

## Asymmetrical Volume Loss in Hippocampal Subfield During the Early Stages of Alzheimer Disease: A Cross Sectional Study

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### Abstract

Hippocampal atrophy is a well-established imaging biomarker of Alzheimer disease (AD). However, hippocampus is a non-homogenous structure with cytoarchitecturally and functionally distinct sub-regions or subfield, with each region performing distinct functions. Certain regions of the subfield have shown selective vulnerability to AD. Here, we are interested in studying the effects of normal aging and mild cognitive impairment on these sub-regional volumes. With a reliable automated segmentation technique, we segmented these subregions of the hippocampus in 101 cognitively normal (CN), 135 early mild cognitive impairment (EMCI), 67 late mild cognitive impairment (LMCI) and 48 AD subjects. Thereby, dividing the hippocampus into hippocampal tail (tail), subiculum (SUB), cornu ammonis 1 (CA1), hippocampal fissure (fissure), presubiculum (PSUB), parasubiculum (ParaSUB), molecular layer (ML), granule cells/molecular layer/dentate gyrus (GCMLDG), cornu ammonis 3(CA3), cornu ammonis 4(CA4), fimbria and hippocampal-amygdala transition area (HATA). In this cross sectional study of 351 ADNI subjects, no differences in terms of age, gender, and years of education were observed among the groups. Though, the groups had statistically significant differences ( $p < 0.05$  after the multiple comparison correction) in the Mini-Mental State Examination (MMSE) scores. There was asymmetrical volume loss in the early stages of AD with the left hemisphere showing volume loss in regions that were unaffected in the right hemisphere. Bilateral parasubiculum, right cornu ammonis 1, 3 and 4, right fimbria and right HATA regions did not show any volume loss till the late MCI stages. Our findings suggest that the hippocampal subfield regions are selectively vulnerable to AD and also that these vulnerabilities are asymmetrical especially during the early stages of AD.

**Keywords:** Hippocampal subfield, Alzheimer's disease, Cognitively normal, Early mild cognitive impairment, Late mild cognitive impairment

### 1. Introduction

Accounting of an estimated 60-80% of all cases, Alzheimer's disease (AD) is the most common form of dementia that cannot be prevented, slowed or stopped. Although, with the identification of AD related biological markers or biomarkers in the recent years, the knowledge and comprehension of the disease has moved from symptom based to one based on the brain

changes. These changes in the brain begin to occur very early and prior to any clinical symptoms show up. Hence, early diagnosis of AD based on these biomarkers would have prominent personal and financial benefits on the patient, their family and the society<sup>[1]</sup>. Hippocampal atrophy is one such highly approved biomarker that is listed among the criteria for the diagnosis of AD<sup>[2,3]</sup>. Until recently, due to the lack of reliable and consistent segmentation techniques and the limitations of the structural magnetic resonance imaging (sMRI), the researchers have considered the hippocampus as a single structure<sup>[4,5]</sup>. However, hippocampus is a non-homogenous structure with histologically distinct sub-regions or subfields like subiculum and presubiculum, cornu ammonis (CA1-4), fimbria and dentate gyrus (DG) and others, that are believed to be functionally discrete performing functions related to learning and memory, certain aspects of motor control, regulation of

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emotional behavior and regulation of hypothalamic functions among others<sup>[6]</sup>. With the recent advances in the segmentation techniques – quick, reliable and automated segmentation of the hippocampus into its various subfields is possible<sup>[7]</sup>. It is shown that the process of normal aging and the AD associated aging have varying effects on subfields. Additionally, adverse effects of various neuropsychological disorders are selective on the subfields and not diffuse on the whole hippocampus<sup>[8,9]</sup>. Since asymmetrical cerebral atrophy in AD and hippocampal asymmetry characterized by differences in subfield structure and function has been reported<sup>[10-12]</sup>. Left and right hemispheric volumes were analyzed individually. It is instrumental to understand the hippocampal subfields atrophy – 1) associated with normal aging, 2) associated with AD and 3) associated with various early stages of AD.

In the present study, we investigate the volumetric differences in the hippocampal subfields among the cognitively normal (CN), early mild cognitive impairment (EMCI), late mild cognitive impairment (LMCI) and Alzheimer disease (AD) with the intent to understand the variations in subfield volumes that might shed some light on the pathological changes associated with AD.

## 2. Materials and Methods

### 2.1. Participants

Data used in the preparation of this article were obtained from the Alzheimer's disease Neuroimaging Initiative (ADNI) database ([adni.loni.usc.edu](http://adni.loni.usc.edu)). The ADNI was launched in 2003 by the National Institute on Aging (NIA), the National Institute of Biomedical Imaging and Bioengineering (NIBIB), the Food and Drug Administration (FDA), private pharmaceutical companies and nonprofit organizations, as a \$60 million, 5-year public-private partnership. The primary goal of ADNI has been to test whether serial MRI, positron emission tomography (PET), other biological markers, and clinical and neuropsychological assessment can be combined to measure the progression of MCI and early AD. Determination of sensitive and specific markers of very early AD progression is intended to aid researchers and clinicians to develop new treatments and monitor their effectiveness, as well as lessen

the time and cost of clinical trials.

The principal investigator of this initiative is Michael W. Weiner, MD, VA Medical Center and University of California, San Francisco. ADNI is the result of efforts of many coinvestigators from a broad range of academic institutions and private corporations, and subjects have been recruited from over 50 sites across the United States and Canada. The initial goal of ADNI was to recruit 800 subjects, but ADNI has been followed by ADNI-GO and ADNI-2. To date these 3 protocols have recruited over 1500 adults, aged 55-90, to participate in the research, consisting of CN older individuals, people with early or late MCI, and people with early AD. The follow-up duration of each group is specified in the protocols for ADNI-1, ADNI-2, and ADNI-GO. Subjects originally recruited for ADNI-1 and ADNI-GO had the option to be followed in ADNI-2. For up-to-date information, see [www.adni-info.org](http://www.adni-info.org).

For this study, high-resolution T1-MRI was selected from the subset of the ADNI 2 & GO participants. Clinical categorization of study participants into CN, early MCI (EMCI), late MCI (LMCI), and AD were as described in detail at [www.adni-info.org](http://www.adni-info.org) and used standard clinical criteria for MCI and AD<sup>[2,13,14]</sup>. MCI patients were all amnesic, either single domain or multi-domain. Division into early and late MCI groups was based solely on education adjusted scores for the delayed paragraph recall sub-score of the Wechsler Memory Scale-Revised Logical Memory II. Thus, EMCI patients straddled the boundary between normal memory and LMCI. Demographic and clinical characteristics of the subjects are described in Table 1.

### 2.2. MRI Image Acquisition

All subjects were scanned with a T1-MRI protocol optimized for best contrast to noise in a feasible acquisition time<sup>[15,16]</sup>. Raw data had an acquisition matrix of  $192 \times 192 \times 166$  and voxel size  $1.25 \times 1.25 \times 1.2 \text{ mm}^3$ . Zero-filled reconstruction (i.e., sinc interpolation) resulted in a  $256 \times 256$  matrix and voxel size of  $0.9375 \times 0.9375 \times 1.2 \text{ mm}^3$ . The MRI sequence parameters were repetition time/echo time 8020/50 ms,  $0.4 \times 0.4 \times 2.0 \text{ mm}^3$  resolution, minimum 24 slices, and acquisition time: 8.1 minutes. Further details on ADNI imaging protocols can be found at <http://adni.loni.usc.edu/methods/documents/mriprotocols/>.

**Table 1.** Demographic features

	CN	EMCI	LMCI	AD
Number of subjects (n)	101	135	67	48
Age <sup>a,b</sup>	70.10 ± 2.76	70.10 ± 2.77	70.88 ± 2.70	71.13 ± 2.54
Male gender (in percentage) <sup>c</sup>	44.55	60.74	53.73	47.91
Education level (years) <sup>a,d</sup>	16.41 ± 2.62	16.11 ± 2.55	16.62 ± 2.71	15.77 ± 2.71
MMSE <sup>a,e</sup>	29.08 ± 1.08	28.36 ± 1.61	27.70 ± 1.87	23.70 ± 2.13

Values are expressed as mean ± standard deviation (SD).

Key: CN, Cognitively normal; EMCI, Early MCI; LMCI, Late MCI; AD, Alzheimer's disease; ANOVA, analysis of variance; MMSE, Mini Mental State Examination

<sup>a</sup>The *p*-values were calculated using general linear model; Bonferroni post hoc test was also performed when F-test was significant.

<sup>b</sup>Main interaction among groups:  $F_{3,351} = 2.66$ ,  $p = 0.04$ ; post hoc: no pairwise significance against groups (Age)

<sup>c</sup>The *p*-value were calculated using the  $\chi^2$  test:  $\chi^2 = 6.65$ ,  $p = 0.08$ .

<sup>d</sup>Main interaction among groups:  $F_{3,351} = 1.74$ ,  $p = 0.15$ .

<sup>e</sup>Main interaction among groups:  $F_{3,351} = 122.69$ ,  $p = 5.34E-54$ ; post hoc: CN versus EMCI, 0.01; CN versus LMCI, 2.00E-6; CN versus AD, 2.45E-52; EMCI versus LMCI, 0.03; EMCI versus AD, 5.57E-46; LMCI versus AD, 2.44E-30.

### 2.3. MRI Processing

All the images were processed using the Freesurfer software package (Athinoula A. Martinos Center for Biomedical Imaging, Harvard University, Cambridge, MA, USA) (v5.3.0). The process of cortical and subcortical reconstruction and segmentation were performed which involved procedures including motion correction, non-uniform intensity normalization, image registration using affine transformation, skull-stripping and removal of non-brain tissues, topology correction and labeling of subcortical structures, brain stem, cerebellum, and cerebral cortex. An exclusive documentation of the pipeline and methodologies can be found elsewhere<sup>[17,18]</sup>. We used a novel fully automated technique that was incorporated with the Freesurfer (v6.0.0) processing stream and facilitates the reliably reproducible segmentation of the hippocampal formation at the subfield levels in the ultra-high resolution MRI data<sup>[7]</sup>. The segmentation process was able to reliably identify and divide the hippocampal formation into: Hippocampal tail (tail), Subiculum (SUB), Cornu ammonis 1 (CA1), Hippocampal fissure (fissure), Presubiculum (PSUB), Parasubiculum (ParaSUB), Molecular layer HP (ML), Granule cells in the molecular layer of the Dentate gyrus (GC-ML-DG), Cornu ammonis 3 (CA3), Cornu ammonis 4 (CA4), Fimbria, Hippocampal-Amygdala-Transition-Area (HATA) and Whole hippocampus.

### 2.4. Statistical Analysis

All statistical analysis was performed using IBM SPSS Statistics (Version 23.0. Armonk, NY: IBM Corp.) All the analysis was two-tailed and controlled for covariates age, sex, years of education and estimated total intracranial volume. One-way Analysis Of Variance (ANOVA) and Bonferroni post hoc correction for multiple comparisons was used for continuous demographic variables and Chi-squared test was performed on categorical demographic variables. Differences among the diagnostic groups were tested using Analysis of covariance (ANCOVA) and when the ANCOVA was significant (greater than the value of adjusted multiple comparison, Table 2&3), pairwise Bonferroni post hoc was applied to check the between groups differences. We considered *p*-values <0.05 as significant. All analyses were separately performed for the left and right hemispheres.

## 3. Results and Discussion

### 3.1. Demographics

The complete demographics and the statistical analysis results of the respective features were summarized in Table 1. A total of 351 subjects were recruited, 101 CN, 135 EMCI, 67 LMCI and 48 AD subjects. As expected, the MMSE scores of AD groups were significantly lower than LMCI, EMCI and CN groups

( $p < 0.0001$ ). No differences in terms of age, gender, and level of education were observed among the groups.

*Subfield volumetry of left hemisphere – group comparisons*

All group comparisons of the left hemisphere subfields except fissure showed statistically significant differences between groups after adjusting for Bonferroni multiple comparisons ( $p = 9.61E-4$ ) (as shown in table 2). The analysis was adjusted for covariates age, gender, education level and total intracranial volume. The com-

parison of CN and EMCI groups revealed subfield volume loss in the SUB, CA1, ML, GC-ML-DG, CA3, CA4 and HATA ( $p < 0.05$ ) whilst comparison of EMCI and LMCI groups revealed subfield volume loss in SUB, CA1, ML, GC-ML-DG, CA4 similar to CN and EMCI groups. Contrary to CN and EMCI groups, volume loss in CA3 and HATA were not significant between EMCI and LMCI groups. Additionally, volume loss in tail and fimbria were observed during comparison of the EMCI and LMCI groups. All subfields

**Table 2.** Volume ( $\text{mm}^3$ ) and statistical analysis for left hippocampal subfields

Regions	CN	EMCI	LMCI	AD	ANCOVA <sup>a</sup>	Bonferroni pairwise post hoc			
						CN versus EMCI	EMCI versus LMCI	LMCI versus AD	CN versus LMCI
Tail	477.86 ± 67.42	463.26 ± 72.13	414.59 ± 69.40	370.52 ± 68.97	<b>F = 33.30,</b> <b>p = 9.61E-4</b>	0.20	<b>2.20E-5</b>	<b>0.01</b>	<b>8.34E-9</b>
Sub	402.18 ± 47.76	384.68 ± 51.49	346.07 ± 69.03	289.51 ± 54.12	<b>F = 55.20,</b> <b>p = 9.61E-4</b>	<b>0.01</b>	<b>1.90E-5</b>	<b>1.00E-6</b>	<b>7.35E-11</b>
CA1	587.07 ± 77.59	569.20 ± 73.09	537.64 ± 97.54	467.94 ± 74.87	<b>F = 31.95,</b> <b>p = 9.61E-4</b>	<b>0.01</b>	<b>0.04</b>	<b>3.50E-5</b>	<b>5.00E-6</b>
Fissure	160.47 ± 26.23	162.88 ± 30.23	159.52 ± 26.72	153.22 ± 30.83	F = 1.73, p = 0.16	NA	NA	NA	NA
PSUB	289.39 ± 37.69	279.69 ± 45.34	247.48 ± 55.61	204.63 ± 41.88	<b>F = 46.01,</b> <b>p = 9.61E-4</b>	0.23	<b>2.50E-5</b>	<b>8.00E-6</b>	<b>1.24E-8</b>
Para SUB	62.04 ± 12.41	61.37 ± 12.49	56.28 ± 15.87	47.77 ± 13.98	<b>F = 15.21,</b> <b>p = 9.61E-4</b>	1.00	0.06	<b>0.01</b>	<b>0.01</b>
ML	526.20 ± 61.66	503.35 ± 64.65	464.03 ± 82.55	393.66 ± 64.21	<b>F = 51.58,</b> <b>p = 9.61E-4</b>	<b>2.72E-3</b>	<b>5.68E-4</b>	<b>5.82E-7</b>	<b>5.25E-10</b>
GC-ML-DG	282.65 ± 36.21	268.32 ± 36.08	252.70 ± 39.77	218.90 ± 36.32	<b>F = 40.01,</b> <b>p = 9.61E-4</b>	<b>4.08E-4</b>	<b>0.02</b>	<b>1.60E-5</b>	<b>1.73E-8</b>
CA3	204.69 ± 31.62	196.26 ± 32.27	188.69 ± 30.28	166.40 ± 32.56	<b>F = 19.54,</b> <b>p = 9.61E-4</b>	<b>0.01</b>	0.49	<b>3.09E-3</b>	<b>2.71E-4</b>
CA4	243.87 ± 30.48	233.99 ± 30.87	220.15 ± 33.04	192.90 ± 31.54	<b>F = 37.23,</b> <b>p = 9.61E-4</b>	<b>2.97E-3</b>	<b>0.01</b>	<b>4.90E-5</b>	<b>5.76E-8</b>
Fimbria	84.22 ± 20.39	78.28 ± 21.72	68.44 ± 26.24	53.13 ± 18.45	<b>F = 22.88,</b> <b>p = 9.61E-4</b>	0.15	<b>0.02</b>	<b>3.67E-3</b>	<b>4.00E-5</b>
HATA	62.72 ± 10.38	58.57 ± 10.46	54.47 ± 12.37	45.26 ± 9.18	<b>F = 30.13,</b> <b>p = 9.61E-4</b>	<b>4.00E-3</b>	0.09	<b>1.40E-4</b>	<b>3.00E-6</b>

Values are expressed as mean ± standard deviation (SD).

Key: CN, Cognitively normal; EMCI, Early MCI; LMCI, Late MCI; AD, Alzheimer's disease; ANCOVA, analysis of covariance.

<sup>a</sup>ANCOVA followed by Bonferroni correction was carried out to test the differences among groups (adjusted significance threshold:  $p = 0.05/13$  structures/4 groups =  $9.61E-4$ ). When the ANCOVA was significant, pairwise Bonferroni post hoc was applied. Bold characters indicate significant results.

Bonferroni pairwise post hoc  $p$ -values for all subfields (except hippocampal fissure) were significant ( $p < 0.001$ ) in the pairwise comparison between CN versus AD and EMCI versus AD suggesting no new findings. Hence, is not shown in the table.

showed significant volume loss in pairwise comparisons of LMCI-AD, CN-LMCI, EMCI-AD and CN-AD (as shown in table 2).

*Subfield volumetry of right hemisphere – group comparisons*

Similar, to the left hemisphere, the group comparisons of all subfields except fissure showed statistically significant differences between groups after adjusting for Bonferroni multiple comparisons ( $p=9.61E-4$ ) (as shown in table 3). The analysis was adjusted for covari-

ates age, gender, education level and total intracranial volume. Although, the pairwise comparison revealed that the volume loss in many subfields in the right hemisphere were preserved. The comparison of CN and EMCI groups showed volume loss in SUB, ML and GC-ML-DG ( $p<0.05$ ) whilst comparison of EMCI and LMCI groups showed volume loss in tail, SUB, PSUB and ML. Similar to the left hemisphere, all subfields showed significant volume loss in pairwise comparisons of LMCI-AD, CN-LMCI, EMCI-AD and CN-AD (as

**Table 3.** Volume (mm<sup>3</sup>) and statistical analysis for right hippocampal subfields

Regions	CN	EMCI	LMCI	AD	ANCOVA <sup>a</sup>	Bonferroni pairwise post hoc			
						CN versus EMCI	EMCI versus LMCI	LMCI versus AD	CN versus LMCI
Tail	494.82 ± 69.74	483.05 ± 82.37	432.64 ± 85.70	383.11 ± 69.00	<b>F = 30.90,</b> <b>p = 9.61E-4</b>	0.29	<b>7.00E-5</b>	<b>0.01</b>	<b>6.65E-8</b>
Sub	403.07 ± 50.14	386.10 ± 58.92	350.27 ± 66.92	294.67 ± 51.61	<b>F = 45.84,</b> <b>p = 9.61E-4</b>	<b>0.02</b>	<b>3.39E-4</b>	<b>5.00E-6</b>	<b>4.74E-9</b>
CA1	622.11 ± 81.62	604.92 ± 84.74	575.08 ± 104.95	491.07 ± 75.37	<b>F = 29.65,</b> <b>p = 9.61E-4</b>	0.06	0.17	<b>5.00E-6</b>	<b>1.86E-4</b>
Fissure	166.76 ± 25.82	166.67 ± 32.15	171.59 ± 28.23	165.74 ± 33.13	<i>F = 0.60,</i> <i>p = 0.60</i>	NA	NA	NA	NA
PSUB	274.23 ± 41.85	265.66 ± 42.21	239.90 ± 45.78	201.70 ± 31.84	<b>F = 40.95,</b> <b>p = 9.61E-4</b>	0.15	<b>2.53E-4</b>	<b>1.70E-5</b>	<b>9.64E-8</b>
Para SUB	60.83 ± 11.57	59.24 ± 12.46	55.73 ± 15.64	47.28 ± 10.24	<b>F = 15.66,</b> <b>p = 9.61E-4</b>	0.67	0.30	<b>3.56E-3</b>	<b>9.65E-3</b>
ML	544.87 ± 64.42	523.92 ± 70.35	487.85 ± 87.47	413.72 ± 63.02	<b>F = 43.11,</b> <b>p = 9.61E-4</b>	<b>0.01</b>	<b>5.96E-3</b>	<b>8.75E-7</b>	<b>1.18E-7</b>
GC-ML-DG	293.62 ± 36.60	282.97 ± 38.47	269.42 ± 47.95	236.18 ± 40.91	<b>F = 25.89,</b> <b>p = 9.61E-4</b>	<b>0.02</b>	0.17	<b>2.00E-4</b>	<b>4.90E-5</b>
CA3	221.88 ± 34.22	216.16 ± 35.26	209.35 ± 38.21	186.96 ± 36.28	<b>F = 12.54,</b> <b>p = 9.61E-4</b>	0.23	1.00	<b>0.01</b>	<b>0.01</b>
CA4	253.81 ± 30.52	246.46 ± 32.75	234.49 ± 39.78	208.03 ± 34.96	<b>F = 24.49,</b> <b>p = 9.61E-4</b>	0.051	0.10	<b>5.23E-4</b>	<b>6.40E-5</b>
Fimbria	82.44 ± 20.89	75.69 ± 23.33	70.24 ± 25.06	53.51 ± 19.36	<b>F = 16.89,</b> <b>p = 9.61E-4</b>	0.058	1.00	<b>1.30E-3</b>	<b>5.04E-3</b>
HATA	61.98 ± 10.33	59.79 ± 9.90	56.61 ± 12.90	47.12 ± 8.58	<b>F = 23.70,</b> <b>p = 9.61E-4</b>	0.11	0.46	<b>2.90E-5</b>	<b>1.83E-3</b>

Values are expressed as mean ± standard deviation (SD).

Key: CN, Cognitively normal; EMCI, Early MCI; LMCI, Late MCI; AD, Alzheimer's disease; ANCOVA, analysis of covariance.

<sup>a</sup>ANCOVA followed by Bonferroni correction was carried out to test the differences among groups (adjusted significance threshold:  $p = 0.05/13$  structures/4 groups =  $9.61E-4$ ). When the ANCOVA was significant, pairwise Bonferroni post hoc was applied. Bold characters indicate significant results.

Bonferroni pairwise post hoc *p-values* for all subfields (except hippocampal fissure) were significant ( $p<0.001$ ) in the pairwise comparison between CN versus AD and EMCI versus AD suggesting no new findings. Hence, is not shown in the table.

shown in table 3).

#### 4. Discussion

In the current study, using a contemporary reliable method, we investigated the differences in the hippocampal subfields volume and how they vary between the two hemispheric volumes in control and various risk stages of AD. We observed an asymmetrical pattern of atrophy in the subfields starting from the very early stages of AD. The results also suggested the predominance of left first atrophy pattern in the early stages of the AD which may be the result of pathological mechanism associated with AD. There were two primary findings from the present study. First, the atrophy of the hippocampus was not whole but diffuse. That is, there was atrophy in certain subfields but not others. Second, the atrophy of the subfields was not symmetrical between the two hemispheres.

Though, many studies consider the hippocampus as a single unitary entity, researchers have long been performing studies by manually delineating the hippocampus into its respective sub-regions based on the differences in the structure and function to better understand one of the major targets of AD. Since, the process of manual delineation is labor intensive and can only be performed by trained experts. Extensive large scale studies are very limited. Additionally, prior performed studies lack consistency in the methodological procedures, study protocols and parameters resulting in differences in the findings<sup>[19-21]</sup>. With the advent of the high resolution MRI and automated segmentation procedures, it is now possible to form a consensus in the procedures, protocols and parameters to be used. Despite the variations in the earlier studies, volume loss of the CA1 and the subiculum regions<sup>[22,23]</sup> has been consistently reported in studies including those performed on autopsied brain samples<sup>[24,25]</sup>.

In line with the prior studies, in the current study, we observed volume loss in bilateral subiculum region in the EMCI and LMCI stages, which are considered to be early stages in the progression of AD. Significant volume loss along the CA1 region was observed only in the left hemisphere. Here, we observed volume loss in bilateral subiculum but not along the bilateral CA1 region. Nonetheless, similar discrepant finding has been reported earlier<sup>[26]</sup>. It has been reported that the volume

loss begins anteriorly around the CA1 and the subiculum regions progressively affecting the other CA regions<sup>[27,28]</sup>. The possible reason for the discrepancy of unilateral atrophy of CA1 region in the current study may be due to the atrophy initiating site around the subiculum. The atrophy may begin around the subiculum and then sequentially affecting the CA regions. The atrophy may affect the left hemisphere severely prior to the right hemisphere. In line with previous structural imaging studies<sup>[29,30]</sup>, in the present study, we observed bilateral atrophy in all subfields between the CN and AD. However, the atrophy in these subfields was not found bilaterally in the early stages of AD. Additionally, the atrophy in CA regions were severe than the subiculum regions. These findings have not been reported earlier or have been over looked in the prior studies. Animal studies have discussed the functions of the CA regions<sup>[31,32]</sup>, suggesting functions relating to spatial and contextual memory. Similarly, attempts to explain the functions of subiculum have also been made earlier<sup>[33]</sup> revealing functions related to processing of information about space, movement and memory. It is a well-established factor that the memory is affected in the AD. Though, asymmetrical volume loss as observed here brings forward a question if the subfields of the two hemispheres have different functions. Studies to further substantiate the results obtained here and to understand the singular functions of each subfield might help comprehend the pathological mechanism of AD.

The vital questions beyond the reach of the present study are: what are the reasons for the severe atrophy of the CA regions than the subicular complex and severe atrophy of the left hemisphere than the right hemisphere. Brain asymmetries in human are observed in the structure, function and behavior influencing hereditary, developmental, and pathological factors among others. Notable reports on some neurodegenerative diseases progressing asymmetrically are also available<sup>[34]</sup>. The asymmetrical volume loss in AD are an area that has received limited focus except certain studies demonstrating an abnormal increase in functional asymmetry in AD<sup>[35]</sup>. Though, asymmetries in other regions of the brain are report earlier. Reports on hippocampal asymmetry are scarce. Here, we have observed a clear asymmetry in the hippocampal subfield volume loss. Multiple neuroimaging and longitudinal studies have reported presence of significant damage to

the hippocampal region years before the diagnosis of dementia. Certain studies reported damage to the region even before the stages of MCI or ten years before the dementia diagnosis<sup>[36-38]</sup>. Thus, hippocampal atrophy especially atrophy in certain subfields if detected very early may aid in the identification or investigation of the crisis. We have used a state-of-the-art automated segmentation technique that uses reliable atlas for the analysis. This has revealed volume loss in multiple subfields and not the whole hippocampus. Volume loss in certain subfield in one hemisphere and not in the other indicates that the pathology associated with AD affects the subfields of the two hemispheres in different asymmetrical pattern.

Imaging studies using conventional nuclear medicine imaging techniques have reported metabolic brain asymmetry inclined to the impairment of the left hemisphere<sup>[39,40]</sup>. Similarly, in the present study, there was predominance in the atrophy of subfields in the left hemisphere than the right. Left hippocampal subfield atrophy based on the volumetric analysis of the rather non-invasive sMRI technique if observed in the early years might constructively act as a beckon for the prospective danger of AD.

The present study has some limitations: Lack of analysis on the neuropsychological data to understand the correlation with the volume, cross sectional data and unforeseen selection bias that may have been caused as a result of random selection. Finally, the study could not help in bringing about a conclusion on the dynamics of the asymmetry only with cross sectional study design.

In conclusion, the current study might kick start the dormant area of study of asymmetry in AD for early diagnosis and further very large scale longitudinal studies involving multi-faceted data may support the present notions and bring relevance to the study.

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