Original research

Differentiating healthy and mesial temporal lobe epileptic (MTLE) brains by analyzing the adjusted volume of the hippocampus using Magnetic Resonance Imaging (MRI).

L.R. S. Rathnakumara[1], B. G. S. Nadeeshan[1], I. U. Wickramarathne[1], Sahan M. Vijithananda[2], Anil De Silva[3], Manjula de Silva[4], Padma B. Hewavithana[5], Mohan L. Jayatilake[1*]

[1] Department of Radiography/Radiotherapy, Faculty of Allied Health Sciences, University of Peradeniya, Peradeniya, Sri Lanka

[2] Department of Anatomy, Faculty of Medicine and Allied Sciences, Rajarata University, Sri Lanka

[3] Epilepsy Unit, National Hospital of Sri Lanka, Colombo, Sri Lanka

[4] Department of Physics, Faculty of Science, University of Ruhuna, Sri Lanka.

[5] Department of Radiology, Faculty of Medicine, University of Peradeniya, Sri Lanka.

Abstract

Epilepsy is one of the most affecting neurological diseases that occur due to an abnormal, uncontrolled firing of a group of neurons in the brain. The temporal lobe is the most common epileptogenic site of humans, with mesial temporal lobe epilepsy (MTLE) being the most common type in adults. MRI plays a major role in diagnostic and therapeutic procedures of MTLE. This study focused on calculating the adjusted volume of human hippocampus by measuring the volumes of the cerebrum and hippocampus and utilizing them to identify the correlation of adjusted volume distribution patterns among MTLE and healthy subjects.

The study was carried out using MR images of ten subjects, five MTLE and five healthy, who are within the 20-30 age range. All the MR Digital Imaging and Communications in Medicine (DICOM) images were acquired from a 3-Tesla Siemens MRI facility, and each subject's pathology was confirmed by radiological reports.

The study has acquainted a semi-automated method to investigate volume differences in hippocampus between healthy and MTLE subjects using hippocampal volume as a potential biomarker. The adjusted volumes of hippocampus were calculated as a proportion of the brain volume. Finally, the volume values received for each category were tested with a one-tailed *t*-test.

The mean adjusted hippocampal volume of epilepsy and normal subjects were reported as 0.01011 ± 0.00127 and 0.011709 ± 0.000659 respectively. The *t*-test revealed that there is a significantly low, mean adjusted hippocampal volume for MTLE subjects than the healthy population; *t*-stat (2.56) > *t*-critical (2.13). Therefore, the study suggests the application of adjusted hippocampal volume as a potential biomarker to identify MTLE patients.

Keywords: Epilepsy, Mesial Temporal Lobe Epilepsy (MTLE), Hippocampus, Adjusted volume of hippocampus

Funding: None
Competing interest: None
Received: 17.08.2022 **Accepted revised version:** 14.03.2023 **Published:**30.04.2023

*✉**Correspondence:** jayatiml@pdn.ac.lk https://orcid.org/0000-0002-7979-9147

Cite this article as: Rathnakumara LRS *et al*. Differentiating healthy and mesial temporal lobe epileptic (MTLE) brains by analyzing the adjusted volume of the hippocampus using Magnetic Resonance Imaging (MRI). Anuradhapura Medical Journal 2023; 17 (1): 54-61, DOI: http://doi.org/10.4038/amj.v17i1.7739

Introduction

Epilepsy is a common neurological disorder that can occur at any age. It is caused by abnormal brain activity, resulting in convulsions or periods of unusual behaviour, sensation, or loss of awareness [1-4]. Epilepsy is characterized by a person's risk of frequent seizures due to chronic and underlying processes. However, a single or recurrent seizure that occurs due to known and avoidable circumstances does not necessarily constitute epilepsy. A seizure is an excessive electrical discharge of nerve cells in different parts of the brain. The cleavage of the regulated balance between excitatory and inhibitory neuronal activity within the brain may lead to epileptic seizures [5]. Epileptogenesis can be driven by various factors, such as genetic tendency, developmental dysfunction, and neurological affront [6]. Generalized epilepsy involves bilateral and symmetrical electrical discharges in the brain, while focal epilepsy affects a specific area of the brain [2, 7-10]. Focal epilepsy can be subdivided based on the confined area of the hyperexcitable neuronal network, and its manifestation may differ. However, the exact cause of almost 50% of epileptic cases cannot be identified in the current clinical environment [7].

However, the most common focal epilepsy type in adults is Mesial Temporal Lobe Epilepsy (MTLE) (70% of all cases of temporal lobe epilepsy and 20% of all cases of epilepsy) which is usually combined with hippocampal sclerosis (HS) [5, 11-13]. MTLE is a chronic neurological disorder that can cause seizures and other symptoms that can affect a person's daily life.

The etiology of MTLE is also not fully understood, but it is thought to be multifactorial, involving both genetic and environmental factors. Brain damage due to head injury, infection, or brain tumor can also cause MTLE. Other potential causes include stroke, brain malformations, and certain genetic disorders (11).

Diagnosis of MTLE typically involves a combination of medical history, physical examination, and diagnostic tests such as an electroencephalogram (EEG), a magnetic resonance imaging (MRI) scan, and a positron emission tomography (PET) scan. The EEG is especially useful in detecting seizure activity in the brain. MRI and PET scans can help identify structural abnormalities in the brain that may be causing seizures (11-13).

The treatment of MTLE is mainly based on the severity of the condition and the individual's symptoms. The most common treatment for MTLE is medication, which can help control seizures and other symptoms. Surgery may be an option if medication is not effective. Other treatment options may include dietary changes, counseling, or psychotherapy.

The medial aspect of the temporal lobe also known as the mesial temporal lobe, comprises three anatomical entities; the hippocampal formation, the amygdaloid body and the parahippocampal cortices, which is the most superficial component that forms the external surface of the hemisphere [13]. The relationship between epilepsy and the hippocampus is well known and very important as the hippocampus is often involved in seizures, even if they are not generated in the hippocampus [14-16].

Hippocampus is a C-shaped complex brain structure located deep in the temporal lobe, appearing as a ridge of light grey shade in T2 weighted MRI images. It is mainly composed of neurons cells and glial cells. Hippocampus consists of two parts: Cornu ammonis (CA) and dentate gyrus. These two parts are separated by a hippocampus fissure which curves into each other. Hippocampal CA consists of four main parts: CA1, CA2, CA3, and CA4 [15,17]. However, the dentate gyrus , CA1, and CA4 parts of the hippocampus has more involvement to occur MTLE events meanwhile the CA3 sections of the hippocampus has lesser involvement [18].

However, Magnetic Resonance Imaging (MRI) is considered as the most suitable neuroimaging modality due to its sensitivity and specificity of imaging [16]. In MRI, the number of protons (hydrogen atoms) contained within the area being scanned, applied magnetic properties (gradient fields) and radio frequency (RF) pulses to the imaging field are responsible for the contrast of the resultant image. By altering these gradient fields and radio frequency (RF) pulses, MRI produces images with particular contrast and spatial resolution known as MRI sequences; T1, T2, Proton Density, Diffusion Weighted, Flow Sensitive sequences).

Multiple sequences are utilized in the diagnostic process and the particular combination of MRI sequences are referred to as an MRI protocol. The standard MRI protocol to investigate temporal lobe abnormalities is acquiring coronal volumes, which are perpendicular to the long axis of the hippocampus; coronal high-resolution T2-weighted, inversion

recovery, fluid attenuation, and inversion recovery (FLAIR) images, and axial FLAIR images [19-22].
Hippocampus sclerosis (HS) is a common pathology in MTLE patients and according to some studies, it is possible to identify and differentiate HS by observing several characteristic features of MR images such as shrinkage of the hippocampus, elevated signal intensity on T2-weighted images, and disturbed internal architecture are considered in identifying (HS) in current clinical radiology setup [23,24].

This study addresses the lack of a reference value to define the volume of the healthy hippocampus and that of patients with mesial temporal lobe epilepsy (MTLE). Therefore, the study focuses on developing a standard method to measure the hippocampus volume as a fraction of the entire cerebrum (referred to as the "adjusted hippocampal volume") and evaluate the correlation of adjusted volume differences of the hippocampus between healthy and MTLE subjects. By conducting this research, we hope to contribute to the advancement of knowledge regarding hippocampal volume and its role in MTLE.

Materials and Methods

This descriptive study was carried out using five MTLE and five healthy subjects, who were presented to the MRI facility at the Epilepsy Unit, National Hospital of Sri Lanka (NHSL) and Teaching Hospital, Anuradhapura (THA) within three months of the period. The MRI Digital Imaging and Communications in Medicine (DICOM) data, radiological reports and patient medical history were collected retrospectively after obtaining ethical clearance from the ethical review board of the Faculty of Allied Health Sciences, University of Peradeniya, Sri Lanka (AHS/ERC/2021/058) and informed consent from each subject. All the selected subjects were within the age range of 20-30 years. The gender of subjects was not considered due to the adjusted volume calculation process. The radiological report and patient's past medical history (PMH) confirmed the pathological condition of each MTLE patient. As the control group, subjects with healthy brains (without a PMH of MTLE or other brain diseases such as brain tumours, trauma, congenital malformations, etc.,) who were presented to the MRI facility were selected. However, according to the inclusion and exclusion criteria of the study plan, the subjects with confirmed other pathological conditions such as brain tumours (malignant/benign), Intracranial hemorrhage, head trauma and hydrocephalus also the subjects on certain medication (diuretics) were excluded from the data set.
3T MR system with head coli was utilized to acquire all the MR images. The acquired images were sorted according to the sequence name, and both T1 weighted Turbo Inversion Recovery Magnitude (T1 TIRM) and T2 weighted Turbo Spin Echo (T2 TSE) sequences were used for the data acquisition. Here, T1 TIRM was utilized to acquire the cerebrum volume and the T2 TSE sequence was utilized to acquire the volume of the hippocampus of each patient (Figure 1). The MATLAB Simulink 2019 a software was utilized in all the image processing, region of interest (ROI) drawing and volume calculation steps in this study. Here, manually selected the region of interest (ROI) in terms of reducing the potential errors occur in image processing steps (Figures1, 2 and 4).

The sequence which was used to extract cerebrum (T1 TIRM) has parameter values of TR- 2000s, TE-9.5s, FOV-252mm, TI-900s, Slice Thickness-1mm and Matrix Size-320×320mm and the sequence used to extract the hippocampus (T2 TSE) has parameter values of TR-5230s, TE-99s, FOV-252, Slice Thickness-1mm and Matrix Size-320×320mm.

Image slices within the selected sequences were re-arranged in ascending order according to their slice location. Then, the cerebrum of each T1 TIRM image of each patient was separated from the skull by manually drawing an ROI surrounding the cerebrum (Figure 1). The researchers were careful enough to exclude all the intensities generated by cerebrospinal fluid (CSF) while drawing the ROI. Also, the MRI sagittal T2 TSE sequence was utilized to select the hippocampal area by drawing an ROI surrounding the hippocampal region and storing the drawn ROIs in a unique place for each subject (Figure 2). However, the purpose of using sagittal images to draw ROIs surrounding the hippocampus is, it is the most suitable way to draw and tract the hippocampus in areas such as the posterior body and the tail sections of the hippocampus. The researchers drew all the ROI under the supervision and guidance of two board-certified consultant radiologists.

The saved 2D images of the cerebrum and the hippocampus were converted into 3D by visualizing all the slices simultaneously and aligning the slices according to their slice locations without keeping spaces between them. .

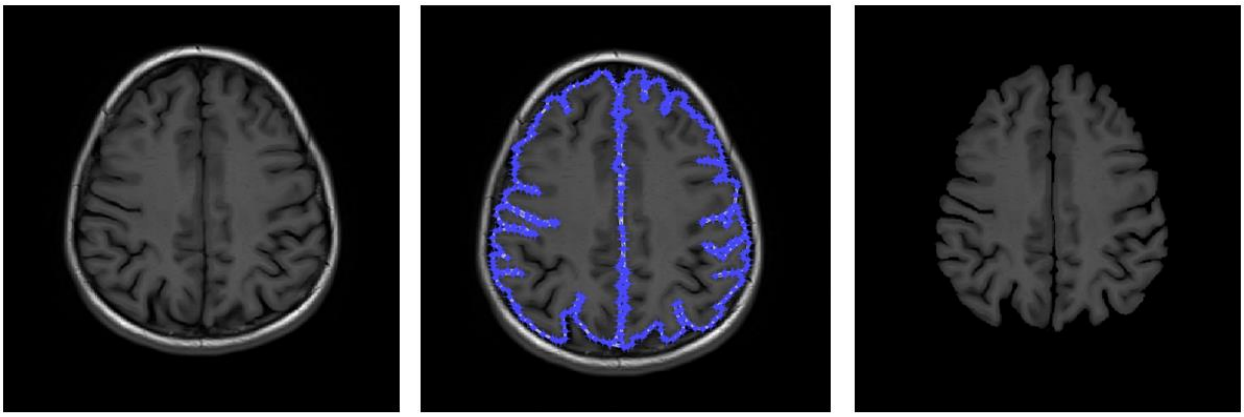

Figure 1: Drawing region of interest (ROI) surrounding the cerebrum. The Image illustrates the steps of extraction of the cerebrum from an MR axial T1 weighted Turbo Inversion Recovery Magnitude (T1 TIRM) Image of the head. The image was taken from a 25 years old male patient presented with a short history of mesial temporal lobe epilepsy. (**A**). Brain with the skull, (**B**). ROI mapping of the brain, (**C**). Extracted 2D ROI of the brain.

.

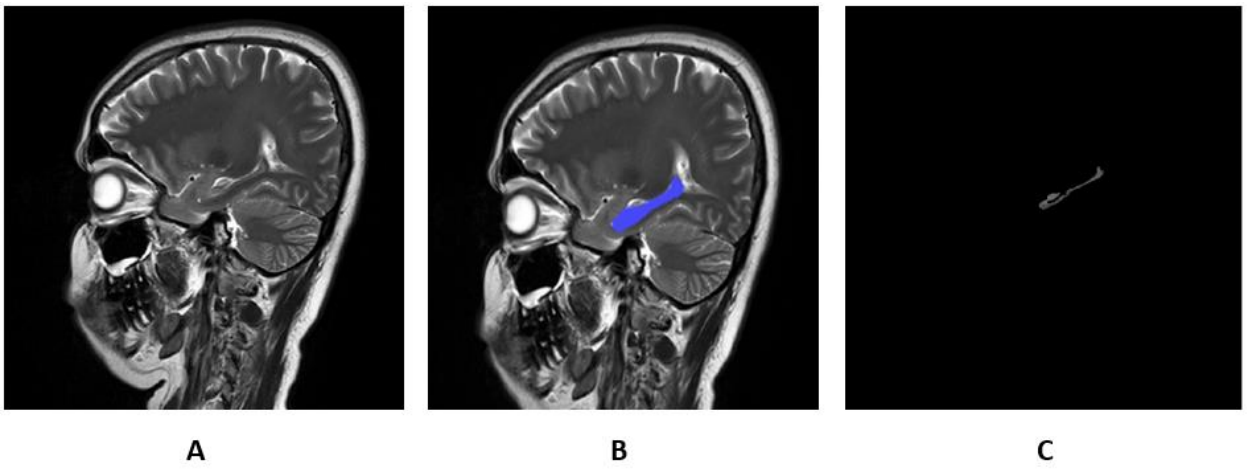

Figure 2: **Drawing region of interest surrounding the hippocampus.** The Image illustrates the steps of extraction of the hippocampus from an MR sagittal T2 weighted Turbo Spin Echo (T2 TSE) image of the head. The image was taken from a 25 years old male patient presented with a short history of mesial temporal lobe epilepsy. (**A**). Sagittal Image that visualizes left hippocampal region, (**B**). ROI mapping of the hippocampus, (**C**). 2D Image of the extracted hippocampus.

The converted 3D Image was saved as a 3D matrix file manually. The brain cerebrum and hippocampus volumes were calculated based on voxels. The above process was iterated over all the selected subjects, and the volume information of both the cerebrum and the hippocampal region of each subject was gathered separately.

According to the body type (height and weight) of each subject, the volumes of their cerebrum and hippocampus were unique. To avoid this uniqueness of the brain according to their size (in cubic centimeters (cm^3)), the volume information was adjusted by dividing the volume of the hippocampus by the volume of the cerebrum (Equation 1).

$$V_{Adjusted} = \frac{V_{Hippocampus\ (cm^3)}}{V\ cerebrum\ (cm^3)}$$

Equation 1: *This equation illustrates the method of volume normalization of cerebrum. Here, $V_{adjusted}$ represents the adjusted volume calculated by dividing the volume of hippocampus ($V_{Hippocampus}$) by the volume of the cerebrum ($V_{Cerebrum}$).*

The mean adjusted hippocampal volumes for healthy and epileptic categories were tested for hypothesis

using two sample t-test with a 95% confidence level by assuming the human brain volume in the population distributed in normal distribution pattern. Although the selected sample sizes are small enough to utilize nonparametric statistical analysis i.e., Mann Whitney U-test for the data analysis, by considering the above assumption and the power of the analysis, a parametric statistical analysis; student t-test, for this hypothesis

testing. The result of the hypothesis testing was implemented to make a conclusion and reject or accept the null hypothesis (H_0) of this study; there is no significant difference in mean volumes of the hippocampus between MTLE and healthy subjects. All the statistical analyses have been performed using MINITAB 20.4 (beta version) statistical software.

Results

According to the adjusted volumes of the hippocampus of healthy subjects (Table 1), the value of the mean adjusted volume was 0.011709 and the mean adjusted hippocampus volume of MTLE subjects was 0.010111 (Table 1, Table 2, and Table 3). The distribution of adjusted hippocampal volume in both MTLE and healthy subjects is illustrated in Figure 3. According to the Figure 3, adjusted hippocampal volumes of healthy subjects shows higher mean values than the mean values of adjusted hippocampal volumes of epilepsy subjects.

Table 1: Brain and the hippocampal volumes with adjusted hippocampal volumes of healthy subjects

Subject	Brain volume (cm^3)	Hippocampal volume (cm^3)	Adjusted hippocampal volume
1	1070.4359	13.0864	0.0122253
2	1207.9025	14.4290	0.0119455
3	765.4484	8.8048	0.0115028
4	1170.0003	14.2960	0.0122188
5	1140.6326	12.1523	0.0106540

Table 2: Brain and the hippocampal volumes with adjusted hippocampal volumes of epilepsy subject

Subject	Brain volume (cm^3)	Hippocampal volume (cm^3)	Adjusted hippocampal volume
1	1140.6155	13.3241	0.0116815
2	1265.7011	13.5630	0.0107158
3	1210.6445	10.5113	0.0086824
4	1277.6298	11.4192	0.0089378
5	1129.7102	11.9050	0.0105381

The result shows that there was a negative difference between both populations mean values as shown in Table 3. According to the t-statistics, a significant difference between population means of adjusted hippocampal volumes of healthy and epileptic categories were found; t-stat (2.5639) > t-critical (2.1318). According to that, the null hypothesis (H_0) was rejected and alternative hypothesis (H_1) was not rejected. As the accepted result is within 95% confidence level, there is an Observable difference between population mean values of adjusted hippocampal volumes of healthy and epilepsy subjects (Figure 3).

Discussion

Although previous studies have shown a significant loss of volume of the hippocampus in MTLE patient, but none have measured the volume of the hippocampus compared to the volume of the cerebrum [24,26]. In this study, we have developed a universally applicable method to measure and compare the hippocampal volume as a ratio (Adjusted hippocampal volume) (Equation 1). Using this adjusted volume calculation process, clinicians can have a clear idea about the volume of the hippocampus without affecting the volume (size) of the cerebrum. It is practically illustrated using subject number 3 in the Table 1. When compared to the other subjects on Table 1, subject 3 has a comparatively low hippocampal volume and the cerebrum.

Table 3: Descriptive statistics of adjusted hippocampal volumes of healthy and epilepsy subjects

Sample	N	Mean	SD	SE Mean	Mean Difference	95% CI for Difference
Epilepsy	5	0.01011	0.00127	0.00057	0.001598	0.003162, 0.000034
Healthy	5	0.011709	0.000659	0.00029		

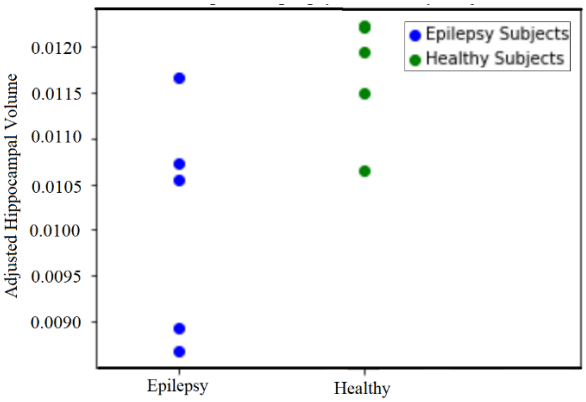

Figure 3: Hippocampal volume distribution. The dot plot visualizes the distribution pattern of adjusted hippocampal volumes of healthy and epilepsy subjects.

However, the adjusted hippocampal volume of the same subject was expressed the value that floating around the mean of the adjusted hippocampal volume of the other four subjects; mean adjusted volume of all five healthy subjects. 011709 (SD - 0.000659): adjusted hippocampus volume of the third healthy subject - 0.0115028 (Table 1).

This study utilized a semi-automated method to measure the volume of hippocampus and in addition to that, the hippocampal volume was adjusted to avoid the effect of body mass index on the global brain and the hippocampus volume of subjects (Equation 1) [25]. The study found a significant difference of mean adjusted hippocampal volumes between healthy and MTLE subjects.

Therefore, this study was focused on utilizing the adjusted hippocampal volume as a potential biomarker to differentiate MTLE patients and healthy subjects using a study sample consisting of five epilepsy subjects and five healthy subjects. The bilateral hippocampal volume and cerebrum volumes of each subject were measured. To reduce the errors in drawing the ROIs, researchers were utilized manual technique instead of using automated image segmentation algorithm. However, images were zoomed to its maximum before starting the ROI selection in order to avoid potential mistakes in the process (Figure 4).

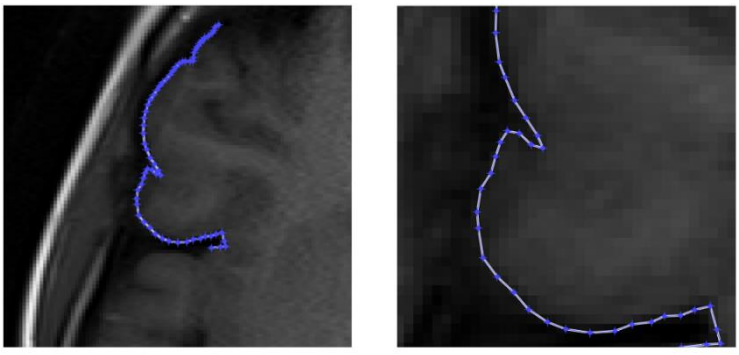

Figure 4: Measures taken to reduce the errors that occur in ROI selection. A) ROI selection by including each gyrus and sulci of the brain cortex. **B)** Illustrates the technique (magnified Image and close ROI points) used to draw ROI visualized in Figure 4.A.

However, there were two main limitations occurred during this study. The first one is ROI selection. The ROI of each image slice for cerebrum and hippocampus was drawn manually and it was a time-consuming process. As the second one, the age range of the selected population to the study was highly limited; from 20 to 30 years and a small sample size was chosen without considering the gender of the subjects.

Increasing the sample size, patients with a wide range of age, considering the gender of the patient and both manual and automated techniques for the segmentation can be used to improve the results of this study further. This study can be expanded to study other anatomical structures that may be affected by atrophy in MTLE such as the amygdala and parahippocampal gyrus. Moreover, the concept of adjusted hippocampal volume, developed in this study can be applied to study the adjusted volumes of other anatomical structures within the brain such as basal ganglia (putamen, globus pallidus, caudate nucleus, thalamus etc.), the ventricles etc.

Conclusion

The developed semi-automated technique in this study can be used to estimate the brain volume, hippocampal volume and adjusted hippocampal volume. The results show a significant difference between the population mean values of adjusted hippocampal volumes of healthy and MTLE subjects. The population mean of the adjusted hippocampal volume of epilepsy subjects was significantly lower than that of healthy subjects. Hence, this study concludes that adjusted hippocampal volume is a potential morphological biomarker to discriminate MTLE subjects from healthy subjects.

Acknowledgement

We acknowledge the support provided by the Department of Radiography/Radiotherapy, Faculty of Allied Health Sciences, University of Peradeniya (Sri Lanka), the National Hospital of Sri Lanka, Teaching Hospital Anuradhapura, and all the staff members including consultants of the departments of radiology of the above-mentioned hospitals for their guidance and the immense support provided in the data collection process.

References

1. Beghi E. The epidemiology of epilepsy. Neuroepidemiology. 2020;54(2):185–91.
2. Thijs RD, Surges R, O'Brien TJ, Sander JW. Epilepsy in adults. The Lancet. 2019 Feb 16;393(10172):689-701.
3. Moshé SL, Perucca E, Ryvlin P, Tomson T. Epilepsy: new advances. Lancet. 2015 7;385(9971):884–98.
4. Stafstrom CE, Carmant L. Seizures and Epilepsy: An Overview for Neuroscientists. Cold Spring Harb Perspect Med. 2015;5(6):a022426.
5. Pitkänen A, Lukasiuk K, Edward Dudek F, Staley KJ. Epileptogenesis. Cold Spring Harb Perspect Med. 2015;5(10):a022822.
6. Aidan Neligan, Willard A. Hauser, Josemir W. Sander, Chapter 6 - The epidemiology of the epilepsies, Editor(s): Hermann Stefan, William H. Theodore, Handbook of Clinical Neurology, Elsevier, Volume 107, 2012, Pp 113-133.
7. Stafstrom CE, Carmant L. Seizures and epilepsy: an overview for neuroscientists. Cold Spring Harbor perspectives in medicine. 2015 Jun 1;5(6):a022426.
8. Bromfield EB, Cavazos JE, Sirven JI. Basic mechanisms underlying seizures and epilepsy. 2006 [cited 2021 Dec 27]; Available from: https://www.ncbi.nlm.nih.gov/books/NBK2510/
9. WHO. EPILEPSY : A manual for medical and clinical officers in Africa and clinical officers. World Heal Organ. 2002;124p.
10. Ben-Ari Y, Holmes GL. Effects of seizures on developmental processes in the immature brain. Lancet Neurol. 2006 Dec 1;5(12):1055–63.

11. Engel J. J. Mesial temporal lobe epilepsy: what have we learned? Neuroscientist. 2001;7(4):340–52.
12. Engel J. Mesial Temporal Lobe Epilepsy : What Have We Learned ? 2001;7(4):340–52.
13. Shinnar S. Febrile Seizures and Mesial Temporal Sclerosis. Epilepsy Curr.;3(4):115–8.
14. Van Hoesen GW. Anatomy of the medial temporal lobe. Magn Reson Imaging. 1995 ;13(8):1047–55.
15. Tarkka R, Pääkkö E, Pyhtinen J, Uhari M, Rantala H. Febrile seizures and mesial temporal sclerosis. Neurology. 2003;60(2):215–8.
16. DeFelipe J, Fernández-Gil MÁ, Kastanauskaite A, Palacios Bote R, Gañán Presmanes Y, Ruiz MT. Macroanatomy and Microanatomy of the Temporal Lobe. Semin Ultrasound, CT MRI. 2007 ;28(6):404–15.
17. Labate A, Ventura P, Gambardella A, Le Piane E, Colosimo E, Leggio U, et al. MRI evidence of mesial temporal sclerosis in sporadic "benign" temporal lobe epilepsy. Neurology. 2006; 66(4):562–5. 2
18. Brodtkorb E, Samsonsen C, Sund JK, Bråthen G, Helde G, Reimers A. Treatment non-adherence in pseudo-refractory epilepsy. Epilepsy Res. 2016 1;122:1–6.
19. Chan S, Erickson JK, Yoon SS. Limbic system abnormalities associated with mesial temporal sclerosis: a model of chronic cerebral changes due to seizures. https://doi.org/101148/radiographics1759308104. 1997;17(5):1095–110.
20. Camacho DLA, Castillo M. MR Imaging of Temporal Lobe Epilepsy. Semin Ultrasound, CT MRI. 2007 ;28(6):424–36.
21. Martinez-Rios C, McAndrews MP, Logan W, Krings T, Lee D, Widjaja E. MRI in the evaluation of localization-related epilepsy. J Magn Reson Imaging. 2016;44(1):12–22.
22. Spencer. The Relative Contributions of MRI, SPECT, and PET Imaging in Epilepsy. Epilepsia. 1994;35:S72–89.
23. Briellmann RS, Berkovic SF, Syngeniotis A, King MA, Jackson GD. Seizure-associated hippocampal volume loss: A longitudinal magnetic resonance study of temporal lobe epilepsy. Ann Neurol. 2002;51(5):641–4.
24. Bernasconi A, Cendes F, Theodore WH, Gill RS, Koepp MJ, Hogan RE, et al. Recommendations for the use of structural magnetic resonance imaging in the care of patients with epilepsy: A consensus report from the International League Against Epilepsy Neuroimaging Task Force. Epilepsia. 2019 Ju;60(6):1054–68.
25. Tsai SY, Sajatovic M, Hsu JL, Chung KH, Chen PH, Huang YJ. Body mass index, residual psychotic symptoms, and inflammation associated with brain volume reduction in older patients with schizophrenia. Int J Geriatr Psychiatry. 2020;35(7):728–36.