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Assessment of Atherosclerotic Changes in a Sample of Egyptian Multiple Sclerosis patients

Esraa G. Ghander[1], Tamer M. Belal[2], Ahmed M. Tawfik[3], Asmaa M. Borg[4], Ayman Abdelnaby[5]

1.Assistant lecturer of Neurology, Mansoura university, Mansoura, EGYPT
2. Professor of Neurology, Mansoura university, Mansoura, EGYPT
3. Professor of Radiology, Mansoura university, Mansoura, EGYPT
4. Lecturer of Clinical Pathology, Mansoura university, Mansoura, EGYPT
5.Assistant Professor of Neurology, Mansoura university, Mansoura, EGYPT.

Corresponding author: Esraa G. Ghander
Email: esraa.gamal155@yahoo.com

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Abstract:

Background: Recently, the presence of vascular abnormalities in patients with multiple sclerosis had been reported. Endothelial dysfunction secondary to inflammatory activity and global cerebral hypoperfusion which might predispose them to the development of ischemic stroke may be a proposed mechanism. **Objectives:** To assess atherosclerotic changes in a Sample of Egyptian MS patients using different laboratory and radiological markers as well as correlating these changes with patients' clinical symptoms. **Patients and Methods:** 45 multiple sclerosis patients and 45 healthy controls were enrolled. They underwent clinical assessments using cognitive scales, EDSS. Serum IL17A as common inflammatory marker and structural and vascular imaging were done. Results: Serum IL17 analysis revealed higher levels in cases group compared to control group and these findings revealed statistacal significant difference (ρ-value <0.05). Also, there is a statistically significant difference in the Arterial spin labeling results and vascular ultrasonography between the two groups. **Conclusion:** Numerous atherosclerotic abnormalities are present in MS patients, which highlight the vascular component of the illness and provide an overview of the pathophysiological alterations that are brought about by the disease.

Keywords: Atherosclerosis, ASL, Serum IL17A, Multiple Sclerosis

1. Introduction

Multiple sclerosis (MS) is a chronic demyelinating disease of the central nervous system (CNS), which is characterized by the recurrence of focal neurological episodes associated with inflammation, axonal injury, and gliosis. In the later stages of the disease, axonal dissection and Wallerian degeneration, the age-associated cerebral small vessel disease, and vascular comorbidities are suggested as important factors for development of MS neurodegeneration.[1]
In recent years, several studies have reported the presence of vascular abnormalities in patients with multiple sclerosis. First, findings from epidemiological studies suggested that patients with MS had a higher risk for ischemic stroke than people who do not have MS. The underlying mechanism is unknown but might involve endothelial dysfunction secondary to inflammatory disease activity and Interleukin-17-expressing CD4+T helper 17 (Th17) cells are considered as critical regulators of disease activity either in MS or atherosclerosis. Second, patients with MS had global cerebral hypoperfusion, which might predispose them to the development of ischemic stroke. Previous studies suggested that MS lesions might have an ischemic origin, and there appeared to be a link between decreased white matter perfusion and cognitive impairment in MS. [2]
In this study we would hopefully assess atherosclerotic changes in a Sample of Egyptian MS patients using different laboratory and radiological markers as well as correlating these changes with patients' clinical symptoms.

Aim of the work

In this study we would hopefully assess atherosclerotic changes in a Sample of Egyptian MS patients using different laboratory and radiological markers as well as correlating these changes with patients' clinical symptoms.

2. Patients and Methods

This is a case control study. The study was carried in Neurology department, Mansoura university hospital. Ninety Egyptian individuals were enrolled during the study period. The Institutional Research Board - IRB Ethics Committee under the code of (MD.21.07. 494.R1.R1.R2) approved the research protocol. All patients or their surrogated had clearly read the study protocol and an informed written consent were collected from them.
The study enrolled 45 patients with MS diagnosed according to the 2017-revised McDonald criteria regardless whether they would be receiving disease modifying therapy or not. They were recruited from the Neurology outpatient's clinic Department, Neurology department, Mansoura University Hospital between September 2021 and September 2023. They were of both sex and between the ages of 20 and 50.
The exclusion criteria were: Individuals with an EDSS score of greater than five, relapses, or corticosteroid treatment within three months after study enrollment, individuals with a prior medical history of peripheral artery disease, diabetes mellitus, hypertension, stroke and any other

vascular risk factors, individuals with dyslipidemia, Individuals who did not want to comply. Individuals who were using medicines such as sildenafil, beta blockers, calcium channel blockers, ACE inhibitors, and non-steroidal anti-inflammatory drugs (NSAIDs) that potentially change vascular reactivity, Individuals who have a history of verified psychiatric conditions, mood disorders, or serious depressive disorder, moms who are nursing and pregnant , Situations where a person's cognitive impairment of any kind keeps them from understanding Survey Questionnaire. Control group included 45 healthy individuals; age and sex matched subjects with matched with cases and underwent the same exclusion criteria of the cases.

At initial visit, gender, age and body mass index (BMI) were recorded. Then we collected data about comorbid medical diseases as hypertension, diabetes, peripheral artery disease, stroke, any other vascular risk factors, psychiatric illness and medication exposure history.

Then the patient was subjected to psychometric assessment using Mini-mental State Examination (MMSE) as a screening tool for cognitive impairment [3]as well as using Montreal Cognitive Assessment Arabic version to monitor the course of the cognitive function over time.[4]

Then evaluation of the degree of disability by using Expanded Disability Severity Scale (EDSS).[5]

Each individual provided a blood sample that weighed roughly three milliliters of venous blood. The sample was centrifuged at a speed of about 3000 rpm for fifteen minutes. Serum samples were kept at -40°C until analysis was performed to measure human IL17A levels in the serum.

IL17A ELISSA KIT (Wuhan Fine Biotech co., Wuhan, China) Catalogue No.: EH3267) was used to detect serum IL17A in subjects' sera in accordance with manufacturer's instructions (Detection method: sandwich, Double antibody). with kit's ideal detection range (31.25-2000pg/ml). Serum Human IL17A concentrations were obtained via infinite F50 ELISA Reader (TECAN, Mannedorf, Switzerland). Additionally, all subjects underwent routine lab work that included a complete blood picture, an automated international normalized ratio (INR), random blood glucose testing, kidney and liver function tests, and specific lab work such as a complete lipid profile ,a manual latex test for C-reactive protein (CRP), and Neutrophil Lymphocyte ratio as a marker for atherosclerosis. Samples were analyzed at the Clinical pathology Laboratory, Mansoura University Hospital.

Then we perform a vascular ultrasound examination to all subjects. Extracranial carotid artery duplex and transcranial color-coded duplex sonography were performed at the Mansoura Neurology Department at the Mansoura University Neurosonology Unit (CUNU) using an ultrasonography machine of type GELOGIQE9.

Following that, patients had neuroimaging All MR images were acquired with a 1.5 Tesla MR device (Siemens Magnetom Aera, Germany) and Every patient got standard T1-weighted images (TR/TE of 800/15 ms) and T2-weighted fast spin-echo images (TR/TE of 6000/80 ms) with a 256 X 224 acquisition matrix, a 6 mm slice thickness, and a field-of-view (FOV) of 25–30 cm. A multi-phase arterial spin labeling method utilizing the FEEPI sequence was used to produce control and labeled images.

Statistical Analysis

Sample size calculation was based on Mean CIMT as indicator of atherosclerosis between cases with MS and control group retrieved from previous research. Using G*power version 3.0.10 to calculate sample size based t test=2.06, 2-tailed, α error =0.05 and power = 80.0% with effect size (1.158), the total calculated sample size will be 13 in each group at least.
Data will be analyzed using SPSS (statistical package for social sciences) version 22. Qualitative data will be presented as number and percent, Quantitative data will be tested for normality by Shapiro-Wilk test then described as mean and standard deviation for normally distributed data and median and range for non-normally distributed. The appropriate statistical test will be applied according to data type with the following suggested tests: Chi-square for categorical variable, Student t test.

3. Results

Ninety Egyptian participants, 45 of them had multiple sclerosis (MS) and the other 45 were in good condition. multiple sclerosis (MS) cases were diagnosed using the revised McDonald criteria from 2017. The Kurtzke Expanded Disability Status Scale (EDSS) was used to assess neurological impairment and determine clinical scores. Between september2021and September 2023, subjects were recruited for our study from the Mansoura University Hospital's Neurology Outpatient Clinic. The age distribution of the population under study was 31.89±9.38 years for the cases group and 35.0±6.25 years for the control group. Regarding marital status, there were differences between the cases group (married 31(68.9%), not married 14(31.1%)) and the control group (married 27(60.0%), not married 18(40.0%)). In the cases group, the gender distribution was Male 16(35.6%), Female 29 (64.4%), and Female 33(73.3%) and Male12(26.7%) in the control group. Education (Educated 43(95.6%), Non Educated 2(4.4%))in cases group ,but all control group was Educated 45(100%) ,Special habits (Smoker14(31.1%) ,Non smoker31(68.9%) in cases group ,but in control group (Smoker 11(24.4%) Nonsmoker 34(75.6%) ,No past history of any medical condition in both groups (45(100%) ,Working (Working 33(73.3%) ,Not Working 12(26.7%))in cases group but in control group (Working 35(77.8%) , Not working10(22.2%) , As regard BMI (in cases group Normal 26(57.8%),Overweight 10(22.2%) ,Obese8(17.8%) ,Underweight 1(2.2%)) but In Control group (Normal29(64.4%),Overweight10(22.2%),Obese5(11.1%), Underweight 1(2.2%) as shown in Table (1).

Table (1): ***Comparison of sociodemographic characteristics between studied groups:***

	Cases group n=45	**Control group n=45**	**test of significance**
Age / years of studied group	**31.89±9.38**	**35.0±6.25**	**t=1.85 p=0.067**
Marital status Not married Married	**14(31.1) 31(68.9)**	**18(40.0) 27(60.0)**	**χ2=0.776 p=0.378**
Sex			

Female **Male**	**29(64.4)** **16(35.6)**	**33(73.3)** **12(26.7)**	**χ2=0.829** **p=0.362**
Education **Not educated** **Educated**	 **2(4.4)** **43(95.6)**	 **0** **45(100)**	 **FET=2.05** **p=0.494**
Special habits **Non smoker** **Smoker**	 **31(68.9)** **14(31.1)**	 **34(75.6)** **11(24.4)**	 **χ2=0.498** **p=0.480**
Medical history **No**	 **45(100)**	 **45(100)**	
Occupation **Not working** **Working**	 **12(26.7)** **33(73.3)**	 **10(22.2)** **35(77.8)**	 **χ2=0.241** **p=0.624**
BMI **Normal** **Overweight** **Obese** **Underweight**	 **26(57.8)** **10(22.2)** **8(17.8)** **1(2.2)**	 **29(64.4)** **10(22.2)** **5(11.1)** **1(2.2)**	 **χ2=0.856** **p=0.836**

t: Student t test **χ^2= Chi-Square test**
FET: Fisher exact test **BMI: body mass index**

Analysis of routine laboratory results among the two groups revealed no statistical significant differences as regrad N/LRatio, ESR,CRP and Lipid profile(Table 2). Serum IL17 analysis revealed higher levels in cases group compared to control group and these findings revealed statistacal significant difference (ρ-value <0.05). (Table2) and Figure (1) and Figure (2), with valid cut off point 31.057 (Table3).

Table (2): ***Comparison of laboratory findings between studied groups:***

	Cases group n=45(%)	**Control group n=45(%)**	**test of significance**
N/L RATIO **Normal** **high**	 42(93.3) 3(6.7)	 45(100) 0	 FET=3.10 P=0.242
ESR **Normal** **high**	 34(75.6) 11(24.4)	 45(100) 0	 χ^2=12.53 p<0.001*
CRP **Normal** **high**	 44(97.8) 1(2.2)	 45(100) 0	 FET=1.01 p=1.0
Lipid profile **Normal**	 45(100)	 45(100)	
IL17 average **Mean ± SD** **Median (min-max)** **IQR)**	 103.43±47.23 91.14(22.49-229.79) (72.55-125.11)	 14.34±8.38 13.73(1.47-31.47) (6.74-20.88)	Z=8.09 P=0.001*

Z: Mann Whitney U test ESR: Erythrocyte Sedimentation Rate
FET: Fisher exact test CRP:C Reactive Protein
χ2= Chi-Square test N/L RATIO: Neutrophil /Lymphocyte ratio

*statistically significant (if p<0.05) IL17: interleukin 17
IQR: interquartile range
p value: probability value

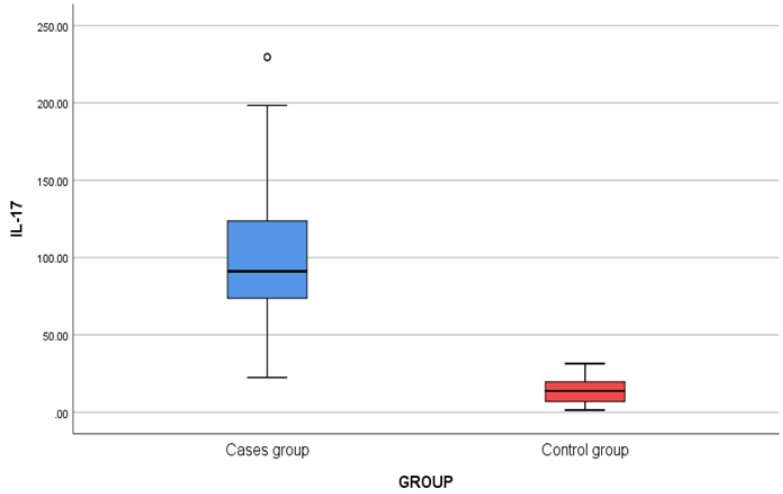

Figure (1): Box& Whisker plot showing median IL-17 among studied group

Table (3): ***Validity of IL-17 in differentiating cases from control group:***

	AUC (95%CI)	P value	cut off point	Sensitivity	Specificity	PPV	NPV	Accuracy
IL-17	0.995 (0.985-1.0)	0.001*	31.057	97.8%	97.8%	97.8%	97.8%	97.8%

AUC: Area under curve p value: probability value
PPV: Positive predictive value
NPV: Negative predictive value

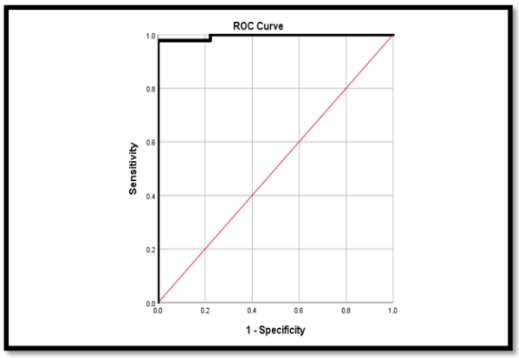

Figure (2): ROC curve of IL-17 in differentiating cases from control group

As can be seen in the table4 and Figure 3, there is a statistically significant difference (p = 0.001*) between the cases group and the control group when comparing the AV brain, AV musculature, and rCBF in ASL.

Table (4): ***Comparison of av brain, av muscle and rCBF among studied groups:***

	Cases group n=45	Control group n=45	test of significance
av. Brain	28.28±3.99	53.79±7.37	t=19.3 p=0.001*
av Muscle	17.12±2.39	23.33±6.08	t=5.91 p=0.001*
rCBF	1.67±0.32	2.42±0.48	t=8.17 p=0.001*

t: Student t test
*statistically significant (if p<0.05)
p value: probability value
ASL: arterial spin labeling

av. Brain: average brain
av Musc: average muscle
rCBF; relative cerebral blood flow

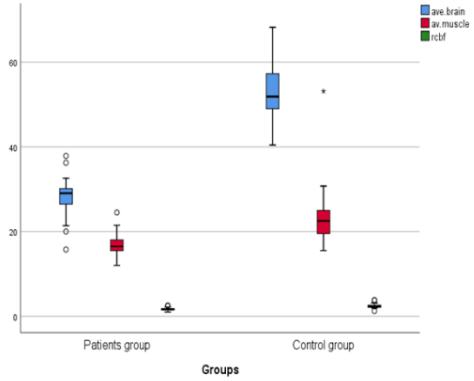

Figure (3): Box& Whisker plot showing av brain, av muscle and rCBF among studied groups

For more further evaluation, There's statistically significant difference between cases group and control group as regard extracranial doppler findings in RT CCA PSV, RT CCA EDV, RT ECA PSV, LT CCA EDV, LT VA PSV and LT PI. With p value (P=0.002*) in RT CCA PSV , p value (P=0.001*) in RT CCA EDV, p value(p=0.029*) in RT ECA PSV, p value (p=0.001*in LT CCA EDV, p value (p=0.046*in LT PI) and p Value (p=0.04*in LTVA PSV) as shown in table5.

Table (5): ***Comparison of extracranial doppler findings between studied groups:***

	Cases group n=45	Control group n=45	test of significance
Rt.CCA.PSV	79.24±20.05	89.67±8.98	t=3.18 p=0.002*
Rt.CCA.EDV	21.27±4.49	24.38±2.66	t=3.99 p=0.001*
RT.PI	1.23±0.44	1.17±0.13	t=0.840 p=0.403
RT.RI	0.700±0.0	0.70±0.0	t=0.0 p=1.0

RT.ICA.PSV	62.11±17.32	64.71±14.11	t=0.781 p=0.437
RT.ECA.PSV	68.62±12.86	74.80±13.52	t=2.22 p=0.029*
RT.VA.PSV	38.69±11.77	35.24±6.87	t=1.69 p=0.093
LT.CCA.PSV	76.64±12.01	75.33±7.34	t=0.625 p=0.534
LT.CCA.EDV	21.49±4.13	24.38±0.68	t=4.63 p=0.001*
LT.PI	1.17±0.53	1.01±0.025	t=2.03 p=0.046*
LT.RI	0.70±0.0	0.70±0.0	t=0.0 p=1.0
LT.ICA	59.64±0.37	63.62±14.09	t=1.53 p=0.131
LT.ECA.PSV	67.76±11.13	73.20±15.03	t=1.95 p=0.054
LT.VA.PSV	40.91±10.85	37.42±3.27	t=2.07 p=0.04*
IMT CCA right **Normal** **Plaque**	 43(95.6) 2(4.4)	 45(100) 0	
IMT CCA left **Normal** **Plaque**	 41(95.3) 2(4.7)	 45(100) 0	

P value: probability value
t: Student t test
*statistically significant (if p<0.05)
PSV: peak systolic velocity
EDS: End diastolic velocity
PI: pulsatility index
RI: resistivity index
IMT: intimal medial thickness
CCA: common carotid artery
ICA: internal carotid artery
VA: vertebral artery

Our research revealed a statistically significant difference (P Value = 0.015*) in transcranial doppler in LT ACA between the cases group and control group, as indicated by the table 6 and Figure 4.

Table (6): ***Comparison of transcranial doppler finding between studied group:***

	Cases group n=45	**Control group n=45**	**test of significance**
RT.MCA.PSV	65.84±14.25	65.93±13.58	t=0.03 p=0.976
RT.ACA.PSV	52.76±10.89	49.62±4.02	t=1.81 p=0.074
RT.P1.PSV	59.73±6.16	57.73±4.27	t=1.79 p=0.08
RTP2PSV	59.0±3.88	59.0±3.87	t=0.244 p=0.808
BA.PSV	44.60±11.48	43.47±7.44	t=0.555 p=0.580
RT_VA_PSV	39.62±7.29	38.73±6.54	t=0.608

			p=0.544
LT_MCA_PSV	100.80±10.09	100.7±10.64	t=0.153 p=0.879
LT_ACA_PSV	42.44±9.36	39.0±0.0	t=2.47 p=0.015*
LTP1PSV	40.62±8.64	38.60±2.45	t=1.51 p=0.134
LTP2.PSV	35.44±6.20	34.02±4.27	t=1.27 p=0.209
LT_VA__PSV	39.33±8.66	38.82±8.23	t=0.287 p=0.775

t: Student t test MCA: middle cerebral artery
*statistically significant (if p<0.05)
RT: RIGHT
LT: LEFT
BA: basilar artery
ACA: anterior cerebral artery
VA: vertebral artery
PSV: peak systolic velocity
EDV: end diastolic velocity

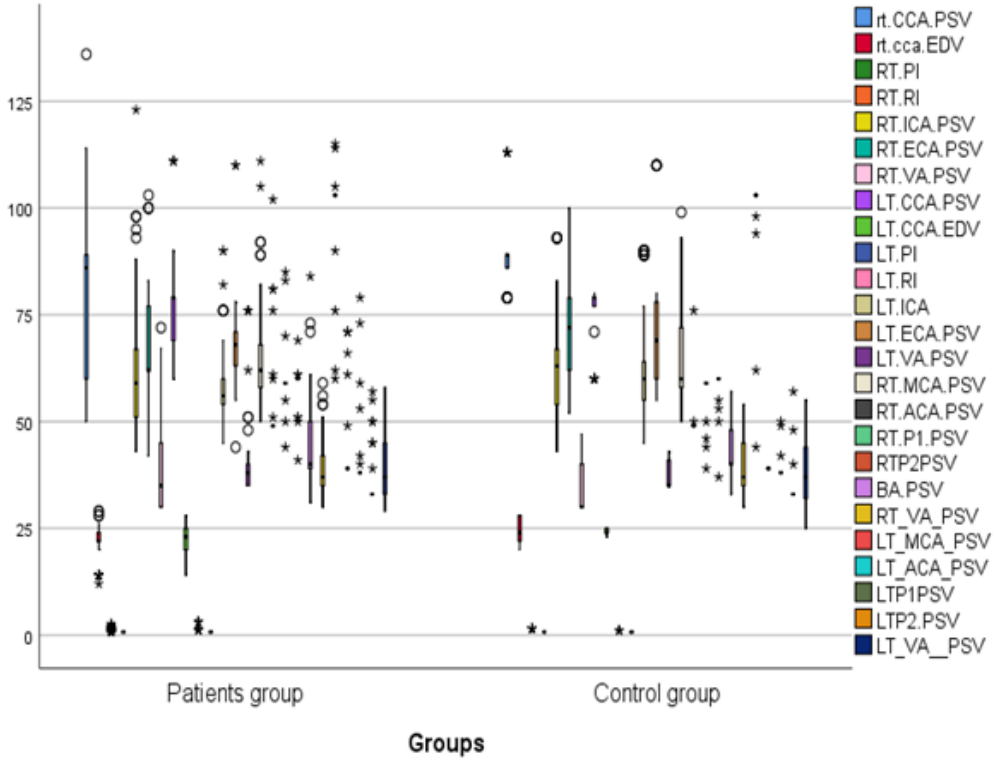

Figure (4): Box& Whisker plot showing comparison of transcranial doppler finding between studied group

4. Discussion

Multiple sclerosis (MS) is a chronic neurological disease characterized by inflammation and demyelination in the central nervous system (CNS), leading to neurodegeneration and severe axonal loss, and is the most common nontraumatic disabling neurologic condition in young adults. The clinical course of MS is heterogeneous, but patients accumulate a significant burden of clinical disability over time. [6]

In this current study we aimed to assess atherosclerotic changes in a Sample of 45 Egyptian MS patients and 45 Egyptian Heathy controls using different laboratory and radiological markers as well as correlating these changes with patients' clinical symptoms. This is through clinical evaluation and correlate data with clinical, Minimental state examination (MMSE), Montreal Cognitive Assessment (MOCA)Arabic version to monitor the course of the cognitive function over time, Expanded Disability Status Scale (EDSS) with Calculation OF BMI (kg/m2) for all subjects using their height and weight data to detect their nutritional status.

According to the current study, there is no statistically significant variation in the sociodemographic data between the analyzed groups. The small sample size and the exclusion criteria for patients which exclude individuals with dyslipidemia, any vascular risk factors, cognitive impairment, an EDSS of more than five, or those who are experiencing a relapse may be responsible for this outcome. Pozzilli and colleagues' earlier research supports these findings, as they failed to find an association between gender differences in MS patients. [7] Hakim and his colleagues also found that there has been no change in the married status since the disease's beginning, but that the disease's direct effects have caused them to quit their occupations. [8] Furthermore, there is no connection between MS risk and schooling. [9,10] Furthermore, smoking had no effect on the outcome.[11]

Zakaria and his colleagues discovered, in contrast to our data, that MS was more prevalent in Egyptian women, with RRMS being the most common manifestation. [12] Additionally, Hamdy and his associates discovered that 2.8% of workers changed their tasks and 32.5% of workers quit as a result of the illness. Consequently, MS imposes a significant financial burden on both individuals and society at large. Additionally, this study showed a high prevalence of divorce (9.9%), suggesting that MS patients also experience a significant social impact. [13] Prior research shown that men experience severe relapses more frequently than women, as do older singles who smoke and are on OCPS. [14]

Eating a lot of fat increases the risk of MS via increasing systemic inflammation, according to other studies.[15] Additionally, it was shown that MS patients with an EDSS of ≥3 were older, had higher BMIs, and were more likely to have lower levels of education.[16]

In our investigation, there was a statistically significant difference in the IL17 average across the analyzed groups, with a cut-off value of 31. 057.This is due to the fact that multiple sclerosis is a crippling inflammatory disease of the central nervous system that causes neurodegeneration and demyelination. The continuous cytokine-regulated autoimmune process appears to have a major impact on the development and recurrence of multiple sclerosis. in contrast to patients who are in remission and healthy controls. This is consistent with past studies that found higher serum levels of IL-17 in MS patients compared to healthy controls. [17, 18]

Furthermore, a prior study showed that the concentration of IL-17 was significantly lower in individuals taking methylprednisolone, interferon-β (IFN-β), or both.[19] Regarding additional research, levels of N/L RATIO, ESR, and CRP only rise during relapses and revert to normal during remissions as a result of our patients' treatment.[20].Several studies have assessed the clinical significance of NLR, ESR, and CRP in MS patients. [21]

The average brain volume, average muscle, and relative cerebral blood flow (rCBF) of the MRI data (ASL) between the case group and the control group in our research revealed a statistically significant difference. This discovery, which also aligns with other studies, regarding inflammation may be the cause of the reduced cerebral perfusion. [22, 23] The limitations of ASL, in contrast to our investigation, may be due to post-processing errors, which may affect our results, especially in situations of small artery disease and white matter disorders like multiple sclerosis.[24]

In our investigation, the extracranial doppler results show a statistically significant difference between the cases group and the control group. Additionally, transcranial doppler velocities in LT ACA provide statistical support. The patient's exclusion criteria, which include the fact that the majority of patients do not smoke and that dyslipidemia has any vascular risk factors during relapse, explain this result. This is consistent with recent research that shows arterial brain flow velocities (BFVs) decreased more in MS patients than in controls during head-up tilt, especially in more disabled individuals. All of these findings suggested that autonomic nerve system dysfunction hampered brain autoregulation mechanisms in MS patients.[25] In a different trial, another study found no difference in CIMT among MS patients. This disparity could be the consequence of various research methodologies, varying sample sizes, and possible ethnic variations that are addressed in this investigation.[26]

CIMT appears to be impacted by age and the disease itself in MS patients, according to other research, which contradicts the findings of our study. Therefore, CIMT rather than Hs-CRP may be a better indicator of the propensity for subclinical atherosclerosis. [21, 27] The end-diastolic and peak systolic blood flow velocities of the left internal carotid artery and the right vertebral artery were also found to be negatively correlated in other studies. Biochemical and lifestyle factors significantly influenced these associations.[28]

5. Summary and Conclusion

Numerous atherosclerotic abnormalities are present in MS patients, which highlight the vascular component of the illness and provide an overview of the pathophysiological alterations that are brought about by the disease and are identified by various laboratory and radiographic markers.

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