Hippocampal volumetry: Normative data in the Indian population

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Abstract

Background: Mesial temporal sclerosis (MTS) is the most common cause of temporal lobe epilepsy. Quantitative analysis of the hippocampus using volumetry is commonly being used in the diagnosis of MTS and is being used as a marker in prognostication of seizure control. Although normative data for hippocampal volume (HV) is available for the western population, no such data is available for the Indian population. **Aim:** The aim of the study was to establish normative data for HV for the Indian population, which can aid in the accurate diagnosis of MTS. **Materials and Methods:** Magnetic resonance imaging (MRI) scans of 200 healthy volunteers were acquired using a 3 Tesla (3T) MRI scanner. Manual segmentation and volumetry was done using Siemens Syngo software. The data was analyzed using two tailed *t*-test to detect associations between HV and age, gender, and education. The data so obtained was also correlated with the data available from the rest of the world. **Results:** A mean HV of 2.411 cm³ (standard deviation -0.299) was found in the study, which was significantly smaller when compared to the data from the western population. The right hippocampus was larger than the left, with a mean volume of 2.424 cm³ and 2.398 cm³, respectively. HV was detected to be significantly higher in males. No association was found between HV and age and education. **Conclusion:** The values obtained in this study may be adopted as a standard in the evaluation of patients with intractable epilepsy.

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Introduction

Complex partial-onset epilepsies account for about 60% of all adult epilepsy cases. Temporal lobe epilepsy (TLE) is the most common type of complex partial-onset epilepsy referred for epilepsy surgery and is often refractory to antiepileptic drugs.[1] Mesial temporal sclerosis (MTS) is the most common cause of temporal lobe epilepsy.[2] MTS was introduced as a term to encompass sclerosis, including the hippocampus and involves adjacent medial structures, such as the amygdala. MTS is a specific pathological diagnosis and the histological findings consist of hippocampal formation atrophy, selective hippocampal subfield neuronal loss, and mesial temporal gliosis.[3,4] At times, subjective interpretation of size and signal

changes need to be quantified, especially in cases with bilateral sclerosis, which accounts for 19%[5,6] of cases, and magnetic resonance (MR) normal intractable epilepsy, which forms 15% of cases.[7] Quantitative analysis of the hippocampus using volumetry and T2 relaxometry is commonly being used in difficult situations and also in research.[8] Quantifying hippocampal atrophy through magnetic resonance imaging (MRI) volumetry provides an independent source of information on seizure lateralization, expected postoperative outcome, and it may aid in appropriately selecting patients for invasive preoperative monitoring studies.[9]

In addition, hippocampal atrophy has been detected in other conditions such as major depression and Alzheimer's disease. There are studies demonstrating hippocampal volume (HV) loss in chronic and recurrent depression. It has also been shown to occur in early depression and therefore, volume assessment has the potential in aiding early diagnosis.[10] Atrophic changes in the hippocampus have been detected in the early stages of development of Alzheimer's dementia and this highlights its potential use in its diagnosis.[11]

There have been efforts made to establish normative data for HV in the western population. We are commonly faced

with a dilemma in clinical practice, where there is a need for lateralization of epilepsy on MRI, in cases of bilateral sclerosis and MR normal MTS. Detecting a size difference when compared to the apparently normal hippocampus, fails in these situations. It then becomes essential to compare the values with a normative data. The mean HV obtained from the western population varies from 2.78 cm^3 to 3.91 cm^3,[12-16] which we found to be very high for our patients. However, there have been no studies done to assess normal HV in the Indian population. Hence, we undertook this study; to assess HV in a normative data of 200 volunteers, using a manual method of segmentation, which is the most commonly used method in clinical practice. Similar studies have been done in other Asian countries and the mean volumes reported varied from 1.99 cm^3 to 2.91 cm^3.[17-19]
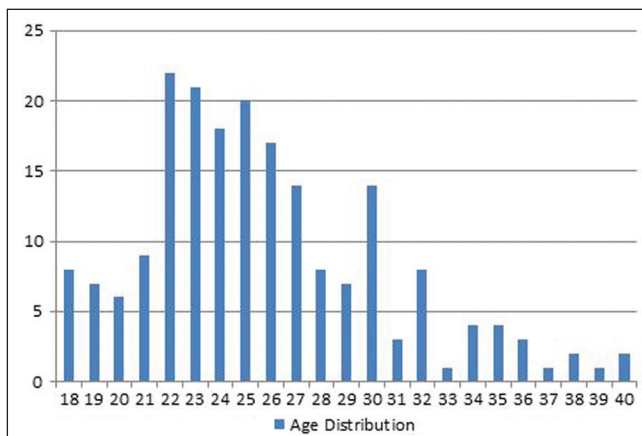
Materials and Methods

Study population characteristics

This is a study involving 200 healthy volunteers with no history of neurological disease, substance abuse, or injury. A mini-score assessment was done to rule out psychiatric diseases. As the study was based in a tertiary level neurocenter in the south, the study population was drawn from this region. Despite this, many of the volunteers were students and professionals, who had migrated from different states in India and therefore, the results of the study are relevant to the whole Indian population. The age of the volunteers ranged from 18 to 40 years, with a mean age of 25.75 years. A breakup of the age of the volunteers is given in Figure 1. There were 134 males and 66 females. One hundred and forty-two volunteers were college educated and the remaining 58 were educated up to high school. MRI scans were done using a 3T Siemens Skyra MRI scanner. The relevant anatomical data was separated and transferred to a Siemens Syngo workstation, where it was assessed by a single observer under the guidance of a neuroradiologist, with more than 10 years of experience.

Segmentation method

The HV was assessed manually with Siemens Syngo software, on T1-weighted MP RAGE images, using the coronal planes. Images of slice thickness 1 mm and interslice gap of 1 mm were used. Based on the review conducted by Konrad *et al.*,[20]

a protocol was setup to manually segment the hippocampus for volumetry. The white matter structures, alveus, and fimbria were excluded. Internal landmarks were used wherever possible and external landmarks were used only where necessary, in combination with internal landmarks.

Superiorly — The alveus and the cerebrospinal fluid (CSF) within the lateral ventricle.

Inferiorly — The white matter of the parahippocampal gyrus below the subiculum.

Laterally — The CSF in the lateral ventricle.

Superomedially — CSF in the cisterna ambiens.

Inferomedially — Extend the inferior border of the cornu ammonis medially, with a straight horizontal line and considered all tissue above as hippocampus and below as parahippocampal cortex.

Anteriorly — The alveus, as an internal landmark, in combination with the appearance of CSF of the lateral ventricle, as an external landmark, to delineate the hippocampus from the amygdala.

Posteriorly — The lateral ventricle is used as an external landmark and the posterior end is localized in the slice, where an ovoid grey matter starts to appear inferomedial to the trigone of the lateral ventricle.

This is illustrated in Figures 2, 3, and 4. Segmentation was done manually using a mouse cursor. To assess intraobserver variability, 20 of the subjects were selected randomly and hippocampal segmentation and volumes were reassessed by the same observer.

Statistical method

A paired *t*-test analysis was done to compare the two sets of volumes to assess intraobserver variability. The mean and standard deviation (SD) for right HV (RHV) and left HV (LHV)

Figure 1: An illustration of the breakup of age among the volunteers
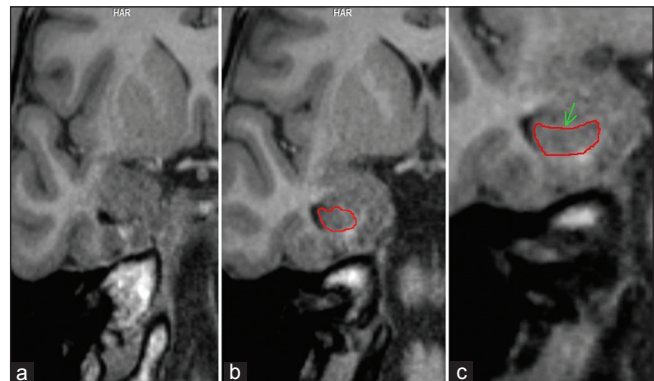
Figure 2: Hippocampus head. Red outline indicates segmented hippocampus. (a) Slice before the appearance of hippocampus head. (b) First slice showing the hippocampus head. (c) The *arrow* indicates the alveus, which separates the hippocampus from the amygdala
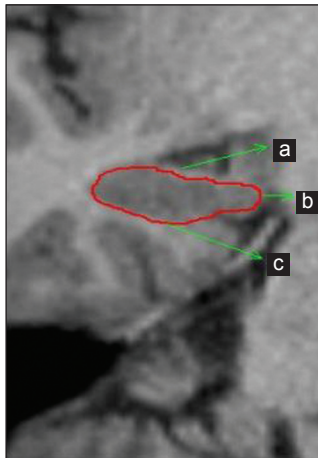
Figure 3: Hippocampus body. Red outline indicates segmented hippocampus. (a) Superior border delineated by alveus and cerebrospinal fluid (CSF) of lateral ventricle. (b) CSF of the cisterna ambiens forming medial border. (c) White matter of the parahippocampal gyrus forming inferior border

Figure 4: Hippocampus tail. Red outline indicates segmented hippocampus. (a) Last slice showing tail of hippocampus as an ovoid grey mass medial to lateral ventricle. *Arrow* indicates lateral border of hippocampus delineated by CSF in lateral ventricle. (b) Slice beyond the hippocampus tail
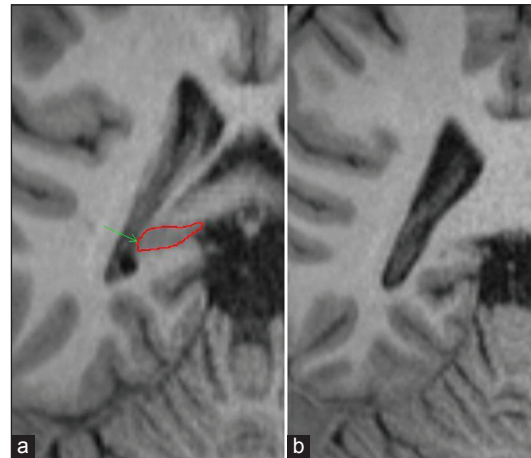
were assessed. The upper and lower limits were calculated using 95% confidence intervals (2 SD), on either side of the mean. We used *t*-test analysis to understand the effect of age, sex, and education on HV. We divided the volunteers into two groups based on age (18-29 and 30-40), to study the effect of age.

Results

In the sample of 200 healthy volunteers from the Indian population that we assessed, the HV followed normal distribution with no skew. The mean HV was found to be 2.411 cm^3 (SD - 0.299). The mean RHV was 2.424 cm^3 and the mean LHV was 2.398 cm^3. The upper and lower limits for HV, calculated using 95% confidence intervals (2 SD), were 3.009 cm^3 and 1.813 cm^3, respectively. Equal variances were assumed when comparing gender and HV. A mean RHV of 2.491 in males and 2.288 in females was obtained, with the difference being statistically significant ($P < 0.001$). This was mirrored in the LHV, with a mean of 2.447 in males and 2.298 in females ($P < 0.001$). When comparing education with HV, equal variances were not assumed with respect to the distribution of age between the two groups. There was no statistically significant difference in mean volumes between college educated and school educated volunteers. Equal variances were assumed when comparing the two age groups with the HV and no statistically significant variation was detected. The details of the statistical analysis are outlined in Table 1. The paired samples *t*-test, done to assess variation between the two volumes obtained from the 20 randomly selected volunteers, did not show a statistically significant variation; thus, indicating good intraobserver agreeability. This is indicated in Table 2.

Discussion

The primary findings in MTS are hyperintensity of hippocampus and hippocampal atrophy, which is detected by quantitative volumetry.[21-24] This is best appreciated on the coronal FLAIR sequence, which suppresses out the CSF signal from the uncal recess and the choroidal fissure, thus avoiding false

Table 1: Statistical analysis with *t*-test

	t-test for equality of means			
	Mean	T	Df	Sig. (2-tailed)
RHV with gender				
Male	2.49134	4.830	198	<0.001
Female	2.28752			
LHV with gender				
Male	2.44694	3.336	198	<0.001
Female	2.29830			
RHV with education				
C E	2.44363	1.466	198	0.144
S E	2.37622			
LHV with education				
C E	2.41860	1.514	198	0.132
S E	2.34719			
RHV with age				
18–29 years	2.43892	1.358	198	0.176
30–40 years	2.36988			
LHV with age				
18–29 years	2.41370	1.410	198	0.160
30–40 years	2.34016			

Df = Degrees of freedom, Sig. = significance, CE = College educated, SE = School educated, RHV = Right hippocampal volume, LHV = Left hippocampal volume, T = *t* test value

Table 2: Intraobserver variability assessment with paired sample *t*-test

	Mean	N	T	Sig.(2-tailed)
Pair 1				
RHV 1st assessment	2.33810	20	−0.129	0.899
RHV 2nd assessment	2.33975	20		
Pair 2				
LHV 1st assessment	2.37245	20	−1.088	0.290
LHV 2nd assessment	2.38240	20		

Sig. = Significance, RHV = Right hippocampal volume, LHV = Left hippocampal volume, N =Number of subjects , T = *t* test value

positive high signal changes.[25] In a study performed by Berkovic *et al.*, in 1995, the sensitivity of MRI for MTS was as high as 97%, and specificity was 83%.[26] Other studies have shown that visual MRI interpretation of these features has sensitivities of 87-100%.[21,24] Therefore, MRI is the investigation of choice for diagnosing MTS. In a study conducted by Coan *et al.,* HV identified hippocampal sclerosis in 95% of electroencephalography (EEG) confirmed MTS patients. The quantification of HV and T2 signal can increase the detection of signs of MTS in approximately 28% of patients with mesial temporal lobe epilepsy (MTLE).[8] Studies have shown that a close correlation exists between histologically identified cell loss in the hippocampus and atrophy detected by MRI volume measurements, and that quantitative MRI measurements can act as a surrogate for pathological identification of presence and severity of hippocampal sclerosis.[27,28] Volumetry correctly lateralizes the side of MTS in 93% of patients.[29] The technique is a useful adjunct in a multidisciplinary, preoperative epilepsy evaluation, when T2-weighted MRIs do not reveal an epileptogenic mass lesion.[9]

Cole *et al.,* conducted a meta-analysis, assessing studies demonstrating hippocampal atrophy in patients with the first episode of depression. Cumulative analysis revealed atrophy in patients with first episode major depressive disorder compared to controls for both left and right hippocampi (*P* - 0.03 and *P* - 0.01, respectively). The average volume reduction was –4.0% for the left and –4.5% in the left hippocampus.[10] Apostolova *et al.,* conducted a study involving 17 patients, where they assessed the utility of HV assessment in predicting the development of Alzheimer's dementia. When compared to the controls, patients who went on to be diagnosed with mild cognitive impairment and Alzheimer's dementia, had lower HV both at baseline and a 3-year follow-up. The drop in volume was significant for both left (*P* - 0.001) and right (*P* < 0.02). The loss of volume mainly occurred in the subicular region and cornu ammonis.[11]

HV may be assessed by manual, semiautomated, or automated methods. Automated methods are user-independent and utilize geometric template matching methods to extract brain size and shape parameters. There are many types of software such as Analysis of Functional NeuroImages (AFNI), Freesurfer and FMRIB (functional MRI of the brain) software library (FSL). The main drawback of automated software is the loss of anatomic specificity, which may lead to errors. The drawback of manual methods is that it is time consuming and labor intensive.[30] Pardoe and Jackson[31] conducted a study comparing manual segmentation and automated methods for HV assessment, in reply to the study conducted by Coan *et al.*[8] They used the Freesurfer software for automated assessment. Manual HV detects hippocampal sclerosis with a sensitivity and specificity of 72 and 52%, respectively, with the area under the curve being 0.9. Automated volumetry had sensitivity and specificity of 63 and 19%, repectively, with the area under the curve being 0.85.[31] Therefore, despite automated methods being less time consuming, they are not as accurate as manual methods. Moreover, manual methods can be used in centers which have access to MRI machines, without possessing the required software.

The protocol used to segment the hippocampus is an important aspect of its volumetric assessment and the criteria used differs between institutions. The most contentious aspects of its delineation are the anterior and posterior limits along with the inferomedial border, along the extent of the hippocampus. In the protocol outlined above, we have used criteria which have been used most commonly in literature. Reliable internal landmarks such as the alveus and cornu ammonis were used whenever possible, with external landmarks used only where necessary and even then in combination with an internal landmark. Anatomical decisions that are not based on the intrinsic anatomy of the hippocampus, depend on the position of the hippocampus relative to external structures, rather than to the hippocampal anatomy, and thus can lead to a lack of uniformity while segmentation.[20]

We have attempted to define normative data for HV in the Indian population. We have found the mean HV to be 2.411 cm³, with the mean RHV as 2.424 cm³ and the mean LHV as 2.398 cm³. Using 2 SD, a lower limit of 1.813 cm³ can be set for the HV and values below this may be considered to be abnormal. From the analysis of our data, we did not detect any significant associations between age and education and HV. We did detect a small but statistically significant association between gender and HV, with males having slightly larger volumes than females. We have performed the segmentation on software that is available in most laboratories and is easy to use.

Literature on western population, with comparable segmentation criteria had mean HV ranging from 2.78 cm³ to 3.91 cm³.[12-16] When compared with the data we have obtained, the HV in the Indian population are smaller than those in the west. This indicates that using the data established in other population groups, to set limits for identifying disorders such as MTS, major depression, and Alzheimer's dementia in the Indian population, may lead to erroneous diagnosis. It highlights the need for an established normative data set against which suspected patients may be compared. There have been some studies done in Asian literature with values ranging from 1.99cm³ to 2.91 cm³.[17-19] We have found higher values for the right hippocampus when compared with the left and this is something that is reflected in the majority of articles on the topic. The higher volume in males is something that is mirrored in other studies as well.[13] There have not been any studies done to assess the effect of education on HV and our analyses indicate that there is no significant association. Our study population included volunteers between 18 and 40 years and no variation was detected across this age-group, which is the most common age-group to undergo surgery for intractable epilepsy. With progression of age, HV regresses due to age-related atrophy of the brain.[19] This highlights the fact that the normative data will differ in elderly people. As already discussed above, there is increasing value of HV as a biomarker for Alzheimer's disease and there is a need for establishing normative data in the Indian population in this age-group as well.[11] More studies need to be done in this area.

Limitations

We acknowledge that the study is limited by the fact that the volumes have been assessed by only one observer. There is a need for validation of these findings with further studies.

Conclusion

The mean HV in this study is lesser than those found in literature based on the western population. The values obtained here may be adopted as a standard in the evaluation of patients with intractable epilepsy.

Acknowledgment

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