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ORIGINAL ARTICLE

Comparison of point counting and planimetry methods for the assessment of cerebellar volume in human using magnetic resonance imaging: a stereological study

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Abstract The cerebellum is involved in motor learning and cognitive function in human. Many studies have been conducted to assess the cerebellar volume. To the best of our knowledge, there is no cerebellar volume study evaluating the efficiency and the accuracy of point-counting and planimetry methods of the Cavalieri principle in the literature. In this study, the volume of cerebellum was estimated in 53 Turkish young volunteers (26 males and 27 females), aged between 20 and 25 who are free of any neurological symptoms and signs, using serial magnetic resonance (MR) images. The cerebellar volumes of subjects were determined on MR images using the point-counting and planimetry methods. The mean results of planimetry method were 116.69 ± 10.1 and 114.41 ± 9.3 cm^3 in males and females, respectively. The mean results of point-counting method were 116.34 ± 10.6 and 113.48 ± 8.8 cm^3 in males and females, respectively. Our results revealed that female subjects had less cerebellar volumes compared with males, although there was no statistical significant difference between genders ($P > 0.05$). Total cerebellar volumes obtained by two different methods were not statistically different ($P = 0.189$) and they were correlated well to each other ($r = 0.935$). We found that the point-counting method takes less time than the planimetric method (mean 8 ± 3.6 vs. 15 ± 5.5 min). Thus, while planimetric and stereological approaches yield very similar results, the stereological method has the advantage of greater speed and, therefore, efficiency.

Keywords Cerebellar volume · Magnetic resonance imaging · Stereology · Cavalieri principle · Point-counting · Planimetry

Introduction

The use of computerized tomography (CT) and magnetic resonance (MR) imaging has resulted in a revolution in the morphologic evaluation of the brain and substructures in vivo. Technically, MR imaging provides much more precise data than CT because of its better tissue contrast and absence of bone artifact observed in latter. Furthermore, MR imaging has made it possible to attempt accurate in vivo volumetric measurements of whole brain and substructures. Therefore, the measurements obtained by MR imaging study are increasingly being considered as diagnostic measures in many neurological diseases, such as basal ganglia and caudate volume in schizophrenia, amygdale volume in dementia, hippocampal volume in Alzheimer disease and epilepsy [4, 8–10].

It is generally assumed that the cerebellum is involved in motor learning and cognitive function in human [18]. However, the relationship between gender of the subject and cerebellar size is not clear and there are conflicting reports in the literature [6, 19, 21]. In some samples, in

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comparison to women, men had larger cerebellar hemispheres [6, 11, 21].

Recent studies demonstrated that the volume of organs or structures can be obtained using the Cavalieri principle of stereological approaches [5, 22]. 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 and CT scanning techniques [23, 24]. From stereological point of view, planimetry and point-counting are two different methods for estimating volume based on the Cavalieri principle. Out of these methods, 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. The sum of the measured areas of sections obtained by planimetry is multiplied by the section thickness, and the volume of the structure is estimated. The point-counting method consists of overlying each selected section with a regular grid of test points, which is randomly positioned. After each superimposition, the number of test points hitting the structure of interest on the sections is counted, and the volume of the structure is estimated by multiplying section thickness, total number of points, and the representing area per point in the grid [7, 24].

In the current study, we stereologically evaluated the effects of gender on human cerebellar volume and specifically, compared total cerebellar volume using planimetric and point counting approaches, and then investigated the reliability and efficiency of each technique.

Materials and methods

We carried out the present study on 53 subjects consisting of 27 females and 26 males. They were normal volunteers and written informed consent was obtained. The official permissions are taken from the university and state hospital administrators. All procedures were fully explained to the subjects. Through history, taking alcohol consumption as well as physical and neurological examinations, the individuals with possible neurological abnormalities were excluded.

We analyzed neurologically intact cranial MR images of the all subjects. We used the protocol, which was used for the accumulated MR imaging data. T2-weighted sagittal images using a 0.5 Tesla MR machine (Gyroscan T5 II Vision, Philips, Netherlands) were obtained. The following parameter were used for the imaging process; TR/TE: 3,000/120; two excitations, FOV: 250/1.1, 5-mm thickness with a 0.1 mm gap between slices and 250 × 256 matrix.

Cerebellar volume was estimated using the Cavalieri principle as the combination of planimetric and point-counting methods. The same sections were used for both volume estimation methods.

Point-counting method

The MR images of a section series with 5 mm thicknesses were used to estimate cerebellar volume. These images were printed on films in square frames measuring 6 × 8 cm. The films were placed on a negatoscope and the transparent square grid test system with $d = 0.15$ cm between test points was superimposed, randomly covering the entire image frame (Fig. 1). The points hitting the cerebellar sectioned surface area were counted for each section and the volume of the cerebellar volume was estimated using the modified formula for volume estimations of radiological images [24].

$$V = t \times \left[\frac{\mathrm{SU} \times d}{\mathrm{SL}}\right]^2 \times \sum P \qquad (1)$$

where "t" is the section thickness of consecutive sections, "SU" the scale unit of the printed film, "d" the distance between the test points of the grid, "SL" the measured length of the scale printed on the film and "ΣP" is the total number of points hitting the sectioned cut surface areas of the cerebellum.

The mean time for the volume estimations was also provided. The coefficient of error (CE) of the point-counting method was calculated using the formula described in previous studies [2, 25]. Calculation of cerebellar volume, CE of estimates and other related data were obtained as a spreadsheet using Microsoft Excel. After initial setup and preparation of the formula, the point counts, the number of point and other data were entered for each scan and the final data were obtained automatically.

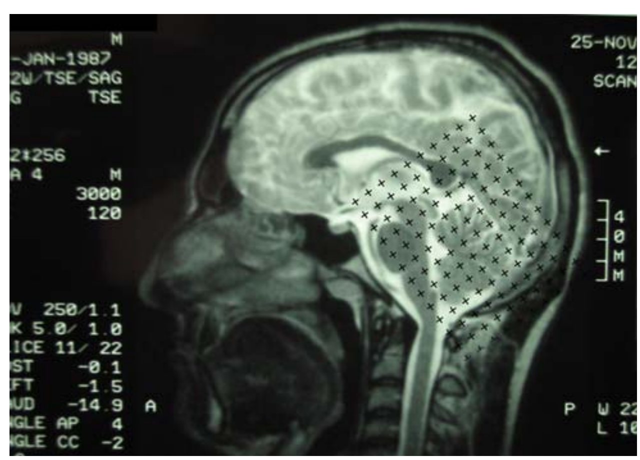

Fig. 1 A sagittal MRI scan with a point counting grid superimposed on it for the estimation of CV

Planimetry method

The cross-sectional surface area measured by means of the planimetry method using software namely Dicom Works (DicomWorks computer program. Version 1.3.5. France. http://dicom.online.fr.) (Fig. 2).

A previously described [17], using hand-held mouse, the rater traced around the area of interest within slice. The software calculate number of pixels enclosed within the traced area and process was repeated for each slice. Since the pixel dimension and the slice thickness were known, total cerebellar volume can be estimated:

Total cerebellar volume = the sum of the areas (cm^2) × slice thickness (cm)

For the planimetry measurements, pictures were taken from the MR images that hold a millimetric scale for the calibration. Each measurement was done using the tools of software to the nearest millimeter at least three times and the average was considered for calculation.

The sum of the areas multiplied by the section thickness provided the cerebellar volume. The CE of planimetric volume estimations was calculated using the formula described in previous studies [1, 13, 26].

The mean time for the volume estimations was also provided. Calculation of cerebellar volume, CE of estimates and other related data were obtained as a spreadsheet using Microsoft Excel as given point-counting method.

Statistics

Values are expressed in terms of the mean and standard deviation (SD). The volumes of cerebellum were compared between the genders using independent *t* test. The differences of the estimated volumes obtained by two different approaches, namely point-counting and planimetry, were compared using paired *t* test to check the methodological differences. Then, Pearson correlation test was also applied to see the relation between the results of two different approaches. A "*P*" value lower than 0.05 was accepted as being statistically different.

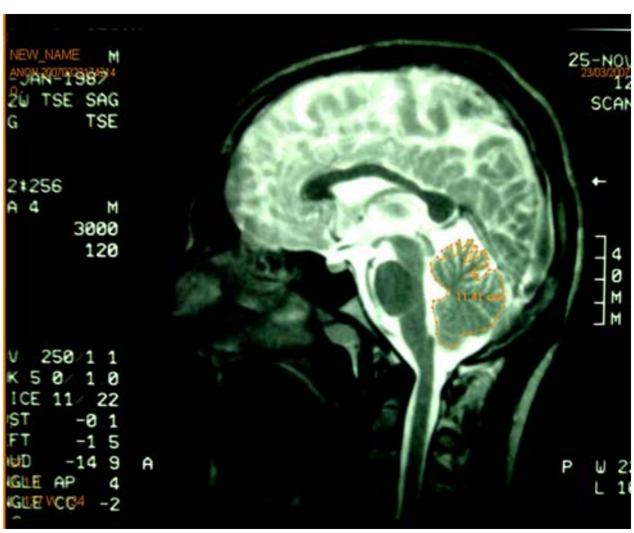

Fig. 2 Delineation of the contour of cerebellum for planimetry

Results

Mean values for cerebellar volume calculated according to planimetric and stereological point-counting methods are listed in Table 1. We found that total cerebellar volumes obtained by two different methods were not statistically different ($P = 0.189$) and well correlated with each other ($r = 0.935$). The point-counting technique did, however, take less time than planimetry for estimating cerebellar volume from MR images. Moreover, the mean time (±SD) needed to estimate the cerebellar volume using the point counting technique was 8 ± 3.6 min, with a range of 7–12 min; planimetry was 15 ± 5.5 min, with a range of 12–19 min. The mean CEs for the CV estimates derived from the technique of point-counting and planimetry were 2 and 1%, respectively.

Cerebellar volume data obtained by the application of two different methods in both sex are shown in Table 2. The mean results of planimetric method were 116.69 ± 10.1 and 114.41 ± 9.3 cm^3 in males and females, respectively. The results of point counting method were 116.34 ± 10.6, 113.48 ± 8.8 cm^3 in males and females, respectively. Our results demonstrated that female subjects had smaller cerebellar volumes than males. However, the difference between the genders did not statistically significantly different ($P > 0.05$).

Discussion

The results of our study investigating cerebellar volume in normal young adults clearly demonstrated that total cerebellar volumes obtained by two different methods were not statistically different but correlated well with each other. Furthermore, we also found that the point-counting method takes less time than the planimetric method.

In a previous study, Escalona [6], measuring from MRI, cerebellar volume can be estimated using the Cavalieri principle but not calculated a coefficient of error.

Stereological estimate can be implemented alongside planimetric methods as commercial software systems are becoming readily available. As a cheap, reliable alternative one could replace the stereological package with a simple set of transparent sheets upon which are copied grid points at different intensities. These could be placed over the

Table 1 Statistical comparisons and agreements of two methods (paired *t* test)

Methods	N	Mean (cm^3)	SD	*t*	Significance	Correlation	Significance
Point counting	53	114.88	9.7	−1.330	0.189	0.935	0.000
Planimetry	53	115.53	9.7				

Table 2 Mean ± SD both sexes (cm^3) and statistical significance (independent *t* test)

Methods/genders	Point-counting	Planimetry	Significance
Male	116.34 ± 10.6	116.69 ± 10.01	$P = 0.291$
Female	113.48 ± 8.8	114.41 ± 9.3	$P = 0.400$

images and viewed using a standard light box, thus allowing simple point counting. Furthermore, both planimetry and point counting, may be compared with the newly developing automated computer-thresholding techniques, so as to confirm that algorithm-dependent procedures are reliable in boundary delineation. Indeed, boundary delineation is a problem even when applying planimetric and point-counting methods, as it boundary is inherently subjective. Unfortunately, in this investigation, the absence of a reference measurement of brain volume (such as by fluid displacement) makes it impossible to judge whether the planimetric or the point-counting method gives values closer to the true volume.

Some researchers speculated that sexual dimorphism in cerebellar size can be attributed to the effects of sex hormones [12, 20], although empirical support for that supposition is still lacking. Present-day imaging techniques, such as CT and MR imaging, do not have the degree of resolution that would enable us to assess human Purkinje cell number in vivo. Using human cerebellar tissue at autopsy, Nairn et al. [16] reported that brain imaging estimates of cerebellar volume may be an indirect measure of Purkinje cell number.

Several studies have found associations between cerebellar atrophy and neuropsychiatric symptomatology. The cerebellum is known to be involved in such disease as alcoholism and ataxia [3, 30]. Generally known cerebellar disease such as olivopentocerebellar atrophy, cerebellar system-inherited ataxias (Friedreich's ataxia, autosomal dominant cerebellar ataxia type 1, ataxia-telangiectasia), acute cerebellitis and cerebellar infarcts may result in changing volume of cerebellum [14, 15, 17, 29]. Cerebellar volume changes due to supratentorial lesions or injury are rare, but unilateral cerebral lesions have been associated with atrophy of the contralateral cerebellar hemisphere [28]. Bilateral cerebellar atrophy has been described in the setting of temporal lobe epilepsy [27]. So that it is important to study cerebellum volume. We might use two techniques to calculate cerebellar volume.

Good agreement was found between results obtained with the point-counting and planimetry techniques. We found that the major difference distinguishing the planimetric and point counting methods is time. While the point-counting approach takes less time, planimetry provides more accurate results. As the point-counting method can, be applied to any sets of printed MR images, this approach allows one to perform retrospective and prospective studies, and the MR machines and their PC accessories do not have to be engaged. Moreover, the procedure of manually tracing boundaries of cerebellum in all MR sections using planimetry is tedious, requiring experience. Finally, a practical volume estimation for cerebellum can large enough to be meaningful for a clinic neuroradiologist.

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