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

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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 settings5. 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 growing4-5.

Several factors like depression5-6, diabetes7 hypertension8, and seizures9 have been known to be counterproductive to hippocampus in general. Also, their role in patients with dementia has been under investigation10. 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 lacking10. 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-seziures, 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 anticubital 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 ^{0}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-B1214 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)15 was used to divide patients into mild moderate and severe category.
2. Depression was evaluated using Cornell Scale for Depression in Dementia (CSDD)3, 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 hippocampus16. In the current study, hippocampal volume has been measured using 1.5 Tesla Magnetic Resonance (Megnatom 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 paramaters 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 subiculum16.

Hippocampus was delineated using the following anatomical landmarks: In the first slide, coronal section-T1 weighted images where hippocampus is first visualized and in the area bordering amygdale, this was considered to be the most anterior part of the hippocampus. Alveus was used as a landmark to separate amygdale from hippocampus. Precaution was taken not to include part/s of amygdale. Three dimension (3D) viewing images were used to clearly define hippocampal boundaries and confirm the location of hippocampus on MRI console in coronal, axial and sagittal plane. Alevus, was visualized as a band of white matter and used as the border between hippocampus and amygdale. Hippocampal volume was calculated by summing up the area that has been delineated using the manual cursor on both sides of hippocampus. Area thus obtained was multiplied by 0.15 (1mm slice thickness and 0.5 mm inter-slice gap). This gives values in cubic centimeters. The regression analysis between hippocampal volumes and intracranial volumes was done to see the extent to which the variances in hippocampal volumes are explained by head size. An intra-rater and inter-rater reliability was calculated using Cohen's kappa between two raters.

Image processing

All image processing steps were performed as standard protocol16. The borders of the hippocampi were manually traced sequentially with a mouse-driven cursor on each slice from the posterior to anterior till the entire length of hippocampus. On a 2-dimensaional plane, hippocampal anatomic boundaries were defined to include the CA1 to CA4 sectors of the hippocampus proper, the dentate gyrus, and the 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

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

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).

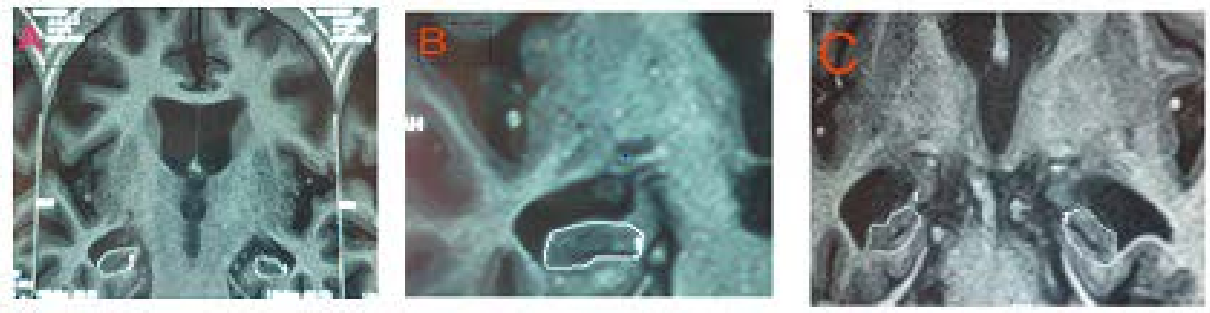

Figure-1: The manual delineation of hippocampal volumes on both right and left side. In the figure-A, 78Y male has mild dementia, while in figure B (80M), moderate and figure C (75F) had severe dementia.

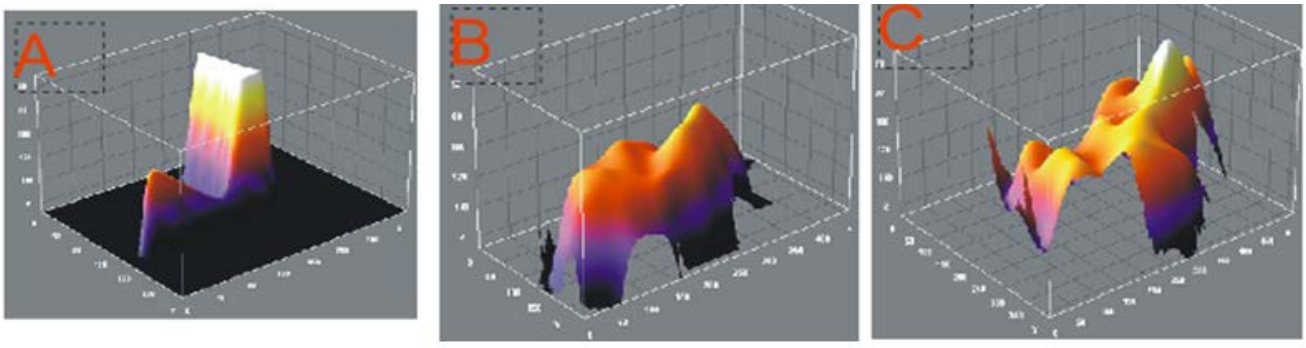

Figure-2: Three Dimensional (3-D) outlines of three hippocampal images (Pseudoimages generated by image-J, National Institute of Health, USA, downloaded free by http://imagej.nih.gov/ij. Figures-A, B & C have been drawn from present patients to show comparison of normal hippocampus (Figure-A) in healthy 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

Cognitively normal individuals (n=30)		AD with risk factors (n=30)		AD without risk factors (n-20)		p-value between groups (ANOVA)
Right	Left	Right	Left	Right	Left	<0.0001
2.73±0.53	2.77±0.6	1.64±0.55	1.59±0.55	1.84±0.5	1.73±0.5	

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

Depression		Seizures		Hypertension		Diabetes	
Left	Right	Left	Right	Right	Left	Left	Right
1.2700±0.29	1.1863±0.32	1.2550±0.38	1.3825±0.40	1.76±0.27	1.70±0.25	1.62±0.04	1.68±0.04

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

Cortisol		Vitamin D3		Serum Homocysteine		Serum B12	
Left	Right	Left	Right	Right	Left	Left	Right
1.59±0.19	1.63±0.22	1.79±0.24	1.76±0.24	1.79±0.21	1.75±0.0.17	1.62±0.04	1.68±0.04

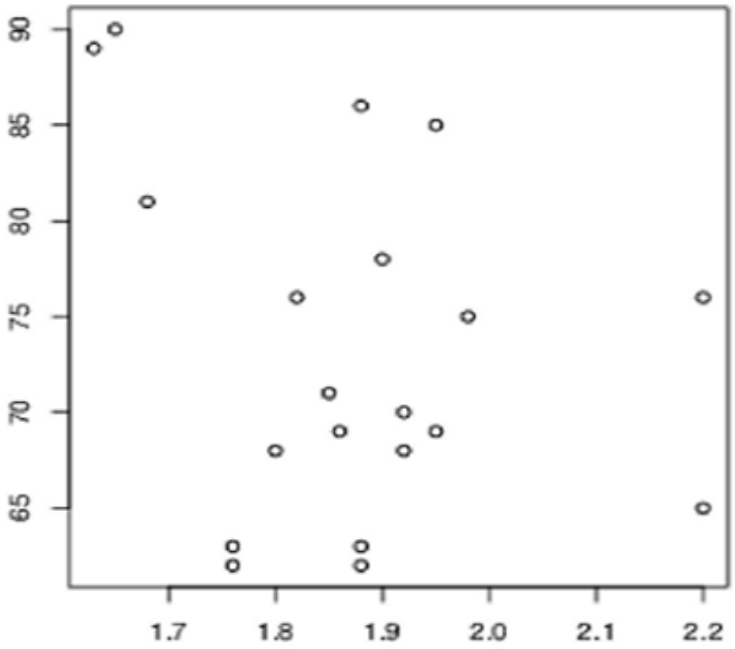

Figure-3: Correlation between the age and hippocampal volumes in the patients without risk factors (Pearson's Correlation Coefficient=-0.29).

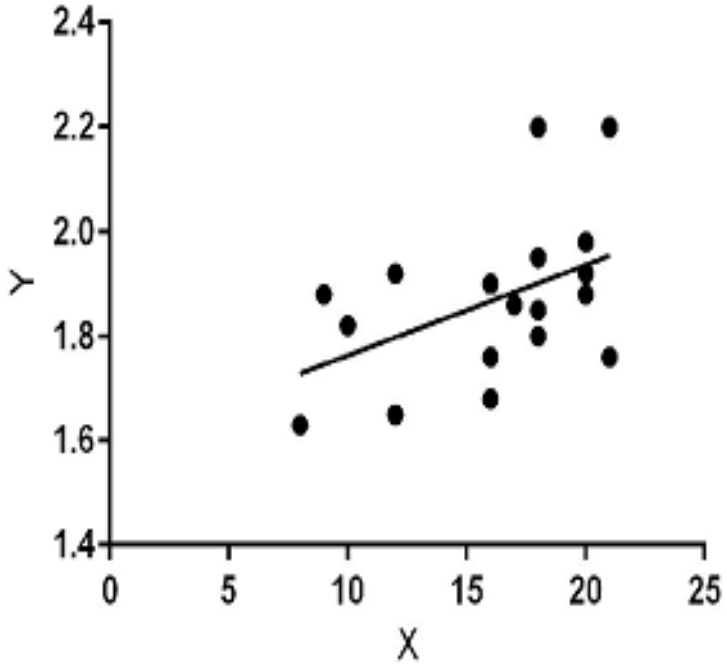

Figure-4: Linear regression between MMSE and hippocampal volume in the patients with AD without risk factors (r2=0.22) showing deviation from the horizontal as significant with a p-value <0.05.

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 one1. Though atrophy of hippocampus is considered primarily a neurodegenerative disease; the role of vascular and other factors in causing hippocampal atrophy has been growing4-5. 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 etc1. 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 memory31-33. 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-degeneration25, 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 elderly28 and presence of diabetes accelerates the rate of cognitive decline in AD patients29. 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 30. Loss of responsiveness to insulin could render neurons more susceptible to neurotoxic insults. As the protective effect of insulin; diminishes apoptosis, neurodegeneration and the resultant38-39 cognitive decline occurs38-40.

Seizures and hippocampal atrophy

New onset epileptic seizures occur in AD and are more common than seen in general population41. These are more common in patients with severe disease42. 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 41.

Depression and hippocampal atrophy

It has been postulated based on animal data that elevated levels of glucocorticoids can damage hippocampus leading to its atrophy42. Hippocampus expresses high number of glucocorticoid receptors2 and is responsive to circulating steroids43. Considering the negative relationship between high levels of stress or elevated cortisol levels and hippocampal atrophy in animals44; high cortisol levels presumably may adversely affect AD patients as well44, 46. Depression has been associated with hippocampal atrophy in young patients with major depressive disorders44. Relevance of depression in causing hippocampal atrophy of dementia of AD is not known45. 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 46.

Hippocampal atrophy and cortisol

Studies suggest that disturbances exist in the central regulation of the hypothalamic-pituitary-adrenocortical axis in advanced AD at multiple levels2, 47-48. Elevated glucocorticoid concentrations, decreased hippocampal volume and frontal atrophy with poor cognitive function have been reported in the elderly but not extensively in AD 41. Plasma cortisol in AD has been recommended as a component of a panel of biochemical markers for AD23, 49.

Homocysteine and hippocampal atrophy

The role of homocysteine in brain atrophy50 associated with AD is not completely understood51. Limited evidence available so far indicates both kinds of outcomes: positive and negative50, 52. Elevated total plasma homocysteine levels are associated with cognitive dysfunction, in which changes in the hippocampus plausibly play a pivotal role51. Patients with AD may have higher plasma homocysteine levels than controls52, but it is uncertain whether higher plasma homocysteine levels are involved in the pathogenesis of the disease24. 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 AD52.

Hippocampal atrophy and vitamin D

AD patients are at risk of nutritional deficiencies because of physiological and psychological factors53. 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 20-21.

Vitamin B12 and hippocampal atrophy

Deficiency of vitamin B12 is known to be deleterious to neural structures17,57. 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

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

1. Dhikav V, Anand K. Potential predictors of hippocampal atrophy in Alzheimer's disease. Drugs Aging. 2011 Jan 1;28(1):1-11. doi: 10.2165/11586390-000000000-00000. Review.

2. Dhikav V, Anand KS. Glucocorticoids may initiate Alzheimer's disease: a potential therapeutic role for mifepristone (RU-486). Med Hypotheses. 2007; 68(5):1088-92.

3. Dhikav V, Sethi M, Anand KS. Medial temporal lobe atrophy in Alzheimer's disease/mild cognitive impairment with depression. Br J Radiol. 2014; 87(1042):20140150.

4. Rathakrishnan BG1, Doraiswamy PM2, Petrella JR3. Science to practice: translating automated brain MRI volumetry in Alzheimer's disease from research to routine diagnostic use in the work-up of dementia. Front Neurol. 2014 9; 4:216.

5. O'Brien JT, Desmond P, Ames D, Schweitzer I, Chiu E, Tress B. Temporal lobe magnetic resonance imaging can differentiate Alzheimer's disease from normal aging, depression, vascular dementia, and other causes of cognitive impairment. Psychol Med. 1997; 27(6):1267–7510.

6. Burton EJ, Barber R, Mukaetova-Ladinska EB, Robson J, Perry RH, Jaros E, et al. Medial temporal lobe atrophy on MRI differentiates Alzheimer's disease from dementia with Lewy bodies and vascular cognitive impairment: a prospective study with pathological verification of diagnosis. Brain 2009; 132(1):195–20310.

7. Dhikav V, Anand KS. Are vascular factors linked to the development of hippocampal atrophy in Alzheimer's disease? J Alzheimers Dis. 2012;32(3):711-8. doi: 10.3233/JAD-2012-120928.

8. Dhikav V, Verma M, Anand KS. Is hypertension a predictor of hippocampal atrophy in Alzheimer's disease? Int Psychogeriatr. 2009 Aug;21(4):795-6. doi: 10.1017/S1041610209009168.

9. Dhikav V, Anand KS. Hippocampal atrophy may be a predictor of seizures in Alzheimer's disease. Med Hypotheses. 2007;69(1):234-5. Epub 2007 Feb 5.

10. Donix M, Scharf M, Marschner K, Werner A, Sauer C, Gerner A, Nees JA, Meyer S, Donix KL, Von Kummer R, Holthoff VA. Cardiovascular risk and hippocampal thickness in Alzheimer's disease. Int J Alzheimers Dis. 2013;2013:108021. doi: 10.1155/2013/108021.

11. Zhang D, Wen X, Wu W, Guo Y, Cui W. Elevated homocysteine level and folate deficiency associated with increased overall risk of carcinogenesis: meta-analysis of 83 case-control studies involving 35,758 individuals. PLoS One. 2015 May 18;10(5):e0123423. doi: 10.1371/journal.pone.0123423. eCollection 2015.

12. Kennedy DM, Selby C, Lawson N. Measurement of urinary free cortisol using the Acs:180 serum cortisol chemiluminescen-

timmunoassay. Ann Clin Biochem. 2000 Jul;37 (Pt 4):520-8.

13. Goswami R, Kochupillai N, Gupta N, Goswami D, Singh N, Dudha A. Presence of 25(OH) D deficiency in a rural North Indian village despite abundant sunshine. J Assoc Physicians India. 2008 Oct;56:755-7.

14. Dhonukshe-Rutten RA, Pluijm SM, de Groot LC, Lips P, Smit JH, van Staveren WA. Homocysteine and vitamin B12 status relate to bone turnover markers, broadband ultrasound attenuation, and fractures in healthy elderly people. J Bone Miner Res. 2005 Jun;20(6):921-9. Epub 2005 Feb 7.

15. Folstein MF, Folstein SE, McHugh PR. "Mini-mental state". A practical method for grading the cognitive state of patients for the clinician. J Psychiatr Res. 1975 Nov;12(3):189-98.

16. Anand KS, Dhikav V, Doraswamy S, Garga UC Issues with normative data of hippocampal volumetry in Indian population. Ann Indian Acad Neurol. 2015 Apr-Jun;18(2):259. doi: 10.4103/0972-2327.150607.

17. Dolek N, Saylisoy S, Ozbabalik D, Adapinar B. Comparison of hippocampal volume measured using magnetic resonance imaging in Alzheimer's disease, vascular dementia, mild cognitive impairment and pseudodementia. J Int Med Res. 2012;40(2):717-25.

18. Sun Y, Lu CJ, Chien KL, Chen ST, Chen RC. Efficacy of multivitamin supplementation containing vitamins B6 and B12and folic acid as adjunctive treatment with a cholinesterase inhibitor in Alzheimer's disease: a 26-week, randomized, double-blind, placebo-controlled study in Taiwanese patients. Clin Ther. 2007;29(10):2204-14.

19. Douaud G, Refsum H, de Jager CA, Jacoby R, Nichols TE, Smith SM, Smith AD. Preventing Alzheimer's disease-related gray matter atrophy by B-vitamin treatment. Proc Natl Acad Sci U S A. 2013;110(23):9523-8.

20. Annweiler C, Llewellyn DJ, Beauchet O. J Alzheimers Dis. 2013;33(3):659-74. doi: 10.3233/JAD-2012-121432. Low serum vitamin D concentrations in Alzheimer's disease: a systematic review and meta-analysis. J Alzheimers Dis. 2013;33(3):659-74. doi: 10.3233/JAD-2012-121432.

21. Annweiler C, Montero-Odasso M, Llewellyn DJ, Richard-Devantoy S, Duque G, Beauchet O. Meta-analysis of memory and executive dysfunctions in relation to vitamin D. J Alzheimers Dis. 2013 Jan 1;37(1):147-71. doi: 10.3233/JAD-130452.

22. Aszalós Z. [Cerebral complications of diabetes mellitus] Orv Hetil. 2007 16;148(50):2371-6.

23. Zvěřová M, Fišar Z, Jirák R, Kitzlerová E, Hroudová J, Raboch J. Plasma cortisol in Alzheimer's disease with or without depressive symptoms. Med Sci Monit. 2013;19:681-9.

24. Gallucci M, Zanardo A, Bendini M, Di Paola F, Boldrini P, Grossi E. Serum Folate, Homocysteine, Brain Atrophy, and Auto-CM System: The Treviso Dementia (TREDEM) Study. J Alzheimers Dis. 2013 Sep 12. [Epub ahead of print]

25. Dolek N, Saylisoy S, Ozbabalik D, Adapinar B. Comparison of hippocampal volume measured using magnetic resonance imaging in Alzheimer's disease, vascular dementia, mild cognitive impairment and pseudodementia. J Int Med Res. 2012;40(2):717-25.

26. Vijayakumar A, and Vijayakumar A. Comparison of Hippocampal Volume in Dementia Subtypes. ISRN Radiology 2013. http://dx.doi.org/10.5402/2013/174524.

27. Teipel SJ, Meindl T, Grinberg L, Heinsen H, Hampel H. Novel MRI techniques in the assessment of dementia. Eur J Nucl Med Mol Imaging. 2008;35 Suppl 1:S58-69.

28. Teipel SJ, Grothe M, Lista S, Toschi N, Garaci FG, Hampel H. Relevance of magnetic resonance imaging for early detection and diagnosis of Alzheimer disease. Med Clin North Am.

29. den Heijer T, Vermeer SE, van Dijk EJ, Prins ND, Koudstaal PJ, Hofman A, Breteler MM. Type 2 diabetes and atrophy of medial temporal lobe structures on brain MRI. Diabetologia. 2003;46(12):1604-10.

30. den Heijer T, Vermeer SE, Clarke R, Oudkerk M, Koudstaal PJ, Hofman A, Breteler MM. Homocysteine and brain atrophy on MRI of non-demented elderly. Brain. 2003;126(Pt 1):170-5.

31. den Heijer T, Launer LJ, Prins ND, van Dijk EJ, Vermeer SE, Hofman A, Koudstaal PJ, Breteler MM. Association between blood pressure, white matter lesions, and atrophy of the medial temporal lobe. Neurology. 2005;64(2):263-7.

32. den Heijer T, van der Lijn F, Koudstaal PJ, Hofman A, van der Lugt A, Krestin GP, Niessen WJ, Breteler MM. A 10-year follow-up of hippocampal volume on magnetic resonance imaging in early dementia and cognitive decline. Brain. 2010;133(Pt 4):1163-72.

33. Henneman WJ, Sluimer JD, Barnes J, van der Flier WM, Sluimer IC, Fox NC, Scheltens P, Vrenken H, Barkhof F. Hippocampal atrophy rates in Alzheimer disease: added value over whole brain volume measures. Neurology. 2009;72(11):999-1007.

34. Maestú F, Arrazola J, Fernández A, Simos PG, Amo C, Gil-Gregorio P, Fernandez S, Papanicolaou A, Ortiz T. Do cognitive patterns of brain magnetic activity correlate with hippocampal atrophy in Alzheimer's disease? J Neurol Neurosurg Psychiatry. 2003;74(2):208-12.

35. de Leeuw FE, Barkhof F, Scheltens P. Progression of cerebral white matter lesions in Alzheimer's disease: a new window for therapy? J Neurol Neurosurg Psychiatry. 2005;76(9):1286-8.

36. Korf ES, Scheltens P, Barkhof F, de Leeuw FE. Blood pressure, white matter lesions and medial temporal lobe atrophy: closing the gap between vascular pathology and Alzheimer's disease? Dement Geriatr Cogn Disord. 2005;20(6):331-7.

37. Korf ES, van Straaten EC, de Leeuw FE, van der Flier WM, Barkhof F, Pantoni L, Basile AM, Inzitari D, Erkinjuntti T, Wahlund LO, Rostrup E, Schmidt R, Fazekas F, Scheltens P. Diabetes mellitus, hypertension and medial temporal lobe atrophy: the LADIS study. Diabet Med. 2007;24(2):166-71.

38. Ylikoski R, Jokinen H, Andersen P, Salonen O, Madureira S, Ferro J, Barkhof F, van der Flier W, Schmidt R, Fazekas F,Scheltens P, Waldemar G, Salvadori E, Pantoni L, Inzitari D, Erkinjuntti

T; LADIS Study Group. Comparison of the Alzheimer's Disease Assessment Scale Cognitive Subscale and the Vascular Dementia Assessment Scale in differentiating elderly individuals with different degrees of white matter changes. The LADIS Study. Dement Geriatr Cogn Disord. 2007;24(2):73-81.

39. de la Monte SM, Wands JR. Alzheimer's disease is type 3 diabetes-evidence reviewed. J Diabetes Sci Technol. 2008;2(6):1101-13.

40. Qiu WQ, Folstein MF. Insulin, insulin-degrading enzyme and amyloid-beta peptide in Alzheimer's disease: review and hypothesis. Neurobiol Aging. 2006;27(2):190-8.

41. Pearlson GD, Harris GJ, Powers RE, Barta PE, Camargo EE, Chase GA, Noga JT, Tune LE. Quantitative changes in mesial temporal volume, regional cerebral blood flow, and cognition in Alzheimer's disease. Arch Gen Psychiatry. 1992;49(5):402-8.

42. Larner AJ. Epileptic Seizures in AD Patients. Neuromolecular Med. 2009 Jun [Epub ahead of print]

43. Sousa N, Cerqueira JJ, Almeida OF. Corticosteroid receptors and neuroplasticity. Brain Res Rev. 2008;57(2):561-70.

44. Sheline YI, Wang PW, Gado MH, Csernansky JG, Vannier MW. Hippocampal atrophy in recurrent major depression. Proc Natl Acad Sci U S A. 1996;93(9):3908-13.

45. Bierman EJ, Comijs HC, Jonker C, Scheltens P, Beekman AT. The effect of anxiety and depression on decline of memory function in Alzheimer's disease. Int Psychogeriatr. 2009;21(6):1142-7..

46. Panza F, D'Introno A, Colacicco AM, Capurso C, Del Parigi A, Caselli RJ, Frisardi V, Scapicchio P, Chiloiro R, Scafato E, Gandin C, Vendemiale G, Capurso A, Solfrizzi V. Temporal Relationship between Depressive Symptoms and Cognitive Impairment: The Italian Longitudinal Study on Aging. J Alzheimers Dis. 2009 [Epub ahead of print]

47. Elgh E, Lindqvist Astot A, Fagerlund M, Eriksson S, Olsson T, Näsman B. Cognitive dysfunction, hippocampal atrophy and glucocorticoid feedback in Alzheimer's disease. Biol Psychiatry. 2006 Jan 15;59(2):155-61.

48. Elgh E, Lindqvist Astot A, Fagerlund M, Eriksson S, Olsson T, Näsman B. Cognitive dysfunction, hippocampal atrophy and glucocorticoid feedback in Alzheimer's disease. Biol Psychiatry. 2006;59(2):155-61.

49. Huang CW, Lui CC, Chang WN, Lu CH, Wang YL, Chang CC. Elevated basal cortisol level predicts lower hippocampal volume and cognitive decline in Alzheimer's disease. J Clin Neurosci. 2009;16(10):1283-6.

50. Morra JH, Tu Z, Apostolova LG, Green AE, Avedissian C, Madsen SK, Parikshak N, Hua X, Toga AW, Jack CR Jr, Schuff N, Weiner MW, Thompson PM; Alzheimer's Disease Neuroimaging Initiative. Automated 3D mapping of hippocampal atrophy and its clinical correlates in 400 subjects with Alzheimer's disease, mild cognitive impairment, and elderly controls. Hum Brain Mapp. 2009;30(9):2766-88.

51. Siuda J, Gorzkowska A, Patalong-Ogiewa M, Krzystanek E, Czech E, Wiechuła B, Garczorz W, Danch A,Jasińska-Myga B, Opala G. From mild cognitive impairment to Alzheimer's disease - influence of homocysteine, vitamin B12 and folate on cognition over time: results from one-year follow-up. Neurol Neurochir Pol. 2009;43(4):321-9.

52. Shimomura T, Anan F, Masaki T, Umeno Y, Eshima N, Saikawa T, Yoshimatsu H, Fujiki M, Kobayashi H. Homocysteine levels are associated with hippocampus volume in type 2 diabetic patients. Eur J Clin Invest. 2011;41(7):751-8.

53. Joko T, Washizuka S, Sasayama D, Inuzuka S, Ogihara T, Yasaki T, Hagiwara T, Sugiyama N, Takahashi T, Kaneko T, Hanihara T, Amano N. Patterns of hippocampal atrophy differ among Alzheimer's disease, amnestic mild cognitive impairment, and late-life depression. Psychogeriatrics. 2016 Jan 12. doi: 10.1111/psyg.12176. [Epub ahead of print]

54. Ossenkoppele R, Cohn-Sheehy BI, La Joie R, Vogel JW, Möller C, Lehmann M, van Berckel BN, Seeley WW, Pijnenburg YA, Gorno-Tempini ML, Kramer JH, Barkhof F, Rosen HJ, van der Flier WM, Jagust WJ, Miller BL, Scheltens P, Rabinovici GD. Atrophy patterns in early clinical stages across distinct phenotypes of Alzheimer's disease. Hum Brain Mapp. 2015 Nov;36(11):4421-37. doi: 10.1002/hbm.22927. Epub 2015 Aug 11.

55. Li JQ, Tan L, Wang HF, Tan MS, Tan L, Xu W, Zhao QF, Wang J, Jiang T, Yu JT. Risk factors for predicting progression from mild cognitive impairment to Alzheimer's disease: a systematic review and meta-analysis of cohort studies. J Neurol Neurosurg Psychiatry. 2015 May 22. pii: jnnp-2014-310095. doi: 10.1136/jnnp-2014-310095. [Epub ahead of print]

56. Clerx L, van Rossum IA, Burns L, Knol DL, Scheltens P, Verhey F, Aalten P, Lapuerta P, van de Pol L, van Schijndel R, de Jong R, Barkhof F, Wolz R, Rueckert D, Bocchetta M, Tsolaki M, Nobili F, Wahlund LO, Minthon L, Frölich L, Hampel H, Soininen H, Visser PJ. Measurements of medial temporal lobe atrophy for prediction of Alzheimer's disease in subjects with mild cognitive impairment. Neurobiol Aging. 2013 Aug;34(8):2003-13. doi: 10.1016/j.neurobiolaging.2013.02.002. Epub 2013 Mar 27.

57. van de Pol LA, van der Flier WM, Korf ES, Fox NC, Barkhof F, Scheltens P. Baseline predictors of rates of hippocampal atrophy in mild cognitive impairment. Neurology. 2007 Oct 9;69(15):1491-7.