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Testing the effects of pre-processing on voxel based morphometry analysis*

Chaitanya CV, Koirala N, Mideksa KG, Anwar AR, Schmidt G, Deuschl G, Groppa S, Muthuraman M,

Abstract— Voxel based morphometry (VBM) is an automated analysis technique which allows voxel-wise comparison of mainly grey-matter volumes between two magnetic resonance images (MRI). Two main analysis processes in VBM are possible. One is cross-sectional data analysis, where one group is compared with another to depict see the regions in the brain, which show changes in their grey-matter volume. Second is longitudinal data analysis, where MRIs, taken at different time points, are compared to see the regions in the brain that show changes in their grey matter volume for one time point with respect to another time point. Both types of analyses require pre-processing steps before performing the statistical analysis. In this study, we examined grey matter differences for patients with blepharospasmus (BFS) before and after treatment, at two different time points. The main evidence base therapy for this condition is the “botulinum toxin” injection in the respective muscles. The main aim of this study was to look at the effects of different pre-processing steps, namely, normalization and smoothing on the results of the longitudinal data analysis. A second aim was to analyze structural grey-matter differences before and after the treatment. Our results showed that the DARTEL normalization and the lower width for smoothing as preprocessing steps delivered pathophysiological plausible results. The longitudinal analysis revealed significant temporal differences after the injection of the botulinum toxin injection mainly in patients with BFS.

I. INTRODUCTION

The identification of how structural brain changes evolve in the grey-matter volume over time and which structural correlates are associated with a specific treatment could help us in understanding the pathophysiology of the disease and help us to pinpoint the treatment for the disease. Magnetic resonance imaging (MRI) of the brain allows us to identify and quantify structural brain changes. The traditional ways of analysing changes in the brain included visual assessment by experienced radiologists and performing manual region of interest analysis, which are very time consuming. Voxel based morphometry analysis is a statistical framework [1]

which has the ability to quantify grey and white matter changes between different group of subjects or statistical changes in time. This can be applied to track brain structural changes over time due to a specific treatment. The steps that are involved in any kind of VBM analyses are pre-processing, quality check, smoothing [2], and statistical analysis. The purpose of pre-processing is to prepare the MRI-data to the proper statistical analysis. The pre-processing steps play an essential role, because the results will vary depending on the pre-processing steps performed. The pre-processing steps used in this study are realignment [3], bias correction, normalization, segmentation [4], and applying deformations. Normalization is an important step because the anatomy varies across the subjects, and the position of the heads inside the scanner will vary among the analysed subjects. The most common method is performing a 12-parameter affine transformation followed by a non-linear registration using a mean squared difference matching function [1]. The second method is called Diffeomorphic Anatomical Registration Using Lie Algebra (DARTEL) normalization which does not use tissue-probability-maps [5]. Another important step is the smoothing which makes the data close to the Gaussian field model, which is an important assumption in VBM, and reduces inter-subject variability [6]. BFS is a neurological disorder, which has uncontrolled muscle contractions in eyelid. The temporary treatment for this disorder is the use of botulinum toxin injections. Recently, one study reported that use of botulinum toxin injection in cervical dystonia patients caused changes in the grey matter [7]. But the structural grey matter volume change due to botulinum toxin treatment in BFS patients is still unclear. In the present study, we used VBM analysis algorithms for pre-processing and to perform analysis in grey-matter volume changes in BFS patients before and after botulinum toxin treatment.

II. METHODS

A. Data acquisition and preprocessing

13 patients with BFS (age at first time point \pm s.d: 65.04 \pm 6.21 years, 4 males), were included in this study. The 3T MRI, Philips Achieva, with sensehead-8 coil is used to acquire the longitudinal data at two time points. The study protocol was approved by the local ethics committee and all patients gave their informed written consent. The first time point was before the botulinum toxin treatment and the second time point is one month after the treatment. The isotropic voxel resolution of 1 mm and sagittal slice orientation of repetition time (TR) - 7.7 ms, echo time (TE) -

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3.6 ms, and 160 slices were used. The pre-processing was done using voxel based morphometry toolbox (VBM) [1] and smoothing and further statistical analysis were performed using Statistical Parametric Mapping (SPM8, University College London, UK). The region of interest analysis masks was created using Automated Anatomical Labelling (AAL) atlas in WFU_PickAtlas toolbox [8, 9]. The VBM analysis pipeline is shown in Figure 1. It has four major steps, namely, pre-processing, quality check, smoothing, and statistical analysis. In the pre-processing there are five different steps, namely, realignment, bias correction, normalization, segmentation, and applying deformations. The main aim of realignment is to remove the head movement artifact in the magnetic resonance images. In this study, taking the mean of all the magnetic resonance images so as to align all the images with respect to the mean image did the realignment. The realignment can be done using rigid body transformations. The rigid body transformation is a method, which uses translations and rotations. The segmentation is the process of dividing an MR image into grey-matter, white matter, and cerebrospinal fluid. Partial volume effect, bias field, and noise mostly affect segmentation. Partial volume effect means a single voxel contains two different tissue types. Bias field is a very smooth undesired signal, and is produced because of inhomogeneity in the magnetic field produced by the MRI machine [10]. Here partial volume effect - maximum a-posterior (PVE-MAP) framework is considered which includes partial volume effect, bias field, tissue class and image intensities in the model [11]. This framework is followed by denoising methods to remove the noise effects. Estimating the mixtures, instead of labeling each voxel a different tissue class based on hard segmentation procedure, can reduce partial volume effect.



Figure 1. Shows the flowchart of the VBM analysis pipeline used in this study. The data was pre-processed using VBM8.

B. Normalization and smoothing

The normalization step involves registering the brains considered in our analysis to a standard template, so that removing the effect of different brain sizes in a group can do comparison. The mapping parameters can be determined, which transform one point in the image to another point in template image. Two kinds of normalization procedures can be implemented within the vbm8 toolbox. They are default normalization and DARTEL normalization. The default normalization is well used in the context of unified segmentation. Unified segmentation performs bias correction, segmentation, and normalization using the iterative model. In this process, the iterative model needs the tissue-probability-maps for the grey-matter, white matter,

and cerebrospinal fluid. The probabilistic way of performing unified segmentation is provided in [4]. The DARTEL technique [12] does not need the tissue-probability-maps like the default normalization. The DARTEL T1 template used in this study was developed from 550 healthy subjects. The DARTEL normalization is estimated based on deformation fields. There are two kinds of frameworks, which are small-deformation framework and large deformation framework. The large deformation framework is used in DARTEL normalization, because of the properties such as preservation of topology, closure, associativity, inverse, and identity [5]. The DARTEL normalization is also dependent on the segmentation algorithms used. Segmentation is first performed and is followed by the normalization. Mathematical algorithms can be applied to find out mapping between the given image and the standard template and is explained in [5]. The final step is application of deformations, which is done to compensate the volumetric differences caused by the normalization procedure. In the normalization procedure, some subject's brain regions can be expanded or contracted to match to the standard template. Thus, if the volume of the brain region is changed, then the volume of the grey-matter and white matter that is present in that specific region will also change. Applying deformations step involves multiplying the segmented grey and white matter images with the relative volumes after normalization, so that the total amount of grey-matter and white matter changes due to normalization is corrected and remains constant. Suppose that a particular point (x, y, z) in the brain is mapped to $(\hat{x}, \hat{y}, \hat{z})$ after normalization, then the gradient of deformation field at a particular coordinate is given by the Jacobian matrix ' J ' which is given as follows:

$$J = \begin{bmatrix} \frac{dx}{d\hat{x}} & \frac{dx}{d\hat{y}} & \frac{dx}{d\hat{z}} \\ \frac{dy}{d\hat{x}} & \frac{dy}{d\hat{y}} & \frac{dy}{d\hat{z}} \\ \frac{dz}{d\hat{x}} & \frac{dz}{d\hat{y}} & \frac{dz}{d\hat{z}} \end{bmatrix} \quad (1)$$

The determinant of the matrix $|J|$ gives the relative volume, which is multiplied at each voxel so that the total amount of the grey-matter and white matter remains unchanged after normalization. The main purpose of smoothing is to reduce the noise and compensate the inaccuracies caused by the normalization.

In this step, each voxel is convolved with a Gaussian kernel, so that each voxel becomes the weighted average of the surrounding voxels. The width of the Gaussian kernel varies from 8 mm to 14 mm. The lower width of the Gaussian kernel is suited for observing focal changes, whereas higher width of Gaussian kernel is used for observing distributed changes. In this study, we used the width of 8 mm Gaussian kernel, so that focal changes are well detected. Smoothing improves the t-statistics value [13].

C. Statistical analysis

The statistical analysis is done using general linear model (GLM). The mathematical representation of the GLM is given as:

$$Y = X \cdot \beta + \zeta \quad (2)$$

Here Y is the matrix of the measurements, X is a design matrix, β is the set of parameters to be estimated, and ζ is the error. The statistical tests are very sensitive to the pre-processing steps performed. The pre-processing is changed with respect to the statistical method used. The hypothesis here is to see the regions that show changes in their grey-matter concentration because of botulinum toxin treatment in BFS patients. The statistical method used here is the flexible factorial design that suits the hypotheses. The advantage of using flexible factorial over full factorial design is that, the user has the flexibility in choosing the specific interactions (for ex: between subjects and time point) in the design, where as in full factorial all the interactions are tested. In this analysis, flexible factorial design technique is used to create a design matrix. In the case of within group analysis, there are 13 rows in the design matrix. Each row represents one scan from each patient and each column represents either the subject factor or the time factor. In this study, the estimation of the general linear model parameters is done using the classical estimation scheme which is restricted to the maximum likelihood procedure [14]. For this analysis, t-contrast is used to see the regions that show either an increase or decrease in their grey- matter concentration. If the analysis is to be done in specific regions of the brain, masks can be provided. The 'p' value can be adjusted either to corrected family wise error rate (FWE) or uncorrected. For the corrected FWE, the 'p' value is 0.05 and for the uncorrected it is 0.001. The FWE corrected results does not have any false positives, whereas uncorrected results with above mentioned 'p' value have an acceptable 1% false positives. The extent threshold used in this study was 10 voxels, which allows us to see the regions where the specified number of voxels has survived satisfying the conditions for a specified contrast and for the correction method used.

III. RESULTS

A. Normalization and smoothing differences

To understand the effect of normalization on further analysis and results, both default normalization and DARTEL normalization were presented here for the specific contrast 'BSFpre>BSFpost'(pre-before treatment and post-after treatment). In both cases, after pre-processing, the data is smoothed using 8 mm Gaussian kernel and statistical analysis is performed using flexible factorial design technique to see the effect of normalization technique. Figure 2A shows brain regions for the specific contrast 'BSFpre>BSFpost', which are obtained by using default (upper three slices) and DARTEL normalization (lower three slices). In the case of default normalization, the global maxima is at the 'precentral-left', whereas in the case of

DARTEL the global maxima is at the 'cingulum-ant-left'. The average cluster size was higher in DARTEL normalization and a smaller number of clusters was showed. And also the identified clusters are pronounced in the case of DARTEL normalization and not smeared into different brain regions as in the case of the default normalization. Next, the effect of varying width of Gaussian kernels in the smoothing step was tested. The results are shown in figure 2 B for the width of 8 mm (upper three slices) and 12 mm (lower three slices) Gaussian kernel for default normalization and the specific contrast considered is 'BSFpre>BSFpost'. It can be observed that more smoothing leads to results which are distributed and spreaded over the other neighboring brain regions.

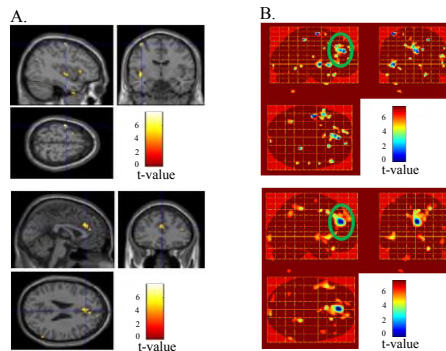


Figure 2. A) Shows the results from the two normalization techniques. The upper 3 slices show the results from the default normalization and the lower 3 slices show the results from the DARTEL normalization. B) The upper 3 slices show the results from the 8 mm smoothing and the lower 3 slices show the results from the 12 mm smoothing. The colorbar represents the t-values in both A and B.

B. Region of interest analysis results

Figure 3A shows the whole brain analysis results that was obtained for the specific longitudinal contrast 'BSFpre > BSFpost'. From figure 3A, it was concluded that grey-matter is reducing mainly in prefrontal lobe, frontal lobe, parietal lobe, and temporal lobe due to botulinum toxin treatment in BFS patients. The first region of interest analysis is performed for 90 regions, excluding cerebellum from the whole brain analysis. One mask is created for these 90 regions. From figure 3 B, it is observed that grey-matter is mainly reduced in prefrontal lobe, parietal lobe, temporal lobe, and occipital lobe because of botulinum toxin treatment in BFS patients. The second region of interest analysis is performed only over the four regions namely, basal ganglia, cerebellum, thalamus, and frontal cortex. One mask is created for these 4 regions and further analysis is done to see the grey-matter volume changes only within these four regions. In all the three cases, the global maxima was found at the 'cingulum-ant-left'. From figure 3 C, it is observed that grey-matter is mainly reduced in prefrontal lobe because of botulinum toxin treatment in BFS patients. It is observed that the global maxima remained the same in whole brain analysis and region of interest analyses. By comparing the obtained results taking into consideration the clusters size and p-maxima in the statistical tables, we found that the probability of finding a specific pathophysiological

relevant way is higher in the region of interest analyses than the whole brain analysis without an a priori hypothesis.

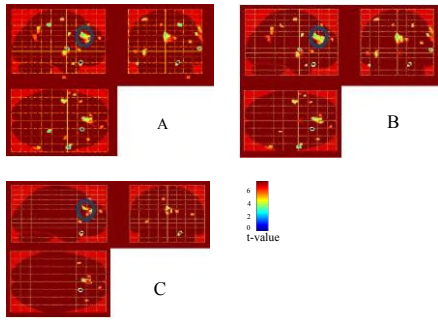


Figure 3. A) Shows the result from the whole brain analysis B) shows the results from the consideration of 90 ROI's (excluding cerebellum) of the AAL atlas. C) shows the results by considering 4 regions of interest, namely, basal ganglia, cerebellum, thalamus, and frontal regions. The colorbar represents the t-values in A, B, and C.

IV. DISCUSSION AND CONCLUSION

In this study, the whole brain analysis is done using both default and DARTEL normalization. The advantage of using DARTEL normalization in voxel based morphometry is that it is more accurate in localizing the changes in the regions of the brain in comparison to default normalization. The effect of both normalization techniques on VBM analysis is discussed in [5, 12]. The whole brain analysis in voxel based morphometry is done using both Gaussian kernels of width 8 mm and 12 mm. The effect of smoothing on VBM results is discussed elsewhere [6, 15]. The lower width of Gaussian kernels allows us to find the changes that are focal, whereas larger widths of Gaussian kernels allow us to find the changes that are spatially smeared. The longitudinal analysis showed that botulinum toxin treatment in BSF patients is associated with grey-matter volume reduction in wide-spread cortical regions, whereas grey-matter volume increases in sub-cortical structures. In conclusion, in this study we were able to show longitudinal structural changes at two time points in this cohort of patients. The DARTEL and 8 mm smoothing was optimal for this patient cohort and for this specific pathophysiological question.

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