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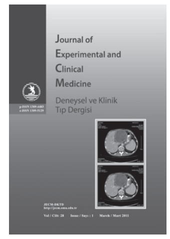

Experimental Research

Evaluation of the intra-rater variation for the estimation of volume of cerebral structures using the cavalieri principle on magnetic resonance images

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ABSTRACT

Measurement of brain volume is regarded as an objective marker of neurodegenerative diseases. The purpose of this study is to evaluate intra-rater variation for the estimation of volume of cerebral structures using the Cavalieri principle on magnetic resonance (MR) images to determine its reproducibility. The MR images of 30 cases were analyzed using the same standard protocols of the Cavalieri principle in two sessions with one month intervals. The structural MR images were analyzed using the ImageJ software by the same observer. The planimetry and threshold process was used for the cut surface area assessments. The volume of hemispheres, total brain, gray and white matters were and lateral ventricles estimated by means of the multiplication of cut surface are by the section interval. The same sections were used in both sessions. The results of two sessions were compared using Wilcoxon Signed Rank test. The mean total brain, right and left hemispheres volumes of first session were 1089.53, 544.82 and 544.71cm^3, respectively. The mean total brain, right and left hemispheres volumes of second session were 1086.62, 543.99 and 542.63cm^3, respectively. There was no statistically significant difference between the data ($p>0.05$). The mean total gray and white matters volumes were 553.55, 535.98 and 549.32, 537.31cm^3 for the first and second sessions, respectively. There was no statistically significant difference for the gray and white matters results ($p>0.05$). Our results showed that the reproducibility of the obtained data is good. The volume of cerebral structures could be estimated using the Cavalieri principle on MR images for comparative studies. We are planning to evaluate inter-rater difference in advance.
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1. Introduction

Human brain morphometry advanced dramatically by the invention of the modern neuroimaging methods. Magnetic resonance (MR) imaging techniques make it possible to abstract quantifiable information from the slices taken through different parts of the living body. This image slicing approach is directly comparable to the physical or mechanical slicing which is routine in anatomy and pathology both at macroscopic and microscopic levels.

The volume of organs or structures can be obtained using the Cavalieri principle of stereological approaches (Roberts et al., 1993; Cruz-Orive et al., 1997). The requirement for the application of this method is an entire set of two-dimensional slices through the object, provided they are parallel, separated by a known distance, and begins randomly within the object, criteria that are met by standard MR imaging techniques (Roberts et al., 2000; Sahin and Ergur, 2006). In the Cavalieri principle the sum of the sectional surface area of the slices is multiplied by the section thickness and the volume is estimated. The sectional surface area of the slabs could be assessed both the point-counting and planimetry methods of the principle. Planimetry, which involves manually tracing the boundaries of objects of interest on images of sections, is the most commonly used technique for estimation of volume (Gong et al., 1999; Sahin and Ergur, 2006).

There are some studies in the literature evaluating the inter/intra-rater variations of estimates of different organs and structures (Sahin et al., 2003; Odaci et al., 2005). However, we have not seen a study evaluating intra-rater changes of the volume estimates of brain and its components using planimetry technique and MR images.

The purpose of this study is to evaluate intra-rater variation for the estimation of brain volume using the Cavalieri principle on MR images in the living human brain and to determine its reproducibility.

2. Experimental Procedure

We carried out the present study on 30 subjects. They were selected blindly from 88 normal volunteers of the study of the "Quantitative Evaluation of the Cerebral Hemispheres and the Corpus Callosum in Schizophrenic Patients Using Planimetric and Stereological Methods on Magnetic Resonance Images". Subjects age range from 20 to 40. The official permissions are taken from the university and state hospital administrators. All procedures were fully explained to the subjects and written informed consent was obtained. Subjects with head trauma, drug abuse and central neurological disorders were excluded.

Structural MR imaging was done to all subjects in the department of radiology in the National Ribat University/ SUDAN. The scanner is Siemens 1.5 Tesla Magnetom Avanto Vision System. T1-weighted images were obtained using three-dimensional acquisition by Magnetization Prepared Rapid Acquisition (MP-RA); it produces good grey/white contrast in a very short acquisition time. Slice distance is 1.0mm, the field of view is 250 read, 192mm phase, TR=1657ms, TE=2.95ms, band width 180Hz/pixel, flip angle 15°, ECHO spacing=7.5ms, phase resolution=100%, slice resolution=50%, and acquisition time = 5 minutes & 18 seconds. The images are in coronal section. This T1-weighted sequence is part of the standard clinical protocol for qualitative and quantitative analysis of the whole brain in patients with epilepsy. The whole procedures take five minutes, without contrast media.

Morphometric measurements were conducted blind to clinical data using ImageJ software. The ImageJ is produced and distributed by the National Institute of Health of USA. The software is in the public domain and was downloaded from the Internet (available at the site: http://rsb.info.nih.gov/ij/). It runs on any computer systems. Measurements from images can be stored separately. This software provides valid and reliable measurements of specific structures using a delineation approach.

The Dicom images of patients were transferred to the software and converted into stack. Systematic random sampling was done since the number of slices in coronal plane about 192 sections. The sampling fraction was 1/10 and 1/5 for the brain and ventricles, respectively. This mean, for example take the 5th section as first than go on 15, 25, 35 for the brain. Therefore, the section interval for the brain was 1 cm and for the ventricle was 0.5 cm. Finally 15 to 17 sections containing brain images and 14 to 20 sections containing ventricles were obtained.

The midline of the brains and possible bluer borders of the hemispheres were drawn on the images to split them from the surrounding tissues (Fig. 1A). The outer boundaries of the hemisphere were manually delineated (Fig. 1B). Threshold toll of the program was used to delineate the boundaries of the WM then the wand tool of the software was used to delineate the boundaries of hemispheres (Fig. 1C). It was done for the ventricles also (Fig. 1D). The sectional cut surface of the interested structure was measured by the software automatically or semi-automatically. The volume of cerebral hemispheres, ventricles and WM was estimated by the multiplication of total sectional surface area with the section interval (i.e. 1cm and 0.5cm for the hemisphere and ventricle, respectively) at its shown in the formula below. The total brain volume was estimated by multiplying the volume of both hemispheres and GM volume was obtained by subtracting the volume of white matter (WM) and ventricles from the total hemispheric volume.

$V=\sum a \times t$

Where, V is the volume, $\sum a$ is the total sectional area of the structure and t is the interval between the examined sections.

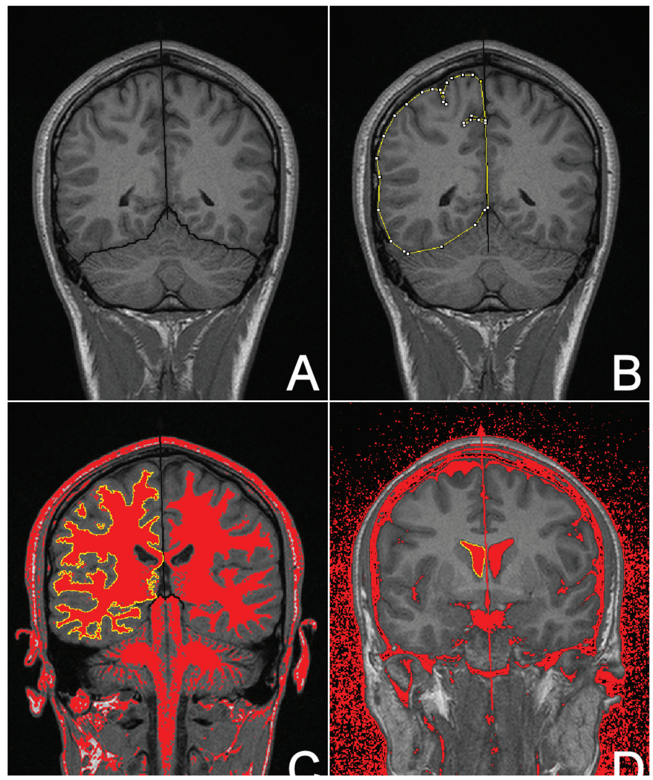

Fig. 1. Image processing in ImageJ for the volume assessment.
A: Delineation of the borders of brain.
B: Manual planimetry for total brain volume.
C: Tresholding for white matter.
D: Tresholding for lateral ventricles.

Two week later the same procedure was applied and the volume data were obtained by the same observer.

The calculations and the coefficient of error (CE) values were done using Microsoft Excel. A spreadsheet was prepared and the surface area data were transferred from ImageJ to the Excel. Finally, all the calculations were done automatically. Wilcoxon's signed-rank test was applied to compare the results of first and second sessions. Pearson correlation and Bland & Altman tests were done to see the relation between two session values. A p value equal or less than 0.05 was accepted statistically significant.

3. Results

The mean total brain volumes of 30 cases were 1089.5 and 1086.5 cm^3 for the first and second sessions, respectively. The mean total WM volumes were 536 and 537.3 cm^3, for the first and second sessions, respectively. They were 553.6 and 549.3 cm^3 for the total GM volumes in two sessions, respectively (Fig. 2). The values for the total ventricular volumes were same for both sessions, 14.3cm^3. The details of the hemispheres, GM, WM and ventricles were given in the statistical analysis of the data obtained in the first and second sessions did not differ from each other (p<0.05).

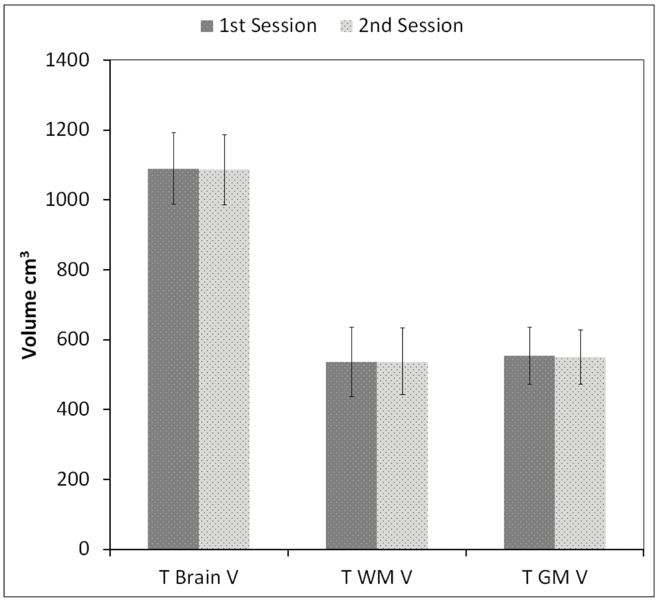

Fig. 2. The mean volumes of total brain (T Brain V), white matter (T WM V) and gray matter (T GM V) obtained in the first and second sessions in cm^3.

Table 1. The volume data of cerebral structures for the first and sec ond sessions. R; right, L; left, T; total, V; volume, Hem; hemisphere, WM; white matter, GM; gray matter, Vent; ventricle.

	Minimum		Maximum		Mean	
	1st Session	2nd Session	1st Session	2nd Session	1st Session	2nd Session
R Hem V	440.6	446.8	665.9	660.4	544.8	544.0
L Hem V	450.1	455.1	655.6	653.4	544.7	542.6
T Brain V	890.7	901.9	1321.5	1313.8	1089.5	1086.6
R WM V	184.2	180.6	393.5	383.3	277.4	278.0
L WM V	172.9	169.4	369.8	359.9	258.6	259.3
T WM V	357.1	349.9	763.4	743.2	536.0	537.3
R GM V	193.2	199.4	373.3	365.3	267.5	266.0
L GM V	204.2	204.4	374.3	369.8	286.1	283.3
T GM V	402.3	403.9	747.6	735.1	553.6	549.3
R Vent V	3.4	3.7	15.0	14.9	6.8	6.8
L Vent V	3.3	3.4	12.5	13.3	7.5	7.5
T Vent V	7.0	7.1	27.5	27.6	14.3	14.3

The correlation analysis of the estimates showed that there were high correlations between the first and second sessions. The correlation coefficient (r) for the total brain, total WM, total GM and total ventricles were 0.994, 0.987, 0.979 and 0.987, respectively.

We also applied the Bland & Altman analysis to see the agreement between to session (Fig. 3). There was good agreement between the sessions.

The mean CE of volumes estimates were 0.79, 1.43, 2.69 for the hemispheres, WM and ventricles, respectively.

4. Discussion

Unbiased and efficient estimation of volume of organs and structures could be done using the Cavalieri principle of the stereological methods with a known precision (Roberts et al., 1993; Cruz-Orive et al., 1997). This method requires sectioning the whole structure with a series of parallel planes. To avoid bias, the first section must be placed at a uniform and random position in a constant interval of length (t) i.e. to start the scanning always at, for example, one cm from the right tip of the object will introduce an unknown amount of bias in general. Moreover, the series of sections must encompass the object entirely. The direction of cutting does not affect the unbiasedness property, but will affect the estimation precision in general (Mackay et al., 1999).

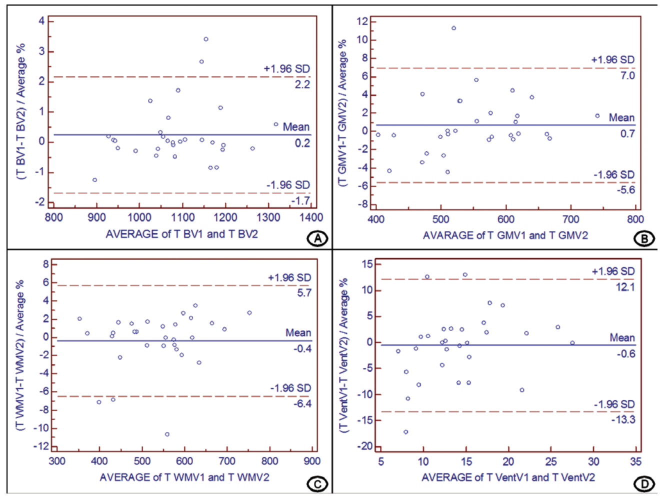

Fig. 3. Results of Blant & Altman analysis. T; total, V; volume, B; brain, WM; white matter, GM; gray matter, Vent; ventricle.

Previous studies showed that the obtained volumes using the Cavalieri principle on sectional radiological images showed little inter-rater variation (Sahin et al., 2003; Odaci et al., 2003; Akbaş et al., 2004). However, these studies are not conducted on the brain or its components.

There are little studies evaluating intra-rater variation of the estimation of brain volume using the MR images. Ronan et al. (2006) reported that the limits of estimates for the volume of brain were felt within ±2 SD's of the population and there was a good agreement between repeated measures for the total brain volume data. They used point counting method for the assessment of brain volume in the mentioned study. Bermel et al. (2003) developed a semi-automated measure of brain parenchymal fraction using commercially available edge-finding and thresholding software. Their intra-rater variability was very low and they concluded their software could be used to quantify the brain structures within 30 minutes. We used both the combination of manual delineation and semi-automated planimetry. However, the intra-rater repeatability was similar to the findings of both studies.

Rest of the other available studies evaluates the intra-rater variation for the estimation of different part of the brain. Giesel et al. (2009) proposed a new semi-automated method for the estimation of ventricle volume on MR images. Their data revealed that there was great agreement for intra-rater reliability as it is the case for our study. Dawant et al. (1999) also evaluated the volume of brain structure using manual planimetry on MR images. Their findings also showed low intra-rater variability.

The result of point-counting, manual planimetry and semi-automated planimetry there is good agreement for the intra-rater comparisons. Although previous studies used very different strategies for measuring brain volumes in neurodegenerative diseases, they all achieved relatively low intra-rater variability, with mean COVs ranging from 0.2 to 2.9% (Rovaris, 2000).

Beside the intra-rater variation, there are some points which must be regarded for the estimation of brain and its structures using the MR images. The section thickness and windowing procedures may contribute bias to the obtained data.

Partial voluming artifact is the result of sectioning and the thickness of sections in MR imaging. The finite resolution of images is recognized as an important problem of the MR imaging technique. The intensity of signal cannot be separated within each voxel when it arises from different tissue compartments and this produces what is referred to as partial voluming artifact. García-Fiñana et al. (2003) recommends that the relatively high resolution is not sufficient to solve the tissue boundaries ambiguity. To fix the contribution of effect of section thickness standard section thickness should be used for the research studies (Sahin and Ergur, 2006).

Windowing is an adjustment for the best visualization of frames of MR images. During the windowing of frames, different levels of settings to obtain best view are chosen. Windowing adjustments are related to the nature of scanned structure and the imaging technique. Moreover, Diederichs et al. (1996) showed that a proper windowing must be chosen to obtain maximum intensity projections. In the living subjects, the skull is filled up with brain, meninges and cerebrospinal fluid. During the scanning all those structures absorbs or reflects magnetic resonance signals in different degrees. Standard windowing parameters may eliminate the effect of windowing for the estimation of volumes of structures.

As a conclusion, we may say that the estimation of volume of brain and its structures do not affected by the applicant when using the manual or semi-automated planimetry techniques. Therefore, the studies having data obtained by the same observer may have reliable and acceptable data. However, inter-rater differences and the other factor such as the section thickness, the number of sections used in the study and the windowing procedures must be taken in to account.

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