Investigating the volume of hippocampus and corpus callosum in Iranian multiple sclerosis patients using magnetic resonance imaging: a retrospective study

Soltani Reza[1], Aghajanpour Fakhroddin[2], Torabi Abolfazl[2], Afshar Azar[2], Kolivand Masoumeh[3], Dehghani Nejad Ali[4], Movassaghi Mahdiyeh[5], Mohammadzadeh Ibrahim[6], Kaedi Hossein[7], Norouzian Mohsen[2]

[1] Student Research Committee, Faculty of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran

[2] Department of Cell Biology and Anatomical Sciences, School of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran

[3] Department of Anatomical Sciences, School of Medicine, Kashan University of Medical Sciences, Kashan, Iran

[4] Department of Anatomical Sciences, School of Medicine, Qazvin University of Medical Sciences, Qazvin, Iran

[5] Master student of Clinical Psychology, Faculty of Clinical Psychology, Allameh Tabatabaei University, Tehran, Iran

[6] Skull Base Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran

[7] Department of Radiology, Loghman Hakim Hospital, Shahid Beheshti University of Medical Sciences, Tehran, Iran

SUMMARY

Multiple Sclerosis (MS) is one of the most common forms of the acquired autoimmune demyelinating disorder affecting the brain. The neurological symptoms of MS are often presented in a relapsing-remitting manner. This study aims to investigate volume changes in the hippocampus and Corpus Callosum (CC). A cross-sectional study was conducted on 200 MS patients, 100 males and 100 females, aged 20-56 years. According to McDonald's 2017 criteria,100 patients were in the MS group, and 100 were in the control group. This study assessed volume changes in the hippocampus and CC with ITK-SNAP 4.0 software. Our study revealed that the volumes of the right Hippocampus ($P<0.0001$), left Hippocampus ($P<0.05$), and corpus callosum ($P<0.001$), were significantly decreased in MS group compared to control group, regardless of the sex of the patients. Additionally, our results showed that the volume of these three areas has no significant difference with the age of MS patients. This research shows that some brain regions, including the hippocampus and corpus callosum, can be essential landmarks in determining MS disease.

Key words: Multiple sclerosis – Hippocampus – Corpus callosum

Shared corresponding authors:
Dr Mohsen Norouzian. Department of Cell Biology and Anatomical Sciences, School of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran. E-mail: norozian93@gmail.com

Submitted: October 18, 2023. **Accepted:** February 20, 2024

https://doi.org/10.52083/VAFU2967

INTRODUCTION

Multiple Sclerosis (MS) is the most common form of the acquired autoimmune demyelinating disorder of the brain. In 2007, the global population of individuals with multiple sclerosis (MS) reached 2 million, and the prevalence of the disease has been on the rise in various regions across the world in recent years (Doerksen et al., 2007; Koch-Henriksen and Sorensen, 2011). The incidence rate of MS has shown an upward trend in many areas, including the Middle East and Iran. Ecological studies have estimated the prevalence to be 24.6/100,000 in Iran in 2006, increasing to 44.53/100,000 in 2011 (Dehghani et al., 2015). Urban lifestyle, and more significantly, smoking, have been identified as contributing factors (Dehghani et al., 2015). The neurological symptoms of MS are often presented in a relapsing-remitting pattern, encompassing a range of presentations, including blurred vision, diplopia, focal weakness, sensory disturbance, ataxia, bladder dysfunction, impaired cognitive abilities of the brain, or even psychiatric symptoms, which are common (Garg et al., 2015; Honeycutt and Smith, 1995).

Multiple Sclerosis (MS) is a chronic condition characterized by a variable natural history. In Relapsing-Remitting MS (RRMS), clinical relapses mark the onset, with subsequent periods of recovery that may be either complete or partial. However, over time, disability tends to accumulate, and the recoveries often become incomplete. Approximately 20% of individuals with RRMS eventually experience a transition to Secondary-Progressive MS (SPMS) during the course of the disease (Cree et al., 2016). In contrast, a minority of patients, around 15%, exhibit a progression of disability from the outset, a condition known as Primary-Progressive MS (PPMS). Notably, recent advancements in Disease-Modifying Treatments (DMTs) have contributed to an improvement in life expectancy and outcomes for individuals diagnosed with MS in more recent cohorts (McGinley et al., 2021; Marrie et al., 2015).

When a patient presents with neurological manifestations suggestive of MS, brain Magnetic Resonance Imaging (MRI) becomes pivotal for diagnosing and disease monitoring. This emphasis on MRI for diagnosis and monitoring has been underscored by the 2017 McDonald Criteria (Kalincik et al., 2012).

Among numerous diagnostic landmarks for MS, the Corpus Callosum (CC) holds a special place. CC is a commissural structure connecting the cortices of the two cerebral hemispheres. The CC is divided into rostrum, genu, body, and splenium in the sagittal plane from anterior to posterior. Recent advances in imaging technologies have revealed a lobe-specific structural connectivity gradient along the CC, reshaping our understanding of this brain region (Garg et al., 2015). The specific arterial supply to the CC renders it relatively resistant to chronic small vessel ischemia, as the many penetrating arterioles contributing to the circulation of CC show resistance to atherosclerosis (Garg et al., 2015). This makes the lesions in this region often specific to inflammatory processes, including MS. Abnormalities within the CC are observed in 55% to 95% of the patients. These CC lesions are diverse, but progressive CC atrophy in MS is known to correlate with disability and cognitive dysfunction (Garg et al., 2015; Kalincik et al., 2012; Llufriu et al., 2019; Honeycutt and Smith, 1995).

The hippocampus, situated in the temporal lobe, is a complex structure with distinct anatomical and functional features. It plays an important role in learning and memory (Anand and Dhikav, 2012). Studies utilizing MRI have enabled the specification of hippocampal morphology and its connections with other brain regions, linking these features to clinical and behavioural performances (Mey et al., 2023).

While there are relatively few studies investigating hippocampal alterations in MS, understanding the radiologic characteristics of the hippocampus in the progress of the disease may provide valuable insights into the relationships between hippocampal subfield changes and neurological presentations, particularly cognitive impairments. Furthermore, research has consistently demonstrated that age, sex, and race can influence various aspects of brain anatomy, including the hippocampus and corpus callosum (Caldito et al., 2018; Tokarska et al., 2023).

Given the limited number of studies conducted in the Iranian population, our study aims to com-

pare the volumes of the corpus callosum and hippocampus in the brain MRI of Iranian MS patients relative to healthy individuals. The results of this study may pave the way for future research, contributing to the development of radiological standards for accurate diagnosis and the assessment of MS severity while enhancing our understanding of the disease's nature.

MATERIALS AND METHODS

This cross-sectional study was conducted in the Radiology Department of Shahid Beheshti University of Medical Sciences. This study extended between March 2022 to March 2023 and received approval from the Student Research Committee at Shahid Beheshti University of Medical Sciences (IR.SBMU.RETECH.REC.1401.777).

Participants

We recruited a total number of 100 MS patients, maintaining an equal gender distribution, with participants aged between 20 and 56 years. Inclusion criteria encompassed individuals aged 18 years and older who met the McDonald's 2017 criteria. Exclusion criteria involved patients with positive imaging findings, as well as those with neurological or cognitive disorders stemming from conditions such as brain or spinal cord ischemia, tumours, infectious diseases impacting the neurological system, genetic disorders, systemic autoimmune disorders, brain or spinal trauma, or drug abuse affecting the neurological system. All images were reviewed and approved by experienced radiologists. The individuals responsible for brain region size measurement and image analysis were blinded to the disease, state, gender, and other personal information regarding the participants. Additionally, we randomly selected 100 healthy patients who had sought medical attention for headaches as the control group.

Magnetic Resonance Imaging (MRI) of the Brain

MRI scans were conducted in our Imaging Department using the Siemens 1.5T system from Siemens Medical Systems, Germany.

Routine brain MRI protocols included axial (T1WI and T2WI), coronal PDWI, and sagittal T1WI. A 3D T1WI sequence was performed with a slice thickness of 0.5 mm and slice spacing of 0 mm. The study images were converted into DICOM format and transferred to a personal computer (PC) workstation equipped with ITK-SNAP 4.0 software. ITK-SNAP 4.0 is an open-source software package designed for visualization and computation on medical images. It was developed by student teams led by Guido Gerig at the University of North Carolina, in collaboration with NYU Tanden School of Engineering. Notably user-friendly, this tool finds specific application in the field of image segmentation. Its primary function is to facilitate the reliable morphometry of structures of interest through manual methods.

Image Analysis

The structures scrutinized in this study included the total part of Hippocampus and CC. Volumetric analysis was conducted on the 3D T1-weighted images in both axial and sagittal planes. Manual tracing was drawn around the boundaries of the hippocampi and CC (Fig. 1). These boundaries were established according to anatomical landmarks on each slice based on accepted conventions derived from those used in the literature and from comparison with standard brain atlases (Morelli et al., 2020). The area within the boundary was calculated and multiplied by slice thickness to obtain volumetric measurement (cm^3).

Statistical analysis

All statistical analyses were executed using SPSS version 23, and graphs were plotted with Graph Pad Prism 9. Data are presented as mean ± SD. The normality of all data was assessed using Kolmogorov–Smirnov test.

A two-way ANOVA and Tukey's post-hoc test was employed to evaluate mean differences between groups, categorized based on two independent variables, and to assess the interaction effect between these variables—specifically, sex and the state of disease. The correlation between variables, the volume of regions and the age of MS patients, were examined using the Pearson correlation coefficient (PCC). A significance level of $P < 0.05$ was considered to determine statistical significance in the analyses.

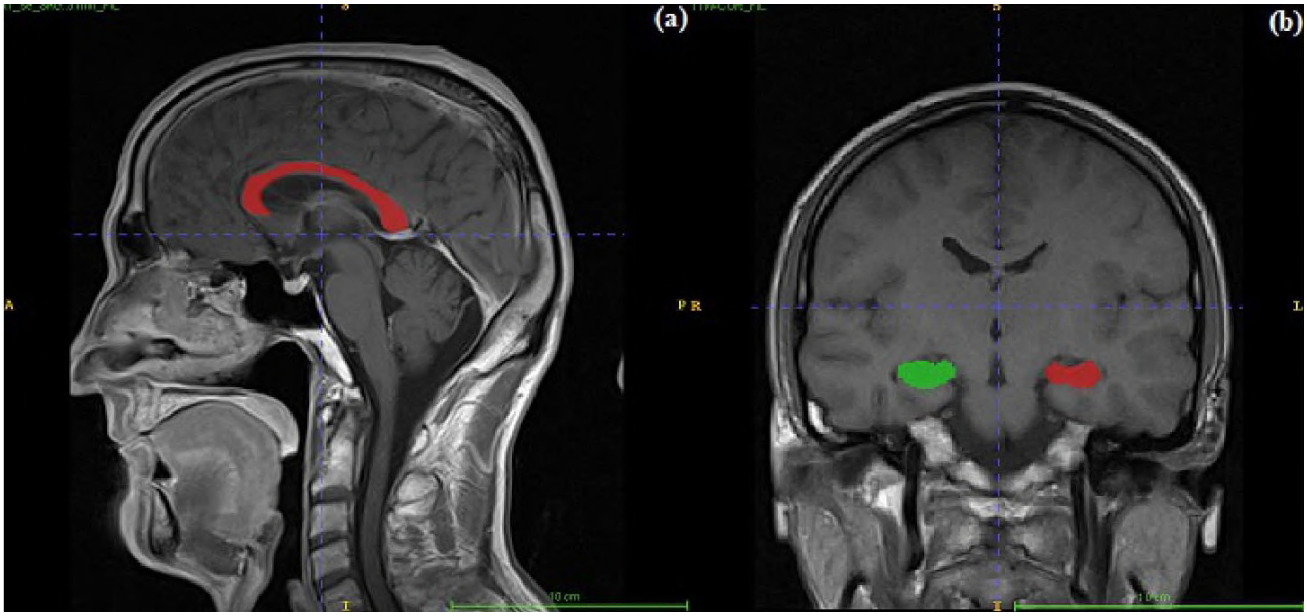

Fig. 1.- Example of manual segmentation in the control group. **a)** sagittal view of the corpus callosum segmentation. **b)** coronal view of the hippocampus segmentation.

RESULTS

Participants

All MS patients were included in the study based on McDonald's 2017 criteria. Th analysis included 100 healthy patients (50 males and 50 females) and 100 MS patients (50 males and 50 females) were analysed. The mean ± SD age of patients in the control and MS group was (39.02±11.87) and (39.46±9.67) years, respectively (Table1).

Table 1. Demographic statistics.

Variables		Mean±SD	
		Control	MS
Age		39.02±11.87	39.46±9.67
Gender	Male	50 (38.34±9.34)	50 (38.82±8.99)
	Female	50 (39.7±13.98)	50 (40.10±10.36)
Total		100	100

Volumetric assessment in experimental groups

In this study, the volume of the right hippocampus in the control and MS groups was (3.18±4.56) and (2.42±5.58) cm^3, respectively. Moreover, the volume of the left hippocampus in the control and MS group was (3.16±1.97) and (2.75±4.52) cm^3, respectively. Also, the volume of the corpus callosum in the control and MS groups was (16.54±5.30) and (12.40±10.4) cm^3, respectively. The Results from the volumetric assessment indicated a significant decrease in the volume of the right hippocampus (F=140.767, df=1, P=0.000), left hippocampus (F=4.083, df=1, P<0.045), and corpus callosum (F=12.668, df=1, P=0.000) in the MS group compared to the control group (Table 2).

Table 2. Results and comparison of mean volume three brain regions in experimental groups (cm^3).

Regions	Mean±SD		F (df=1)	p
	Control (n=100)	MS (n=100)		
Right Hippocampus Volume	3.18±4.56	2.42±5.58	140.767	0.000
Left Hippocampus Volume	3.16±1.97	2.75±4.52	4.083	<0.045
Corpus Callosum Volume	16.54±5.30	12.40±10.4	12.668	0.000

Volumetric assessment in both sexes

Volumetric assessment was done in both sexes. This assessment showed that the right hippocampus volume in the males and females was (3.07±45.63) and (2.53±45.63) cm^3, respectively. Moreover, the left hippocampus volume in the males and females was (3.01±14.40) and

(2.90±14.40) cm^3, respectively. Furthermore, the corpus callosum volume in males and females was (13.63±82.08) and (15.61±82.08) cm^3, respectively. A significant sex difference was observed in the volume of the right hippocampus (F=69.956, df=1, P=0.000) and corpus callosum (F=3.885, df=1, P<0.050), with the right hippocampus volume being lower in females and the corpus callosum volume being lower in males. However, no significant difference (F=0.340, df=1, P<0.561) was detected in the left hippocampus volume between males and females (Table 3).

Table 3. Results and comparison of mean volume three brain regions in both sexes (cm^3).

Regions	Mean±SD		F (df=1)	P
	Male (n=100)	Female (n=100)		
Right Hippocampus Volume	3.07±45.63	2.53±45.63	69.956	0.000
Left Hippocampus Volume	3.01±14.40	2.90±14.40	0.340	<0.561
Corpus Callosum Volume	13.33±82.08	15.61±82.08	3.885	<0.050

Interaction Effect of sex and pathology in Volume regions

The two-way ANOVA test results indicated no significant difference in the interaction effect of sex and pathology on the volume of the right (F=0.707, df=1, P<0.40) and left hippocampus (F=0.297, df=1, P<0.58) and corpus callosum (F=0.970, df=1, P<0.32; Table 4).

Assessment of the correlation between volume regions and age patients

This assessment aimed to determine the correlation between the volume of regions and the age of MS patients. The correlation between the volume of the right hippocampus (r= 0.0494), left hippocampus (r=0.0646), and corpus callosum (r=0.0016) with age patients was investigated using Pearson's correlation coefficient. This assessment showed that the volume of these three areas has no significant difference with the age of MS patients (P >0.05; Table 5).

Table 5. Pearson correlation between volume regions and age patients.

Regions	Age	
	R	P
Right Hippocampus Volume	0.0494	0.62
Left Hippocampus Volume	0.0646	0.52
Corpus Callosum Volume	0.0016	0.98

DISCUSSION

In this study, we did a volumetric analysis of brain images of patients with MS to investigate the sizes of two cortical brain structures, namely, the corpus callosum and the hippocampus, compared to those in healthy controls. In our current study, we had undertaken an analysis to explore the connection between the volume of the hippocampus and corpus callosum among individuals of varying ages and genders.

The findings of this study will provide some valuable intuitions into the alteration of these regions in the background of MS, shedding light on potential implications of the pathophysiology of the disease.

Table 4. Results of interaction effect of sex and pathology in volume regions (cm^3).

Regions	Mean±SD				F (df=1)	P
	Male (n=100)		Female (n=100)			
	Control (n=50)	MS (n=50)	Control (n=50)	MS (n=50)		
Right Hippocampus Volume	3.42±64.53	2.71±64.53	2.94±64.53	2.12±64.53	0.707	<0.40
Left Hippocampus Volume	3.17±20.36	2.86±20.36	3.16±20.36	2.63±20.36	0.297	<0.58
Corpus Callosum Volume	15.96±11.60	10.69±11.60	17.11±11.60	14.12±11.60	0.970	<0.32

A pivotal finding in our study was the notable reduction in the measure of the right and the left hippocampus in the MS group, which aligns with many other previous researches (Mey et al., 2023; Morelli et al., 2020; Naghavi et al., 2023). The observed hippocampal atrophy signifies the intrinsic neural alterations, encompassing demyelination and neural degeneration, that transpire throughout the progression of multiple sclerosis (Mey et al., 2023).

Pelletier et al. (2001) demonstrated that hippocampal atrophy may indicate a progressive cognitive decline, particularly in the left hippocampus. The hippocampal volume is linked to cognitive reserve measurements. Throughout the disease, the correlation between atrophy in hippocampal subfields and Information Processing Speed (IPS) appears to intensify (Planche et al., 2018; Platten et al., 2022; Sotgiu et al., 2022).

Another notable finding from our investigation was the shrinkage of the corpus callosum. This outcome also aligns with the existing body of evidence (Sparaco et al., 2021; Llufriu et al., 2019). Prompt identification of callosal atrophy in patients during their initial demyelination episode can predict their progression to MS (Sumowski et al., 2016; Thompson et al., 2018). The precise impact of corpus callosum (CC) atrophy on the disability of patients with MS remains elusive. While specific studies posit a correlation between CC atrophy and disability, contrasting perspectives exist (Yaldizli et al., 2010).

The fundamental mechanisms underlying hippocampal atrophy in the brains of patients with MS are intricately complex, involving factors such as the depletion of synaptic proteins and microglial responses triggered by complement activation in response to axonal demyelination and injury, among other contributing elements. This notable hippocampal atrophy, particularly in the CA1 region, has been observed to align with a decline in performance on memory-related tasks. The capacity of learning is also compromised (Zhao et al., 2021). Corpus callosum atrophy is also attributed to distinct inflammatory cellular responses, which precipitate the demise of oligodendrocytes (Zheng et al., 2022).

Our study found that the volume of the right hippocampi differed significantly between males and females. Specifically, the hippocampi in males were larger than those in females. Indeed, this discovery aligns with the findings of other studies that have explored sex-specific differences in the brains (Ruigrok et al., 2014). Researchers have identified that factors, including menstrual cycle, hormonal therapy, genotype, and testosterone levels, can affect the hippocampus volume in males and females (Lisofsky et al., 2015; Everaerd et al., 2012). However, we have not found significant differences in the left hippocampus volume between males and females.

Our study results indicated that CC volume in females was more significant than in males. This discovery aligns with other research finding that CC volume in females was more significant than in males (Shiino et al., 2017). However, Luders et al. (2014) have shown the opposite results. Several studies have shown that the gender difference in the volume of the corpus callosum can be due to the size of the brain (Ardekani et al., 2013).

The results of the literature corroborate our findings, suggesting that compromised hippocampal tissue integrity early in the course of MS results in diminished whole and regional hippocampal volumes, although our study specifically assessed the whole hippocampal volume (Sicotte et al., 2008; Longoni et al., 2015; Ciolac et al., 2021).

When assessing the interaction effect of sex and the disease state (MS or control) on the volume of the regions of interest, our statistical analysis revealed no significant differences in the volume of the right and left hippocampus, as well as the corpus callosum. In simpler terms, within our study cohort, there was no significant difference in the impact of disease status on regional volumes between females and males.

Studies on sex differences in corpus callosum volumetric indices in patients with MS are currently lacking.

In a paper published in 2021, Ciolac et al. evaluated the morphometric networks of the hippocampus, its anatomic compartments, and their impact on cognitive performance in both gen-

ders. The results of their study indicate a more clustered architecture in females with MS compared to males, both at baseline and after a 2-year follow-up. Interestingly, other available studies on sex differences in brain networks in MS patients did not report any significant distinctions between males and females (Ciolac et al., 2021; Schoonheim et al., 2012). It is worth noting that there may be regional, sex-specific changes in the hippocampus for both males and females, as highlighted by Ciolac et al. (2021).

Notably, there is a gap in the literature regarding studies on sex differences in callosal volume in MS patients. Among the limited research available, the results have shown a consistent pattern of similarity in terms of sex differences in the corpus callosum among MS patients with our study (Khasawneh, 2023).

Regarding age, our research found no significant correlation between the volume of the three areas of interest in MS patients. The relationship between a patient's age and the size of the brain regions involved in the disease is not established. Further investigations are needed to explore this relationship, as there is currently a lack of comprehensive literature on the subject.

While our study offers valuable insights into the volumetric measurements of the hippocampus and corpus callosum in patients with MS compared to a healthy population, we acknowledge the limitations inherent to our study design. Firstly, the small sample size may limit the generalizability of our results. Additionally, our study exclusively focused on size measurements of the regions of interest, potentially constraining our understanding of the underlying pathophysiology driving these changes.

Unfortunately, our study did not specifically explore subregional variations within the hippocampus. Furthermore, exploring the natural progression of the disease would enhance our insights into the relationship between Multiple Sclerosis and the morphological changes in the hippocampus and corpus callosum.

Conducting longitudinal studies with larger and more diverse cohorts and delving into molecular and cellular-level investigations could significantly enhance our comprehension of the underlying nature of the disease in the future.

In summary, it is evident that patients with MS exhibit a decrease in the volume of both the Hippocampus and Corpus Callosum. The outcomes of this study, in conjunction with findings from other investigations, contribute to the advancement of our comprehension regarding the underlying disease mechanisms. This collective knowledge holds the potential to refine prognostic assessments and pave the way for innovative therapeutic approaches.

CONCLUSION

The results of our study show that some of the brain regions, including the hippocampus and corpus callosum, can be essential landmarks in determining MS disease. These structures can relate to variables including age and sex, which can give doctors a better view of the disease. Moreover, it can provide neurologists with comprehensive information about some diseases, including Alzheimer's.

ACKNOWLEDGEMENTS

This study relates to project NO 1401/59304 From the Student Research Committee, Shahid Beheshti University of Medical Sciences, Tehran, Iran. We also appreciate the "Student Research Committee" and "Research & Technology Chancellor" at Shahid Beheshti University of Medical Sciences for their financial support of this study.

REFERENCES

ANAND KS, DHIKAV V (2012) Hippocampus in health and disease: An overview. *Ann Indian Acad Neurol*, 15: 239.

ARDEKANI BA, FIGARSKY K, SIDTIS JJ (2013) Sexual dimorphism in the human corpus callosum: an MRI study using the OASIS brain database. *Cereb Cortex*, 23(10): 2514-2520.

CALDITO NG, SAIDHA S, SOTIRCHOS ES, DEWEY BE, COWLEY NJ, GLAISTER J, FITZGERALD KC, AL-LOUZI O, NGUYEN J, ROTHMAN A (2018) Brain and retinal atrophy in African-Americans versus Caucasian-Americans with multiple sclerosis: a longitudinal study. *Brain*, 141: 3115-3129.

CIOLAC D, GONZALEZ-ESCAMILLA G, RADETZ A, FLEISCHER V, PERSON M, JOHNEN A, LANDMEYER NC, KRÄMER J, MUTHURAMAN M, MEUTH SG, GROPPA S (2021) Sex-specific signatures of intrinsic hippocampal networks and regional integrity underlying cognitive status in multiple sclerosis. *Brain Commun*, 3(3): fcab198.

CREE BA, GOURRAUD PA, OKSENBERG JR, BEVAN C, CRABTREE-HARTMAN E, GELFAND JM, GOODIN DS, GRAVES J, GREEN AJ, MOWRY E, OKUDA DT, PELLETIER D, VON BUDINGEN HC, ZAMVIL SS, AGRAWAL A, CAILLIER S, CIOCCA C, GOMEZ R, KANNER R, LINCOLN R, LIZEE

A, QUALLEY P, SANTANIELLO A, SULEIMAN L, BUCCI M, PANARA V, PAPINUTTO N, STERN WA, ZHU AH, CUTTER GR, BARANZINI S, HENRY RG, HAUSER SL (2016) Long-term evolution of multiple sclerosis disability in the treatment era. *Ann Neurol*, 80: 499-510.

DEHGHANI R, YUNESIAN M, SAHRAIAN MA, GILASI HR, KAZEMI MOGHADDAM V (2015) The evaluation of multiple sclerosis dispersal in iran and its association with urbanization, life style and industry. *Iran J Public Health*, 44: 830-838.

DOERKSEN SE, MOTL RW, MCAULEY E (2007) Environmental correlates of physical activity in multiple sclerosis: a cross-sectional study. *Int J Behav Nutr Phys Act*, 4: 49.

EVERAERD D, GERRITSEN L, RIJPKEMA M, FRODL T, VAN OOSTROM I, FRANKE B, FERNÁNDEZ G, TENDOLKAR IJN (2012) Sex modulates the interactive effect of the serotonin transporter gene polymorphism and childhood adversity on hippocampal volume. *Neuropsychopharmacology*, 37(8): 1848-1855.

GARG N, REDDEL SW, MILLER DH, CHATAWAY J, RIMINTON DS, BARNETT Y, MASTERS L, BARNETT MH, HARDY TA (2015) The corpus callosum in the diagnosis of multiple sclerosis and other CNS demyelinating and inflammatory diseases. *J Neurol Neurosurg Psychiat*, 86: 1374-1382.

HONEYCUTT NA, SMITH CD (1995) Hippocampal volume measurements using magnetic resonance imaging in normal young adults. *J Neuroimaging*, 5: 95-100.

KALINCIK T, VANECKOVA M, TYBLOVA M, KRASENSKY J, SEIDL Z, HAVRDOVA E, HORAKOVA D (2012) Volumetric MRI markers and predictors of disease activity in early multiple sclerosis: a longitudinal cohort study. *PloS One*, 7: e50101.

KHASAWNEH R (2023) The impact of multiple sclerosis on the size and morphology of corpus callosum: an MRI-based retrospective study. *Int J Morphol*, 41: 422.

KOCH-HENRIKSEN N, SORENSEN PS (2011) Why does the north-south gradient of incidence of multiple sclerosis seem to have disappeared on the northern hemisphere? *J Neurol Sci*, 311: 58-63.

LISOFSKY N, MÅRTENSSON J, ECKERT A, LINDENBERGER U, GALLINAT J, KUHN SJN (2015) Hippocampal volume and functional connectivity changes during the female menstrual cycle. *Neuroimage*, 118: 154-162.

LLUFRIU S, ROCCA MA, PAGANI E, RICCITELLI GC, SOLANA E, COLOMBO B, RODEGHER M, FALINI A, COMI G, FILIPPI M (2019) Hippocampal-related memory network in multiple sclerosis: a structural connectivity analysis. *Mult Scler J*, 25: 801-810.

LONGONI G, ROCCA MA, PAGANI E, RICCITELLI GC, COLOMBO B, RODEGHER M, FALINI A, COMI G, FILIPPI M (2015) Deficits in memory and visuospatial learning correlate with regional hippocampal atrophy in MS. *Brain Struct Funct*, 220: 435-444.

LUDERS E, TOGA AW, THOMPSON PM (2014) Why size matters: differences in brain volume account for apparent sex differences in callosal anatomy: the sexual dimorphism of the corpus callosum. *Neuroimage*, 84: 820-824.

MARRIE RA, ELLIOTT L, MARRIOTT J, COSSOY M, BLANCHARD J, LEUNG S, YU N (2015) Effect of comorbidity on mortality in multiple sclerosis. *Neurology*, 85: 240-247.

MCGINLEY MP, GOLDSCHMIDT CH, RAE-GRANT AD (2021) Diagnosis and treatment of multiple sclerosis: a review. *JAMA*, 325: 765-779.

MEY GM, MAHAJAN KR, DESILVA TM (2023) Neurodegeneration in multiple sclerosis. *WIREs Mech Disease*, 15: e1583.

MORELLI ME, BALDINI S, SARTORI A, D'ACUNTO L, DINOTO A, BOSCO A, BRATINA A, MANGANOTTI P (2020) Early putamen hypertrophy and ongoing hippocampus atrophy predict cognitive performance in the first ten years of relapsing-remitting multiple sclerosis. *Neurol Sci*, 41: 2893-2904.

NAGHAVI S, ASHTARI F, ADIBI I, SHAYGANNEJAD V, RAMEZANI N, POURMOHAMMADI A, DAVANIAN F, KARIMI Z, KHALIGH-RAZAVI S-M, SANAYEI M (2023) Effect of deep gray matter atrophy on information processing speed in early relapsing-remitting multiple sclerosis. *Mult Scler Relat Disord*, 71: 104560.

PELLETIER J, SUCHET L, WITJAS T, HABIB M, GUTTMANN C, SALAMON G, LYON-CAEN O, CHERIF AA (2001) A longitudinal study of callosal atrophy and interhemispheric dysfunction in relapsing-remitting multiple sclerosis. *Arch Neurol*, 58: 105-111.

PLANCHE V, KOUBIYR I, ROMERO JE, MANJON JV, COUPE P, DELOIRE M, DOUSSET V, BROCHET B, RUET A, TOURDIAS T (2018) Regional hippocampal vulnerability in early multiple sclerosis: Dynamic pathological spreading from dentate gyrus to CA 1. *Human Brain Mapping*, 39: 1814-1824.

PLATTEN M, OUELLETTE R, HERRANZ E, BARLETTA V, TREABA CA, MAINERO C, GRANBERG T (2022) Cortical and white matter lesion topology influences focal corpus callosum atrophy in multiple sclerosis. *J Neuroimaging*, 32: 471-479.

RUIGROK AN, SALIMI-KHORSHIDI G, LAI M-C, BARON-COHEN S, LOMBARDO MV, TAIT RJ, SUCKLING J (2014) A meta-analysis of sex differences in human brain structure. *Neurosci Biobehav Rev*, 39(100): 34-50.

SCHOONHEIM MM, HULST HE, LANDI D, CICCARELLI O, ROOSENDAAL SD, SANZ-ARIGITA EJ, VRENKEN H, POLMAN CH, STAM CJ, BARKHOF F, GEURTS JJ (2012) Gender-related differences in functional connectivity in multiple sclerosis. *Mult Scler*, 18:164-173.

SHIINO A, CHEN Y, TANIGAKI K (2017) Sex-related difference in human white matter volumes studied: inspection of the corpus callosum and other white matter by VBM. *Sci Rep*, 7: 3-9.

SICOTTE NL, KERN KC, GIESSER BS, ARSHANAPALLI A, SCHULTZ A, MONTAG M, WANG H, BOOKHEIMER SY (2008) Regional hippocampal atrophy in multiple sclerosis. *Brain*, 131: 1134-1141.

SOTGIU M, PIGA G, MAZZARELLO V, ZARBO I, CARTA A, SADERI L, SOTGIU S, CONTI M, SABA L, CRIVELLI P (2022) Corpus callosum volumetrics and clinical progression in early multiple sclerosis. *Eur Rev Med Pharmacol Sci*, 26(1): 225-231.

SPARACO M, LAVORGNA L, BONAVITA S (2021) Psychiatric disorders in multiple sclerosis. *J Neurol*, 268: 45-60.

SUMOWSKI JF, ROCCA MA, LEAVITT VM, RICCITELLI G, SANDRY J, DELUCA J, COMI G, FILIPPI M (2016) Searching for the neural basis of reserve against memory decline: intellectual enrichment linked to larger hippocampal volume in multiple sclerosis. *Eur J Neurol*, 23: 39-44.

THOMPSON AJ, BANWELL BL, BARKHOF F, CARROLL WM, COETZEE T, COMI G, CORREALE J, FAZEKAS F, FILIPPI M, FREEDMAN MS (2018) Diagnosis of multiple sclerosis: 2017 revisions of the McDonald criteria. *Lancet Neurology*, 17: 162-173.

TOKARSKA N, TOTTENHAM I, BAAKLINI C, GAWRYLUK JR (2023) How does the brain age in individuals with multiple sclerosis? A systematic review. *Front Neurol*, 14: 1207626.

YALDIZLI Ö, ATEFY R, GASS A, STURM D, GLASSL S, TETTENBORN B, PUTZKI N (2010) Corpus callosum index and long-term disability in multiple sclerosis patients. *J Neurol*, 257: 1256-1264.

ZHAO Z, LI T, DONG X, WANG X, ZHANG Z, ZHAO C, KANG X, ZHENG R, LI X (2021) Untargeted metabolomic profiling of cuprizone-induced demyelination in mouse corpus callosum by UPLC-orbitrap/ms reveals potential metabolic biomarkers of CNS demyelination disorders. *Oxid Med Cell Longev*, 2021: 7093844.

ZHENG F, LI Y, ZHUO Z, DUAN Y, CAO G, TIAN D, ZHANG X, LI K, ZHOU F, HUANG M (2022) Structural and functional hippocampal alterations in multiple sclerosis and neuromyelitis optica spectrum disorder. *Mult Scler J*, 28: 707-717.