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Anatomical changes of brain of amyotrophic lateral sclerosis patients: Viewed from MRI

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Abstract
Introduction: Amyotrophic Lateral Sclerosis (ALS) is a progressive, fatal disorder, which signs of motor neuron degeneration in one or more anatomic regions. Post-processing image techniques could be useful to reveal anatomic brain changes in those patients.
Objective: Describe brain changes of a group of ALS patients through post-processing MRI techniques and correlate it with clinical abnormalities.
Experimental Procedure: Thirty sporadic ALS patients and thirty healthy subjects were recruited, they were paired in age and sex with patients. T1, T2, FLAIR and DTI weighted 3T MRI scans were acquired. Tractography, Voxel-Based Morphometry (VBM) and cortical thickness processing analysis were done. Between group comparison and regression analysis were applied.
Results: Tractography revealed that corticospinal tract and optic radiation volumes of ALS patients is lower than healthy subjects (p=0.03), (p=0.04). A number of fibers of evaluated tracts diminishes when disease duration increasing (p=0.00) and when ALSFRS score is worse. VBM revealed brain atrophy on cortical areas, cerebellum, brainstem, deep gray nuclei, corpus callosum, corticospinal tract, internal and external capsule, lower and middle longitudinal fascicle and optical radiation of ALS patients. Cortical thickness analysis showed diminish of mean thickness on bilaterally pre-central gyrus and parietal posterior areas (p=0.03) on ALS patients. It diminishes when disease duration increases (p=0.00) and when ALSFRS score is worse (p=0.00).
Conclusions: We demonstrated that ALS patient's brain have abnormalities in corticospinal tract, optic radiation, corpus callosum, internal and external capsule, lower and middle longitudinal fascicle; pre and post-central, occipital and medial cortical areas, cerebellum, brainstem and deep gray nuclei.

Keywords: ALS, tractography, voxel-based morphometry, cortical thickness

Introduction
According to the ICD-11, Amyotrophic Lateral Sclerosis (ALS, code: 8B60.0) is a progressive, fatal disorder in which progressive signs of lower and upper motor neuron degeneration are seen in one or more regions: bulbar, cervical, thoracic and lumbosacral [1]. Median survival time is 2-4 years from onset of symptoms and it is the third more frequent neurodegenerative disease [2-3].
Electrophysiological studies may be required to confirm it and to exclude alternative causes. MRI, may be performed to exclude other causes that might explain clinical and electrophysiological features [2-5].
Incidence rate have been reported 2-3 per 100 000 person-years in European population, 0.7-0.8 per 100 000 person-years in Asian population and 0.55 per 100 000 person-years in Cuba, for that reason it is considered as a rare disease, but the number of patients is increasing [6].
Recently have changed the widely held belief that ALS affects only the motor neuron system, because evidence suggests that ALS is a multisystem neurodegenerative disease that involves sensory and extrapyramidal systems [2-3].
Nevertheless, new images methods have showed abnormal brain structures and function in ALS patients [1-2]. With this research, we intend to describe the brain structural changes in ALS patients through post-processing MRI studies and we propose correlate these changes with clinical abnormalities.

Material and Methods

Subjects

A total of 30 consecutive sporadic ALS patients were recruited between 2018 and 2020, according to the revised El Escorial criteria for clinically definite or probable. The control group consisted of 30 healthy subjects. Participants' characteristics are summarized on Table 1.

No clinical diagnosis of frontotemporal dementia (FTD) and no cognitive impairment symptoms were found in these patients.

Ethical considerations

The study protocol was approved by Ethical Committee of Cuban Neuroscience Centre and all of the patients and healthy subjects were agree with the evaluation and signed informed consent. All procedures of the study were in accordance with the ethical standards of the institution and with the 1964 Helsinki declaration.

Exclusion criteria

Persons with contraindications for MRI and who deny participating.

Clinical measurements

ALS Functional Rating Scale Reviewed (ALSFRS-R) was used to evaluate the functional status of ALS patients based on 12 items, functional disability scores range from 0 (Maximum disability) to 48 (Normal) points.

Image acquisition

MRI was carried out using a 3T Allegra system scanner (Siemens) device with a standard quadrature head coil.

High-resolution three-dimensional whole-brain T1-weighted MRI scans were acquired using a magnetization-prepared rapid gradient echo sequence, were obtained as a volumetric three-dimensional spoiled fast gradient echo with the following parameters: repetition time (TR)= 2000 ms, echo time (TE)= 2.6 ms, inversion time (TI)= 900ms, slice thickness = 1.0 mm; flip angle = 9 Ê, field of view (FOV) = 230 × 230 mm, 1 × 1 × 1 mm^3 voxel size. The volume consisted of 192 contiguous coronal sections covering the entire brain, acquisition matrix: 256x256, slice thickness: 1.

T2-weighted MRI scans were acquired using the following parameters: TR=3500 ms, TE= 354 ms.

FLAIR MRI scans were acquired using the following parameters: TR=5000 ms, TE= 353 ms, TI=1800 ms.

The DWI data were acquired using a diffusion-weighted spin echo imaging sequence with the following parameters: 80 volumes, slice thickness 2.0 mm, representing 80 gradient directions, FOV= 230 mm, TR=86 ms, TE= 8000 ms, slice thickness = 2.0 mm; flip angle = 90, b =1000 s/mm^2 and two scan with gradient 0 (b = 0), a resolution was 1 × 1 × 1.

Image processing

DICOM to nifty image format converted with dcm2nii tool, Chris Rorden's dcm2nii: 4 AUGUST 2014 32bit (https://www.nitrc.org/projects/dcm2nii/).

DTI processing: DTI images were processed using DSI Studio software tool (https://dsi-studio.labsolver.org/).

Images were reoriented into oblique axial slices aligned parallel to the anterior-posterior commissural axis with the origin set to the anterior commissure, Eddy currents distortions were corrected, diffusion tensor was estimated, scalar maps were constructed, fibre tracking was done and tensor were visualized.

After images were converted, src files were created, reconstruction was done, t fibre tracking was done and tractography-based analysis (automatic) was done.

Based on Montreal Neurologic Institute (MNI) maps, ROIs were placed at left (Cortical spinal tract, arcuate fascicle and optic radiation) with a volume size of 2.7e+04 mm cubic. An ROI was placed at right (Cortical spinal tract, arcuate fascicle and optic radiation) with a volume size of 2.9e+04 mm cubic. A seeding region was placed at the whole brain. The anisotropy threshold was randomly selected. The change threshold was 20%. The angular threshold was randomly selected from 15 degrees to 90 degrees. The step size was randomly selected from 0.5 voxel to 1.5 voxels. Tracks with length shorter than 26.9531 or longer than 269.531 mm were discarded. A total of 50000 seeds were placed.

Once tracts were constructed, the following quantitative metrics related with the tracks were analysed:

- Number of tracts (called too number of fibres or count of fibres): It is the number of streamlines generated by the algorithm (n).
- Tract length or mean longitude: It was calculated by multiplying number of coordinates in the streamlines with the distance between the coordinates. It was calculated trough the following equation:

$$\frac{1}{n}\sum_{i=1}^{i=n}\sum_{t=1}^{t=mi-1}\|vi(t)-vi(t+1)\|_2$$

n is the total number of tracks, vi(t) is a sequence of 3D coordinates representing the trajectory of a track, t is a discrete variable from 1 to mi, where mi is the number of the coordinates.

- Tracts volume (in mm cubic): It was calculated by multiplying number of voxels passed by all streamlines with the voxel size (N × voxel volume),

N is the total number.

- **Diameter (in mm):** It was calculated trough the following equation: $2\sqrt{\frac{volume}{\pi\ x\ lenght}}$
- Total surface area= N_s × voxel spacing^2, $\mathbf{N_s}$ is number of surface voxels.
- Irregularity: Is conceptually similar to convexity and concavity. It is the opposite of compactness or roundness defined in computer vision. It was calculated

  Through the following equation: $\frac{surface\ area}{\pi\ x\ diameter\ x\ lenght}$

- Fractional Anisotropy (FA): It is a scalar measure of the preferential axis of diffusion motion. It is related to the integrity of the myelin, the density and the parallelism of the fibres, it has a value ranged from 0 (isotropic) to 1 (totally anisotropic). It is high on myelinated fibres.
- **Mean Diffusivity (MD):** It is the average displacement of water within a voxel in the main axes. It representing total diffusion within a voxel.
- **Axial Diffusivity (AD):** It quantifies how fast water diffuses along the axonal fibres. It reveals axonal damage; it is low on axonal damage.
- **Radial Diffusivity (RD):** It evaluates the perpendicular component of water diffusion to axons [8].

Voxel-Based Morphometry (VBM) analysis

VBM was a fully automated, whole-brain technique that enables measurement of regional brain volumes based on voxel-wise comparison of grey and white matter volumes and the DARTEL registration method; it was done using Statistical Parametric Mapping 8 software, running on Matlab 2017a. Grey (GM) and white matter (WM) of ALS patients and healthy subjects were compared using t-test statistical analysis, with $p<0.05$.

Cortical thickness processing

Computational Anatomy Toolbox (CAT), version CAT12.6-rc1 (1430) was used, it runs within SPM12. This software tools are freely available at http://dbm.neuro.uni-jena.de/cat/.

CAT uses a fully automated method that allows the measurement of cortical thickness and reconstruction of the central surface in one step. It uses a tissue segmentation to estimate the WM distance and then projects the local maxima onto other GM voxels using a neighbouring relationship described by the WM distance [9-10].

Statistical analysis

Descriptive statistical measures were applied.

Between groups mean comparison (t-test) was done to compare the mean parameters of tractography and between ALS and healthy subjects.

Regression analysis was done in order to evaluate the relation of number of fibres of all of analysed tracts and cortical thickness with disease duration and with ALSFRS-R score in ALS patients.

By group automated analysis (based on t-test) was done for VBM and group cortical thickness, and maps of signification were construed.

We applied a statistical threshold of $p<0.05$ in all statistical analysis.

Results

Clinical characteristics

Clinic details of all of participants could be seen on Table 1.

Tractography

Tractography revealed that corticospinal tract and optic radiation volumes of ALS patients is lower than healthy subjects. See Figure 1.

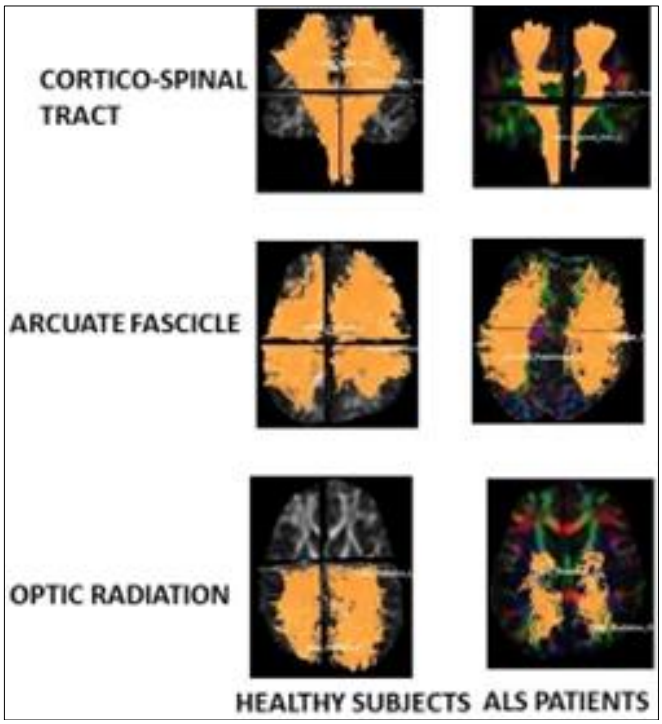

Fig 1: Tractography.

Note diminish of fibers number apparent on cortico-spinal tract and optic radiation on ALS patients in relation with healthy subjects

A. Comparison of tractography parameters between groups (ALS patients and healthy subjects)

Corticospinal Tract (CST) statistical analysis

It showed diminish of mean number of tracts (p=0.03), diameter (p=0.03) and volume (p=0.04) of ALS patients in relation with healthy subjects. See table 2.

Arcuate Fascicle (AF) statistical analysis

It showed that any mean parameters were statistically significant to comparison between ALS patients in relation with healthy subjects.

Optic Radiation (OR) statistical analysis

It showed diminish of mean total area (p=0.03), RD (p=0.03) and increase of mean MD (p=0.03) of ALS patients in relation with healthy subjects. See Table 3.

B. Regression analysis

Analysis of number of tracts in relation with disease duration

It showed that number of tracts of CST, OR and AF diminishes when disease duration increases (p=0.00). See Table 4a). The rest of parameters of tractography of all evaluated tracts were not statistically significant in relation with the increase in disease duration.

Analysis of number of tracts in relation with ALSFRS score

It showed that a number of tracts of CST diminishes when ALSFRS score is worse (p=0.04). See table 4b).

The rest of parameters of tractography of CST tracts were not statistically significant in relation with ALSFRS score.

Any parameters of tractography of OR and AF were statistically significant in relation with ALSFRS score.

Voxel-based morphometry

Patterns of brain atrophy in grey matter

We observed reduced grey matter density in ALS patients in relation with healthy subjects in cingulate gyrus, anterior portion of occipital lobe, paracentral lobe, precuneus, cerebellum, uncus, gyrus Parahippocampal, gyrus lingual, medulla oblongata, tectum mesencephalic, frontal and parietal lobes, insula, claustrum, thalamic nucleus, globe pallidum and putamen nucleus and amygdalin nucleus. (Figure 2).

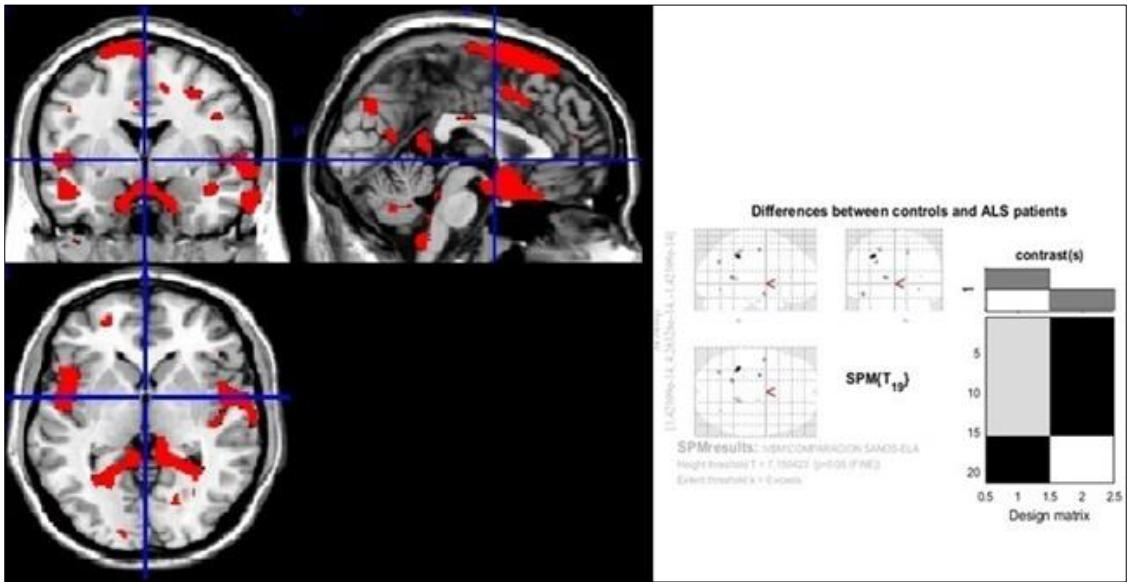

Fig 2: Generic MRI brain slices with superimposed areas showing statistically significant regions of grey matter atrophy ($p<0.05$, corrected for multiple comparisons) in a group of 30 ALS patients compared with 30 healthy age-matched subjects using voxel-based morphometry.

It shows grey matter atrophy on cingulate gyrus, anterior portion of occipital lobe, paracentral lobe, precuneus, occipital areas, cerebellum, uncus, gyrus parahipocampallis, gyrus lingual, medulla oblongata, tectum mesencephalic, frontal and parietal lobes, insula, claustrum, thalamic nucleus, globe pallidum, putamen and amygdalin nucleus. The colored bar represents the T score.

Patterns of brain atrophy in white matter

We observed reduced white matter density in ALS patients in relation to healthy subjects in corpus callosum, corticospinal tract (at midbrain, pons and medulla oblongata), internal and external capsule, optical radiation, lower and middle longitudinal fascicle, white matter of frontal, parietal and occipital areas. (Figure 3).

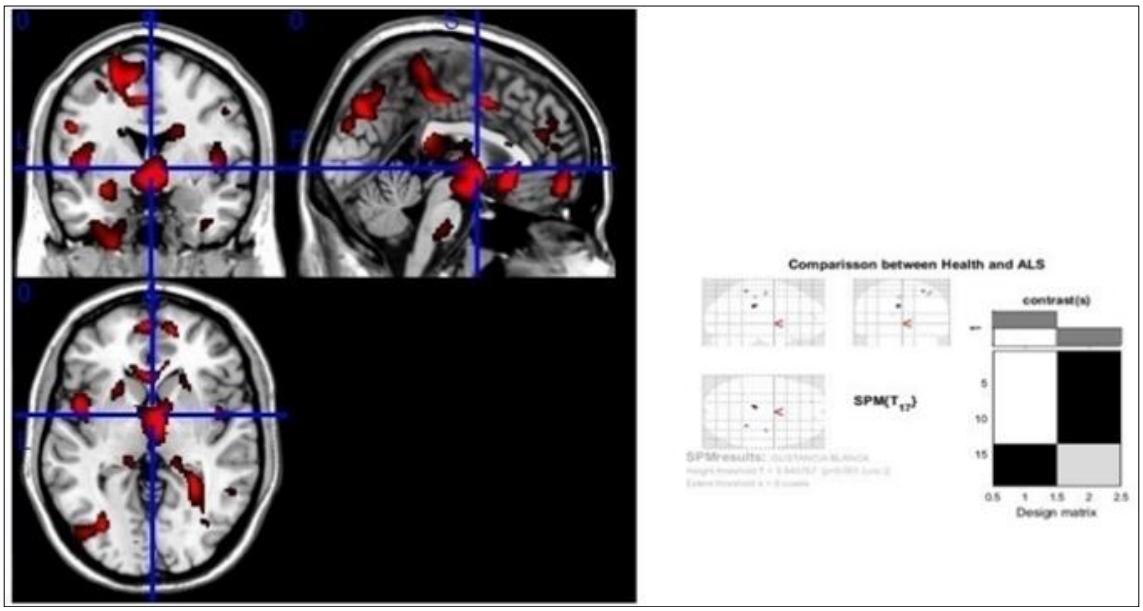

Fig 3: Generic MRI brain slices with superimposed areas showing statistically significant regions of white matter atrophy ($p<0.05$, corrected for multiple comparisons) in a group of 30 ALS patients compared with 30 healthy age matched subjects using voxel-based morphometry.

It shows white matter atrophy on corpus callosum, corticospinal tract (at midbrain, pons and medulla oblongata), internal and external capsule, optical radiation, lower and middle longitudinal fascicle, white matter of frontal, parietal and occipital areas. The colored bar represents the T score.

Cortical thickness

A. Comparison between groups (ALS patients and healthy subjects)

ALS patients showed diminish of mean global cortical thickness (p=0.03). See figure 4.

Group statistical analysis of cortical thickness showed differences between ALS and healthy subjects: ALS patients had diminished of cortical thickness on parietal area and posterior and superior sites of frontal lobe in relation with healthy subjects, surface-based statistical maps showing clusters of significance. See figure 5.

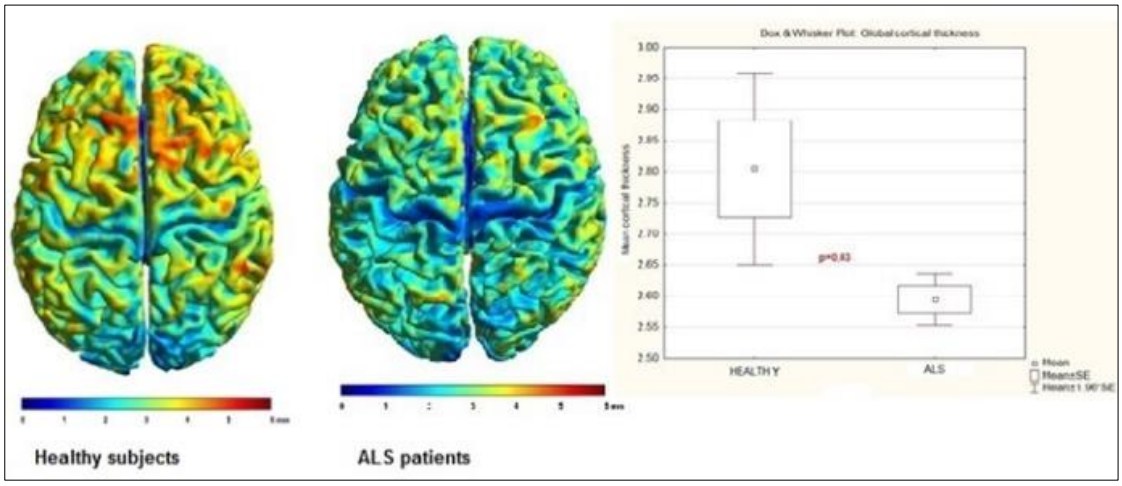

Fig 4: Comparison of individual mean cortical thickness between healthy subjects and ALS patients. Notice it is diminished in ALS patients. p=0.03

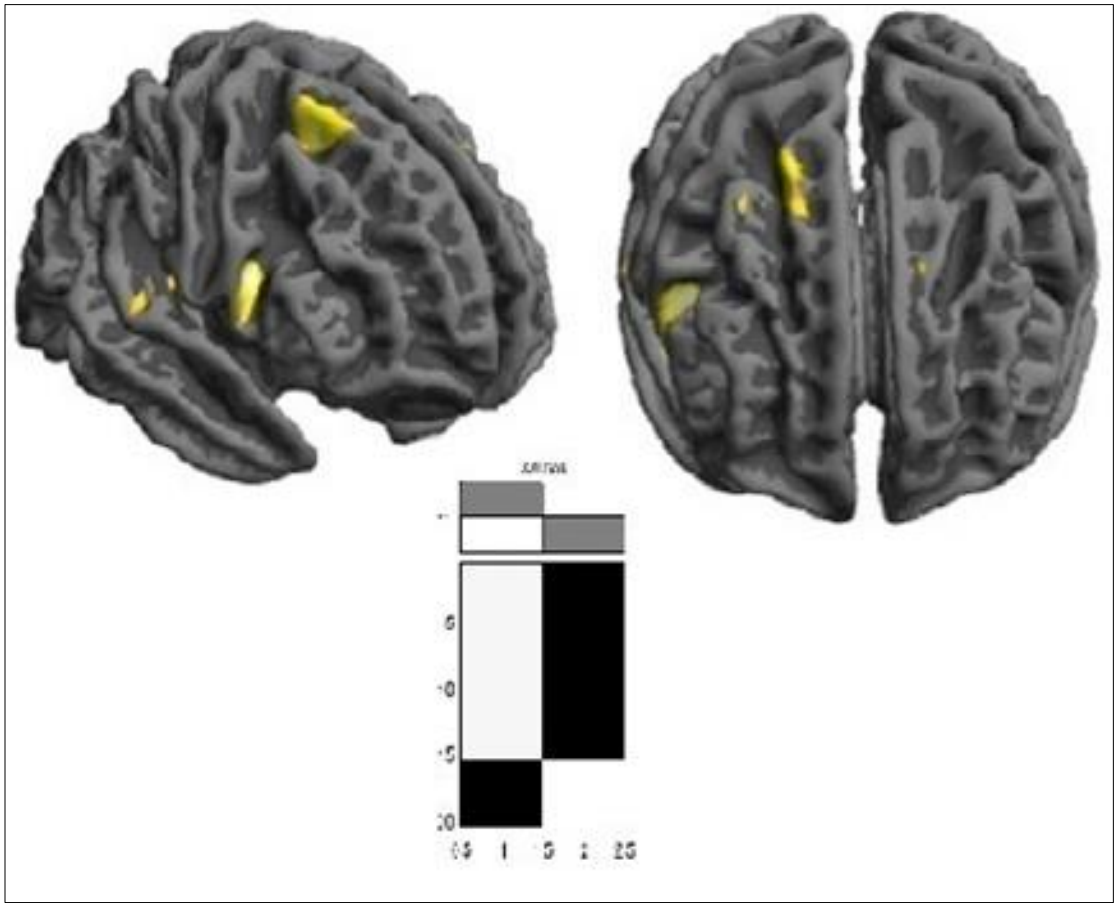

Fig 5: Cortical thickness group differences between ALS vs healthy subjects. Surface-based statistical maps showing clusters of significant cortical thinning on ALS patients

B. Regression analysis

Analysis of cortical thickness in relation to disease duration

It showed that cortical thickness diminishes when disease duration increases (p=0.00). See Table 5a).

Analysis of cortical thickness in relation with ALSFRS score

It showed that cortical thickness diminishes when ALSFRS score is worse (p=0.00). See table 5b).

Table 1: Clinic characteristics of ALS patients and healthy subjects

	ALS patients (n=30)	Healthy subjects (n=30)	t/X^2	p-value
Age mean and SD in years (range)	53.83±10.54 (34-66)	50.60±12.31 (32-64)	1.443	0.165
Sex (M/F)	16/14	15/15	0.895	0.370
Disease duration mean and SD in months (range)	12.00± 2.17 (2-24)	NA	NA	NA
Score of ALSFRS-R mean and SD (range)	39.60± 5.59 (32-47)	NA	NA	NA
Spinal/Bulbar/Both onset	20/10/0	NA	NA	NA
Laterality onset R/L	15/15	NA	NA	NA
Diagnostic category Definite/Probable	20/10	NA	NA	NA

Table 2: Mean comparison of CST parameters between healthy subjects and ALS patients

Variable	Healthy subjects	ALS	t	p
Number of fibres	1638.5	1118.0	2.46107	0.03 *
Mean longitude	67.1	66.6	0.14549	0.88
Diameter	65007.4	40077.3	2.32762	0.03*
Irregularity	14.6	17.0	0.55733	0.58
FA	0.4	0.4	-0.96036	0.35
MD	0.9	0.9	-0.38089	0.71
AD	1.3	1.3	-0.59265	0.56
RD	0.7	0.6	0.45364	0.65

Table 3: Mean comparison of OR parameters between healthy subjects and ALS patients

Variable	Healthy subjects	ALS	t	p
Number of fibres	703.50	466.6	1.48414	0.16
Mean longitude	67.95	66.4	0.33832	0.74
Diameter	24.49	21.3	0.98534	0.34
Irregularity	15.71	15.0	0.21240	0.83
FA	0.39	0.4	0.01145	0.99
MD	0.90	1.0	-2.42499	0.03*
AD	1.28	1.4	-2.14382	0.05
RD	0.8	0.71	-2.23692	0.04*

Table 4: a) Regression summary for dependent variable: number of the tract on ALS patients in relation with:

Left corticospinal tract	**R=.15505220 R2=.02404119 Adjusted R2=----- F (1,17)=.41877 *p*<.52619 Std Error of estimate: 430.80**				
		b	**Std Error**	**t**	p
	Disease duration	-1322.98	142.76	9.26	0.00*
Right corticospinal tract	R=.30777864 R2=.04426524 Adjusted R2=----- F (1,17) =.78736 *p*<.38728 Std Error of estimate: 459.87				
		b	Std Error	t	p
	Disease duration	-1311.22	152.40	8.60	0.00*
Left arcuate fascicle	R=.14774127 R2=.02182748 Adjusted R2=----- F (1,17) =.37935 *p*<.546111 Std Error of estimate: 376.19				
		b	Std Error	t	p
	Disease duration	-1328.48	124.67	10.65	0.00*
Right arcuate fascicle	R=.37274846 R2=.13894141 Adjusted R2=0.8829091 F (1,17) =2.7431 *p*<.11601 Std Error of estimate: 309.99				
		b	Std Error	t	p
	Disease duration	-959.78	102.73	9.34	0.00*
Left optic radiation	R=.02291464 R2=.00052508 Adjusted R2=----- F (1,17) =.00893 *p*<.92581 Std Error of estimate: 307.28				
		b	Std Error	t	p
	Disease duration	-453.41	101.83	4.45	0.00*
Right optic radiation	R=.02291464 R2=.00052508 Adjusted R2=----- F (1,17) =.00893 *p*<.92581 Std Error of estimate: 307.28				
		b	Std Error	t	p
	Disease duration	-398.53	75.46	5.28	0.00*

Notice it diminishes as disease duration increase

Table 4: b) disease duration and ALSFRS-R score

Left corticospinal tract	**R=.48232575 R2=.23263813 Adjusted R2=----- F (1,3)=.90950 *p*<.41060 Std Error of estimate: 352.86**				
		b	**Std Error**	**t**	**p**
	ALSFRS-R score	382.22	1258.75	0.30	0.78
Right corticospinal tract	R=.68003288 R2=.046244472 Adjusted R2=0.28325963 F (1,3)=2.5808 *p*<.20652 Std Error of estimate: 222.95				
		b	Std Error	t	p
	ALSFRS-R score	5578	17.00	3.27	**0.04***
Left Arcuate fascicle	R=.34705073 R2=.12044421 Adjusted R2=----- F (1,3)=.41081 *p*<.56716 Std Error of estimate: 292.37				
		b	Std Error	t	p
	ALSFRS-R score	846.19	1042.95	0.81	0.47
Right Arcuate fascicle	R=.47990701 R2=.23031073 Adjusted R2=----- F (1,3)=.89768 *p*<.41330 Std Error of estimate: 264.55				
		b	Std Error	t	p
	ALSFRS-R score	71.77	795.30	-0.09	0.93
Left optic radiation	R=.06038905 R2=.00364684 Adjusted R2=----- F (1,3)=.01098 *p*<.92316 Std Error of estimate: 485.29				
		b	Std Error	t	p
	ALSFRS-R score	572.02	1731.13	0.33	0.76
Right optic radiation	R=.47990701 R2=.23031073 Adjusted R2=----- F (1,3)=.89768 *p*<.41330 Std Error of estimate: 264.55				
		b	Std Error	t	p
	ALSFRS-R score	1520.27	943.71	1.61	0.20

Notice it diminishes when ALSFRS-R score is worse on right cortico-spinal tract. It is not significate on the rest of evaluated tracts

Table 5: a) Regression summary for dependent variable: mean cortical thickness over the entire brain surface on ALS patients in relation with:

R=.30777864 R2=.9472769 Adjusted R2=----- F (1,3=.31392 *p*<61440 Std Error of estimate: 12565				
	B	Std Error	t	p
Disease duration	-2.47	0.08	28.85	**0.00***

Notice it diminishes when disease duration increase

Table 5: b) disease duration and) ALSFRS-R score

R=.37587119 R2=.14127915 Adjusted R2=----- F (1,3=.49357 *p*<53295 Std Error of estimate: 12265				
	b	Std Error	t	p
ALSFRS-R score	2.81	0.43	6.45	0.00*

Notice it is better when ALSFRS-R score is better

Discussion

Stämpfli in 2017 demonstrated a significant deterioration of the white matter integrity in ALS patients, as reflected by reduced fibre density (FD) or fibre number and mean diffusion signal (MDS) values. Significant decreases of both parameters were found along CST and thalamic radiation, body of the corpus callosum as well as the forceps, uncinated fasciculus, superior longitudinal fasciculus, inferior longitudinal fasciculus, and inferior frontal-occipital fasciculus [11].

Geraldo in 2018 demonstrated that MD and AD maps showed significant differences between ALS patients and healthy controls in the CST and in the prefrontal white matter in the right cerebral hemisphere (p<0.05). They also found an increase of MD, AD, and RD in extra-motor frontal areas of the right cerebral hemisphere in ALS patients relative to controls [12].

They suggested that MD, AD, and RD might be more sensitive than FA to pathological changes subjacent to the neurodegenerative process associated with ALS, especially when patients are in the initial phase of the disease [12].

Our results are according to these results.

Verber in 2019 showed that MD elevation has to be considered as a marker in ALS diagnosis. It is according to our results [13].

In ALS, FA reduction in the corticospinal tracts and corpus callosum is a consistent finding [14], which correlates with clinical measures of disease progression [15]. Nevertheless in our cases it was not significate in any of the evaluated tracts.

Bao in 2018 showed significate increased RD in premotor, primary motor, primary and secondary somatosensory cortical regions, corticospinal tract, superior corona radiata, posterior limb of internal capsule and cerebral peduncle; body of corpus callosum and part of bilateral external capsule. They found significate correlations between ALSFRS-R scores and the values of RD indicating that this DTI indices might serve as an imaging biomarker in evaluating the disease severity of ALS, and that the CST could be a reliable region for clinical monitoring [16]. Diagnostic sensitivity and specificity of 68 and 73%, respectively [17-19].

They did not find any significant alteration in AD in the study. Previous studies have reported diverse results for AD in ALS patients, with some demonstrating increased AD in varying regions [19-20], decreased AD in one study [21], no significant change AD in the majority of research findings [21-23].

Bao demonstrated that the results of RD of the CST are more convincible and suitable for detection of microstructural changes for early-stage non-demented ALS [16]. Previous studies have consistently demonstrated significant changes in the CST in ALS patients compared with healthy volunteers [24-27].

According to Pallebage-Gamarallage: DTI showed significant degree of white matter damage to the uncinated fasciculus, inferior and superior longitudinal fasciculus, genu of corpus callosum, forceps minor and cingulum bundle [28].

In relation to white matter analysis, Qiu showed significantly decreased FA along the left corticospinal tract

as well as the body of corpus callosum in patients with ALS [29].

In post-mortem studies they showed through the structural MRI lateral corticospinal tract hyperintensity along the scanned segment of the spinal cord. The latter MRI signal change was concomitant with the degeneration of the lateral corticospinal tract histologically demonstrated with marked axonal myelin and neurofilament loss together with heightened inflammatory response on a segment of the cervical cord, quantitative evaluation of the same region on the corresponding MRI plane demonstrated a significant decrease in average FA value (p<0.01) and a significant increase in average MD values (p< 0. 01) in comparison to the normal-appearing white matter region [27-28].

Other post-mortem studies have demonstrated absence of Betz cells from Layer 5 of the precentral gyrus in patients with ALS and that the remaining pyramidal cells were significantly smaller than those seen in healthy controls [2-30].

Stämpfli showed significant changes in the fibre density values with disease progression in the left hemisphere. The fibre density values were decreased in parts of the CST, the thalamic radiation, the body of the corpus callosum, arcuate and uncinated fasciculus and in various association fibres [11]. Our results are according to Stämpfli´s results.

Stämpfli explained that fibre density may be more sensitive to white matter changes than the FA value and may permit evaluating pathological processes at an earlier stage of the disease.

In our opinion number of tracts or fibre density is a robust parameter to evaluate neurodegenerative diseases, at the beginning and in the diseases progress.

In relation to correlation with clinic abnormalities, Bao showed there was a negative correlation between the mean RD values and ALSFRS-R scores (r= -0.439, p=0.011) on the left CST of ALS patients [16].

Stämpfli´s analysis did not reveal significant correlations of the diffusion parameters with disease severity as measured by the ALSFRS-R [11].

Sarsılmaz demonstrated that there was a significant correlation between FA abnormalities with symptoms/disease duration and upper and lower motor neuron scores (p<0.05). Statistical analysis indicated a strong correlation with *El Escorial* criteria, r-ALSFRS, and disease duration (p<0.01) [31].

Qiu found focal grey matter atrophy in the left precentral gyrus in patients with ALS in VBM analysis [29].

Steinbach showed that ALS patients had significantly decreased GM density in the medial, inferior-frontal, and temporal lobes; in the parietal, occipital and cerebellar and sub-cortical regions (p< 0.01). WM density was decreased in patients primarily in the bilateral frontal and parietal regions, extending down to the brainstem; in the bilateral temporal lobes, but not within cerebellar projections (p<0.01) [32].

Trojsi demonstrated GM atrophy (p<0.05) in the left precentral gyri, left frontal pole, right supramarginal gyrus, right inferior temporal gyrus and putamen [33].

In recent years imaging in ALS confirmed extensive extra-motor pathology in cerebellar, extrapyramidal, subcortical, hippocampal hypothalamic, brainstem, and frontotemporal involvement. Compared to HCs, patients with ALS showed at baseline regional [34-40].

Our results are according with other author´s results.

Tanja in 2019 reported a case of ALS with posterior cortical atrophy in conventional MRI images. Posterior regions seem to be affected in ALS, it has been reported by some authors and in our patients, posterior areas were very affected, it was demonstrated by different techniques [41].

Wirth in 2018 had done a cortical thickness analysis focused on precentral and postcentral regions of interest. They showed cortical thickness was not significantly different between ALS patients and healthy controls (p=0.258) [42].

Meyer and other authors have done post-mortem and MRI studies and they have shown approximately 1.5 times greater cortical thickness of the precentral compared to the postcentral cortex in ALS patients [43-45].

Meadowcroft in 2015 observed cortical thinning in ALS patients with faster progression or advanced stage of disease [46]. Kropf and other authors have demonstrated that the significant cortical thinning of the postcentral gyrus occurs in ALS, and is correlated with disease severity [47-48].

Abidi described the right paracentral gyrus exhibited volumetric (p=0.05) and thickness (p=0.049) reduction in the upper motor neuron predominant cohort compared to the lower motor neuron predominant group [49].

This analysis demonstrated abnormalities in motor and non-motor cortical zones too.

Wirth in 2018 showed that ALSFRS-R sum scores correlated with cortical thickness of the precentral ventral cortex (p=0.009) and the postcentral ventral region (p=0.032).

In summary

There are different brain structures that could be affected in ALS patients:

Grey matter

Motor structures: frontal and parietal lobes, cerebellum, medulla oblongata, tectum mesencephalic, claustrum, globe pallidum, putamen and amygdalin nucleus.

Non-motor

The occipital lobe, cingulate gyrus, paracentral lobe, precuneus, uncus, gyrus Parahippocampal, gyrus lingual, insula, thalamic nucleus

White matter

Motor structures: corticospinal tract at midbrain, pons and medulla oblongata, internal capsule, white matter of frontal and parietal areas.

Non-motor structures

corpus callosum, external capsule, optical radiation, lower and middle longitudinal fascicle, white matter of occipital areas.

Axonal degeneration seems to be the primary abnormality of main tracts, they shows diminish of fibre numbers, diameter, volume, total area, RD and increase of MD. The tracts appear more affected with disease duration increase and when ALSFRS score gets worse.

Cortical thickness is globally diminished in ALS patients, predominantly on pre-central gyrus, parietal posterior areas and posterosuperior part of the frontal lobe. It is more affected with disease duration increase and when ALSFRS score gets worse.

Conclusions

We demonstrated that ALS patient’s brain have abnormalities in different motor and non-motor structures, supporting the theory that ALS is a multisystem disease.

Disclosure statement

The authors report no conflicts of interest.

The authors report there are no competing interests to declare.

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