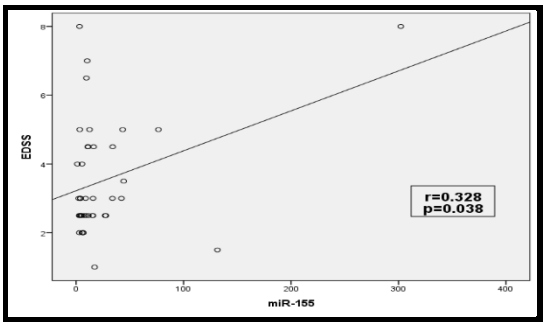
Regarding disease duration, YKL-40 showed a negative correlation in the RRMS group, and miR-155 showed a positive correlation in the SPMS group.

Table 3: Correlation of measured biomarkers with disease duration and EDSS among patient groups:

	miRNA-150	**miRNA-155**	**NFL Conc.**	**YKL-40 Conc.**
	rho* (p-value)			
MS Group				
• **DD/months**	-0.091 (=0.317)	0.222 (=0.119)	-0.125 (=0.255)	-0.199 (=0.146)
• **EDDS**	0.095 (=0.308)	**0.328 (=0.038)**	**0.475 (=0.004)**	0.097 (=0.304)
RRMS Group				
• **DD/months**	-0.015 (=0.472)	0.008 (=0.468)	-0.322 (=0.067)	**-0.365 (=0.048)**
• **EDDS**	0.119 (=0.119)	0.147 (=0.252)	-0.184 (=0.200)	-0.023 (=0.417)
SPMS Group				
• **DD/months**	0.296 (=0.219)	**0.630 (=0.039)**	-0.259 (=0.278)	0.445 (=0.159)
• **EDDS**	**0.458 (=0.049)**	**0.657 (=0.041)**	-0.279 (=0.272)	**0.483 (=0.045)**

DD= Disease duration, EDSS= expanded disability status scale, MS= multiple sclerosis, RRMS=relapsing remitting multiple sclerosis, SPMS=secondary progressive multiple sclerosis, sNFL=serum neurofilament light chain, miR=microRNA
***Spearman's ranked correlation**

A



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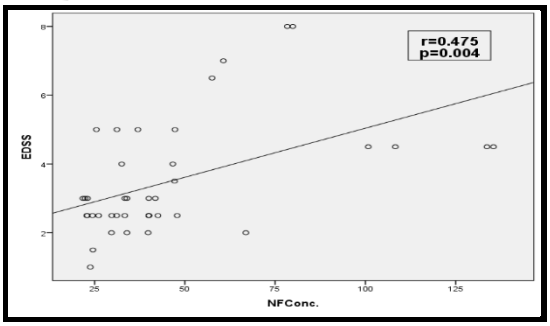

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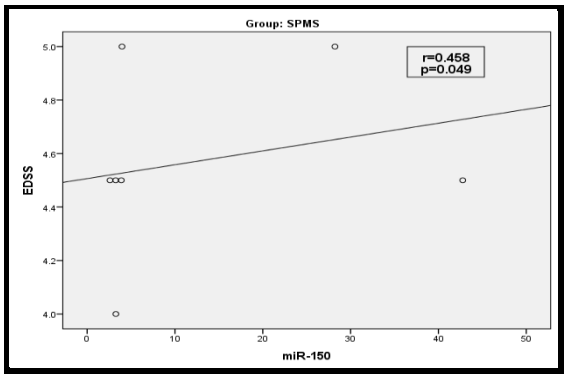

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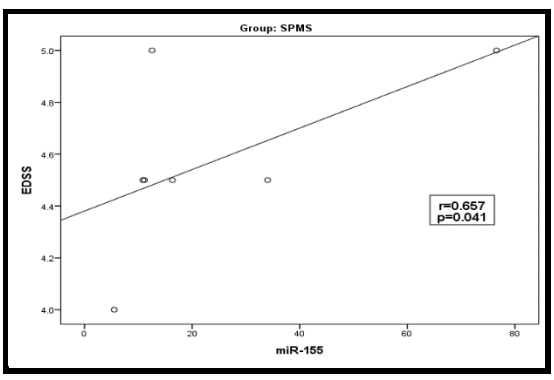

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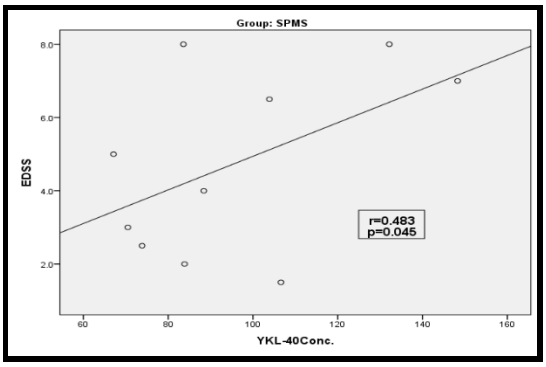

Figure 1: Correlation of measured biomarkers with EDSS among patient groups:

Figure 1: A. Correlation between miR-155 and EDSS (MS), B. Correlation between NFL and EDSS (MS), C. Correlation between miR-150 and EDSS (SPMS), D. Correlation between miR-155 and EDSS (SPMS), E. Correlation between YKL-40 and EDSS (SPMS)
miR=microRNA, EDSS= expanded disability status scale, MS= multiple sclerosis, sNFL=serum neurofilament light chain SPMS=secondary progressive multiple sclerosis

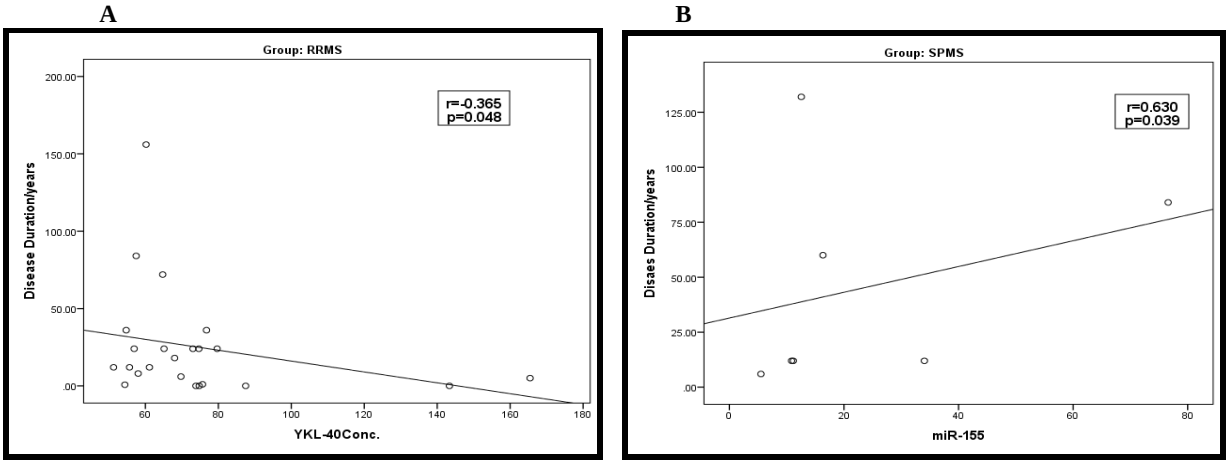

Figure 2: Correlation of measured biomarkers with disease duration among patient groups

Figure 2: A. Correlation between YKL-40 and DD (RRMS), B. Correlation between miRNA-155 and DD (SPMS).
miR=microRNA, ,RRMS= relapsing remitting multiple sclerosis, SPMS=secondary progressive multiple sclerosis

Diagnostic role of the four measured biomarkers

To assess the diagnostic role of our measured biomarkers, we conducted an ROC curve, which showed that the four biomarkers had good diagnostic accuracy for predicting MS and each of its two subtypes, RRMS and SPMS, from healthy controls, as shown in Table 4.

Table 4: Performance of biomarker expression levels in diagnosing patient groups

Biomarker	AUC (95%CI)	Sensitivity	Specificity	P-value
MS vs. HC				
miR-150	0.927 (0.860 -0.994)	97%	90%	< 0.001
miR-155	0.869 (0.778 - 0.961)	100%	78%	< 0.001
sNFL	0.775 (0.665 - 0.884)	80%	70%	< 0.001
sYKL-40	0.791 (0.679 - 0.904)	87%	75%	< 0.001
RRMS vs HC				
miR-150	0.997 (0.989-1.000)	100%	93%	< 0.001
miR-155	1.000 (1.000-1.000)	100%	100%	< 0.001
sNFL	0.914 (0.841-0.988)	100%	70%	< 0.001
sYKL-40	0.986 (0.962-1.000)	96%	96%	< 0.001
SPMS vs. HC				

miR-150	1.000 (1.000-1.000)	100%	100%	< 0.001
miR-155	1.000 (1.000-1.000)	100%	100%	< 0.001
sNFL	0.981 (0.944-1.000)	100%	93%	< 0.001
sYKL-40	0.983 (0.945-1.000)	100%	84%	< 0.001

AUC=Area under the Curve, CI=Confidence Interval, sNFL=serum neurofilament light chain, miR= microRNA, RRMS=relapsing remitting multiple sclerosis, SPMS=secondary progressive multiple sclerosis, HC=healthy control.

Diagnostic role of the assayed biomarkers in distinguishing MS subtypes

We conducted another ROC curve to assess the four biomarkers' possible role in differentiating the two MS subtypes. MiR-155, NFL, and YKL-40 could distinguish between RRMS and SPMS. However, the NFL appeared to be the best-performing single biomarker for distinguishing, with an AUC of 0.801, a sensitivity of 86%, and a specificity of 66%. Combined NFL and YKL-40 showed promise with an AUC of 0.807, 86% sensitivity, and 74% specificity. The combination of miR-155/NFL/YKL-40 also performed well, with an AUC of 0.820 and 86% sensitivity and 79% specificity (Table 5, Figure 3).

Table 5: Performance of biomarker expression levels in distinguishing patient groups

Biomarker	AUC (95%CI)	Sensitivity	Specificity	P-value
RRMS vs SPMS				
• miR-150	0.384 (0.198 -0.668)	38%	67%	= 0.807
• miR-155	0.669 (0.502-0.996)	71%	55%	= 0.039
• NFL	0.801 (0.605-0.997)	86%	66%	= 0.017
• YKL-40	0.766 (0.551-0.901)	78%	65%	= 0.022
• miR-150/155	0.596 (0.344-0.849)	72%	52%	= 0.464
• miR-150/ NFL	0.814 (0.608-1.000)	72%	87%	= 0.028
• miR-150/YKL-40	0.571 (0.307-0.876)	72%	48%	= 0.544
• miR-155/ NFL	0.857 (0.670-1.000)	86%	66%	= 0.022
• miR-155/ YKL-40	0.640 (0.389-0.981)	72%	69%	= 0.552
• NFL/YKL-40	0.807 (0.636-0.979)	86%	74%	= 0.044
• miR-150/155/NFL	0.826 (0.609-1.000)	86%	61%	= 0.027
• miR-150/155/YKL-40	0.590 (0.320-0.860)	72%	44%	= 0.746
• miR-150/NFL/YKL-40	0.801 (0.611-0.991)	72%	87%	= 0.040
• miR-155/NFL/YKL-40	0.820 (0.622-1.000)	86%	79%	= 0.034
• miR-155/150/NFL/YKL-40	0.783 (0.656-1.000)	72%	91%	= 0.048

AUC=Area under the Curve, CI=Confidence Interval, sNFL=serum neurofilament light chain, miR= microRNA, RRMS=relapsing remitting multiple sclerosis, SPMS=secondary progressive multiple sclerosis.

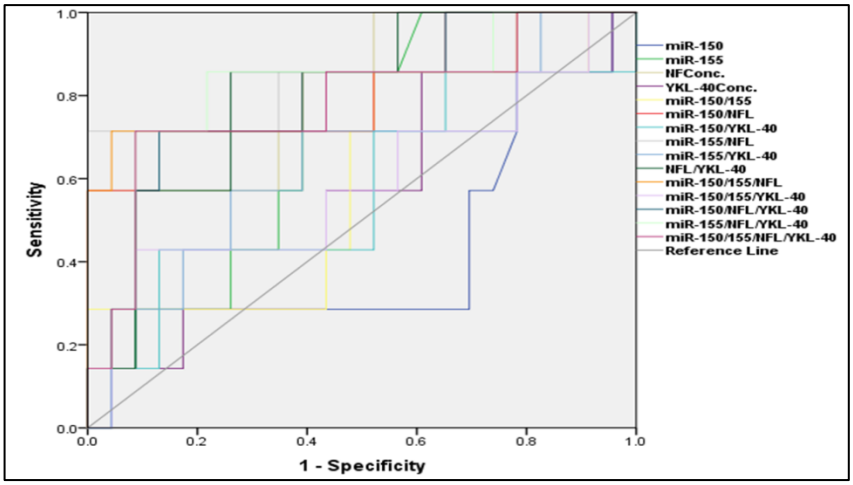

Figure 3: ROC curve for biomarkers for RRMS vs SPMS prediction
sNFL=serum neurofilament light chain, miR= microRNA

Discussion

MS is a chronic autoimmune disease characterized by immune cell infiltration, inflammation, and neurodegeneration and is among the most frequent contributors to young adults′ non-traumatic neurologic impairment (19). Blood-derived biomarkers that can identify and discriminate MS phenotypes, allowing well-timed detection of progressive aspects in the MS continuum disease, find an early fanlight opportunity for efficacious treatment to modify the disease course (4, 20).

Our results regarding miR-150 and miR-155 showed significantly higher expression levels in the MS group. In RRMS and SPMS subtypes vs HC, and by ROC curve analysis, they showed high discriminative power in detecting MS patients and their two subtypes, RRMS and SPMS. That was concordant with many studies that have found that miR-150 and miR-155 were upregulated in the plasma and serum of MS patients, especially during relapse (21-23). De Candia et al. also displayed increased expression of miR-150 in the serum and exosomes from activated lymphocytes, suggesting miR-150 as a sensor for adaptive immune activation (24). It is believed to control gene expression during the immunological response and immune cell differentiation processes. Proinflammatory reactions may be generated and/or amplified due to dysregulation of miR-150 expression (25). MiR-155 enhances Th17 and Th1 differentiation by producing excessive amounts of cytokines, prolonging the inflammatory response, and exacerbating the EAE models' clinical symptoms (26). Shademan et al. showed significantly higher expression of miR-155 in each of RRMS and SPMS vs HC. Their ROC curve analysis evaluating the diagnostic potential of miR-155 demonstrated an AUC value of 0.79 (27).

Although miR-150, miR-155, and YKL-40 showed no differential expression between RRMS and SPMS, which could be due to the small sample size, our ROC curve results showed that miR-155 can be used to distinguish both RRMS and SPMS subtypes with AUC values of 0.669, sensitivity of 71%, and specificity of 55%. Also, adding miR-155 and YKL-40 combined to NFL increased the specificity of NFL, thus possibly decreasing false positive cases more than adding YKL-40 alone. In addition, adding miR-150 to NFL decreased the sensitivity of NFL alone but increased its specificity, indicating a potential role of miR-150 in discriminating RRMS from SPMS. A study by Wesam Sharaf Eldin showed that miR-155, single or combined with miR-23a and miR-575, couldn't differentiate RRMS from SPMS (28). In our study, miR-155 showed a positive correlation with EDSS score in MS patients ($r=0.328$, $p=0.038$), and both miR-150 and miR-155 showed a positive correlation with EDSS among SPMS patients ($r=0.458$, $p=0.049$; $r=0.657$, $p=0.041$). In accordance with us, Elkhodiry et al. and Saridas et al. demonstrated a significant positive correlation between serum expression of miR-155 and EDSS in MS patients (29, 30).

Our results, furthermore, demonstrated a correlation of the inflammatory miR-155 with disease duration in SPMS, suggesting, with other studies, ongoing neuroinflammation with increased disease duration in MS disease and progression to SPMS (32 ,31)

Our results also demonstrated significantly higher serum NFL levels in the MS group and RRMS and SPMS vs HC. Axonal damage is a hallmark feature of MS disease that correlates with disability (33). During axonal damage, neurofilaments are released into the cerebrospinal fluid (CSF) and finally into the blood (34). ROC curve analysis showed high discriminative power for NFL in distinguishing MS and its two subtypes from healthy controls. Moreover, serum NFL was the only marker that showed significant differential levels between both MS groups, and single biomarker analysis by ROC curve demonstrated NFL's best discriminative power among assayed biomarkers individually in separating apart RRMS from SPMS with an AUC value of 0.801, sensitivity 86%, and specificity 66%. Our

results also showed a significant correlation of sNFL with EDSS score in the MS group (r= 0.475, p=0.004), which agreed with several studies (35-37). Many studies also demonstrated elevated NFL levels in MS relapses (38, 39) and showed a correlation to disease progression and disability (15). The findings related to the association of blood NFL with EDSS are conflicting. Cantó and colleagues reported a significant increase in blood NFL level with an increase in EDSS (40), while Anderson et al. didn't find a significant association between EDSS and blood NFL (41).

Our study found a significant difference comparing serum YKL-40 levels in MS patients and MS subtypes RRMS and SPMS compared to HC. ROC curve analysis showed high discriminative power for YKL-40 in identifying whole MS, RRMS, and SPMS patients with AUC values of 0.791, 0.986, and 0.983, respectively. Studies of YKL-40 in the peripheral blood of MS patients are scarce (42). Hinsinger and his colleagues demonstrated an AUC value for serum CHI3L1 of 0.95 for each RRMS and PMS compared to healthy controls, which is very close to our findings (43). CHI3L1 expression in MS is ascribed to reactive astrocytes, the chief contribution, and primed microglial /macrophage cells (44). A study by Matute-Blanch and his colleagues demonstrated increased serum CHI3L1 levels in RRMS patients who were nonresponders to interferon-beta (INF-β) treatment (42). However, a meta-analysis by Floro and his colleagues, in contrast with our findings, demonstrated no significant difference in the serum CHI3L1 levels comparing RRMS to HC in two studies (45), attributing this to the principal production of CHI3L1 intrathecally in CNS inflammations (11). Furthermore, our study demonstrated higher YKL-40 expression levels in SPMS compared to RRMS, but the difference was insignificant. However, ROC curve analysis indicated discriminative power for YKL-40 in distinguishing RRMS from SPMS with an AUC value of 0.766, sensitivity of 78%, and specificity of 65%. Moreover, our results showed that combining YKL-40 with NFL increased the specificity of NFL in distinguishing RRMS from SPMS and is better than using YKL-40 or NFL alone. Gil-Perotin et al. (2019 demonstrated that assessing CHI3L1 and NFL helps differentiate between MS phenotypes and anticipate clinical progression in RRMS patients (46). Cubas-Nufiez et al. reported CHI3L1 to be involved in MS progression; they explained that variation in CHI3L1 levels between RRMS and progressive MS could be attributed to the location of the CNS lesions and the variability in blood-brain barrier permeability (47).

Our study also demonstrated a correlation between YKL-40 levels and EDSS among SPMS patients. Concordant with our findings, Perez-Miralles and his colleagues proved that higher CHI3L1 levels are associated with higher EDSS and neurological disability in MS patients (48).

Our correlation analysis also demonstrated a negative correlation of YKL-40 with disease duration in RRMS, which may be due to reduced overall inflammatory burden owing to decreased relapse rate with the increase in disease duration in RRMS or owing to treatments (49, 50).

Our findings on the potential of those markers as multiple sclerosis biomarkers need further studies with larger cohorts to be validated.

Study Limitations

The small sample size of the SPMS group, which is attributed to the SPMS subtype of MS, is rare compared to RRMS, and the recruitment of participants was limited to a single center; hence, additional multicenter research with larger sample sizes is necessary. Longitudinal studies that involve monitoring biomarker changes over time and correlating them with MRI findings, in addition to the EDSS score to assess the predictive value of the biomarkers for disease progression and response to treatment, are also recommended.

Conclusion

Our preliminary study suggested that serum miR-150, miR-155, NFL, and

CHI3L1 have the potential to be MS biomarkers, and they could serve as non-invasive tools for diagnosing, monitoring, and predicting disease progression in MS. This could lead to earlier and accurate diagnosis, more personalized treatment, and improved patient outcomes. NFL, miR-155, and YKL-40 showed a potential discriminative role in distinguishing RRMS from SPMS. With the best performance for NFL, adding YKL-40 alone or combined YKL-40 and miR-155 to NFL increased the specificity of NFL in discriminating RRMS from SPMS. Moreover, adding miR-150 to serum NFL decreased the sensitivity of serum NFL but considerably increased its specificity. Among SPMS patients, miR-155 showed the strongest positive correlation with EDSS, with a moderate positive correlation for miR-150 and YKL-40.

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