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ORIGINAL ARTICLES

Assessment of the effect of cigarette smoking on regional brain volumes and lesion load in patients with clinically isolated syndrome

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Purpose: Smoking has been associated with an increased risk of developing multiple sclerosis, disease progression and clinical disability. We detected the effects of smoking on regional brain volumes and lesion load in patients with clinically isolated syndrome using quantitative magnetic resonance imaging. **Materials and Methods:** Smoker patients ($n = 16$), smoker healthy controls ($n = 13$), non-smoker patients ($n = 17$) and non-smoker healthy controls ($n = 14$) underwent magnetic resonance imaging and neocortical volumes were measured. Lesion load was calculated in terms of number and volume of white matter hyperintensities. **Results:** Smoking was associated with increased gray matter volumes in several regions of the brain. A tendency towards greater lesion load in smoker patients was found. Smoking duration was significantly negatively correlated with intracranial volume and left hemisphere cortical gray matter volume. There was no relationship between regional brain volumes and clinical disability scores, lesion load duration of the disease and degree of smoking exposure. **Conclusions:** Clinically isolated syndrome related regional brain atrophy might vary in extent and severity with smoking. Despite increased lesion load, less cortical and deep gray matter damage with a possible neuroprotective effect occurs in smoking.

KEYWORDS: clinically isolated syndrome, cigarette smoking, brain volume

Introduction

Multiple sclerosis (MS) is a chronic inflammatory demyelinating disease of the central nervous system (CNS) with a complex etiology that predominantly affects young adults [1].

With limited availability of the epidemiologic MS studies in Turkey, the prevalence of MS seems between 34/1000.000 and 101/100.000 in different districts of Turkey [2,3]. Although patients usually recover from their presenting episode, clinically isolated syndrome (CIS) often converts into MS even with increased rates if associated with several risk factors [4,5]. Of the envi-

ronmental factors, cigarette smoking not only increases the risk of development of CIS to MS, but also modifies the clinical course of the disease [4].

Nicotine has been held responsible for the majority of the detrimental effects of smoking on the CNS [6–8]. It acts as an agonist within the cholinergic neurotransmitter system and nicotinic receptors are widely distributed within the CNS with the highest density in the thalami [6,9,10]. Although there has been some inconsistency in the results, magnetic resonance imaging (MRI) has been used as a tool to demonstrate the smoking related changes on the CNS: smaller gray matter (GM) [7,11,12] and white matter (WM) [13,14] brain volumes as well as greater corpus callosum (CC) volume [8]. Also Gazdzinski et al. found that chronic smoking in alcohol dependence was associated with more temporal WM volume [15].

MRI is an essential part of the diagnostic criteria in MS. Although atrophy of brain tissue is not included in

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MS diagnostic criteria, decreased global or regional GM volumes in patients with CIS have been associated with a higher rate of conversion to MS [16,17].

In this study, it was aimed to determine whether smoking would result in distinct alterations in regional brain volumes of patients with CIS as assessed by quantitative MRI. In addition, the relationship was examined between smoking duration, age at onset of smoking and regional brain volumes. The secondary objective of the study was to evaluate the potential influence of smoking on lesion load in patients with CIS.

Materials and methods

Participants

The institutional review board approved the study. All participants gave written informed consent.

A total of 60 right-handed, equivalently educated subjects participated in our cross-sectional study. Healthy subjects and patients were collected in four age-matched groups according to smoking habits and presence of CIS: CIS_s ($n = 16$; F/M = 8/8; mean age (\pm SD) = 34.3 (\pm 8.9) years), HC_s ($n = 13$; F/M = 6/7; mean age (\pm SD) = 33.7 (\pm 7.4) years), CIS_{ns} ($n = 17$; F/M = 13/4; mean age (\pm SD) = 29.7 (\pm 6.9) years) and HC_{ns} ($n = 14$; F/M = 10/4; mean age (\pm SD) = 29.8 (\pm 7.9) years). Non-smoker participants had smoked no more than five cigarettes in their lifetime.

Twenty-seven healthy volunteers were recruited from e-mail based on advertisement and volunteers from hospital workers. Those individuals underwent systemic and neurological examination and their medical records were reviewed. Healthy participants with any history of either substance abuse or dependence disorders other than nicotine dependence and contraindications to MRI were excluded from the study.

Thirty-three consecutive patients admitted to our institution during the years 2012–2014 were included in the study. All patients fulfilled the McDonald diagnostic criteria for MS but had only one clinical episode containing optic neuritis, cervical and/or thoracic myelitis or brainstem/cerebellar syndrome. Exclusion criteria for the patients were the presence of any systemic or neurological disease other than CIS, receiving steroid therapy or having an attack within 4 weeks of the MRI scan. No cases were converted to clinically definite MS during the study. Same patients also constituted the participants of another MRI study on diffusion tensor imaging alterations.

All patients were evaluated by two neurologists (S. Diker, A. Tuncer) with 4 and 15 years of experience, respectively, unaware of the MRI findings.

Image acquisition and analysis

All the participants had T1-weighted three-dimensional (3D) high resolution images with 0.9 mm isotropic voxels (MPRAGE) (TR/TE: 2600/3.1 ms; matrix: 224 \times 256; NEX: 1; TA: 4.06; number of slices: 176; slice thickness: 1.00 mm; distance factor: 50%; voxel size: 1 mm; in plane resolutions: 1 mm) and double inversion-recovery sampling perfection with application optimized contrasts using different flip angle evolution (DIR SPACE) (TR/TE: 7500/325 ms; matrix: 192 \times 192; NEX: 1; TA: 6.09; number of slices: 144; slice thickness: 1.12 mm without intersection gap; voxel size: 1 mm; in plane resolutions: 1 mm) imaging on a 3-Tesla MR scanner (Magnetom, Tim, Siemens, Germany). DIR SPACE sequence was used for better multiplanar delineation of the lesions.

The imaging data were free of motion artifact and no subject was discarded from the study. The measurements of neocortical volumes (mm³) on 3D MPRAGE T1WI were performed using FreeSurfer Version 4.5.1 (<http://surfer.nmr.mgh.harvard.edu/>). The automated procedures for volumetric measures of the different brain structures were described by Fischl et al. [18]. Following automated segmentation, normalization to total intracranial volume was obtained. The reconstructed cortical surface models for each participant were manually inspected to ensure segmentation accuracy; regions with poor segmentation accuracy due to poor image quality or misregistration were excluded from further statistical analyses. Cortical surfaces were automatically parcellated and combined to create average volume for total GM and for frontal, temporal, parietal and occipital lobar regions. The volume of T1 hypointense lesions was also calculated automatically by this software.

The coordinations of each hyperintense WM lesion on DIR SPACE imaging was recorded using BrainVoyager QX 2.6 for Linux (<http://www.brainvoyager.com>). The lesions were ranged by a bounding box in which all the intensities of voxels were fixed to a threshold for a clear visual distinction of lesions and parenchyma (<http://support.brainvoyager.com/volumespace/107-volume-rendering/314-users-guide-masking-and-cutting.html>). The number of the voxels-of-interest (VOIs) was calculated and the total volume of VOIs where the lesions were ranged by bounding box. In this way, it was aimed to determine the demyelinating hyperintense lesion load in respect of number and volume of the lesions in the brain of patients with CIS. Patients received intravenous gadopentetate dimeglumine (0.01 mmol/kg) contrast material and postcontrast axial T1W 3D MPRAGE and coronal fat saturated images (TR/TE = 550/15 ms; matrix = 256 \times 256 NEX = 2) were obtained at 5 min after injection in all patients to detect active demyelinating lesion.

Table 1. Baseline demographic characteristics, clinical data and smoking histories for all groups. mean (\pm SD).

	CIS _{ns} (<i>n</i> = 17)	HC _{ns} (<i>n</i> = 14)	CIS _s (<i>n</i> = 16)	HC _s (<i>n</i> = 13)	<i>p</i> value
Age (years)	29.7 (\pm 6.9)	29.8 (\pm 7.9)	34.3 (\pm 8.9)	33.7 (\pm 7.4)	$P^1 = 0.226$
Gender (female/male)	13/4	10/4	8/8	6/7	$P^1 = 0.223$
Pack-year	–	–	9.3 (\pm 10.1)	7.7 (\pm 4.7)	$p^2 = 0.904$
Cigarettes per day	–	–	14.3 (\pm 10.6)	12.9 (\pm 6.6)	$p^2 = 0.760$
Duration of smoking (years)	–	–	11.6 (\pm 8.5)	12.6 (\pm 8.8)	$p^2 = 0.680$
Age at onset of smoking (years)	–	–	20.6 (\pm 5.4)	21.0 (\pm 5.8)	$p^2 = 0.716$
Time lapse from clinical episode (months)	9.2 (\pm 7.0)	–	10.8 (\pm 16.5)	–	$p^3 = 0.313$

CIS_s smoker patients with clinically isolated syndrome, HC_s smoker healthy controls, CIS_{ns} non-smoker patients with clinically isolated syndrome, HC_{ns} non-smoker healthy controls.

P^1 values among all groups.

p^2 values between all smoker individuals.

p^3 values between patient groups.

Statistical analysis

Numerical variables were evaluated for normality of data distribution using the Kolmogorov–Smirnov test. Descriptive statistics were stated as mean \pm standard deviation. Independent samples *t*-test was used to compare the means of two groups. The Mann Whitney U test was used to compare two groups for non-parametric data. A Spearman's rho correlation coefficient was used to investigate the relationship between two variables. One-way analysis of variance was applied to compare the differences between groups. Multiple linear regression was performed to assess the association between two or more independent variables and a single continuous dependent variable. The analysis was also accessed to detect whether confounding exists. The effect of group on dependent variables was examined by multiple linear regression model, after adjustment for age and sex. A value of $p \leq 0.05$ was accepted as statistically significant. Data analysis was performed by IBM SPSS Statistics 21 software.

Results

Subjects and clinical characteristics

The patient group included 16 smokers (F/M = 8/8, mean age (\pm SD) 34.3 (\pm 8.9) years) and 17 non-smokers (F/M = 13/4, mean age (\pm SD) 29.7 (\pm 6.9) years). The mean time lapse from clinical episodes for CIS_s and CIS_{ns} were 10.8 (\pm 16.5) and 9.2 (\pm 7.0) months, respectively. CIS_s and CIS_{ns} had a mean expanded disability status scale (EDSS) of 0.06 and 0.18, respectively. Median EDSS of both patient groups is 0 (min = 0, max = 1).

The control group included 13 smokers (F/M = 6/7, mean age (\pm SD) 33.7 (\pm 7.4) years) and 14 non-smokers (10/4 = F/M, mean age (\pm SD) 29.8 (\pm 7.9) years).

The demographic information, clinical data and smoking history are shown in Table 1. There was no significant difference between CIS_s and HC_s or between CIS_{ns} and HC_{ns} in terms of age and gender (all, $p > 0.05$). CIS_s and HC_s showed no difference in exposure to smoking (all, $p > 0.05$). CIS_s and CIS_{ns} were similar in duration of disease and EDSS (all, $p > 0.05$).

Volumetric comparisons in all groups

CIS_s had lower volume of bilateral hemispheric cortical GM, total cortical GM, total GM, right cerebellar cortex, right globus pallidus (GP), ventral diencephalon (VDC), left hippocampus and bilateral thalami than HC_s (all, $p \leq 0.05$). CIS_{ns} had lower volume of subcortical GM, left putamen, right GP and posterior CC than HC_{ns} (all, $p \leq 0.05$). There was no parenchymal cerebral region with a greater volume in patients when patients and control subjects were compared. The volume of right lateral ventricle was higher in the patient groups than in those of the matched controls. Furthermore, compared to HC_{ns}, CIS_{ns} showed significantly increased volume of the cerebral spinal fluid (CSF). The regions with statistically different volumes and their mean volumes between the patients and control subjects are given in Table 2.

When HC_s and HC_{ns} were compared, the volumes of bilateral cerebellar cortex, GP, VDC as well as thalami were significantly greater in the smoking group (all, $p \leq 0.05$) (Table 3).

Among the patients, volume analysis revealed that CIS_s had higher volumes of the intracranial, left cerebellar cortex, left accumbens area and right GP than CIS_{ns}. There was no region of greater brain volume in CIS_{ns} relative to CIS_s (Table 4).

Regional brain volumes and lifetime exposure to nicotine

Within overall smoking individuals, correlation analysis revealed a significant negative relationship between the

Table 2. Brain regions that show significantly different volumes between patients and controls, their mean volumes (\pm SD) and p values.

Brain region	CIS _s mean (\pm SD) (cm ³)	HC _s mean (\pm SD) (cm ³)	p value
Hemispheric cortical GM (R)	222.6 (\pm 23.5)	242.1 (\pm 24.6)	0.017
Hemispheric cortical GM (L)	224.2 (\pm 24.4)	241.7 (\pm 25.2)	0.022
Total cortical GM	443.2 (\pm 47.3)	483.9 (\pm 49.7)	0.017
Total GM	622.8 (\pm 57.7)	681.0 (\pm 64.5)	0.004
Thalamus (L)	6.3 (\pm 0.9)	7.8 (\pm 1.1)	0.001
Thalamus (R)	6.5 (\pm 0.8)	8.3 (\pm 1.6)	0.001
Cerebellar cortex (R)	51.9 (\pm 5.5)	59.5 (\pm 8.0)	0.004
Hippocampus (L)	4.0 (\pm 0.3)	4.4 (\pm 0.3)	0.011
Globus pallidus (R)	1.4 (\pm 0.2)	1.7 (\pm 0.3)	0.003
VDC (R)	3.9 (\pm 0.5)	4.4 (\pm 0.6)	0.011
Lateral ventricle (R)	0.5 (\pm 0.1)	0.3 (\pm 0.1)	0.002
Brain region	CIS _{ns} mean (\pm SD) (cm ³)	HC _{ns} mean (\pm SD) (cm ³)	p value
Subcortical GM	149.4 (\pm 47.5)	180.9 (\pm 16.9)	0.030
Putamen (L)	5.1 (\pm 1.1)	5.9 (\pm 0.6)	0.050
GP (R)	1.3 (\pm 0.1)	1.4 (\pm 0.1)	0.006
Lateral ventricle (R)	0.4 (\pm 0.1)	0.2 (\pm 0.1)	0.009
CSF	1.0 (\pm 0.2)	0.8 (\pm 0.1)	0.032

CIS_s smoker patients with clinically isolated syndrome, HC_s smoker healthy controls, CIS_{ns} non-smoker patients with clinically isolated syndrome, HC_{ns} non-smoker healthy controls, SD standard deviation, GM gray matter, R right, L left, VDC ventral diencephalon, GP globus pallidus, CSF cerebral spinal fluid.

intracranial volume and pack-years (Spearman correlation coefficient = -0.549 ; $p = 0.003$). Pack-years were also significantly negatively correlated with left hemisphere cortical GM (Spearman correlation coefficient = -0.512 ; $p = 0.006$). (Figure 1).

Lesion load comparison between smoking and non-smoking patients

The lesion load on T1W imaging as determined from volumes of WM hypointensities was greater in smoking patients than non-smoking patients (5.3 cm^3 vs. 2.8 cm^3 ; $p = 0.05$). CIS_s had a higher number and volume of WM hyperintense lesion than CIS_{ns} on DIR space imaging, too. However, the difference did not reach statistical significance. (15 vs. 11 and 5.2 cm^3 vs. 2.4 cm^3 ; $p = 0.08$ and 0.5). There was no correlation between DIR-

SPACE T2 hyperintense lesion load and significantly altered regional brain volumes including intracranial, left cerebellar cortex, left accumbens area and right GP.

Discussion

We found that smoking was associated with increased GM volumes in several regions of the brain in both patients and controls. In contrast to the recent articles showing smaller GM volumes [7,11,12,15], we found that healthy smokers had greater GM volumes bilaterally in the cerebellar cortex, thalamus, globus pallidus and VDC than healthy non-smokers. In this study, we found similar effects of cigarette smoking in both healthy controls and in patients with CIS compared to respective matched non-smoking individuals. Our finding of

Table 3. Brain regions with statistically different volumes between non-smoking and smoking healthy controls, their mean volumes (\pm SD), p values.

Brain region	HC _{ns} mean (\pm SD) (cm ³)	HC _s mean (\pm SD) (cm ³)	p value
Cerebellar cortex (L)	49.0 (\pm 6.4)	56.2 (\pm 6.7)	0.026
Cerebellar cortex (R)	51.0 (\pm 6.3)	59.5 (\pm 8.0)	0.016
Thalamus (L)	6.6 (\pm 0.9)	7.8 (\pm 1.1)	0.049
Thalamus (R)	6.8 (\pm 0.9)	8.3 (\pm 1.6)	0.020
GP (L)	1.6 (\pm 0.09)	1.9 (\pm 0.3)	0.036
GP (R)	1.4 (\pm 0.1)	1.7 (\pm 0.3)	0.012
VDC (L)	3.6 (\pm 0.4)	4.3 (\pm 0.7)	0.050
VDC (R)	3.7 (\pm 0.3)	4.4 (\pm 0.6)	0.008

HC_s smoker healthy controls, HC_{ns} non-smoker healthy controls, SD standard deviation, GM gray matter, L left, R right, GP globus pallidus, VDC ventral diencephalon.

Table 4. Brain regions with significantly different volumes in comparison to non-smoking and smoking patients, their mean values (\pm SD), and p values.

Brain region	CIS _{ns} mean (\pm SD) (cm ³)	CIS _s mean (\pm SD) (cm ³)	p value
Intracranial volume	1294.3 (\pm 135.6)	1501.6 (\pm 106.5)	0.0001
Cerebellar cortex (L)	46.7 (\pm 5.0)	51.0 (\pm 6.5)	0.036
Accumbens area (L)	0.6 (\pm 0.1)	0.7 (\pm 0.1)	0.001
GP (R)	1.3 (\pm 0.1)	1.4 (\pm 0.2)	0.032

CIS_s smoker patients with clinically isolated syndrome, CIS_{ns} non-smoker patients with clinically isolated syndrome, SD standard deviation, L left, GP globus pallidus.

greater GM volumes in smoking relative to non-smoking patients with CIS was also observed in schizophrenia cases by Tregellas et al. demonstrating larger GM volumes in lateral prefrontal and superior temporal gyri using voxel-based morphometry. The authors suggested preservation of these areas in smoking group, in addition to other regions belonging to prefrontal and limbic areas with decreased GM in the patients with schizophrenia [19]. Nicotinic acetylcholine receptor (nAChR)s are extensively distributed in the human brain, with the highest density in the thalamus, substantia nigra and cerebral cortex in a decreasing order as well as cerebral vasculature and astrocytes [9,20–22]. Given the associations of brain region and subtype specific attenuations in nAChRs with neuropsychiatric diseases including Alzheimer's and Parkinson's diseases, dementia with Lewy bodies, schizophrenia and autism, studies revealing improvement following nicotine treatment in the animal Parkinsonian models, and larger putamen volumes in individuals with a greater lifetime nicotine exposure, nicotine has been regarded to have a neuroprotective effect [22–24]. Either through mesolimbic system activation by nicotine addiction or binding to mostly concentrated nAChRs in the striatum, volume

changes in striatum with subsite-dependent alterations have been reported. Our study differs in that our small healthy group showed greater volumes of the GP, thalamus cerebellar cortex and VDC than non-smokers. Because our purpose was to study the effect of smoking on brains with CIS, we did not focus on nicotine dependence and measure Fagerström score, rather we noted subjects' lifetime use by pack-years. The finding of greater lifetime exposure associated with lesser left hemisphere cortical GM and total intracranial volume is in line with previous studies showing correlations between pack-years of smoking and GM volume [7,11,25]. Thus, along with previous cognitive study results, these data suggest that the impact of chronic smoking on GM volume may be an attenuation, in contrast to a single one-time measurement compared with non-smokers [26,27].

In the present study, patients with CIS had volume loss compared to matched individuals, in several brain structures. This was more widespread in CIS_s including the thalamus, hippocampus, cerebellum, VDC and hemispheric cortical GM. Enlargement of the lateral ventricle and CSF can be attributed to volume reductions in the patient groups. Thus, in a

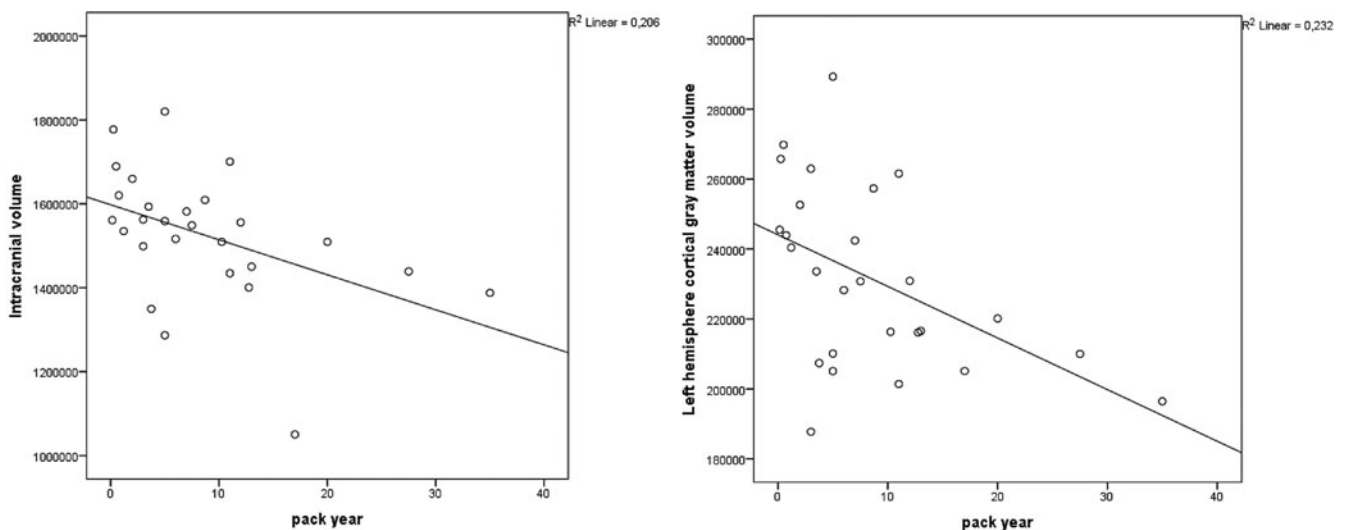


Figure 1. Scatterplot correlation results between regional brain volumes and smoking history in all smoking individuals (pack-years).

longitudinal study of 34 patients with CIS, Raz et al. documented a progressive atrophy at 1-year follow-up [17]. T1 hypointense WM lesion load was greater in smoking patients than in non-smoking patients. We did not find such a significant difference, although lesion load was higher on DIR SPACE imaging, too. We believe these findings may have a clinical implication given that non-enhancing demyelinating lesions on T1WI in CIS patients are associated with a higher risk of conversion to clinically definite MS along with the presence of T2 hyperintense lesions invisible on T1WI [28]. In relapsing–remitting MS, EDSS worsening over 10 years was found to best correlate with the combination of baseline T1W lesion count and increasing T1W lesion volume [29]. One reason why Arikanoglu et al. did not find an increased rate in conversion to MS despite a higher lesion load in smoking CIS patients might have been the measurement of only T2 hyperintense lesions in their study. Smoking was associated with increased T1hypo-, T2 hyperintense and contrast enhancing lesion volumes and reduced regional volumes in MS [30]. In addition to worse disease at baseline, smoker patients with MS were faster in conversion to secondary progressive disease on follow-up [31]. Also, in a 3-year follow-up study of 129 patients with CIS, it was suggested that smoking increased early conversion to MS after a clinically isolated syndrome [4].

Regardless of smoking, we observed atrophy in the thalamus, globus pallidus and putamen in our patients with very low EDSS scores compared to healthy controls as Henry et al. [32]. But, we cannot attribute deep GM atrophy to Wallerian degeneration from WM lesions due to lack of any correlation with neither EDSS nor lesion load in this study. This is in contrast to the findings of Tao et al., which suggested a link between subcortical GM and WM atrophy based on significant positive correlations with demyelinating lesions and clinical disability in MS patients [33]. The reason why we cannot find out any relation similar to Tao et al. is probably lower lesion load and EDSS scores in CIS patients compared to patients with MS.

The major noteworthy limitation in this study is the relative small sample sizes of our patient and control groups. One reason for that was the difficulty of having two matched control groups according to smoking habits of the patients. Due to small sample size we chose to not use Bonferroni correction for statistical analysis. Because, when sample size is small Bonferroni correction gives rise to increase of type II errors [34,35]. But, it will be useful to make Bonferroni correction in the studies which have larger sample size. Also, we could not avoid relative female dominance in the non-smoker patient and control groups, because women usually show fewer tendencies to smoking in our population. To eliminate this limitation, we used statistical sex and age

corrected analysis. Because this study concentrated on CIS, our participants showed relatively younger age, and in turn, less lifetime cigarette smoking compared with previous studies on smoking in literature. These differences might have resulted in some statistical insignificance in the present study. Finally, because this study is a cross-sectional study, it is carried out in a short time and we could not estimate the conversion rate of CIS to MS. Since no patient showed conversion to MS until completion of the study. Future investigations consisting of larger patient and control groups with longer follow-up would give a more comprehensive understanding of smoking-related effects on the occurrence and course of demyelinating diseases.

Conclusion

The present study detected that CIS-related regional brain atrophy varies in extent and severity with cigarette smoking. Despite increased lesion load, less cortical and deep GM damage with a possible neuroprotective effect occurs in smoking.

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Declaration of Interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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