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A Novel Pixel Counting Technique to Assess the Volumetric Changes in Human Brain Morphology

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Morphometric measurements such as volume, thickness and sulcal depth are used to provide valuable information about cortical characteristics in both healthy and diseased conditions of the brain. Relevantly, the focus of this paper is to illustrate the morphometric method of assessing the volume changes in the brain caused by aging and/or pathological condition. Using the T1-weighted magnetic resonance images of the brain, the clustering technique is adopted towards segmenting the image into separate compartments of white and gray matters (WM and GM) and the cerebral-spinal fluid (CSF). The clustering technique pursued includes the traditional K-means and fuzzy C-means algorithm by considering the Euclidean distance metric toward grouping of entities of similar pattern vectors. The method evolved allows the underlying volume measurement of clustered regions by pixel-counting technique. Comparison of volume measurement of segmented cerebral tissues among male and female subjects undergoing ageing process and with cerebral pathogenic states is exercised. The volumetric changes among the male and female subjects are also considered The results reveal distinct details thereof. Specifically, the volumetric assessment indicated proves to be a viable technique toward understanding the gender differences, geriatric changes in the brain as well as the conditions of brain tissues vis-à-vis neuro-related issues. Clinical data gathered and computed results on the proposed method are furnished to illustrate the efficacy of the method and its short comings.

Key words: White matter, Gray matter, Cerebrospinal fluid, K-means and Fuzzy C-means clustering technique, Pixel counting technique.

Knowledge on changes occurring in the brain volume due to gender variations, ageing process and pathological states (both localized and across the gross (overall) brain anatomy) plays an important role in diagnosing and understanding the various neuro-physiological conditions. In general[1] neurodegenerative diseases are characterized by loss of brain tissues. Specifically, The change in hippocampus volume over various aging stages is a potential marker for Alzheimer's disease[2]. Distinctive patterns of accelerated cerebral atrophy are characteristic features of a large number of neurodegenerative and dementing disorders (such as Alzheimer's disease), frontotemporal dementia, Parkinson's disease and others[3].

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The feasibility of using relatively coarse and localized morphological measurements of tissues like white matter (WM), gray matter (GM) or cerebrospinal fluid (CSF) could quantitatively infer the status of diseases like multiple sclerosis, schizophrenia[4] and hydrocephalus conditions of the brain or any other anomalous formation of extra growth/lesions. In addition to pathological states, gender-based alteration observed in the brain has attracted the interest of scientific research and philosophical notions. Typically the anatomical studies indicate that the male brain is larger than the female brain. Relevant perusal of knowing the gender-based distinction in the brain anatomy is still a state-of-the-art interest consistent with the overall structural and volumetric differences found invariably among male and female at large. Further, a study of human brain[5] suggests that the variations in the quantity of brain constituents like GM, WM and CSF in male and female could reveal the underlying physio-anatomical aspects of development of sexual organs as well.

Apart from gender-specific morphologies in the brain, ageing also influence the brain and cognitive functions ascribed with multiple aetiologies. In general, ageing has its effects at the molecular level of cells and vasculature, eventually manifesting as gross morphology and cognitive functions. With ageing[6], the brain shrinks in volume, particularly in the frontal cortex. However, the change does not occur to the same extent in all the regions of the brain.

Neuroimaging studies have been addressed to study various brain morphological features such as changes in sulcal depth and thickness. Typically, assaying the sulcal depth offers a quantitative metric on the morphological features of the cerebral cortex. Measurements on sulcal depth are done using two different approaches namely, the curve-based and the distance-based techniques. The curve-based method includes the manual point-fixing on the convoluted regions; and, the distance-based approach is based on the volume data. However, with highly convoluted regions, these methods provide under-estimated or over-estimated results on the depth. To overcome such limitations, scientists have proposed[7] an automated sulcal depth measurement by considering the so-called adaptive distance transform.

Another Morphometric pursuit refers to assessing the cerebral thickness of a particular structure. This may provide an indication of its functional performance. Several algorithms for measuring the cortical thickness of the human brain using MR image data have been described in the literature. However, the thickness and the sulcal depth morphological details may not provide adequate information pertinent to functional, structural or size of various tissue regions present in the brain. As such, whenever brain structural abnormalities and its related cognitive functions need to be studied, volume computation could provide a better solution as attempted in this study.

Significant extent of anatomical magnetic resonance imaging (MRI) studies on the volume measurement of the human brain has been carried out in neuroscience. The vast majority of such studies refer to simple curve-tracing techniques based on identifying the anatomical boundaries[8]. A mouse-driven cursor is used to trace the test boundary but, the method thereof is a labour-intensive process. Alternatively, a stereologic method[9] was suggested. It is done by placing a graticule of grid-lines on the region of interest so as to estimate the surface area and lateral width and hence the volume of a particular region of the human brain. But, placing of the grid-lines is a tedious process. A method[10] has used an automatic atlas-based volume estimation of human brain regions using MR images and hence estimates the brain volume. In this method, the talairach atlas is adapted and the volume is computed. However, the difficulty lies in transforming the talairach spaces. To overcome this difficulty, they coupled a surface propagation approach via a level-set method[11]. In this approach, the signed distance-function and the level-set formulation are used. But, the non-brain Voxel are also included. The simplest, Voxel based morphometry (VBM) involves a Voxel- wise comparison of local concentration of the GM and it provides the required volume measurement[12]. Determining the hippocampal volume is demonstrated using the so-called Cavalieri method [13]. This Cavalieri method in conjunction with point-counting effort estimates the hippocampal volume. The scientists have estimated the brain volume from serial section measurement using the rectangular, Cavalieri's, parabolic (Simpson's), or trapezoidal rule so as to

integrate numerically a curve of cross-sectional area measurements plotted against the section number[14]. According to their studies,there is no significant difference among various estimates when more number of sections is used. The automated VBM includes the spatial normalization, tissue classification and spatial-smoothing. However, the spatial-smoothing plus the normalization require separate software for the implementation. .

In the present study, the cerebral volume (CV) is computed on both region-wise as well as for the entire brain structure with the help of T1-weighted MR images obtained on axial sections of the brain. Voxel base counting (VBC) method is adopted for volume estimation in conjunction with segmentation procedures.

This paper is organized as follows: Section 2 elaborates data acquisition and detail on segmentation method and volume estimation procedures. In Section 3, the results obtained by applying the volume-based methods adopted are presented; and, the volume-loss (or morphological details) across the compartments versus aging is discussed. In Section 4,concluding remarks are furnished along with open-questions concerning future research directions.

MATERIALS AND METHODS

The CV computation adopted in this study follows the following steps: The image data collected are from MRI T1-weighted sequence, and it is segmented for identifying the regions of WM, GM and CSF using K-means clustering and fuzzy C-means clustering techniques. The overall and region-wise CV is then estimated using pixel-counting method. Subjects of normal and pathogenic states are studied.

Data Acquisition

Images are acquired from volunteers in the age group between 16 and 89 years with no neurological problems (control subjects) and a few volunteers with neurological problems (such as cerebral lesions). Particulars of the subjects are given in Table 1.

The images are acquired from the GE Medical System's 3T Signal HDX Machine with high-resolution, T1-weighted and 3D turbo-flash sequence features. The scanning machine uses adiabatic inversion contrast pulse with the following scan parameters: TR/TI/TE=2311/950/17.2 ms, Spin Echo technique is employed with the Magnification equal to 1.13 and FOV (field-of- view) equal to 19.4 cm, Slice thickness conforms to 5 mm and the pixel matrix is 320*192 (and each pixel corresponds to the 1.2 sq.mm). The axial view of the brain is considered for the quantitative analysis.

Segmentation

Image segmentation refers to the process of partitioning a digital image into multiple segments that is, set of pixels and similar pixels in a region according to some homogeneity criteria such as colour, intensity or texture in order to locate and identify objects and boundaries in an image[15].In the present study, the pixels that have similar attributes are grouped together to get distinctly identifiable segmented regions. Though there are many segmentation methods like clustering, gradient-computation and region-growing are feasible. Some people have suggested that the clustering[16] is one of the simplest and widely used strategies adopted in segmentation of grey-level images and it is used in this study.

Segmentation by clustering

Clustering is an unsupervised learning task, where it suggests that one needs to identify a finite set of categories known as clusters to classify the pixels. This technique attempts to access the relationships between patterns of the pixel data by organizing the patterns into groups (or clusters) such that the patterns within a cluster are more similar to each other than patterns belonging to different clusters. Segmentation by means of clustering is adopted in this study since the numbers of groups needs to be identified from the brain images are known a priori.

K Means Clustering

It is an unsupervised algorithm to classify or to group the objects based on attributes/ features into K number of subsets where, K is a positive integer number. The grouping is done by considering minimum Euclidean distance between the feature data and the corresponding cluster centroid. Given the coordinates of two points (x_1, y_1) and (x_2, y2), the associated Euclidean distance (D) is given by:

$$D = {}_1D = \sqrt{|(x_2 - x_1)^2 + (y_2 - y_1)^2|}$$

... (1)

For instance, any given image that has 'x' regions to be grouped, could be segmented using K-means algorithm by assuming 'x' number of clusters with suitable centroid for each cluster. The centroid could be any feature or even pixel intensity which is normally chosen by observing similar type of image data and or based on prior knowledge on the regions to be segmented. The basic algorithm[17] as shown in the Figure 1.

Fuzzy C Means Clustering

Fuzzy clustering depends on whether a pattern data belongs exclusively to a single cluster or to several clusters with different degrees of correspondence. The fuzzy C-means clustering (FCM) algorithm attempts to partition a total collection of elements of an image into a collection of fuzzy clusters with respect to a set of declared criteria. This algorithm involves assigning membership to each data point corresponding to each cluster center on the basis of distance between the cluster center and the data point. More the data nearer to cluster-center, more is its membership of belongingness towards the particular cluster center. The FCM technique is very similar to the K-means algorithm in all steps excepting assigning a membership function (step 2 mentioned below). The FCM algorithm[18,19] can be summarized by following steps:

Step 1: Fix the number of cluster and initialize the centers by random points from data set (based on observation).

Step 2: Update the membership degrees by using

$$u_{ij} = \frac{1}{\sum_{k=1}^{c} \left[\frac{d_{ij}}{d_{ik}}\right]^{\left(\frac{2}{mt-1}\right)}}$$

...(2)

where u_{ij} represents the membership of i^{th} cluster to j^{th} cluster.

d_{ij} represents Euclidean distance between i^{th} data and j^{th} cluster.

Step 3: Update centers using

$$c_j = \frac{\sum_{i=1}^{N} (u_{ij}^m) * x_i}{\sum_{i=1}^{N} u_{ij}^m}$$

...(3)

Step 4: Repeat steps 2 and 3 until convergence. The convergence of this algorithm will be reached when the change in membership values is less than a given center value (threshold).

Pixel counting technique for volume measurement

The automated pixel counting method is a method of estimating the area of the structure.

The tessellate method is also used to estimate the volume by tracing the boundaries of the region of interest. When compared to the tessellate method, the pixel-counting is simpler and robust for the volume estimation of the human brain.

```
// Pseudocode on K-means clustering

WHILE number of image I for one case ≠0
Initialise K=3 and three centroids c1,c2, c3 ≠ 0
Initialise matrix D1, D2, D3, y1, y2 and y3 of size same as that of I
FOR number of times i = number of rows in I
 FOR number of times j = number of columns in I
                D1(i,j)= sqrt [c1 – I]
                D2(i,j)= sqrt [c2 – I]
                D3(i,j)= sqrt [c3 – I]
                 ENDFOR
                 ENDFOR
FOR number of times m = number of rows in I
FOR number of times n = number of columns in I

                x= min(D1,D2,D3)
                    If x ∈ D1
                     y1(m,n) = I(m,n)
                    If x ∈ D2
                     y2(m,n) = I(m,n)
                          Else
                     y3(m,n) = I(m,n)
                          ENDIF
                          ENDIF
                    ENDFOR
                    ENDWHILE
                    END
```

Fig. 1. Pseudocode of K-means clustering

Table 1. Details of the clinical subjects considered in this study

Number of subjects	Gender	Age in years	Clinical Conditions
3	Male	16,62, 89	Normal
5	Female	16 to 89	Normal
3	Male	27,55, 65	Different types of lesions
1	Female	23	Multiple intracranial granulomatous lesions.

When an organ is scanned, many slices of cross-sectional imaging are performed at different views like sagittal, coronal and axial. Each of the slices gives details about different state/ condition and represents all the tissues present in that cross-sectional view. Each pixel in the slice in turn represents a particular local feature of the tissue. Depending on the resolution of the machine used, each pixel would correspond to the area of the organ in that section, and the slice thickness to thickness of the organ imaged at one instant.

Thus by multiplying number of pixels in one slice with the area it corresponds and with slice thickness would give volume of the organ enclosed in that section. By performing this computation for all the slices and summing up would specify the volume of the organ under scan.

RESULTS

T1-weighted MR images are acquired from 12 volunteers in axial section, totaling to 20 slices for each case. Each slice of the image contains information pertinent to WM, GM and CSF. Since the objective of this study is to estimate CV both region-wise and on overall structure, segmentation is required and clustering technique is used thereof to partition the brain image based on the pixel attribute (which in turn implicitly refers to tissue property). Twenty slices for each case is segmented for WM, GM and CSF using K-means and FCM methods. Then volume is computed for each case using segmented data by means of pixel-counting technique.

Clustering technique as elaborated in Section 2.2.1 is applied to all sections of the images used Since the image data has to be grouped into only three regions, number of clusters are declared as three and suitable mean point (based on gray-level value for each group) is selected. Correspondingly, the algorithm when applied to the acquired T1-weighted MR images results in three segmented images for each slice. Sample outputs as obtained from the two methods are shown in Figures 2 and 3. considers the membership function, there is overlapping between WM and GM found in the segmented output. Therefore, only the results obtained from K-means is used for the next consecutive step of volume measurement

Volumetric measurement

Each Generally, in fuzzy clustering, the data elements may overlap and belong to more than one cluster. As the fuzzy classification MR image obtained shows the cross-sectional view of the brain at different position. Every pixel in the image represents corresponding tissue. Each pixel is mapped as a spatial domain entity of the image;

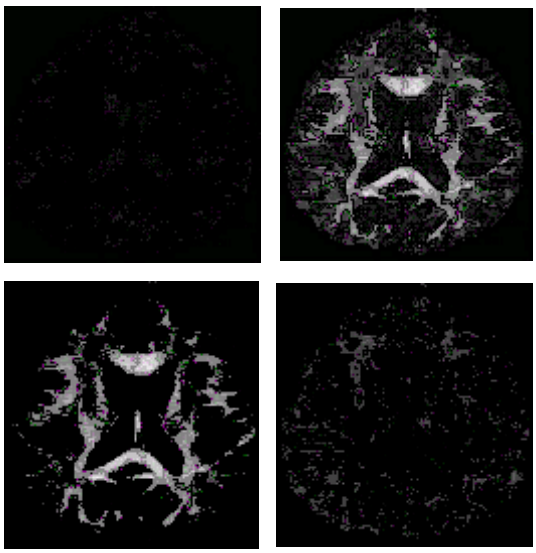

Fig. 2. Segmentation by K-means clustering.
(a) Original image, (b) CSF region,
(c) WM region and (d) GM region

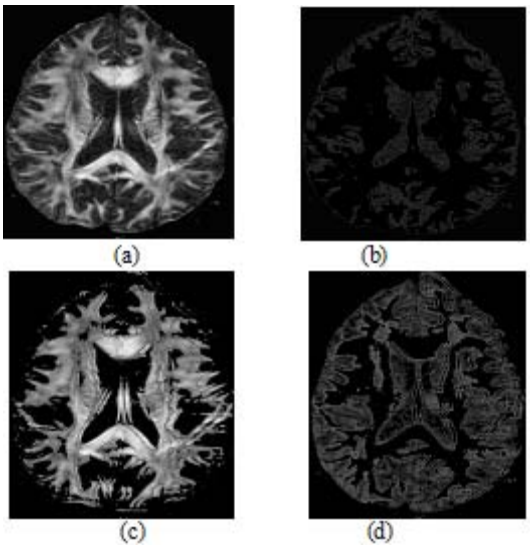

Fig. 3. Segmentation by FCM clustering.
(a) Original image, (b) CSF region,
(c) WM region and (d) GM region

a single pixel signifies about 1.2 mm^2 of area of the object under scan (in the modality used in this study). For instance, to compute the volume of white matter, the cross-sectional area of each WM slice is computed by multiplying the number of pixels in a differential area with a co-efficient 1.2 and summed up. The result is then multiplied by the slice thickness (5 mm) to get the volume of WM. Another study[20] used a similar method for obtaining the hippocampal volume. Likewise the volume of whole brain, GM and CSF are computed for all the volunteers and tabulated in the Table 2. Of the total twelve cases considered here, four are pathogenic state and the remaining are normal

Table 2. Volumetric assessment of twelve volunteers

Age / Gender	CSF volume (cc)	Vol. of WM/CV	Vol. of GM/CV	Vol. of GM/ Vol. of WM	Cerebral volume CV (cc)
89/F	189.018	0.4331	0.3767	0.8696	994.26
70/F	192.726	0.4092	0.4180	1.0215	1186.04
55/F	165.796	0.4537	0.4046	0.8918	1171.31
16/F	149.843	0.4729	0.3695	0.7814	951.471
23/F Case(A)	63.080	0.6286	0.2991	0.4757	873.571
89/M	194.44	0.5917	0.2321	0.3923	1104.31
62/M	287.67	0.5336	0.2285	0.4282	1209.511
16/M	176.904	0.5578	0.2639	0.4730	992.427
65/MCase(D)	101.85	0.4095	0.4782	1.1675	907.76
55/MCase(C)	144.146	0.6267	0.2583	0.4122	1254.46
27/MCase(B)	165.967	0.0837	0.7469	8.923	979.84

Table 3. Gross observations and inference made based on the results and clinical conditions

Age and gender	Clinical condition	Observations	Remarks
23/F	Multipleintracranial granulomatous lesions.	Decrease in CV, CSF and GM/WM ratio. Increase in WM/CV ratio	Damage in GM and WM
27/M	Malignant Lesion in right frontal region of white matter.	Decrease in CV, CSF and drastic changes in all the ratios	White matter damage due to cancerous lesion.
55/M	Right parietal . glioblastoma	Increase in CV and WM/CV ratio	Volume of WM increases due to gliotic changes happening around WM which appears hyperintense when imaged
65/M	Lesion with multilobulated margins predominantly in left temporal and extends to left frontal region.	Decrease in CV and volume of CSF	Disruption of WM. Volume of CSF decreases due to presence of lesion in CSF area.
16 to 89 / F	Normal	Higher percentage of GM in females and CV is less when compared with male subjects.	--------
16 to 89 / M	Normal	Higher percentage of WM among males and CV is high when compared with female subjects	----------

subjects. Clinical information of the subjects who has neuro-related issues is detailed below:

Clinical study of pathogenic states

The clinical study for acquired pathogenic states can be explained as follows

Case A: A 23 year old female subject was diagnosed for lesions in the left perito occipital sulcus during January 2011. She has undergone repeated MR scan during June 2012 and an increase in number and size of perilesional oedema were observed. When the MR image of this case is analysed, relevant results as in Table 2 shows that the CV and volume of CSF are less when compared to the data of controlled subjects.

Case B: Malignant lesion measuring (44 × 41) mm was observed in right frontal periventricular and subcortical WM extending to involve the cortex in the right inferior frontal region in a 27 years old male subject. The estimated volume of WM and CV are found to be significantly less when compared to those of the controlled subjects as shown in Table 2.

Case C: A 55 years male when scanned using MRI, gliotic cavity measuring (23 × 21) mm in the right parietal region with ill-defined T2/FLAIR hyperintensity representing residual edema/gliotic changes surrounding the WM was observed. In this case, the CV is very high but the volume of CSF is very low.

Case D: Third male abnormal case refers to a 65 years old subject with the findings of lesion with multilobulated margins predominantly in left temporal and extends to left frontal region. Lesion is broad-based towards base of the skull and has well-defined CSF and vascular cleft around the lesion. In this case, the CV and the volume of CSF are seen decreased when compared with those of the controlled subjects.

DISCUSSION

A cross-sectional study on CV using T1-weighted MR images is performed to elucidate the relationship between volumes of various brain regions among male and female subjects of different age groups of controlled and pathogenic subjects. The results from Table 2 show that the GM –to- WM volume ratio and GM –to- CV volume ratio under normal condition shows continuous increase in females even upto 70 years and then decrease. However, the CV in female increases upto 70 years and then decreases; and, this increase is widely attributed by CSF rather than other tissues. Hence, the ratio between volumes of WM and CV shows a decreasing trend till 70 years and then starts increasing. Such a decrease is not due to the reduction in WM volume but, as a result of an increase in CV due to CSF. On other hand, in pathogenic state, a 23 years female subject shows decrease in CV due to reduction in CSF and GM, as a result of the presence of lesion in GM which damages that region.

The ratio between the volume of GM and WM of controlled male subjects continuously decreases upto 89 years. As in the case of female, the CV in male also increases initially and then decreases during the later part of the life. This increase in CV is widely contributed by CSF than other tissues. Hence the volumetric ratio between WM and CV decreases upto the first half of eighth decade of life. (The corresponding ratio between WM to CV decreases only till 62 years as per the Table 2; but, it is assumed that this ratio would tend to decrease even during early seventies. This assumption is made based on readings recorded for female subjects and also due to unavailability of male image data in this range of age) and then start increasing from later part of eighth decade of life.

When same type of analysis is extended for pathogenic male subjects, the 27 years male shows drastic increase in the volumetric ratios (GM and WM) and (GM and CV); drastic decrease in ratio between WM to CV suggests the disruption of WM tracts and cortex region due to malignancy. The scan on 55 years male subject shows a significant increase in CV, at the same time the volume of CSF is very less and hence the increase in CV is solely contributed by WM and GM. Therefore, looking into the ratios in this case, much variation is not observed. However increase in ratio between WM to CV suggests that the increase in WM volume is due to gliotic changes around the WM which appears hyperintense when imaged. The image of 65 years male shows that the ratio between GM and WM and between GM and CV are seen increased suggesting an increase in GM volume due to the presence of lesion in the GM region. However, ratio between WM and CV is seen decreased suggesting disruption of fiber

tracts. Since the lesion is towards the base of the skull and occupies the CSF area, volume of CSF shows a marked decrease. Thus, by the volumetric assessment proposed, the status/nature of the tumour among different regions of the brain can be studied.

Another inference from this study is concerned with the gender difference on subjects having no neuro-related issues: Table 2 shows both CV and CSF volume are high in male than in female brain. Due to the physique stature of men, the male brains are in general larger than female brain[21]. It is also suggested that women have higher percentage of GM when compared to men and men have higher percentage of WM when compared to women considering the CV. However the result of this study shows (Table 2) that the ratio of GM and WM and between GM and CV is higher among females at all stages of age, which means that volume of GM in female could be higher than that of male. In controls, the ratio between WM and CV is higher among male subjects when compared with females at all stages of age meaning that the volume of WM and CV is higher in general, in male subjects. All the above discussions and few concluding remarks of this study are summarized in Table 3.

The results as above are in agreement with those due to results obtained by the previous study[22] and implying, that volume of CSF and WM are high in male whereas GM is high in female

CONCLUSION

The results implying, that volume of CSF and WM are high in male whereas GM is high in female.Thus, by the volumetric assessment proposed, the status/nature of the tumour among different regions of the brain can be studied. Therefore it could be concluded that by carefully analyzing the CV and ratios of the volumes of different tissues (like WM and GM) to CV, gender difference,ageing process, status of lesions (benign or malignant) could be inferred.

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