Clinical and Biochemical Predictors of Hippocampal Atrophy in Alzheimer’s Disease

Vikas Dhikav
fellowship cognitive neurology, fellowship neuropsychopharmacology, phd (neurology) director

*Corresponding Author: Vikas Dhika mbbs, md (3-years, aiims, delhi) fellowship cognitive neurology, fellowship neuropsychopharmacology, phd (neurology) director, memory clinic delhi, rohini, secytor-18, new delhi, india, 110089
email:vikasdhikav@hotmail.com

Citation: Vikas Dhika et.al (2017) .Clinical And Biochemical Predictors Of Hippocampal Atrophy In Alzheimer’s Disease Int J Neu & Beh 1:1, 34-43

Copyright: © Vikas Dhika. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Received November 16, 2017; Accepted November 17, 2017; Published November 25, 2017.

Abstract

Aim: Hippocampus is an important brain structure responsible for learning and episodic memory. It is also a plastic and vulnerable structure damaged by a variety of factors. Incidentally, it is among the first and the most severely affected structure that undergoes atrophy in Alzheimer’s disease (AD), the leading cause of dementia worldwide. Though considered neurodegenerative; the role of vascular and other associated factors causing hippocampal atrophy in AD is under investigation. In the present study; an attempt was made to explore the effect of presence of various clinical and biochemical predictors on the hippocampal volumes in patients with AD.

Methods: We screened 280 subjects coming to the Department of Neurology of a Tertiary Care Hospital with memory/and or cognitive complaints starting from July 2012 to December 2015 and enrolled 144 subjects. Those who met the diagnostic Criteria for the diagnosis of probable AD (National Institute of Neurological and Communicative Disorders and Stroke, Alzheimer’s disease related Disease Association criteria (NINCDS-ARDA), were recruited for the present study. Selected patients were segregated into AD-associated comorbidities or biochemical alterations in AD e.g. AD-diabetes, AD-hypertension, AD-depression, AD-seizures categories or AD-hyperhomocysteinemia, AD-hypercortisolemia, AD-vitamin D3 and AD-vitamin B12 deficient patients groups. Selected patients underwent Magnetic Resonance Imaging of the Brain (MRI Brain) and T1 weighted images in a plane perpendicular to long axis of hippocampus were obtained. Hippocampal volumes were measured manually using a standard protocol. The comparison of hippocampal volumes was done among the groups e.g. with the cognitively normal individuals and those patients of AD with or without vascular and non vascular risk factors.

Results: Out of 280 patients screened, 144 met the diagnostic criteria for AD. A total of 8 patients (n=64) per predictor group were selected in the predictor-stratified group. There was a statistically significant difference between the hippocampal volumes of the cognitively normal elderly (n=30), those patients with AD without vascular or other risk factors (n=20), and patients with cumulative risk factors (n=30) [p-value<0.05]. Hippocampal volumes of those with atleast one of the biochemical/vascular or non vascular risk factor for hippocampal atrophy was compared with the patients without risk factors and there was a statistically significant difference between these groups (p-value<0.05). There was a moderately strong correlation between hippocampal volume and MMSE (Pearson Correlation Coefficient=0.56).

Conclusions: Those with the diagnosis of AD and associated seizures, depression and patients of AD with diabetes and hypertension combined together had statistically significant volume reduction of hippocampus compared to AD patients without risk factors for hippocampal atrophy.

Key Words: Alzheimer’s Disease, Predictors, Hippocampal Atrophy, Vascular And Non Vascular Risk Factors, Hippocampal Volumes, Magnetic Resonance Imaging

Introduction

Hippocampus is a plastic and vulnerable structure that plays an important role in episodic memory1-3. It undergoes atrophy in the Alzheimer’s disease (AD), most common cause of dementia in the world1. Hence, the measurements of hippocampal volumes have been used as surrogate markers of cognitive decline in AD clinically, and to track disease progression and monitor potential drug therapies in clinical trials4 and to radiologically differentiate various types of dementias clinically. This has also been used to
predict conversion of Mild Cognitive Impairment (MCI) to dementia in research settings.5 The hippocampus gets damaged and undergoes atrophy in a variety of conditions like MCI, epilepsy, depression, posttraumatic stress disorders, Cushing’s disease and hypertension etc; however, the main condition where it is atrophied is AD3-5, where its role is steadily growing.4-5 Several factors like depression5-6, diabetes7 hypertension8, and seizures9 have been known to be counterproductive to hippocampal volume in general. Also, their role in patients with dementia has been under investigation.10 Similarly, biochemical alterations like cortisol elevation, low vitamin B12 and vitamin D3 and high serum homocysteine have been linked with hippocampal atrophy. The effect of co-morbidities in the pathophysiology of AD has been a matter of investigation for last several years. The combined evidence that the presence of these biochemical alterations or of clinical predictors in patients with AD has any influence on hippocampal volumes in AD is however lacking.10 Despite the high frequency of these comorbidities and/or biochemical alterations in patients with AD; the effect of these factors have not been evaluated on hippocampal volume; which is a surrogate biomarker for AD.

As per the sparsely available literature; there are several predictors of hippocampal atrophy in patients with AD. An attempt was done in the current study to investigate the effect of presence of various clinical and biochemical predictors on hippocampal volume by manually measuring the same and compare the same with those patients of AD who did not have these risk factors.

**Material & Methods**

**Screening**

We screened 280 subjects coming to the Department of Neurology of a Tertiary Care Hospital in starting from July 2012 to December 2015 and enrolled 144 subjects. Those who met the diagnostic criteria for diagnosis of probable AD (National Institute of Neurological and Communicative Disorders and Stroke, Alzheimer's disease related Disease Association criteria (NINCDS-ARDA)) were recruited for the present study. Those who did not meet the diagnostic criteria were excluded from the present study. These included the following patients: Vascular dementias (n=37), Dementia with Lewy body (n=15), Fronto temporal dementias (n=14), Depressive pseudodementias (n=20), Mixed dementias (n=29), Hypothyroid dementias (n=5), B12 deficiency dementias (n=8), Alcohol dementias (n=4), Creutzfeldt Jacobs disease (n=2), HIV dementias (n=2).

**Patient selection and diagnostic evaluation**

Patients who reported to the Department of Neurology of a Tertiary Care Hospital were selected randomly and asked to attend a specialized memory clinic for detailed neuropsychological, radiological and neurological examination for diagnostic evaluation of AD. Routine Laboratory Examinations and work up for exclusion of other dementias was done in all cases. The diagnosis of seizures in AD was done using Alzheimer’s Association Criteria. The selected patients with AD were stratified depending upon the clinical predictors of hippocampal atrophy such as AD-diabetes, AD-hypertension, AD-depression, AD-seizures categories. The patients were then subjected to biochemical analysis (detailed below). Those patients who had alteration in biochemical parameters were segregated into AD-hyperhomocysteinemia, AD-hypercortisolemia, AD-vitamin D3 deficient and AD-vitamin B12 deficient groups. A total of 8 patients per predictors (e.g. AD-diabetes, AD-hypertension, AD-seizures, AD-depression, AD-B12 deficiency, AD-Vitamin D3 deficient, AD-hypercortisolemia, AD-hyperhomocysteinemia) per group out of eight predictors described above (n=64) were selected for comparison of hippocampal volumes.

**Biochemical analysis**

A total of 5-ml blood sample was collected from the antecubital vein of the selected study individuals after an overnight fasting using vacutainer with clot activator gel (BD Biosciences). Samples were immediately kept on ice and serum was separated within half an hour of blood collection. The serum was aliquotted and stored at -80°C till further analysis. Serum homocysteine levels were measured using enzymatic cycling method11 (DiaLab) on fully automated analyzer (Olympus AU-400). The homocysteine assay has analytical sensitivity of 0.4 µmol/L, analytical range 3-50 µmol/L with intra and inter assay coefficient of variation (CV) 4.61% and 5.98% respectively. Reference range for serum homocysteine was taken to be 5-15 µmol/L. Hyperhomocysteinemia was defined as homocysteine value >15 µmol/L. Estimation of serum cortisol12, Vit-B12, 25-OH vitamin-D was done using Enhanced chemiluminescent microparticle immunoassay (VITROS® ECIQ Immunodiagnostic System, Johnson & Johnson, USA). The cortisol assay has analytical range of 4.39-1700 nmol/L with intra-assay and inter-assay variation of 1.7% and 4.5% respectively. The reference range for morning serum cortisol was taken to be 123-626 nmol/L. The 25-OH vitamin-D assay13 has analytical range of 8-150 ng/ml with intra-assay and inter-assay variation of 3.4% and 5.5% respectively. The reference range for 25-OH vitamin-D was taken 30-56 ng/ml. The serum Vit-B12 assay has analytical range of 0-1000pg/ml with intra-assay and inter-assay variation of 1.5% and 5.5% respectively. The reference range for Vit-B12 was taken 231-931pg/ml.

**Healthy controls**

Healthy older adults (M:F=22:8; mean age=62±1 years) were recruited from the staff worker strength of a Tertiary Care Institute. A total of 30 cognitively normal individuals were taken to calculate the reference normative data in this age group. This data was compared with elderly with a diagnosis of AD without the risk factors mentioned. This was then compared with the patients of AD with atleast one risk factor. All the cognitively normal subjects were free from the clinical risk factors or biochemical alterations. Cognitively normal individuals (Healthy controls) were subjected to Magnetic Resonance Imaging of the Brain using the protocol detailed below. A written and informed consent was taken from all study participants. The study was approved by Institutional Ethics Committee.

**Study design**

This is a case control study in which those with the outcome of interest (AD patients with risk factors), having a particular hippocampal volume has been compared with those who do not have the risk factors (AD patients without risk factors). As a reference,
cognitively normal individuals without putative risk factors have been subjected to MRI brain and their hippocampal volumes have been calculated for comparison.

**Scales**

1. Mini Mental Status Examination (MMSE) was used to divide patients into mild, moderate, and severe category.
2. Depression was evaluated using Cornell Scale for Depression in Dementia (CSDD), which is considered to be the gold standard tool to measure depression in dementia. A score >10 was used as depression cut-off.
3. Written records were examined for the diagnosis of Diabetes, Hypertension, and Seizures in patients with AD.

**Radiology protocol**

All subgroups of patients underwent MRI Brain and T1 weighted images in a plane perpendicular to long axis of hippocampus were obtained. Hippocampal volumes were estimated manually using a standard protocol. Volumes have been calculated using region of interest approach (ROI), using manual segmentation magnetization prepared rapid gradient echo sequence (MPRAGE) sequences, coronal oblique, perpendicular to long axis of hippocampus.

In the current study, hippocampal volume has been measured using 1.5 Tesla Magnetic Resonance (Magnatom Symphony 1.5T scanner). Images were acquired in T1, T2, FLAIR sequences in axial, coronal and sagittal plane. A T2 sagittal plane section was used to plan the MPRAGE sequences for estimation of hippocampal volumes.

T1 weighted coronal images were used in all slides wherever hippocampus was visible. Image parameters were as follows: a 3-D image reconstruction was done, using Fast Low Angle Shoot (FLASH). Slice thickness was 1 mm with a repetition time of 14 seconds (Total Scan time=5.22 minutes). Hippocampus was defined as, Coruna Ammonis, dentate gyrus, and subiculum. The posterior boundary of the hippocampus was determined by the oblique coronal anatomic section in which the crura of the fornices were identified in full profile. Intracranial volume was determined by tracing the margins of the inner table of the skull on contiguous images from the sagittal spin echo T1-weighted sequence. Figure-1 and 2 shows manual delineation approach and outlined pseudoimages.

**Statistical analysis**

The latest version of Statistical Package for Social Sciences (SPSS®-SPSS Inc., Chicago, IL) was used for data analysis. Normality of data was checked using Q-Q plot. Correlation and Regression were performed. Analysis of variance (ANOVA) with post hoc analysis was used to analyze the differences between the three groups. Differences between left and right hippocampal volumes were compared using paired t-test. A p-value of <0.05 was considered to be significant. Linear regression was used to know the association between hippocampal volumes, age and MMSE and logistic regression was used for correlation of education and hippocampal volumes.

The right and left hippocampal volumes in each subject were summed up. This summed hippocampal volume was divided by total intracranial volume (i.e., normalized) to control for inter-subject variation in head size.

**Results**

Out of 280 patients screened, 144 met the diagnostic criteria for AD. The demographic information is given in table-1.
Table-1: Demographic and clinical data in the present study

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Mean age (Mean±SD years)</th>
<th>Duration of illness (Mean±SD years)</th>
<th>MMSE</th>
</tr>
</thead>
<tbody>
<tr>
<td>AD without risk factors (n=20)</td>
<td>70±4.1</td>
<td>3.1±0.8</td>
<td>18.1±2.5</td>
</tr>
<tr>
<td>AD with risk factors (n=30)</td>
<td>72±8.1</td>
<td>2.8±1.2</td>
<td>16.1±1.5</td>
</tr>
<tr>
<td>Cognitively normal individuals (n=30)</td>
<td>66.93±3.1</td>
<td>NA</td>
<td>28.1±1.1</td>
</tr>
</tbody>
</table>

The stratification of AD patients into various clinical and biochemical predictors was done. A total of 8 per predictor group were selected and their hippocampal volumes were calculated (Table-2, 3 & 4). There was a statistically significant difference between the hippocampal volumes of the cognitively normal individuals (n=30), and those patients with AD. Also the predictor stratification of AD patients in those with risk factors and without risk factors was done and their hippocampal volumes were estimated. Those patients with AD who did not have risk factors (n=20), and also patients who had cumulative risk factors (n=30) and those with atleast one of the biochemical/vascular or non vascular risk factors (n=8) had a statistical significant difference between the groups (p-value<0.05). Correlation between the age and hippocampal volumes (figure-3) in the patients without risk factors was modest (Pearson’s Correlation Coefficient=-0.29). There was a good correlation between hippocampal volumes and MMSE. Linear regression between MMSE and hippocampal volumes in the patients with AD without risk factors showed deviation from the horizontal as significant (Coefficient of determination; r²=0.22) with a p-value<0.05 (Figure-4).

Figure-1: The manual delineation of hippocampal volumes on both right and left side. In the figure-A, 78Y male has mild dementia, while in figure B (80M), moderate and figure C (75F) had severe dementia.

Figure-2: Three Dimensional (3-D) outlines of three hippocampal images (Pseudoimages generated by image-J, National Institute of Health, USA, downloaded free by http://imagej.nih.gov/ij. Figures-A, B & C have been drawn from present patients to show comparison of normal hippocampus (Figure-A) in healthy individuals with those having early AD and severe AD (Figure C).
A statistical comparison of subgroups of AD cases with seizures (n=8) was done with the patients who did not have risk factors (n=20; p-value<0.05). Similarly, AD patients with comorbid depression (n=8) also had a statistically significant difference in their hippocampal volumes compared to those patients without risk factors (p-value<0.05). The same finding was present in group with diabetes and hypertension. The groups with other predictors did not have a statistically significant difference compared to reference group (p-value>0.05). Normalization of hippocampal volumes with regard to the head size variation was done using the coefficient of determination (r²) to see as to what extent did the variation in head size accounted for the hippocampal size. The calculated r² value was 0.04 indicating that only 4% of the values varied as per head size.

Table-2: Summary characteristics of hippocampal volumes (cm³) in the present study

<table>
<thead>
<tr>
<th>Cognitively normal individuals (n=30)</th>
<th>AD with risk factors (n=30)</th>
<th>AD without risk factors (n-20)</th>
<th>p-value between groups (ANOVA)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Right</td>
<td>Left</td>
<td>Right</td>
<td>Left</td>
</tr>
<tr>
<td>2.73±0.53</td>
<td>2.77±0.6</td>
<td>1.64±0.55</td>
<td>1.59±0.5</td>
</tr>
</tbody>
</table>

Table-3: Hippocampal volumes (cm³) of the AD patients with clinical predictors group

<table>
<thead>
<tr>
<th>Depression</th>
<th>Seizures</th>
<th>Hypertension</th>
<th>Diabetes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Left</td>
<td>Right</td>
<td>Left</td>
<td>Right</td>
</tr>
<tr>
<td>1.2700±0.29</td>
<td>1.1863±0.32</td>
<td>1.2550±0.38</td>
<td>1.3825±0.40</td>
</tr>
<tr>
<td>0.29</td>
<td>0.32</td>
<td>0.38</td>
<td>0.40</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Left</th>
<th>Right</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.76±0.27</td>
<td>1.70±0.025</td>
</tr>
<tr>
<td>0.27</td>
<td>4</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Left</th>
<th>Right</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.62±0.04</td>
<td>1.68±0.04</td>
</tr>
<tr>
<td>4</td>
<td>4</td>
</tr>
</tbody>
</table>

Table-4: Hippocampal volumes (cm³) of the AD with biochemical predictors group

<table>
<thead>
<tr>
<th>Cortisol</th>
<th>Vitamin D3</th>
<th>Serum Homocysteine</th>
<th>Serum B12</th>
</tr>
</thead>
<tbody>
<tr>
<td>Left</td>
<td>Right</td>
<td>Right</td>
<td>Left</td>
</tr>
<tr>
<td>1.59±0.19</td>
<td>1.63±0.22</td>
<td>1.79±0.24</td>
<td>1.62±0.04</td>
</tr>
<tr>
<td>0.2</td>
<td>7</td>
<td>0.24</td>
<td>4</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Left</th>
<th>Right</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.75±0.017</td>
<td>1.68±0.04</td>
</tr>
<tr>
<td>7</td>
<td>4</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Left</th>
<th>Right</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.62±0.04</td>
<td>1.68±0.04</td>
</tr>
<tr>
<td>4</td>
<td>4</td>
</tr>
</tbody>
</table>
**Figure-3:** Correlation between the age and hippocampal volumes in the patients without risk factors (Pearson’s Correlation Coefficient=−0.29).

**Figure-4:** Linear regression between MMSE and hippocampal volume in the patients with AD without risk factors ($r^2=0.22$) showing deviation from the horizontal as significant with a p-value <0.05.
Discussion
Hippocampus is a plastic and vulnerable area of grey matter mass located deep in temporal lobes of the brain. It gets affected by a variety of diseases and AD is the main one. Though atrophy of hippocampus is considered primarily a neurodegenerative disease; the role of vascular and other factors in causing hippocampal atrophy has been growing. The current study reports hippocampal volumes in patients with AD and compares the same with cognitively normal subjects and AD patients with or without risk factors. The patients with AD have been segregated into biochemical and clinical predictors of hippocampal atrophy. The hippocampal volumes of the patients with AD having risk factors have been compared with patients of AD not having risk factors. There are several risk factors that are known to damage hippocampus i.e. stress, depression, seizures, hypertension, depression, diabetes etc. Likewise, evidence is emerging regarding the association of biochemical factors e.g. vitamin D3, serum cortisol, homocysteine with the hippocampal volume loss. Current study has chosen individuals who have normal value of biochemical factors that have been known to cause damage to hippocampus. Likewise, these subjects/patients have also been screened for clinical risk factors potentially or actually damaging hippocampus e.g. diabetes, hypertension, seizures and depression; risk factors that have been putatively to actually shown to cause damage to hippocampus in a variety of studies.
Regarding the biochemical predictors, the evidence is either weak or mixed but still worth investigating considering the association or either or all of them with pathophysiology of AD or physiological ageing in general. For example, the evidence of involvement of serum cortisol with hippocampal atrophy appears to be good; while with the other nutrients like B12, homocysteine and Vitamin D3; the evidence is emerging now. A brief review of the putative risk factors has been given below:

Hypertension and hippocampal atrophy
Hypertension has long been associated with cerebral lesions and has the potential to affect structures vital to learning and memory. Though, the exact mechanism behind these associations is not clear; patho-physiologically, hypertension may lead to vessel wall changes in the brain, leading to hypoperfusion, ischemia and hypoxia. This may cause oxidative stress and consequent neuro-degeneration, which may initiate the pathological process of AD34-36.

Diabetes and hippocampal atrophy
Clinical and epidemiological studies have found that type-2 diabetes, and hyper-insulinaemia, in middle ages increase the risk of developing AD in the elderly. Presence of diabetes accelerates the rate of cognitive decline in AD patients. Neurologically; passive shunting of excess glucose through alternative cellular metabolic pathways induces atherogenic vascular lesions, free radicals, leukoencephalopathy and atrophy of the brain and thus leading to cognitive deficits. Loss of responsiveness to insulin could render neurons more susceptible to neurotoxic insults. As the protective effect of insulin; diminishes apoptosis, neurodegeneration and the resultant cognitive decline occurs38-40.

Seizures and hippocampal atrophy
New onset epileptic seizures occur in AD and are more common than seen in general population. These are more common in patients with severe disease. Seizures may affect the brain adversely. It has been seen that atrophy of temporal lobe (also called as mesial temporal atrophy) precedes cerebral shrinkage in AD. Though exact cause of seizures in AD is not clear but seizure pathophysiology may relate to increased amyloid beta-peptide production, structural alterations in neurons related to cytoskeletal dysfunction, cerebrovascular changes, neurotransmitter dysfunction or combinations thereof.

Depression and hippocampal atrophy
It has been postulated based on animal data that elevated levels of glucocorticoids can damage hippocampus leading to its atrophy. Hippocampus expresses high number of glucocorticoid receptors and is responsive to circulating steroids. Considering the negative relationship between high levels of stress or elevated cortisol levels and hippocampal atrophy in animals; high cortisol levels presumably may adversely affect AD patients as well. Depression has been associated with hippocampal atrophy in young patients with major depressive disorders. Relevance of depression in causing hippocampal atrophy of AD is not known. Based upon available literature; it seems plausible to assume that those with behavioral and psychological dysfunction could have earlier onset of atrophy or have greater degree of hippocampal volume atrophy.

Hippocampal atrophy and cortisol
Studies suggest that disturbances exist in the central regulation of the hypothalamic-pituitary-adrenocortical axis in advanced AD at multiple levels. Elevated glucocorticoid concentrations, decreased hippocampal volume and frontal atrophy with poor cognitive function have been reported in the elderly but not extensively in AD. Plasma cortisol in AD has been recommended as a component of a panel of biochemical markers for AD.

Homocysteine and hippocampal atrophy
The role of homocysteine in brain atrophy is not completely understood. Limited evidence available so far indicates both kinds of outcomes: positive and negative. Elevated total plasma homocysteine levels are associated with cognitive dysfunction, in which changes in the hippocampus plausibly play a pivotal role. Patients with AD may have higher plasma homocysteine levels than controls, but it is uncertain whether higher plasma homocysteine levels are involved in the pathogenesis of the disease. There is a proposal that higher plasma homocysteine levels may be associated with greater degree of atrophy of the hippocampus and cortical regions in elderly at risk of AD.

Hippocampal atrophy and vitamin D
AD patients are at risk of nutritional deficiencies because of physiological and psychological factors. A recent meta-analysis regarding nutritional status in AD has concluded that given the potential role of nutrients in the pathophysiological processes of AD; the utility of nutritional management may currently be underappreciated and offer potential in AD management.

Vitamin B12 and hippocampal atrophy
Deficiency of vitamin B12 is known to be deleterious to neural structures. Evidence suggests that vitamin supplementation can slow down brain shrinkage in patients with AD. But this evidence is not conclusive and there are studies that have refuted this

International Journal of Neuroscience and Behavior Studies
Volume 1 Issue 1, November 2017
11. Therefore, there is a need to investigate effect of low vitamin B12 and hippocampal volume.

In light of the scattered evidence that hippocampal atrophy in general, or perhaps in AD may be connected to nutritional deficiencies/comorbidities; this association was worth exploring using a radiological biomarker.

Major findings of the present study are as follows: The patients with AD had a significant volume loss of hippocampus compared to cognitively normal elderly. The stratification of AD cases into the risk factors and the comparison of their hippocampal volumes with those patients who did not have the risk factors was done. A statistical comparison of subgroups of AD cases with seizures/depression and diabetes and hypertension together was done with the patients who did not have risk factors (p-value<0.05). Similarly, AD patients with comorbid depression also had a statistically significant difference in their hippocampal volumes compared to those patients without risk factors (p-value<0.05). The groups with other predictors did not have a statistically significant difference compared to reference group (p-value>0.05).

The present study has shown the difference in the hippocampal atrophy among cognitively normal, and those with or without risk factors. The pattern of atrophy among different subgroups is getting attention now. A small study consisting of 58 subjects including cognitively normal, AD and MCI have recently been demonstrated in which the pattern of hippocampal atrophy has been found to be different.53 While the current study has focused on the risk factor approach; other authors have taken difference approach in different types of dementias53-54 and focused on different patterns of hippocampal atrophy in dementias. So the current study highlights the possible roles of lot of factors in causing hippocampal atrophy in AD. The presence of predictors in the present study has potential clinical utility as well. A recent study has shown that predictors such as diabetes, hypertension, and depression in MCI are significant predictors of conversion to AD55. The role of hippocampal volume in studying the subtypes of AD and perhaps the risk factors associated appear to be promising with high discriminate sensitivity56-57.

Despite providing the initial evidence that the presence of seizures, depression, diabetes and hypertension or biochemical alterations in putative risk factors e.g. vitamin-D, B12, serum homocysteine and cortisol in patients with AD can have a deleterious influence on hippocampal volume; the present study has several limitations. The sex matching of the study has not been done as there is a well reported sex bias of reporting of patients with AD/memory impairments. Additionally, this is not a follow up study where the direct effect of predictors/factors could have been obtained. So larger follow up studies could provide better evidence of influence of these comorbidities in AD associated hippocampal atrophy.

**Conclusions**

The present study gives the volumes of hippocampus in patients with AD with or without biochemical and clinical predictors of hippocampal volume loss. Percentage of hippocampal volume loss in AD was almost 50% compared to the healthy individuals. In AD group, there was a correlation between decreasing MMSE score and hippocampal volumes. AD patients with depression/seizures and hypertension and diabetes had statistically significant volume reduction of hippocampus compared to those who did not have these risk factors. Those with hypertension and diabetes together had a significant reduction of hippocampus when present together compared to either of them present alone. None of the biochemical predictors groups had influence on hippocampal volumes in the current study.

**References**


