Associations of FAS gene expression, SHMT1 (rs4925166) polymorphism, and serum MOG autoantibodies level with susceptibility and progression of Multiple Sclerosis

Tunis Gamal [a*], Mohamed Ali El-Feky[b], Islam El Malky[c], Mostafa I. EL-Amir[a]

[a]Department of Medical Microbiology and Immunology, Faculty of Medicine, South Valley University, Qena, Egypt.

[b]Department of Medical Microbiology and Immunology Department, Faculty of Medicine, Assiut University, Assiut, Egypt.

[c] Neurology and Psychology Department, Faculty of Medicine, South Valley University, Qena, Egypt.

Abstract

Background: Multiple sclerosis (MS) is a chronic inflammatory demyelinating condition of the central nervous system (CNS). The precise cause of MS remains unknown, but it is believed to result from a combination of genetic and environmental factors.

Objectives: To evaluate FAS gene expression, serine hydroxymethyltransferase 1 (SHMT1) gene polymorphism (rs4925166) and serum myelin oligodendrocyte glycoprotein (MOG) autoantibody levels in MS patients and its role in disease progression.

Patients and methods: The study included 49 MS patients and 50 healthy controls. FAS gene expression was evaluated using RT-PCR. The PCR-RFLP technique was used to detect SHMT1 gene polymorphism (rs4925166). Serum MOG autoantibody levels were measured by ELISA.

Results:-The patients' mean age was 32.49 ± 7.8 years. 69.4% of patients were females. (6.1%) of the patients had a positive family history. According to the Expanded Disability Status Scale (EDSS) 51% of the patients were below 2**.** The median FAS gene expression was significantly higher in MS cases with an EDSS score less than 2, [1.077 (0.3687-3.735)], compared to those with scores ranging from 2 to 4, [0.5304 (0.122-1.725)], (P = 0.043). Regarding SHMT1 gene polymorphism (rs4925166), The TG genotype was present in all participants. Anti-MOG antibodies were significantly higher in patients versus controls (85.7% versus 0%), (P < 0.001). Significantly elevated anti-MOG antibodies in cases with relapses (82.8024 ± 21.7356 ng/ml) than those in remission (25.9481 ± 19.0899 ng/ml), P <0.001.

Conclusion: Insignificant difference in FAS gene expression between MS patients and healthy controls with lower disability (EDSS less than 2) associated with higher median FAS expression indicating the role of the FAS gene in reducing disease progression. Anti-MOG antibodies were significantly higher during relapse compared to remission, highlighting their potential as a biomarker in disease progression.

Keywords: Multiple sclerosis (MS); FAS gene expression; Serine Hydroxymethyltransferase1 (SHMT1); SHMT1 gene polymorphism (rs4925166); Myelin oligodendrocyte glycoprotein (MOG).

*Correspondence: tunis.gamal@gmail.com

DOI: 10.21608/SVUIJM.2025.416786.2255

Received: 1 August, 2025

Revised: 28 August, 2025.

Accepted: 6 September, 2025.

Published: 16 September, 2025

Cite this article as Tunis Gamal, Mohamed Ali El-Feky, Islam El Malky, Mostafa I. EL-Amir. (2025). Associations of FAS gene expression, SHMT1 (rs4925166) polymorphism, and serum MOG autoantibodies level with susceptibility and progression of Multiple Sclerosis. *SVU-International Journal of Medical Sciences*. Vol.8, Issue 2, pp: 530-544.

Introduction

Multiple sclerosis (MS) is a chronic, inflammatory, and demyelinating disease of the central nervous system (CNS), characterized by the degeneration of the myelin sheath, which disrupts nerve signal transmission. MS is a leading cause of disability in young adults, typically affecting individuals between the ages of 20 and 40 presenting with a wide spectrum of neurological symptoms **(Wildner et al., 2020).**

The exact etiology of MS remains unclear, but it is believed to arise from a complex interaction between genetic susceptibility and environmental factors. Infections, vitamin D deficiency, smoking, and geographic location are among the environmental triggers thought to influence disease onset and progression **(Waubant et al., 2019)**. MS is pathologically marked by demyelinating plaques in the CNS's white and gray matter, disrupting nerve conduction and causing symptoms like weakness, sensory loss, visual disturbances, and coordination issues **(Huitema et al ., 2021)**.

MS presents in different clinical forms. Around 15% of patients have primary progressive MS (PPMS), marked by steady worsening of disease manifestation from the beginning without any relapses or remission. Most begin is relapsing-remitting MS (RRMS), featuring episodic relapses and remissions. Many eventually develop secondary progressive MS (SPMS) following an initial relapsing remitting course. The disease is more steadily progressive with or without relapses **(Müller et al., 2023)**.

The FAS cell surface death receptor (FAS /FASL) pathway plays a critical role in immune regulation through removal of auto-reactive T cells by apoptosis. FAS is a trans-membrane receptor of the TNF receptor family. Interaction of FAS with FASL induces apoptosis through activation of intracellular cysteine-dependent aspartate-specific proteases (caspase cascade) **(Sremec et al., 2020)**. In MS, $CD4^+$ and $CD8^+$T cells infiltrate the CNS, causing demyelination and axonal damage. FAS-mediated apoptosis helps regulate these immune cells, and its modulation may offer therapeutic potential by reducing pathogenic T cell populations **(Volpe et al., 2016).** Decrease expression of FAS mRNA associated with unfavorable disease progression in RRMS patients **(Lopatinskaya et al., 2006)**.

The enzyme serine hydroxymethyltransferase 1(SHMT1) has an essential role in folate -mediated one-carbon metabolism through providing one carbon unit from serine catabolism. SHMT1 enzymes catalyze the production of purines and methionine in the cytoplasm in addition to its role in DNA synthesis, repair, and methylation **(Nazari Mehrabani et al., 2019)**. Epigenetic alterations, such as DNA methylation, have been identified in MS patients. The SHMT1 gene is located at 17p11.2. Several single nucleotide polymorphisms (SNPs) were identified in the SHMT1 gene as rs4925166 and rs1979277, which are involved in MS susceptibility through changes in gene expression and alteration in the function of SHMT1 protein **(Andlauer et al.,2016; Manna et al., 2024)**..

Myelin oligodendrocyte glycoprotein (MOG) is a protein implicated in preserving the myelin structure and cell-cell signaling. It is found only in the CNS. It is easily accessible to antibodies as it is expressed on the myelin surface **(Peschl et al., 2017)**. Previous studies suggested the role of MOG antibodies in MS **(Lucchinetti et al., 1996**; **Storch et al., 1998)**. The Level of anti-MOG IgG was estimated in MS patients by different techniques, such as ELISA, Western blotting, and cell-based assays (CBAs). CBAs are now considered as the

golden standard in detecting anti-MOG IgG (**Gastaldi et al., 2020).**

This study aimed to evaluate FAS mRNA expression in blood and investigate the association of the SHMT1 rs4925166 polymorphism and serum MOG autoantibody levels with MS disease in Qena Governorate.

Patients and methods

This cross-sectional study included 49 MS patients and 50 age- and sex-matched healthy controls with no personal or family history of MS or other autoimmune diseases. The study was conducted between December 2023 and November 2024 the by Medical Microbiology and Immunology Department in collaboration with the Neurology and Psychology Department, Qena Faculty of Medicine, South Valley University. The study was approved by the Ethics Committee, Qena Faculty of Medicine, South Valley University**.** The ethical approval code is SVU-MED-MIC007-2-22-6-406.

Inclusion criteria:

- Multiple Sclerosis patient were diagnosed according to the 2017 revision of the McDonald criteria **(Thompson *et al*., 2018)**.
- Informed written consent obtained from all included patient after explanation the aim of the study.

Sample Size Calculation:

The following simple formula was used for calculating the adequate sample size:

$$n=\frac{\left(\frac{z\alpha}{2}+z\beta\right)^2 p*(1-p)*(r+1)}{d^2 r}$$

where n is the number of cases

Zα/2: This depends on the level of significance, for 5% type I error (P<0.05) and 95% confidence interval, it is 1.96.

Zβ: This depends on the power of the study, for 80% this is 0.84

P: Prevalence of exposure in the population = 25%

r = 1 (one control for every one cases)

d = magnitude of difference we want to detect (p1-p0) =0.25

- p1 =risk of exposure in cases = 0.50

- p0 = risk of exposure in controls = 0.25

OR =3

$$\frac{(1.96+0.84)^2 0.25*(1-0.25)*(1+1)}{0.25^2*1}$$

= 47.04

Number of cases =47 and number of controls = 47

To get more informative results, we raised the number of cases to 49 and the number of controls to 50. So, the total Sample size was 99.

Detailed history was taken from all patients, including their age, sex, marital state, and family history. General and neurological examinations including evaluation of cranial nerves, motor and sensory functions, coordination, reflexes, and gait were done with scoring according to the Expanded Disability Status Scale (EDSS) which used to assess the degree of neurological impairment and disability in MS patients. The EDSS evaluates functional systems (pyramidal, cerebellar, brainstem, cerebral ,sensory, bowel and bladder, visual, and other), with scores ranging from 0 (normal neurological examination) to 10 (death due to MS)**(Truong et al ., 2021)** .Magnetic resonance imaging (MRI) brain and spinal cord scans including T1, T2, and post-contrast sequences, were obtained to assess demyelinating lesions consistent with MS.Patients who were suffering from any other neurological and immunological conditions were excluded from this study.

Five milliliters of peripheral venous blood were obtained from patients and controls. For assessment of FAS expression and genotyping of SHMT1 gene polymorphism (rs4925166), three milliliters of peripheral venous blood were placed into a tube containing ethylenediaminetetraacetic

acid (EDTA). The remaining two milliliters were collected into sterile plain tube to determine the level of MOG autoantibodies in patients and control.

For assessment of FAS expression, RNA was extracted from one milliliters of EDTA blood using the MicroRNA Mini Extraction Kit (Applied Biotechnology co, Egypt). The concentration and purity of extracted RNA were measured using NanoDrop (2000 Spectrophotometer; Thermo Scientific, USA) by measuring the absorbance (A) ratio (A260/A280). The sample considered good quality with a ratio of 2-2.2.

Reverse transcription of RNA to generate single stranded cDNA was done using 2X RT Mix (Applied Biotechnology co, Egypt). Ten µl of 2X RT Mix were added to 2 µl of template RNA and 8 µl of nuclease-free water. Each tube was incubated in thermal cycler (Peqstar, VWR International, UK) at 42° C for 30 minutes and then at 70°C for 5 minutes to inactivate the enzyme. DNA was stored at -20^{O}C until real time PCR was performed **(Eftekharian et al., 2017)**.

For detection of FAS gene expression by real-time PCR (Applied Biosystems, Germany), forward and reverse primers were used for FAS and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) genes. The sequence of the forward and reverse primers for the FAS gene were (F: 5'-CACCCCCAAACATGGAAATA-3'); and (R: 5'-GGGTGGGGGAAAAATAAGAA-3') with an amplified PCR product of 186 base pairs (bps). The sequence of the forward and reverse primers for the endogenous control gene GAPDH were (F: 5'CCCCACACACATGCACTTACC3'; R: 5'CCTACTCCCAGGGCTTTGATT3') with an amplified PCR product of 97 bps. Ten µl of reaction mixture for amplification of the FAS gene and GAPDH gene (three µl of cDNA, 5 µl of ABT 2X qRT-PCR Mix SYBR (Applied Biotechnology co., Egypt), 0.2 µl of forward and reverse primers , and 1.6 µl H_2O).

The reaction conditions were as follow: for FAS gene (94°C for 5 min, followed by 40 cycles of 95°C for 20 sec, annealing at 55°C for 15 sec, and extension at 72°C for 20 sec; while for the GAPDH gene 95°C for 5 min; followed by 40 cycles of 95°C for 20 sec, annealing at 62°C for 30 sec, and extension at 72°C for 20 sec). Melt curve analysis was done in order to verify the specificity of the products **(Fig.1)**. Confirmation of the products of the expected size was done by electrophoresis on agarose gel **(Fig.2)**.

The levels of gene expression were assessed by employing the comparative cycle threshold (CT) approach, also known as the ΔCT method. Relative fold-change in expression was computed using $2^{-\Delta\Delta CT.}$

For SHMT1 (rs4925166) genotyping, DNA was extracted from two milliliters of EDTA blood by the Genomic DNA Mini Extraction Kit (Applied Biotechnology co., Egypt). For amplification ofthe S SHMT1 gene, the primers sequences were as follow: the forward primer was 5′AGG TAA ATG CAA TCC AAT GGCT-3′, and the reverse primer was 5′- 5′GCC TCC CAA AGT GCT AGG AT-3′ (Invitrogen, United States). The PCR volume was 25 µl (12.5 µl master mix (Bioline, UK), 1 µl forward primer, 1 µl reverse primer, 2 µl extracted DNA, and 8.5 µl H_2O). The thermal cycler (Peqstar, VWR International, UK) reaction conditions were as follow: an initial denaturation at 95 °C for 6 min, 35 cycles of denaturation at 95 °C for 45 seconds, annealing at 62 °C for one minute, extension at 72 °C for 45 seconds, and finally, final extension at 72 °C for 6 minutes, as previously described by **Nazari Mehrabani *et al*. (2019)**.

A PCR product of 499 bps which was amplified by PCR was separated by gel electrophoresis (Multisub Horizontal Gel

System, Cleaver Scientific, UK). For SHMT1 (rs4925166) genotyping, the restriction enzyme Hpy188I (New England Biolabs, Ipswich, MA, USA) was used. A reaction mixture consisting of 10 µL of PCR product, 1 µL of enzyme, 2 µL of enzyme buffer, and 12 µL of nuclease-free water was utilized. This reaction mixture was then incubated at 37°C for a duration of 1 hour. The genotypes were classified as follows: GG genotype (399 and 100 bps), TG genotype (399, 216, 183, and 100 bp), and TT genotype (216, 183, and 100 bp) **(Fig.3)**.

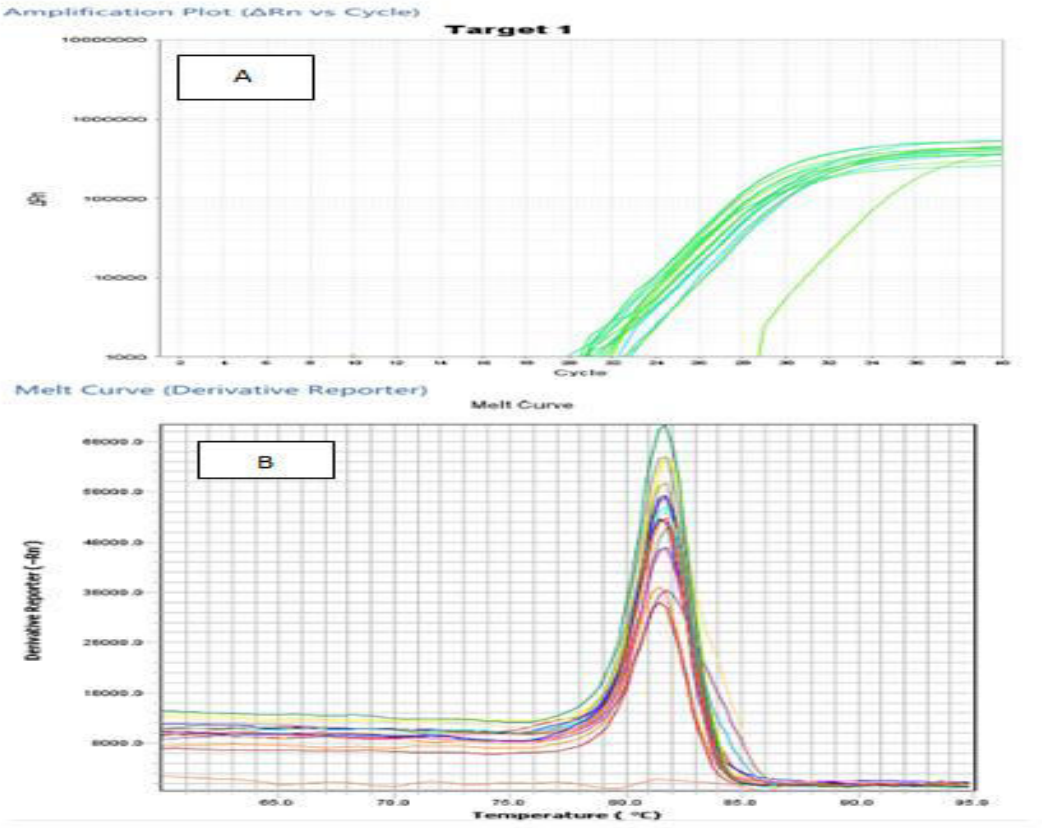

Fig.1. Quantitative assay of FAS expression using RT-PCR; A) amplification plots; B) Melt curve- Raw /Derivative Curve

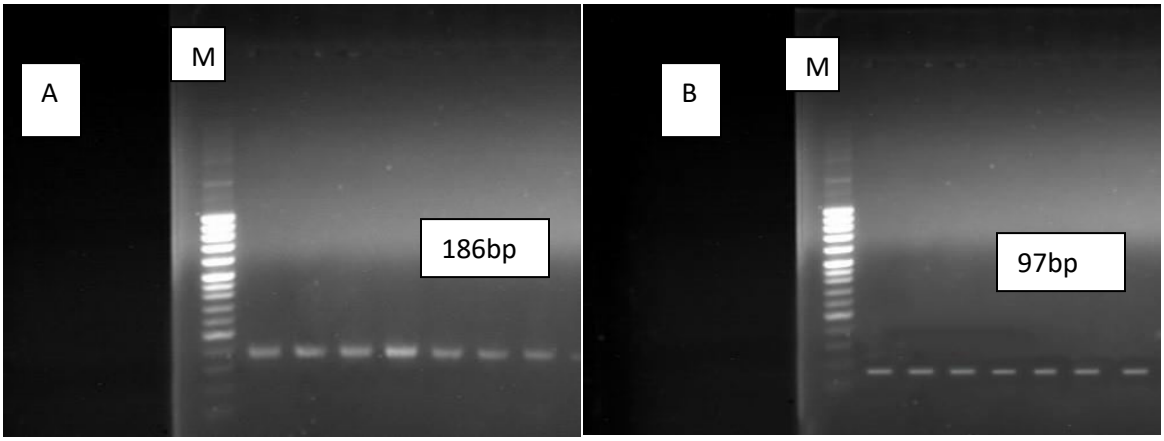

Fig.2. Confirmation of expected size products of FAS gene (A) and GAPDH gene (B) .Lane M shows a 50 bp DNA ladder

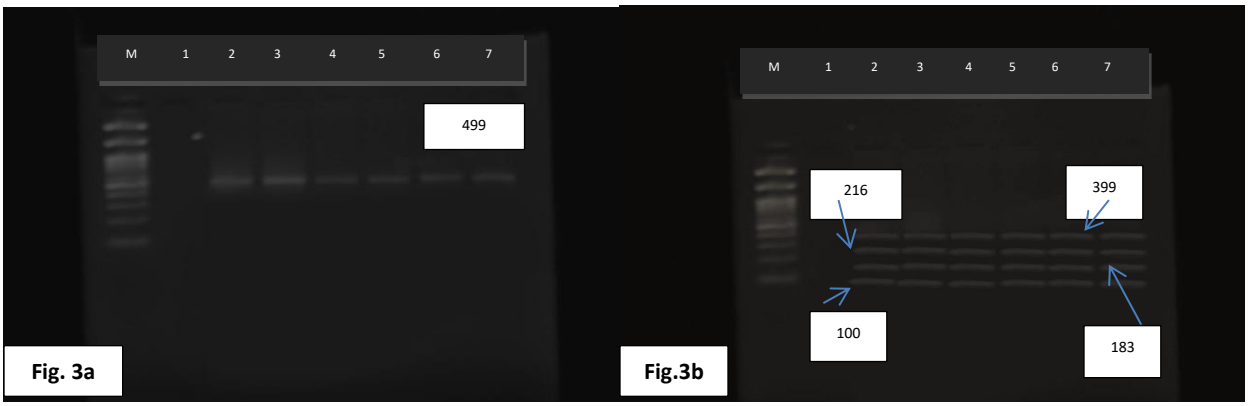

Fig.3. Agarose gel electrophoresis (2%) showing the PCR amplification and (PCR–RFLP) analysis of the SHMT1 rs4925166 SNP. Lanes are denoted by numbers, and Lane M contains the 100 bp DNA ladder. (**Fig.3a**): Lanes 2–7 show the 499 bp PCR product. (**Fig.3b**): Lanes 2–7 show the TG genotype, producing four bands of 399 bp, 216 bp, 183 bp, and 100 bp.

Serum obtained from the remaining venous blood sample was used to determine the level of Anti-Myelin Oligodendrocyte Glycoprotein (MOG) autoantibodies by ELISA Kit provided by ELK Biotechnology CO.,LTD (Wuhan, China) (Catalog No: ELK8083), Sensitivity: 1.21 ng/mL , Detection Range: 3.13-200 ng/mL ,Intra-assay Precision : CV%<8% and . Serum level of antibodies was detected at 450 nm optical density by a microtiter plate reader (Infinite F50, Tecan, Austria). The results were expressed in ng/ml.

Statistical analysis

Analysis was performed using IBM SPSS software version 27. The categorical variables were presented as numbers and percentages and compared using a chi-square test. The data was tested for normality using the Kolmogorov–Smirnov and Shapiro–Wilk tests. The numerical variables with parametric distribution were presented as mean and standard deviations and compared using an independent t-test. The numerical variables with non-parametric distribution were presented as median and interquartile range (IQR) and compared using a Mann-Whitney test. A P-value of 0.05 or less was considered significant. Receiver operating characteristics (ROC) curve analysis was used to assess the diagnostic accuracy of the anti-MOG antibody in multiple sclerosis.

Results

The participants' ages ranged from 20 to 52 years, with a mean age of 32.0 ± 7.63 years. Female participants constituted about two-thirds of all participants (70.7%), and the female-to-male ratio was about 2.4:1. Residence was statistically significantly different between cases and controls, 49% of cases were urban, compared to 70% of controls (p = 0.033). There was a statistically significant difference between cases and controls regarding marital status; 8.2% of cases were divorced, compared to 0% in controls (p = 0.026). Cases had a higher rate of parent consanguinity compared to controls (79.6% vs. 56%, p = 0.012).(**Table.1).**

Table 1. Multiple sclerosis in relation to demographic features

Parameters		Overall	Cases (n = 49)		Controls (n = 50)		p-value
			Number	%	Number	%	
Gender χ	Male	29 (29.3%)	15	30.6%	14	28%	0.775
	Female	70 (70.7%)	34	69.4%	36	72%	
	Female-to-male ratio	2.4:1	2.26:1		2.57:1		
Age (years) t Mean ± SD		32.0 ± 7.63	32.49 ± 7.81		31.52 ± 7.49		0.530
Residence χ	Urban	59 (59.6%)	24	49%	35	70%	0.033*
	Rural	40 (40.4%)	25	51%	15	30%	
Marital status χ	Married	48 (48.48%)	27	55.1%	21	42%	0.026*
	Single	47 (47.47%)	18	36.7%	29	58%	
	Divorced	4 (4.04%)	4	8.2%	0	0%	
Parent consanguinity χ	Positive	67 (67.7%)	39	79.6%	28	56%	0.012*
	Negative	32 (32.3%)	10	20.4%	22	44%	
Family history χ	Positive	3 (3%)	3	6.1%	0	0%	0.076
	Negative	96 (97%)	46	93.9%	50	100%	

*Significant; χ: Chi-square test; t: Independent t-test.

Relapses and remissions were detected in 57.1% versus 42.9% of MS patients, respectively. Relapsing-remitting Multiple Sclerosis was the predominant phenotype (95.9%)**.** Most cases had mild to moderate disability, with 51% scoring below 2 on the (EDSS) and 49% scoring between 2 and 4 (**Table. 2).**

Table 2. Multiple sclerosis Patients' clinical parameters (n=49)

Parameters		**Mean ± SD**	**Median (range)**
Duration of illness (years)		9.06 ± 5.771	8 (1-20)
		Number	**Percentage (%)**
Clinical course	Relapse	28	57.1%
	Remission	21	42.9%
Disease phenotype	RRMS	47	95.9%
	SPMS	2	4.1%
Other autoimmune disease	Yes	0	0%
	No	49	100%
Expanded Disability Status Scale (EDSS)	<2	25	51%
	2-4	24	49%

RRMS: relapsing-remitting Multiple Sclerosis, SPMS: secondary-progressive Multiple Sclerosis, EDSS: Expanded Disability Status Scale

According to FAS gene expression (2−ΔΔCT), there are no statistically significant differences between MS cases and controls (p =0.726). **(Table.3).**

Table 3. FAS gene expression among MS cases and controls

Parameters	MS cases	Controls	P value
	Median (IQR)	Median (IQR)	
FAS gene expression (2−ΔΔCT)	0.7464 (0.184-2.272)	0.825 (0.484-1.19)	0.726#

Mann-Whitney test

FAS gene expression was higher among MS cases with remission than those with relapses. However, this difference was statistically insignificant (p =0.106) **(Table .4)**.

Table 4. Relation between FAS gene expression and MS course

Parameters	Multiple sclerosis course		P value
	Relapses (n=28)	Remission (n=21)	
	Median (IQR)	**Median (IQR)**	
FAS gene expression	0.59 (0.0865-1.293)	1.0196 (0.4659-2.844)	0.106#

Mann-Whitney test

A statistically significantly higher median FAS gene expression among MS cases with EDSS <2 than those with a score of 2 to 4 [1.077 (0.369-3.735) vs. 0.530 (0.122-1.725), respectively (p = 0.043). FAS gene expression was insignificant with gender (p > 0.05), as shown in **(Table.5).**

Table 5. Relation between FAS gene expression, EDSS, and gender among MS cases

Parameter		FAS gene expression (2−ΔΔCT)	p-value
		Median (IQR	
EDSS MWU	<2	1.07 (0.37-3.74)	0.043*
	2-4	0.53 (0.12-1.73)	
Gender MWU	Male	1.07 (0.23-2.93)	0.313
	Female	0.67 (0.14-15)	

*Significant; MWU: Mann-Whitney U test; EDSS: Expanded Disability Status Scale.

FAS gene expression was negatively correlated with the age (r = -0.089, p = 0.545) and anti-MOG antibodies (r = -0.273, p =0.058), as shown in (**Table.6).**

According to SHMT1 (rs4925166) genotyping, the TG genotype was detected in all MS patients and controls (**Table.7)**.

Table 6. Correlation of FAS gene expression with anti-MOG antibodies, disease duration and age.

Variables	FAS gene expression	
	r	**p-value**
Anti-MOG antibodies (ng/ml)**	-0.273	0.058
Duration of disease (years)*	0.088	0.549
Age (years)*	-0.089	0.545

*Pearson; **Spearman; r: correlation coefficient, Bold: significant.

Table 7. Genotype and allele frequency of *SHMT1* rs4925166 among MS cases and controls

Genotype and allele frequencies of SHMT1 (T>G) (rs4925166)	MS patients N = 49 (%)	Healthy controls N = 50 (%)
Allele **T** **G**	49 (100%) 49 (100%)	50 (100%) 50 (100%)
Genotypes **TG**	49 (100%)	50 (100%)

According to anti-MOG antibodies, 85.7% (42/49) of the MS patients were positive for anti-MOG antibodies, while all controls were negative (P **< 0.001**). The median anti-MOG antibodies were significantly higher among MS cases [66.17 (29.65-87.15)] than controls [4.71 (0.04-7.98)] (p<0.001). A statistically significant association was found between the course of MS and the concentration of anti-MOG antibodies, the mean anti-MOG antibodies were significantly higher among MS cases with relapses than those with remission (82.80 ± 21.73ng/ml vs. 25.94 ± 19.08ng/ml, p < 0.001) (**Table .8).**

Table 8. Anti-MOG antibody levels among multiple sclerosis (MS) patients and healthy controls, and their association with the clinical course of MS.

Parameters		MS cases (n=49)		Healthy controls (n=50)		P value
		Number	%	Number	%	
Anti-MOG antibodies (ng/ml)	Positive	42	85.7%	0	0%	**<0.001***
	Negative	7	14.3%	50	100%	
	Median (IQR)	66.17 (29.65-87.15)		4.71 (0.04-7.98)		**<0.001****

Parameters	Multiple sclerosis course		P value
	Relapses (n=28)	Remission (n=21)	
	Mean ± SD	**Mean ± SD**	
Anti-MOG antibodies (ng/ml)	82.80± 21.73	25.94 ± 19.08	**<0.001#**

*Chi-square test, **Mann-Whitney test, #Indpendent t-test

The ROC curve assessed the diagnostic performance of anti-MOG antibodies for prediction of MS. The best cut-off value exceeded 13.305 with 85.71% sensitivity, 100% specificity, 100%PPV, 87.72% NPV, and 92.93% overall accuracy (AUC = 0.944, p <0.001, and 95% CI = 0.900-0.988). **(Table.9).**

Table 9. Diagnostic value of anti-MOG antibody in multiple sclerosis

Parameter	AUC	P value	95% CI		Cut off	Sensitivity	Specificity	PPV	NPV	Accuracy
			Lower	Upper						
MOG antibody	0.944	<0.001	0.900	0.988	13.305 ng/ml	85.71%	100%	100%	87.72%	92.93%

AUC: Area under curve, CI: confidence interval, PPV: Positive predictive value, NPV: Negative predictive value

Discussion

Autoimmune disorders were found to be more prevalent in females, potentially impacting disease progression **(Ashtari, 2021)**. Female sex hormones may contribute to increased susceptibility of females to MS **(Català-Senent et al., 2023).**In our study, 69.4% of the patients were females, and the female-to-male ratio was about (2:1). Similar observation was reported among MS patients in Kuwait **(**female-to-male ratio was 1.95:1) **(Alroughani et al., 2012).** A Tunisian cohort showed a similar trend, with a female-to-male ratio of 2.47, particularly notable in relapsing forms of MS **(Kacem et al., 2022).** Some studies found lower female: male ratios, such as in ratios measured in Saudi Arabia (1.32:1) and China (1.35:1) **(Daif et al., 1998; Wang et al., 2023)**. In addition to environmental factors, variations in statistical reporting methods and case registration systems between regions could influence the difference between female-to-male ratio in different studies.

According to our results, the mean age was 32.49 ± 7.8 years**,** aligning with findings from various studies **(Zakaria et al., 2016; Romero-Pinel et al., 2022)**. Several studies have reported a lower mean age at diagnosis than our study as an Iranian cohort and research from Saudi Arabia were 26.8 ± 9.09 years and 29.3 ± 8.2 years, respectively **(Radmehr et al., 2015; Alsaedi et al., 2022).** These differences likely reflect variations in diagnostic criteria and healthcare practices across regions.

Nearly half of patients came from rural areas (51%). A regional study conducted in Qena reported that 66.6% of patients lived in rural areas and 33.3% in urban areas **(Mohamed et al., 2024).** Urban living has been linked to a higher prevalence of MS in several areas, most likely as a result of a combination of lifestyle modifications, decreased solar exposure, and the hygiene hypothesis. However, despite potentially protective environmental exposures, poor access to healthcare in rural locations may exacerbate long-term disability.

In our, study (6.1%) had a positive family history which similar to the corresponding ratios found in the new registry in Egypt (6%) (**Zakaria et al., 2016)**.However, other studies from different countries found a higher percentage of patients with a positive family history, as in Qatar (32%) **(Deleu et al., 2013)** and Italy (17.2%) **(Esposito et al., 2015).** Our study low percentage of patients with a positive family history could be due to a lack of diagnosis in older generations.

Most cases had mild to moderate disability, with 51% scoring below 2 on the EDSS and 49% scoring between 2 and 4. This finding agreed with a large population-based study from Sweden, United Kingdom, and Canada, which reported that 50.3% of MS patients had EDSS scores between 0–2.5 **(Manouchehrini et al .,2017**), the minimal variation observed in the cut-off points reflects variation in grouping rather than variation in the scale itself**.** However, another study found that EDSS ranged from 0 to 4 in 74.5% of patients **(Young et al., 2025).** This variation is likely due to differences in disease duration, healthcare systems, and patient characteristics.

Regarding the FAS gene expression, Our results show no statistically significant differences between MS cases and controls. This finding is consistent with the study by **Huang et al. (2000)**. This suggesting that FAS Expression may not be altered in MS patients.

Our results show a statistically significantly higher median FAS gene expression among MS cases with EDSS <2 than those with a score of 2 to 4. Similarly, **Lopatinskaya et al., (2006)** found high Fas mRNA levels were associated with long-

term MS courses, indicating the role of FAS gene in reducing disease progression by promoting apoptosis of auto reactive cells. In contrast, decreased FAS L expression in MS patients compared to healthy controls suggests impaired apoptosis, which may contribute to disease progression and higher EDSS scores **(Macchi et al., 2001).**

Regarding SHMT1 gene polymorphism (rs4925166), TG genotype was detected in all MS patients and controls. Another study in the Iranian population found that the GG genotype was significantly higher in MS patients (58.95%) than controls (43%), while the TT genotype was significantly higher in controls than patients **(Nazari Mehrabani et al., 2019)**. The contradictory results observed in our study compared to others may be attributed to several limitations, including differences in ethnicity, geographic diversity, interactions with other genetic or environmental factors, and clinical heterogeneity.

Concerning the concentration of anti-MOG antibodies, a statistically significant difference exists in our study between MS cases and controls suggesting that these antibodies have potential role as an immunological indicators of disease susceptibility and activity. Our findings agreed with another studies **(Gaertner et al., 2004**; **Najah et al., 2014**). In contrast with **Karni et al. (1999),** who reported that higher anti-MOG antibodies are not specific to MS, as they were found in patients with other neurological disorders and healthy controls. Similarly, **Mantegazza et al. (2004)** observed significantly higher anti-MOG levels in both MS and non-inflammatory CNS patients compared to controls ($p < 0.0001$), but not in inflammatory CNS patients ($p = 0.06$). **Klawiter et al. (2010)** found no difference between MS patients and controls.

In our study 85.7% of MS patients were positive for anti-MOG antibodies by ELISA. Similarly, **Egg et al. (2001)** reported that 72% of MS patients were anti-MOG positive using Western blot. This contrasts with the findings of **Lampasona et al. (2004)**, who detected anti-MOG positivity in only 6% of MS patients using a liquid-phase radiobinding assay (RIA). Additionally, **Villacieros-Álvarez et al. (2024)** reported that anti-MOG antibodies were positive in 2.7% of adult patients presenting with a first demyelinating event suggestive of MS, using a live cell-based assay (CBA). Using the same highly specific CBA method, **Cobo-Calvo et al. (2019)** observed a very low frequency (0.3%) of MOG antibody positivity in MS patients. Differences in study results may result from variations in assay methods, patient selection, and disease stage at sampling, along with geographic, ethnic, and genetic factors that affect immune responses.

In our study, the mean anti-MOG antibodies were significantly higher among cases with relapse than those with remission,indicating a correlation between these antibodies and active disease phases. This finding is in agreement with **(Yan et al., 2012; Gastaldi et al., 2023).** However, while high anti-MOG antibody levels correlate with relapse, some studies suggest that not all patients with elevated titers will experience relapses, indicating a need for further research into additional predictive factors **(Singh , 2024).**

The ROC curve analysis demonstrated that the anti-MOG antibodies at the cut-off value of 13.305 ng/ml can discriminate between MS cases and controls with the area under the ROC curve of 0.944, sensitivity of 85.71%, specificity of 100%, diagnostic accuracy of 92.93% and $p < 0.001$. This is in agreement with **Vojdani et al. (2024),** who showed that anti-myelin (including anti-MOG antibodies)

distinguished RRMS from controls with a predictive accuracy of 96.6% (sensitivity = 95.7%, specificity = 95.2%) and an area under the ROC curve of 0.991.

Study limitations: The restricted sample size may limit the applicability of the results, and given that the investigation was carried out at a singular medical institution, it may not adequately represent the wider genetic and demographic heterogeneity. The absence of substantial correlations for the rs4925166 variant of SHMT1 might be ascribed to factors including population stratification, genetic disparities among populations, or the possibility of false-positive or false-negative outcomes.

Recommendations: We recommend larger multi-center studies to validate the significance of FAS gene expression and anti-MOG antibodies as potential biomarkers, and to clarify the impact of the rs4925166 variant of SHMT1 in MS susceptibility and progression.

Conclusion

Our study found no significant differences in FAS gene expression between MS patients and controls, suggesting a complex relationship influenced by disease subtype, stage, and immune cell heterogeneity. Patients with lower disability scores had higher median FAS expression, possibly linked to milder disease. No association was found for the SHMT1 gene polymorphism. Anti-MOG antibodies are useful diagnostic biomarkers of MS disease activity during relapse.

Funding: The study received partial funding from the Faculty of Medicine at South Valley University in Qena, Egypt.

References

- **Alroughani R, Ashkanani A, Lamdhade S. (2012).** Clinical characteristics of multiple sclerosis in Kuwait: data from the new MS registry of Amiri Hospital. International Journal of Neuroscience, 122(2):82-87.
- **Alsaedi EQ, Alsaedi MQ, Mansuri FA, Alsaedi E, Alsaedi M, Mansuri F. (2022).** Clinical profile and the extent of disability in multiple sclerosis patients in Madinah, Saudi Arabia. Cureus, 14(6) :e25851- e25861
- **Andlauer TF, Buck D, Antony G, Bayas A, Bechmann L, Berthele A, et al. (2016).** Novel multiple sclerosis susceptibility loci implicated in epigenetic regulation. Science advances, 2(6): e1501678-e1501690.
- **Ashtari F. (2021).** Gender based epidemiologic survey of multiple sclerosis patients: A report from Isfahan province, Iran. Journal of Vessels and Circulation, 2(2), 41-41.
- **Català-Senent JF, Andreu Z, Hidalgo MR, Soler-Sáez I, Roig FJ, Yanguas-Casás N, et al. (2023).** A deep transcriptome meta-analysis reveals sex differences in multiple sclerosis. Neurobiology of Disease, 181(6): 106113-106129.
- **Cobo-Calvo A, d'Indy H, Ruiz A, Collongues N, Kremer L, Durand-Dubief F, et al. (2019).** Frequency of myelin oligodendrocyte glycoprotein antibody in multiple sclerosis: a multicenter cross-sectional study. Neurology: Neuroimmunology & Neuroinflammation, 7(2): e649-e654.
- **Daif A, Al-Rajeh S, Awada A, Al Bunyan M, Ogunniyi A, AbdulJabar M, et al. (1998).** Pattern of presentation of multiple sclerosis in Saudi Arabia: analysis based on clinical and paraclinical features. European neurology, 39(3): 182-186.
- **Deleu D, Mir D, Al Tabouki A, Mesraoua R, Mesraoua B, Akhtar Net al . (2013).** Prevalence, demographics and clinical characteristics of multiple

sclerosis in Qatar. Multiple sclerosis journal, 19(6):816-819

- **Eftekharian MM, Ghafouri-Fard S, Soudyab M, Omrani MD, Rahimi M, Sayad A, et al. (2017).** Expression analysis of long non-coding RNAs in the blood of multiple sclerosis patients. Journal of Molecular Neuroscience, 63: 333-341.
- **Egg R, Reindl M, Deisenhammer F, Linington C, Berger T. (2001).** Anti-MOG and anti-MBP antibody subclasses in multiple sclerosis. Multiple Sclerosis Journal, 7(5): 285-289.
- **Esposito F, Guaschino C, Sorosina M, Clarelli F, Ferre' L, Mascia E, et al . (2015).** Impact of MS genetic loci on familial aggregation, clinical phenotype, and disease prediction. Neuroimmunology & Neuroinflammation, 2(4): e129-e137.
- **Gaertner S, de Graaf KL, Greve B, Weissert R. (2004).** Antibodies against glycosylated native MOG are elevated in patients with multiple sclerosis. Neurology, 63(12): 2381-2383.
- **Gastaldi M, Foiadelli T, Greco G, Scaranzin S, Rigoni E, et al . (2023).** Prognostic relevance of quantitative and longitudinal MOG antibody testing in patients with MOGAD: a multicentre retrospective study. Journal of Neurology, Neurosurgery & Psychiatry, 94(3): 201-210.
- **Gastaldi M, Scaranzin S, Jarius S, Wildeman B, Zardini E, Mallucci G, et al. (2020).** Cell-based assays for the detection of MOG antibodies: a comparative study. Journal of Neurology, 267(12): 3555-3564.
- **Huang WX, Huang P, Gomes A, Hillert J. (2000).** Apoptosis mediators fasL and TRAIL are upregulated in peripheral blood mononuclear cells in MS. Neurology, 55(7): 928-934.
- **Huitema MJ, Strijbis EM, Luchicchi A, Bol JG, Plemel JR, Geurts JJ, et al . (2021).** Myelin quantification in white matter pathology of progressive multiple sclerosis post-mortem brain samples: a new approach for quantifying remyelination. International Journal of Molecular Sciences, 22(23): 12634.
- **Kacem I, Mrabet S, Souissi A, Gharbi A, Hmissi L, Larnaout F, et al . (2022).** Impact of Gender on Multiple Sclerosis Natural History: Findings from a Tunisian Cohort. Multiple Sclerosis and Related Disorders, 59: 103567- 103567.
- **Karni A, Bakimer-Kleiner R, Abramsky O, Ben-Nun A. (1999).** Elevated levels of antibody to myelin oligodendrocyte glycoprotein is not specific for patients with multiple sclerosis. Archives of neurology, 56(3): 311-315..
- **Klawiter EC, Piccio L, Lyons JA, Mikesell R, O'Connor KC, Cross AH. (2010).** Elevated intrathecal myelin oligodendrocyte glycoprotein antibodies in multiple sclerosis. Archives of neurology, 67(9):1102-1108.
- **Lampasona V, Franciotta D, Furlan R, Zanaboni S, Fazio R, Bonifacio E, et al (2004).** Similar low frequency of anti-MOG IgG and IgM in MS patients and healthy subjects. Neurology, 62(11): 2092-2094
- **Lopatinskaya L, Zwemmer J, Uitdehaag B, Lucas K, Polman C, Nagelkerken L. (2006).** Mediators of apoptosis Fas and FasL predict disability progression in multiple sclerosis over a period of 10 years. Multiple Sclerosis Journal, 12(6):704-709.
- **Lucchinetti CF, Bruck W, Rodriguez M, Lassmann H (1996).** Distinct patterns of multiple sclerosis pathology indicates heterogeneity in pathogenesis. Brain pathology, 6(3): 259-274.

- **Macchi B, Matteucci C, Nocentini U, Tacconi S, Pagnini V, Mastino A, et al.. (2001).** Defective Fas ligand production in lymphocytes from MS patients. Neuroreport, 12(18): 4113-4116.
- **Manna I, De Benedittis S, Porro D . (2024).** A Comprehensive Examination of the Role of Epigenetic Factors in Multiple Sclerosis. International Journal of Molecular Sciences, 25(16): 8921-8942.
- **Manouchehrinia A, Westerlind H, Kingwell E, Zhu F, Carruthers R, Ramanujam R, et al . (2017).** Age related multiple sclerosis severity score: disability ranked by age. Multiple Sclerosis Journal, 23(14): 1938-1946.
- **Mantegazza R, Cristaldini P, Bernasconi P, Baggi F, Pedotti R, Piccini I, et al. (2004).** Anti-MOG autoantibodies in Italian multiple sclerosis patients: specificity, sensitivity and clinical association. International immunology, 16(4): 559-565.
- **Mohamed RB, Rageh TA, Abbas MA, Desoky T, Bsheer MA. (2024).** Demographic and clinical profile of multiple sclerosis in Qena governorate, Egypt. SVU-International Journal of Medical Sciences. *7*(1):564-571.
- **Müller J, Cagol A, Lorscheider J, Tsagkas C, Benkert P, Yaldizli Ö, et al . (2023).** Harmonizing definitions for progression independent of relapse activity in multiple sclerosis: a systematic review. JAMA neurology, 80(11): 1232-1245.
- **Najah Am, Yaseen Aa, Abdulrasool Am. (2014).** Assessment of anti-mog in serum from patients of multiple sclerosis. Mintage Journal of Pharmaceutical and Medical Sciences, 3(1): 7–10.
- **Nazari Mehrabani SZ, Shushizadeh MH, Abazari MF, Nouri Aleagha M, Ardalan A, Abdollahzadeh R, et al . (2019).** Association of SHMT1, MAZ, ERG, and L3MBTL3 gene polymorphisms with susceptibility to multiple sclerosis. Biochemical genetics, 57(3): 355-370.
- **Peschl P, Bradl M, Höftberger R, Berger T, Reindl M. (2017).** Myelin oligodendrocyte glycoprotein: deciphering a target in inflammatory demyelinating diseases. Frontiers in immunology, 8: 529-544.
- **Radmehr M, Meghdadi S, Bahmanzadeh M, Sabbagh S . (2015).** Prevalence, demographics and clinical characteristics of multiple sclerosis in North of Khuzestan Province, Iran Jentashapir J Health Res.. 6(5):e23831-e23838
- **Romero-Pinel L, Bau L, Matas E, León I, Muñoz-Vendrell A, Arroyo P, et al. (2022).** The age at onset of relapsing-remitting multiple sclerosis has increased over the last five decades. Multiple Sclerosis and Related Disorders, 68(12): 104103-10419.
- **Singh YV.(2024).** Myelin Oligodendrocyte Glycoprotein Antibody-associated Disease (MOGAD) (Doctoral dissertation, University of Zagreb. School of Medicine. Department of Neurology).
- **Sremec J, Tomasović S, Tomić Sremec N, Šućur A, Košćak Lukač J, et al. (2020).** Elevated concentrations of soluble Fas and FasL in multiple sclerosis patients with antinuclear antibodies. Journal of clinical medicine, 9(12): 3845.3857
- **Storch MK, Stefferl A, Brehm U, Weissert R, Wallström E, Kerschensteiner M, et al. (1998).** Autoimmunity to myelin oligodendrocyte glycoprotein in rats mimics the spectrum of multiple

sclerosis pathology. Brain Pathology, 8(4):681-694.

- **Thompson AJ, Banwell BL, Barkhof F, Carroll WM, Coetzee T, Comi G, et al . (2018).** Diagnosis of multiple sclerosis: 2017 revisions of the McDonald criteria. The Lancet Neurology, 17(2): 162-173. .
- **Truong CT, Le HV, Kamauu AW, Holmen JR, Fillmore CL, Kobayashi MG, et al. (2021).** Creating a real-world data, United States healthcare claims-based adaptation of kurtzke functional systems scores for assessing multiple sclerosis severity and progression. Advances in Therapy, 38(9):4786-4797.
- **Villacieros-Álvarez J, Espejo C, Arrambide G, Castillo M, Carbonell-Mirabent P, Rodriguez M, et al. (2024).** Myelin oligodendrocyte glycoprotein antibodies in adults with a first demyelinating event suggestive of multiple sclerosis. Annals of neurology, 95(1):116-128.
- **Vojdani A, Almulla AF, Vojdani E, Li J, Zhang Y, Maes M. (2024).** Autoimmune responses to myelin-associated proteins as diagnostic and prognostic biomarkers of relapsing-remitting multiple sclerosis: associations with human herpesvirus-6 and Epstein-Barr Virus reactivation. Journal of Advanced Research, S2090-1232(25):00119-5.
- **Volpe E, Sambucci M, Battistini L, Borsellino G . (2016).** Fas–Fas Ligand: Checkpoint of T Cell Functions in Multiple Sclerosis. Frontiers in Immunology, 7(382): 1-10.
- **Wang H, Zhang X, Li H, Sun Z, Zhong Y . (2023).** Gender differences in the burden of multiple sclerosis in China from 1990 to 2019 and its 25-year projection: An analysis of the Global Burden of Diseases Study. Health Science Reports, 6(12): e1738-e1748
- **Waubant E, Lucas R, Mowry E, Graves J, Olsson T, Alfredsson L, et al. (2019).** Environmental and genetic risk factors for MS: an integrated review. Annals of clinical and translational neurology, 6(9):1905-1922.
- **Wildner P, Stasiołek M, Matysiak M. (2020).** Differential diagnosis of multiple sclerosis and other inflammatory CNS diseases. Multiple sclerosis and related disorders, 37(1):101452-101491.
- **Yan XU, Zhang Y, Liu CY, Bin PE, Wang JM, Zhang XJ, et al. (2012).** Serum antibodies to 25 myelin oligodendrocyte glycoprotein epitopes in multiple sclerosis and neuromyelitis optica: clinical value for diagnosis and disease activity. Chinese Medical Journal, 125(18): 3207-3210.
- **Young CA, Rog D, Sharrack B, Chhetri SK, Kalra S, Harrower T, et al. (2025).** Clinical and socio-demographic characteristics of people with multiple sclerosis at the time of diagnosis: Influences on outcome trajectories. Journal of the Neurological Sciences, 470: 123409-123418.
- **Zakaria M, Zamzam D, Hafeez M, Swelam M, Khater S, Fahmy M, et al. (2016).** Clinical characteristics of patients with multiple sclerosis enrolled in a new registry in Egypt. Multiple sclerosis and related disorders, 10: 30-35.